

Genomic regions associated with grain yield under drought stress in wheat (*Triticum aestivum* L.)

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Abstract Water deficit stress (WDS) is a serious constraint to wheat productivity in rain-fed and limited irrigation environments. Identifying genomic regions responsible for grain yield (GY) under WDS will aid in understanding the genetics of drought tolerance (DT) and development of DT cultivars. A population of 206 recombinant inbred lines derived from WL711/C306 was phenotyped for GY and related traits under water deficit and irrigated conditions in seven different environments to identify genomic regions associated with eleven yield related traits. Both the parents contributed positive alleles for the traits studied. A novel genomic region for GY under WDS, *qGYWD.3B.1* was detected on chromosome 3BS of wheat. The yield enhancing allele under drought stress

at this locus was contributed by DT parent C306. This genomic region explained 18.7 % of phenotypic variation for GY under WDS and co-located with genomic regions for GY components. Another novel, consistent genomic region for GY under WDS, *qGYWD.3B.2* explained 19.6 % of phenotypic variation with positive allele coming from drought susceptible parent WL711. A novel genomic region for drought susceptibility index for GY, *qDSIGY.4A.1* was consistently detected in six of seven environments explaining 15.6 % of phenotypic variation. Other important genomic regions for GY and biomass under WDS were mapped on chromosomes 7BL and 6AS, respectively. Fine mapping of the major QTLs on chromosome 3BS will enable identification of robust markers and candidate genes for marker-assisted breeding for DT in wheat.

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Introduction

Water deficit is a major environmental factor limiting wheat productivity in many parts of the world (Araus et al. 2008). Moreover, drought conditions are expected to worsen because of diminishing water availability and foreseen global climatic changes (Collins et al. 2008; Reynolds and Ortiz 2010). In India, wheat is grown under rain-fed conditions with little access to irrigation in the Central and Peninsular regions which accounts for ~30 % of the total production (Wasson et al. 2012). Breeding for DT is a major aim of many wheat breeding programs around the world and requires identification of germplasm having sustained yield under water deficit environments.

GY is the most important agronomic trait in wheat improvement. Being the final product of many biochemical and physiological processes, it is directly determined by yield component traits and indirectly affected by growth and development traits like phenology and plant architecture. Wheat grain yields have increased substantially since the 1950s (Slafer et al. 1994). The genetic gains as a result of wheat breeding efforts by incorporating dwarfing genes *Rht1* and *Rht2* have been spectacular for irrigated environments.

Breeding for yield improvement in wheat has traditionally relied upon empirical selection methods. However, conventional breeding was generally more effective in achieving yield gains under irrigated rather than WDS environments (Blum 2011). Slower progress in wheat breeding under WDS environments is usually ascribed to the unpredictable and highly variable nature of drought. Selection efficiency for yield under drought is reduced due to significant genotype (G) × environment (E) interactions. However, progress in breeding DT wheat varieties has been rather slow. For instance, C306 a wheat variety released for commercial cultivation in India in 1969 is still grown and considered to be one of the best sources of DT (Sinha et al. 1986; Joshi et al. 2007). C306 is widely adapted to North Eastern, North Western and Central regions of India.

The advent of molecular markers is enabling accumulation of knowledge on the genetics of yield for their use in molecular breeding of crops (Lopes et al. 2013). Molecular marker have several advantages over phenotypic selection as they are easier to score and can be scored at earlier stage, are less affected by environmental factors and may provide

estimates for gene action controlling traits of interest. Development of high density molecular linkage map in conjunction with precise phenotyping provides a tool for dissecting the genetic basis of such complex traits into component quantitative trait loci (QTLs). The identified stable markers can be used in breeding and pre-breeding to increase the selection efficiency for traits that are difficult and costly to phenotype (Campbell et al. 2003).

QTL analyses has identified several chromosomal regions associated with GY under WDS environment in wheat (Quarrie et al. 2005, 2006; Kirigwi et al. 2007; Maccaferri et al. 2008; Peleg et al. 2009; McIntyre et al. 2010; Pinto et al. 2010; Kadam et al. 2012; Bennett et al. 2012a, b; Wu et al. 2012; Alexander et al. 2012). Until now none of the major genomic regions for GY and its component traits under WDS conditions have been cloned in wheat because sequence information for the hexaploid wheat genome has only recently begun to emerge and gene annotation is underway (The International wheat genome sequencing consortium 2012). However, recently genomic region for GY under drought has been identified across studies on chromosome 3BS of wheat (Maccaferri et al. 2008; Campbell et al. 2003; Borner et al. 2002) has been fine mapped and efforts are being made to characterize the candidate genes underlying this genomic region (Graziani et al. 2013, Unpublished). Given the paucity of molecular-genetic knowledge in wheat, some researchers have taken advantage of information of genetic basis of candidate genes important for mechanism of GY formation in rice through synteny (Rustgi et al. 2013).

Wheat cultivars WL711 and C306 were shown to be DS and DT respectively in an earlier study involving *Triticum aestivum*, *T. durum* and *Triticale* varieties (Sinha et al. 1986). These two varieties were crossed in order to obtain a RIL population comprising of 206 RILs (Patil and Khanna-Chopra 2006). Cultivar C306 has several drought adaptive traits such as the ability to maintain better water relations, deep root system and capacity for pre-anthesis assimilate mobilization during post-anthesis phase under WDS conditions (Aggarwal and Sinha 1984; Khanna-Chopra and Selote 2007; Kadam et al. 2012). In the present study we evaluated GY and related traits in the WL711/C306 wheat RIL mapping population under irrigated (IRR) and WDS treatment during grain filling stage under field conditions at three locations and five

seasons in Indian environment. The study was aimed to identify genomic regions associated with GY and yield components under WDS environment.

Materials and methods

Plant material

A mapping population of 206 RILs (F_9 – F_{13}) used in this study was developed from a cross between high-yielding, semi-dwarf, DS wheat variety WL711 ((S308/Chris)/Kalyansona) and a traditional tall, medium-yielding and DT variety C306 (RGB/CSL3//2/C591/3/C217/N14//C281) (Sinha et al. 1986).

Environments

Field trials were conducted at Water Technology Centre, I.A.R.I, New Delhi (77°12'E, 28°40'N; 228.6 M.S.L), Directorate of Wheat Research (DWR), Karnal (76°09'E, 29°60'N; 228.6 M.S.L) and National Research Centre on Soybean (NRCS) Indore (75°50'E, 22°43'N; 529.9 M.S.L). These regions are geographically located within traditionally intensive wheat agroecosystems. From 2007–2011, the two parents and 206 RILs were grown at Delhi. In the 2007–2008 and 2008–2009 wheat growing seasons, the parents and the RILs were also grown at Karnal and Indore, respectively, following a similar layout to Delhi. At Delhi and Karnal, sowing was done in mid-November and harvesting in April while at Indore, sowing was done in early November and harvesting in early March.

Soil characteristics

The soil at Delhi is sandy loam with average bulk density of 1.55 g cm³. The water retention capacity is 18 % at field capacity (−0.02 MPa) and 4.5 % at permanent wilting point (−1.5 Mpa) on w/w basis (Sinha et al. 1986). The contribution of ground water through capillary action is almost negligible because the depth of water table is 4 m. The soil type at Karnal is similar to that at Delhi. The soil at Indore is black clay with average bulk density of 1.45 g cm³. The water retention capacity is 27 % at field capacity and 6.0 % at permanent wilting point on w/w basis and hence requires less irrigation because of its high capacity for moisture retention.

Experimental layout and phenotypic evaluation

Before planting the fields were laser levelled at Delhi and Karnal. In all the three environments, experiments were sown in randomized complete block design (RCBD) pattern. There were two blocks, one for irrigated and other for limited irrigation, with two replicates per treatment. Plot size and seed rate were similar across all experiments at Delhi, Karnal and Indore. Plot size was 2 × 0.5 m for each genotype and the plant density was 200 plants per m². Sowing of the RILs was manually randomized within the replicates at all locations. Pre-sowing irrigation was applied to obtain sufficient soil moisture for germination. Two irrigation regimes were used: full irrigation and limited irrigation leading to WDS. At Delhi and Karnal, irrigation was given at maximum tillering, jointing, anthesis and grain filling stages to the IRR treatment while WDS treatment received only two irrigations at maximum tillering and anthesis stages. At Indore, IRR treatment received two irrigations at anthesis and grain filling stages while WDS treatment received single irrigation at anthesis. During the crop season ~ 360, ~ 370 and ~ 380 mm of total soil water was available in the IRR treatment while ~ 280, ~ 290 and ~ 340 mm of total soil water was available in WDS treatment at 1.2 m depth in Delhi, Karnal and Indore trials, respectively. In all experiments, plots were fertilized with 100:50:50, N:P:K. To avoid rust infestation, foliar spray of 0.2 % solution of fungicide Captan (*N*-trichloromethylthio ~ cyclohexene-1,2-dicarboximide) was done at regular intervals at all locations. Similarly, insecticide Imidacloprid was sprayed at regular intervals at all locations to avoid the attack of aphids. To control termites, a 2 % solution of insecticide Chloropyrifos (20 % EC) mixed with soil was applied to the fields before irrigation. Hence, appropriate measures were taken to keep the crop free from biotic stresses.

Anthesis date was recorded when 50 % of the spikes had anthers extruded. Plant height (PHT) was measured from the base of the plant to the tip of the spike at mid-grain fill stage. GY (gm⁻²) and biomass (BM; gm⁻²) were measured by manually harvesting each plot. Random 500 grains were counted, their weight was recorded and converted to 1,000-grain weight (TGW). In the two trials at Delhi (2007–2008 and 2011–2012), plants from 50 cm length were hand harvested at maturity and these samples were used for the recording of spike number m⁻² (SPN) and tiller number m⁻² (TN). Harvest index (HI) was calculated by the formula: HI = (GY/

BM) \times 100. Number of grains per meter square (GN) was obtained by dividing GY by TGW and multiplied with 1,000. DSI and stress intensity (D) was calculated using the formula of Fischer and Maurer (1978).

$$DSI = (1 - Y/Y_p)/D$$

where, Y is the Mean yield or yield component of a RIL under WDS, Y_p is the Mean yield or yield components under IRR, D is the Stress intensity

$$D = 1 - X/X_p$$

where, X is the Mean Y of all RILs, X_p is the Mean Y_p of all RILs.

DSI < 1 indicates drought tolerance/resistance while DSI > 1 indicates drought susceptible.

Canopy temperature (CT) was measured by infrared thermometer (Model AG42, Tela Temp. Corp, Fullerton, CA) in the 2008–2009 and 2011–2012 trials at Delhi at 15 days after anthesis (DAA) in the afternoon between 1,200 and 1,400 h. Relative water content (RWC) was measured in flag leaves at 15 DAA under both IRR and WDS treatments in 2007–2008 and 2011–2012 trials following the procedure of Barrs and Weatherley (1962).

Statistical analysis

Analysis of variance (ANOVA) was conducted for all traits separately for estimating variance components for evaluation of the significance of genotype, treatment and trial effects and their interactions in the WL711/C306 RIL population. ANOVA was done using three factor factorial analysis of the statistical programme MSTAT-C, version 1.41, Michigan State University, USA. Correlation analysis was performed using MSTAT-C. Broad sense heritability (h_B^2) value was calculated for each trait across environment as

$$h_B^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_{gxe}^2/n),$$

where n is the number of environments,

$$\sigma_g^2 = (MS_{RIL} - MS_{RILxe})/n$$

$$\sigma_{gxe}^2 = MS_{RILxe} \text{ and } MS \text{ is the mean square.}$$

Construction of framework linkage map and QTL mapping

Seeds of parents and 206 RILs were germinated in petri-dishes using germination paper and were grown

at 25 °C in BOD incubator. Fifteen days old etiolated seedlings were collected, immediately frozen with liquid N₂ and ground to a fine powder using sterilized mortar and pestle. DNA was extracted using CTAB method (Murray and Thompson 1980). Total 1,200 additional simple sequence repeats (SSR) markers of the *barc*, *cfa*, *cfb*, *cfp*, *gpw* and *psp* series and 53 expressed sequence tag (EST)-sequence-tagged sites (STS) including markers of *cwem*, *mag*, *cnl* series as described at the GrainGenes website (www.wheat.pw.usda.gov) were tested for polymorphism between the two parents to enrich the existing genetic linkage map of WL711/C306 RIL population having 173 markers covering a total map distance of 3,720.2 cM (Kadam et al. 2012). PCR reactions for SSR markers were performed using GeneAmp PCR system (Applied Biosystems, Foster city, CA, USA) and separated by electrophoresis in 4 % metaphor agarose gel using 1X TBE buffer and those found monomorphic were separated in 10 % polyacrylamide gel for higher resolution. Genotyping of the 206 RILs along with the parents was done using the polymorphic markers and molecular linkage map was constructed using Mapmaker/EXP 3.0 software (Lincoln et al. 1992). Chi squared tests were performed to test the segregation of markers for 1:1 segregation ratios. Kosambi function was used to convert recombination frequencies into centi Morgan (cM) values (Kosambi 1944). Markers were assigned to individual wheat chromosomes based on the microsatellite consensus map (Somers et al. 2004) and the composite wheat map (www.wheat.pw.usda.gov) by giving command “group” with LOD score 3.0, and then “order” to develop the linkage map.

QTL analysis was done using Windows QTL Cartographer version 2.5 (Bioinformatics Research Centre, North Carolina State University, USA). QTL analysis for each trait was carried out for each of the seven trials i.e. five trials at Delhi (DL07, DL08, DL09, DL10, DL11) and one each at Karnal (KL07) and Indore (IN08) separately for WDS and IRR treatments. QTLs were identified via Composite interval mapping (CIM) using forward and backward stepwise regression with window size set at 10 cM and a walk speed of 2.0 cM. The LOD score for significance of QTL was determined by 1,000 permutations and so varied for each trait (Alexander et al. 2012). Any QTL identified in at least two of the seven environments (~ 30 %) studied was considered a consistent QTL (Bogard et al. 2011). The proportion of phenotypic variation (PV) explained by a QTL was

estimated as the coefficient of determination (R^2) using single-factor analysis from a general linear model procedure. Multiple-trait composite interval mapping (MCIM) was conducted for the detection of QTL \times environment (QE) interactions at the main chromosome regions affecting target traits (Jiang and Zeng 1995). The QE interaction was tested using a likelihood-ratio statistic developed for the null hypothesis that $a_1 = a_2 = \dots = a_j$, where a_j is the additive effect of a QTL in the j th environment and implemented in WinQTLCart. The significance of this statistics for few chromosomal regions harboring major QTL was obtained utilizing the $X^2_{(j-1)}$ distribution. Two-locus analysis that identifies epistatic QTL (QQ) and QTL \times QTL \times environment (QQE) interactions was conducted using QTLNetwork Version 2.0.

Wheat-rice comparison

To identify candidate genes and additional markers underlying QTL of interest wheat chromosome 3B-specific sequences were compared with the rice genomic DNA sequences available at various websites (<http://wheat.pw.usda.gov>, <http://ncbi.nlm.nih.gov>, <http://gramene.org/> and <http://www.tigr.org>). The DNA sequence comparisons were made using BLASTn option at an e-value $< 1 \times 10^{-19}$ and score > 100 . Initially, chromosome 3B specific sequences were compared to rice chromosomes 1. BAC/PAC sequences were used to demarcate collinear regions underlying specific QTLs on the rice chromosome. The identified rice BAC/PAC sequences were then compared to wheat BAC end sequences (BES) in order to identify additional markers for each QTL interval. The identified rice BAC/PAC sequences underlying the QTL were manually curated to identify candidate genes. Protein sequences of the identified genes were compared against wheat ESTs using tBLASTn searches to identify wheat orthologues of the candidate genes (e-value $< 1 \times 10^{-25}$).

Results

Effect of water deficit stress on agronomic and physiological traits in WL711/C306 RILs

Mean GY in each of the three environments, total irrigation and ranges for maximum and minimum

temperature, evapo-transpiration and solar radiation during the growing season are given in Table 1. Total rainfall was 30.1 mm during the entire crop season and evaporative demand was averaged 2.8 mm per day across trials. Maximum rainfall occurred in the month of December and January and there was meager rainfall in the month of March and April, during grain filling to maturity. Hence the crop relied on stored soil moisture during the crop season. Total solar radiation was averaged $5.20 \text{ MJ m}^{-2} \text{ day}^{-1}$ across trials and it increased coincidentally in the warmer months of March and April, resulting in increased evaporative demand during grain filling for all trials. In Indore, solar radiation was higher and the wheat crop experienced 2.6 and 1.6 °C higher T_{max} and T_{min} compared to Delhi and Karnal during the crop growth period resulting in slightly higher evapo-transpiration. Mean GY of the RIL population was higher in Indore followed by Delhi and Karnal under both IRR and WDS (Table 1).

WL711 had higher GY, GN and HI than C306 while C306 had higher TGW under IRR across trials (Table 2). C306 was less affected by WDS than WL711, GY reduction being 18 and 40 % respectively. Further, WL711 showed higher reduction in BM, GN, TGW and HI than C306 under WDS. In C306, HI increased under WDS across trials indicating more efficient translocation of the photosynthates from the vegetative parts to the grains. Average GY for the RILs under IRR and WDS was 518.6 and 359.0 gm^{-2} , respectively across trials, the average reduction in GY being 30.8 %. The RIL population showed considerable variation and normal distribution for all traits under IRR and WDS across trials (Table 2, Supplementary Figs. 1, 2). The RIL population showed transgressive segregation for all traits with 23 of the RILs combining yield stability with yield higher than C306. GY reduction of the RIL population across trials varied from 14.7 to 48.5 % under WDS. In the RILs grown under WDS, reduction in GN (29.9 %) was more than TGW (5.5 %) and also BM (24.1 %) was affected more than HI (4.1 %).

C306 is a tall (129 ± 3 cm) while WL711 is a semi-dwarf (102 ± 4 cm) cultivar under IRR conditions (Table 2). The RIL population showed considerable variation and transgressive segregation for PHT, the range being 87–133 cm and 79–124 cm under IRR and WDS respectively. High yielding variety WL711 flowered earlier than C306 both under

Table 1 Mean grain yield (gm^{-2}) of the WL711/C306 RIL population and environmental parameters for the trials in Delhi (2007–2011), Karnal (2007–2008) and Indore (2008–2009)

Trial	T max (°C)	T min (°C)	Rain (mm)	Solar radiation ($\text{M Jm}^{-2} \text{ day}^{-1}$)	Evaporation (mm day^{-1})	Irrigation received (mm) (IRR)	Mean grain yield (IRR)	Irrigation received (mm) (WDS)	Mean grain yield (WDS)
Delhi (2007–2011)	16.2–33.9	4.1–19.1	23.6–34.2	4.37	2.9	160	530.9 \pm 37.9	80	358.9 \pm 24.8
Karnal (2007–08)	17.5–34.4	3.9–17.3	34.1	4.28	2.5	160	404.0 \pm 43.5	80	317.0 \pm 29.6
Indore (2008–2009)	19.6–41.5	7.6–21.2	27.5	5.45	3.0	80	569.0 \pm 16.3	40	402.7 \pm 7.3

All data averaged daily measurements at Delhi, Karnal and Indore

Table 2 Means of agronomic traits studied in the parents and the WL711/C306 RILs under irrigated and water deficit stress treatments across trials in Delhi (2007–2011), Karnal (2007–2008) and Indore (2008–2009)

Traits	IRR					WDS				
	WL711	C306	Population mean	SD	Population range	WL711	C306	Population mean	SD	Population range
GY	671.3	430.8	518.6	103.9	341.8–733.6	402.6	353.2	359	66.9	218.7–537.8
BM	1,796	1,613	1,706	286	1,423–2,217	1,332	1,403	1,295	185	1,068–1,737
GN	19,323	11,120	14,270	2,147	8,863–21,238	12,591	9,316	10,010	1,856	7,523–16,412
TGW	34.3	39.5	36.1	4.0	26.3–46.0	28.2	38.9	34.1	4.9	22.7–41.0
TN	569	650	481	40	327–694	377	562	377	28	217–594
SPN	495	575	455	34	273–620	312	465	325	21	186–564
HI	37.6	25.9	30.12	7.3	25.0–40.4	29	26.3	28.9	6.5	18.56–42.94
PHT	102	129	115	6	87–133	96	121	106	4	79–124
DTF ^a	89	98	91	4	81–100	83	95	87	5	77–97
CT ^b	25.3	24.4	26.1	0.8	22.8–27.5	29.6	26.4	28.1	0.3	25.0–30.7
RWC ^c	85.6	81.6	82.4	1.4	76.8–88.9	71.6	77.8	74.2	0.9	67.3–80.9

All data averaged over years evaluated at Delhi, Karnal and Indore

Air temperature range during measurements was 27.0–30.0 and 26.9–29.9 °C in 2008–2009 and 2011–2012 trials respectively at Delhi

GY grain yield (gm^{-2}), BM biomass (gm^{-2}), GN grain number (m^{-2}), TGW 1,000 grain wt (g), TN tiller no. per m^2 , SPN spike no. per m^2 , HI harvest index (%), PHT plant height (cm), DTF days to flowering, CT canopy temperature (°C), RWC relative water content (%)

^a Data averaged over years evaluated at Delhi 2007–2011

^{b,c} Data averaged at Delhi for two years

IRR and WDS. Earlier flowering was observed under WDS compared to IRR in both the parents and the RIL population. The RIL population flowered between 81 and 100 days after sowing (DAS) and 77–97 DAS under IRR and WDS respectively.

A combined ANOVA was performed over all trials and treatments which indicated statistically significant main effects for genotypes (G), trials (T), treatments

(IRR and WDS), GxT and GxE interactions for different GY and related traits (Table 3). Differences between the parents were statistically significant under IRR condition. The relative magnitudes of variance due to G and GxE interaction varied among traits. Variance due to GxT interaction was substantially lower than variation due to genotype for all the traits except HI where the magnitudes were almost equal,

suggesting that the phenotypic variance for a particular trait was attributable to both genotype and the treatment effects. GN showed highest coefficient of variation (CV) while lowest CV was observed for days to flowering (DTF) across trials. Broad sense heritability was higher under IRR as compared to WDS for all traits except TGW. Under WDS, heritability was high (>0.70) for TGW, DTF, moderately high (0.40 – 0.70) for GY, GN, HI, BM, PHT, SPN and low (<0.40) for TN.

In this study DSI (yield) was used to characterize the parents and RILs for their drought response. $DSI < 1$ indicates drought tolerance/resistance while $DSI > 1$ indicates drought susceptible. As observed earlier, the parental line C306 was DT while WL711 was DS across all the trials (Table 4). The GY stability of C306 was due to relative stability of all the yield component traits. The RIL population showed considerable variation and transgressive segregation for DSI for GY and yield components.

Cultivar C306 maintained higher RWC and cooler canopy than WL711 under WDS (Table 2). Considerable genetic variation and transgressive segregation was observed for RWC and CT as some of the RILs showed higher RWC and cooler CT than C306 and some RILs showed warmer CT than WL711. In Indore and Karnal stations, leaf wilting was observed which indicated prevalence of an effective WDS.

Correlations among the measured traits in the WL711/C306 RIL population

Correlation coefficients among the traits in the RIL population under WDS and IRR across Delhi, Karnal and Indore trials are shown in Table 5 and ESM Table 1, respectively. Significant correlations were observed between GY and its component traits under both the water regimes, although values of the correlation coefficients were higher under IRR than WDS across locations. GY under WDS was highly correlated with GN and HI, and to a lesser extent with BM and TGW. GN appeared to be more important component of GY under WDS than TGW in this population (Table 5). At Indore, the correlation between GY and TGW improved substantially as compared to Delhi and Karnal. It is likely that reduction in GN was compensated by maintaining TGW which is further evident by the higher correlation between GY and HI than with BM. Also, GY was

negatively correlated with DTF, suggesting that early flowering was associated with higher GY under WDS. As expected, GN was negatively correlated with TGW. Thus, in this population, GY under WDS was associated with a combination of traits including GN, HI and BM. Correlation of DSI with GY is significant but poor in both the environments. Positive correlation indicates that it is likely that high yielding varieties may be drought susceptible (Table 5; ESM Table 1). Under IRR, GY was highly correlated with GN, BM and HI across Delhi, Karnal and Indore trials (ESM Table 1).

QTLs for grain yield and related traits under water deficit and irrigated regimes

A total of 173 markers (152 SSR and 21 STS) were added to our earlier published WL711/C306 linkage map (Kadam et al. 2012). The improved map contained 346 markers including 321 SSR, 22 STS, 2 CAPS and 1 DHPLC marker distributed on the 21 wheat chromosomes (Fig. 1). The linkage map of longest wheat chromosome 3B showed the highest number of 35 markers with a total map distance 425.2 cM while chromosomes 6A and 6B showed the lowest number of 9 markers each covering map length of 123.4 and 122.3 cM respectively. Total map lengths of the A, B and D genomes were 1,481.6, 1,679.2 and 1,366.4 with 113, 123 and 110 markers respectively. The A, B and D genome showed 33, 37 and 30 % contributions to the entire genome map length respectively. The total map length was 4,526.8 cM with an averaged marker interval of 12.9 cM which was quite good for QTL analysis (Darvasi et al. 1993).

Significant QTLs for GY and related traits, including BM, HI, GN, TGW, DSI, TN, SPN, DTF, PHT and CT under WDS and IRR were identified (Table 6; ESM Table 2; Fig. 1). The average LOD score of QTLs for GY and related traits varied from 3.1 to 7.3. Multiple QTLs were co-located in a few prominent genomic regions on chromosomes 1B, 2D, 3B, 3D, 5D and 6B. The highest number of QTLs was located in a region on the long arm of chromosome 3B (Fig. 1).

The strongest effect QTL for GY under WDS, *qGYWD.3B.1* was located on chromosome arm 3BS flanked by markers *cfp56-gpw7774* explaining 18.7 % of PV with positive allele coming from the DT parent, C306 (Table 6). This QTL was co-located with QTLs for yield related traits i.e. BM, GN, TGW, HI TN,

Table 3 Analysis of variance for agronomic and morpho-physiological traits of wheat RIL population under irrigated and water deficit stress environments across Delhi, Karnal and Indore trials

Trait code/trials ^a	Df	Mean square values									
		GY (14)	BM (14)	GN (14)	TGW (14)	TN (4)	SPN (4)	HI (14)	PHT (14)	DTF (10)	
Source of variation											
Replicates	2	39,651.4 ns	230,347.7 ns	10,342,542.8n s	1.07 ns	230,347.7 ns	187,651.5 ns	6.5 ns	26,712.2 ns	28.6 ns	
Genotypes (G)	205	47,052.5*	214,226.4*	56,817,289.0*	100.2*	867.0*	675.0*	61.6*	78,251.0*	151.2*	
Treatment (E)	1	26,611,378.9*	23,792,362.4*	4,893,321,789.5*	23,792,362.4*	2,873,667.5*	2,387,651.1*	1,614.8*	15,681,930.0*	28,729.7*	
GxE	205	16,497.6*	92,692.6*	19,786,543.3*	92,692.6*	12,542.7*	10,231.5*	63.4*	97.9*	16.2*	
Trials (T)	6	2,668,746.3*	174,999,907.4*	2,678,356,319.1*	1,749,999,907.4*	235,737.3*	186,285.3*	6,919.8*	1,467,590.0*	20,662.0*	
GxT	1,235	13,323.3*	99,653.3*	2,587,382.8*	25.89*	684.0*	459.0*	64.1*	16,261.8*	39.0*	
ExT	6	262,663.2*	5,262,380.3*	2,436,272,069.3*	5,262,380.3*	213,141.4*	173,849.5*	11,054.3*	1,876,865.7*	2,048.2*	
G xExT	1,235	8,186.5*	43,933.9*	234,567.7*	43,933.9*	5,687.6*	3,543.8*	60.0*	4,579.9*	11.1*	
Error	2,895	1,788.2	14,706.8	10,993,211.3	14,706.8	1,431.4	976.5	47.2	1,357.8	0.62	
Genetic parameters											
CV (%)		7.9	9.7	19	4.2	4.6	2.6	10	5.87	0.90	
CD		40.99	153.6	1,671.20	1.56	14.65	10.56	6.06	5.97	2.0	
h_B^2 (IRR)		0.84	0.67	0.76	0.74	0.40	0.46	0.58	0.62	0.88	
h_B^2 (WDS)		0.68	0.50	0.68	0.87	0.38	0.42	0.52	0.50	0.78	

Genotype-mean heritability (h_B^2) across all trialsGY grain yield (gm^{-2}), BM biomass (gm^{-2}), GN grain number (m^{-2}), TGW 1,000 grain wt (g), TN tiller no. per m^2 , SPN spike no. per m^2 , HI harvest index (%), PHT plant height (cm), DTF days to flowering^a The number of environments in which each trait was assessed, is provided in parenthesis. * Statistically significant ($p \leq 0.05$), ns, non-significant ($p \geq 0.05$)

Table 4 Drought susceptibility index (DSI) for grain yield and its components in the parents and the WL711/C306 RIL population across trials in Delhi (2007–2011), Kamal (2007–2008) and Indore (2008–2009)

Drought susceptibility index (DSI)		Kamal				Indore			
		Delhi	Delhi	Delhi	Delhi	Delhi	Delhi	Delhi	Delhi
Traits	WL711	C306	Population range	WL711	C306	Population range	WL711	C306	Population range
GY	1.32 ± 0.08	0.48 ± 0.04	0.42–1.34	1.19 ± 0.06	0.61 ± 0.04	0.10–1.98	1.44 ± 0.18	0.38 ± 0.08	0.19–1.96
BM	1.32 ± 0.08	0.63 ± 0.05	0.48–1.93	1.96 ± 0.10	0.81 ± 0.08	0.14–2.07	1.12 ± 0.12	0.57 ± 0.11	0.10–1.92
GN	1.23 ± 0.14	0.50 ± 0.09	0.28–1.89	1.17 ± 0.13	0.61 ± 0.08	0.10–1.96	1.38 ± 0.12	0.23 ± 0.10	0.14–1.97
TGW	1.21 ± 0.10	0.42 ± 0.13	0.24–1.68	1.15 ± 0.09	0.24 ± 0.07	0.14–2.04	1.08 ± 0.18	-0.14 ± 0.07	-0.23–2.04
HI	1.65 ± 0.12	-0.07 ± 0.09	-2.5–3.2	1.48 ± 0.14	-0.27 ± 0.08	-0.34–2.8	1.92 ± 0.21	-0.58 ± 0.12	-0.87–2.39

DSI < 1 indicates drought tolerant and > 1 indicates drought susceptible (Fischer and Maurer 1978)

SPN, DTF and CT (Fig. 1) and appears to be an important genomic region for DT in this population. Another major consistent QTL detected for GY under WDS, *qGYWD.3B.2* flanked by markers *gpw7774-cfp3530* with positive allele coming from WL711 was closely linked with the QTL *qGYWD.3B.1*. This QTL explained 19.6 % of PV and was co-located with QTLs for GN, TN and SPN under WDS. A major QTL for GY, *qGYWD.3B.3* with positive allele coming from WL711, explained the highest PV under WDS i.e. 21.8 %. Another major consistent QTL for GY, *qGYWD.7B.1*, flanked by markers *caps149-snp142* explaining 12.5 % of PV with positive allele coming from WL711, was detected in six out of seven trials under WDS. Under IRR, the strongest effect for GY (69.5 gm⁻²) was for QTL *qGYIR.3D.2* explaining 13.9 % of PV with positive allele being contributed by the high yielding parent, WL711 (ESM Table 2). This QTL was co-located with QTLs for BM, GN, TGW, HI, TN and SPN (Fig. 1). This QTL may be an important region for GY under IRR in this population.

The RIL population showed wide variation for DTF and PHT which may cause significant confounding effects and interfere with the detection of genomic regions having significant effects on GY and related traits. Since early flowering RILs might be escapes, an analysis using a subset of medium to late flowering 183 RILs only identified the major QTLs for GY i.e. *qGYWD.3B.1*, *qGYWD.3B.2*, *qGYWD.3B.3* and *qGYWD.7B.1* on chromosomes 3B and 7B with a slight decrease in PV (ESM Tables 3). Also, a minor QTL for DTF that co-located with GY QTL *qGYWD.3B.1* in the first analysis disappeared in both the subsets. Hence, the QTLs identified for GY under drought are important in our population across phenological groups.

PHT in the RIL population varied from 87 to 133 cm. However, we did not find any co-location of the major QTLs identified in this study with *Rht* genes. To ascertain, the RILs were divided into two groups, semi-dwarf (107 RILs) and tall (99 RILs) and each group was subjected to QTL identification. The major QTLs i.e. *qGYWD.3B.1*, *qGYWD.3B.2*, *qGYWD.3B.3* and *qGYWD.7B.1* on chromosomes 3B and 7B were still detected in both the subsets (ESM Tables 4, 5). Hence the major QTLs for GY on chromosome arms 3BS, 3BL and 7BL were consistent across height groups.

Table 5 Correlation coefficients (r) among grain yield and yield component traits in the WL71/C306 RIL population across trials in Delhi (2007–2011), Karnal (2007–2008) and Indore (2008–2009) under water deficit stress treatment

Trial	GY	BM	GN	TGW	HI	TN	SPN	PHT
Delhi (2007–2011)								
BM	0.580**							
GN	0.732**	0.578**						
TGW	0.289**	0.189**	-0.398**					
HI	0.688**	0.109*	0.609**	0.312**				
TN	0.226**	0.367**	0.289**	0.135**	-0.212**			
SPN	0.210**	0.213**	0.326**	-0.178**	-0.145**	0.823**		
PHT	0.034*	0.001n.s.	0.356**	0.423**	0.367**	-0.200**	-0.176*	
DTF	-0.205**	0.002n.s.	0.355**	0.426**	-0.334**	0.098*	0.003n.s.	0.211**
DSI	0.239**	-	-	-	-	-	-	-
Karnal (2007–2008)								
BM	0.334**							
GN	0.640**	0.478**						
TGW	0.126**	0.123**	-0.410**					
HI	0.606**	0.243*	0.587**	0.289**				
PHT	0.024*	0.002n.s.	0.323**	0.410**	0.312**			
DSI	0.213**	-	-	-	-			
Indore (2008–2009)								
BM	0.468**							
GN	0.810**	0.545**						
TGW	0.678**	0.145**	-0.239**					
HI	0.726**	0.334*	0.527**	0.267**				
PHT	0.021*	0.001n.s.	0.312**	0.412**	0.288**			
DSI	0.225**	-	-	-	-			

Correlation is statistically significant at * $P \leq 0.05$ and ** $P \leq 0.01$, n.s., non-significant ($P \geq 0.05$)GY grain yield (gm^{-2}), BM biomass (gm^{-2}), GN grain number (m^{-2}), TGW 1,000 grain wt (g), TN tiller no. per m^2 , SPN spike no. per m^2 , HI harvest index (%), PHT plant height (cm), DTF days to flowering, DSI drought susceptibility index

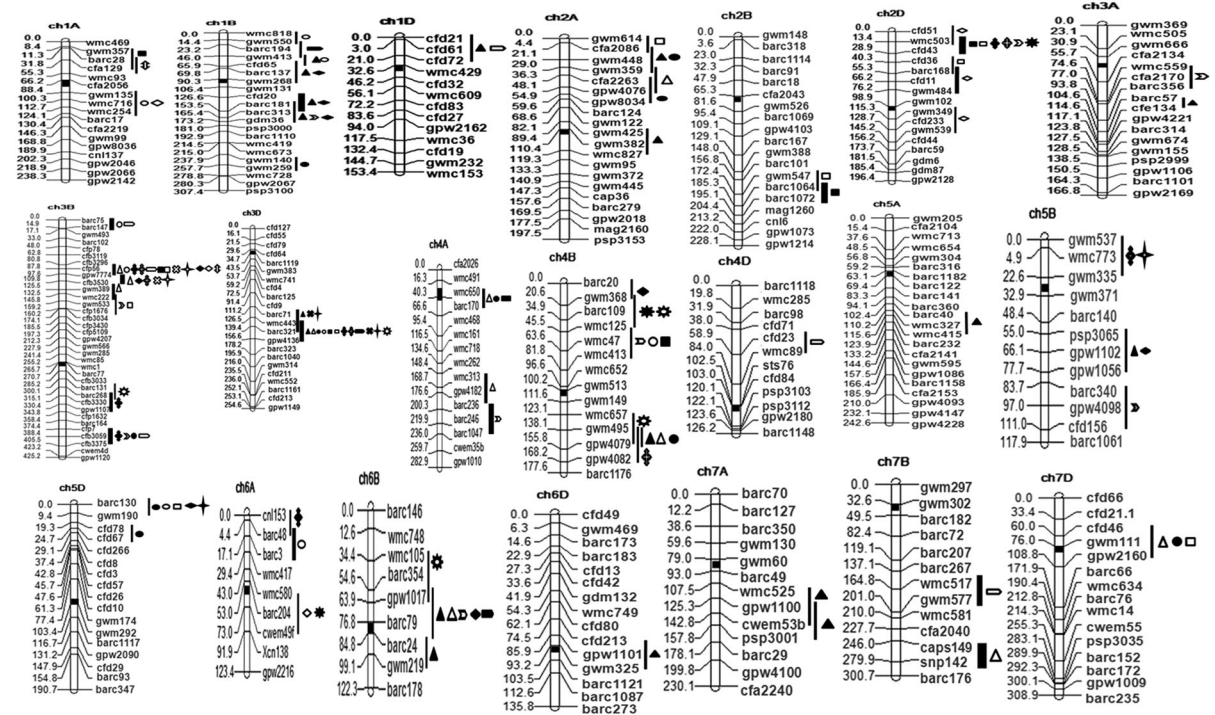


Fig. 1 Quantitative trait loci (QTLs) for GY and associated yield component traits in WL711/C306 wheat RIL population under water deficit stress and irrigated conditions. The vertical bars indicate the QTL confidence intervals. Map distances (cM) are shown on the left side of each chromosome. Open and filled symbols indicate water deficit stress and irrigated treatments.

The largest effect QTL for BM under WDS was *qBMWD.3D.2* flanked by markers *wmc443-gpw4136* detected in the DL09 and IN08 trials explaining 10.4 % of PV with positive allele coming from WL711 (Table 6). This QTL was co-located with QTLs for GY, HI and PHT (Fig. 1). A minor QTL for BM, *qBMWD.6A.1* was detected on chromosome 6AS explaining 9.9 % of PV with positive allele coming from C306 under WDS. Under IRR, the largest additive effect for BM (298.8 gm⁻²) was located on QTL *qBMIR.2A.1* explaining 10.3 % of PV with positive allele coming from WL711 (ESM Table 2).

For GN, the positive effect of the largest effect QTL *qGNWD.3B.2* was coming from C306 explaining 21.4 % of PV under WDS (Table 6; Fig. 1). Under IRR, the largest effect QTL for GN (2,290 m⁻²) was *qGNIR.3B.1* explaining 19.1 % of PV with positive allele coming from WL711, detected in four out of the seven trials namely DL08, DL10, KL07 and IN08 (ESM Table 2). Another major consistent QTL for

GN, *qGNIR.2D.1* explained 20.9 % of PV with positive allele being contributed by WL711. A major QTL for TGW, *qTGWWD.3B.4* with positive allele coming from C306, explained the highest PV under WDS i.e. 11.1 % (Table 6). Another major QTL for TGW, *qTGWWD.3B.5* explained 10.4 % of PV with positive allele coming from C306.

A minor QTL for HI, *qHIWD.3B.5* with positive allele coming from WL711 was co-located with QTL for DSI under WDS (Table 6; Fig. 1). A minor QTL, *qHIWD.3D.2* explaining 9.7 % of PV co-located with QTL for GY, BM and PHT under WDS (Fig. 1). Under IRR, a major consistent QTL for HI, *qHIIR.2B.1* was detected in all the five Delhi trials, explaining 11.3 % of PV with positive allele coming from WL711 (ESM Table 2). A major QTL for DSI of GY, *qDSIGY.4A.1* was detected across all trials except IN08 trial, explaining 15.6 % of PV with positive allele coming from WL711 (Table 6). This QTL was consistent in the subsets analysis also.

Table 6 QTLs for grain yield and related traits identified by composite interval mapping using QTL cartographer software in a population of 206 wheat RILs derived from WL711/C306 under water deficit stress treatment

Traits ^a	QTL	Trial	Flanking markers	A effect ^b	QE ^c	R ² (%)	
						a ^d	QE ^e
GY	<i>qGYWD.3B.1</i>	DL08, DL10, KL07	cfp56-gpw7774	-45.2	-10.5*	18.7	1.8
	<i>qGYWD.3B.2</i>	DL07, DL08, DL11, IN08	gpw7774-cfp3530	43.1		19.6	
	<i>qGYWD.3B.3</i>	DL09, DL10	gwm389-gwm533	40.2		21.8	
	<i>qGYWD.3D.2</i>	DL08, DL10	wmc443-gpw4136	24.0		5.8	
	<i>qGYWD.4A.1</i>	DL09, KL07	wmc313-barc236	26.6		7.1	
	<i>qGYWD.7B.1</i>	DL07, DL08, DL09, DL10, DL11, KL07	caps149-snp142	26.7		12.5	
BM	<i>qBMWD.3B.1</i>	DL10, IN08	cfp56-gpw7774	55.1	31.3*	9.3	1.68
	<i>qBMWD.3B.4</i>	DL07, DL09, DL11, KL07	barc75 – barc147	49.3	22.1*	9.6	1.45
	<i>qBMWD.3D.2</i>	DL09, IN08	wmc443- gpw4136	57.9		10.4	
	<i>qBMWD.6A.1</i>	DL08, DL09, DL10,	barc48-barc3	-47.5		9.9	
GN	<i>qGNWD.2D.1</i>	DL07, DL09, DL11, KL07	wmc503-cfd43	893		14.2	
	<i>qGNWD.3B.1</i>	DL08, DL09	cfp56-gpw7774	1,778		10.9	
	<i>qGNWD.3B.2</i>	DL08, DL10, DL11	gpw7774-cfb3530	-1,791	21.4*	21.4	1.61
	<i>qGNWD.4B.1</i>	DL10, IN08	gwm495-gpw4082	1,039		10.5	
TGW	<i>qTGWWD.1D.1</i>	DL08, DL10, KL07	cfd61-cfd72	-1.9		10.4	
	<i>qTGWWD.3B.1</i>	DL08, DL10, KL07	cfp56-gpw7774	-1.8	0.21*	9.4	0.44
	<i>qTGWWD.3B.4</i>	DL07, DL11	barc75 – barc147	-1.7		11.1	
	<i>qTGWWD.3B.5</i>	DL11, IN08	cfb3059 cwem4d	-1.2		10.4	
	<i>qTGWWD.7B.1</i>	DL07, DL09, DL11	wmc517-gwm577	-1.5		9.3	
TN	<i>qTNWD.3B.1</i>	DL07, DL11	cfp56-gpw7774	-32.7		12.8	
	<i>qTNWD.3B.2</i>	DL07, DL11	gpw7774-cfb3530	-28.7		14.1	
SPN	<i>qSPNWD.3B.1</i>	DL07, DL11	cfp56-gpw7774	-12.6		16.4	
	<i>qSPNWD.3B.2</i>	DL07, DL11	gpw7774-cfb3530	-13.5		15.7	
HI	<i>qHIWD.2B.1</i>	DL08, IN08	gwm547-barc1064	1.7		8.2	
	<i>qHIWD.3B.1</i>	DL09, DL11	cfp56-gpw7774	1.3		8.6	
	<i>qHIWD.3B.5</i>	DL10, KL07, IN08	wmc222-cfp1676	1.8		8.7	
	<i>qHIWD.3D.2</i>	DL08, DL09, DL10, DL11	wmc443-gpw4136	1.5	0.92*	9.7	1.02
DSI	<i>qDSIGY.3B.4</i>	DL08, DL10	cfb3059-cfb3375	0.27		12.7	
	<i>qDSIGY.3B.5</i>	DL07, DL11	wmc222-cfp1676	0.39		15.5	
	<i>qDSIGY.4A.1</i>	DL07, DL08, DL09, DL10, DL11, KL07	barc236-barc1047	0.18	0.02*	15.6	0.14
PHT	<i>qPHTWD.3B.3</i>	DL07, DL11	cfp3430-gpw4207	-3.5		13.4	
	<i>qPHTWD.3D.2</i>	DL07, DL11, KL07	wmc443-gpw4136	-3.2		10.0	
	<i>qPHTWD.4B.1</i>	DL08, DL10	gwm368-wmc125	-2.9		15.1	
DTF	<i>qDTFWD.2D.1</i>	DL10, DL11	barc168-gwm484	-1.5	-0.13*	11.4	0.90
	<i>qDTFWD.3B.1</i>	DL08, DL09	cfp56-gpw7774	-1.7		7.2	
CT	<i>qCTWD.1A.1</i>	DL08, DL11	barc28-wmc93	-0.35		9.0	
	<i>qCTWD.3B.1</i>	DL08, DL11	cfp56-gpw7774	-0.1		13.2	

A positive value of the additive main effects (*a*) indicates that WL711 contributes allele to increase the trait, and a negative value means that C306 provides allele to increase the trait

^a GY-grain yield, BM-biomass, GN-grain number per m², TGW-1,000-grain weight, TN-tiller number per m², SPN-spike number per m², HI-harvest index, DSI-drought susceptibility index, PHT-plant height, DTF-days to flowering, CT-canopy temperature

^{b,c,d,e} *a* additive main effects, QE QTL × environment interaction, R² (*a*) % phenotypic variation explained by *a* effects, R² (QE) % phenotypic variation explained by QE effects

* *P* < 0.05; ** *P* < 0.01. Only significant effects are listed, many non-consistent minor QTLs have not been listed in this table

A major QTL for DTF, *qDTFWD.2D.1* was detected on chromosome 2DS flanked by markers *barc168-gwm484* explaining 11.4 % of PV with positive allele coming from the late flowering parent C306 (Table 6). A major QTL for PHT, *qPHTWD.4B.1* flanked by markers *gwm368-wmc125* was detected under both WDS and IRR treatments, explaining 15.1 % of PV and positive allele coming from the tall parent C306 (Table 6; ESM Table 2). This QTL corresponds to the major PHT gene *Rht1*.

QTL × environment interactions and epistatic QTL

The results of the QTL × environment interactions (QE) of QTLs under WDS and IRR are shown in Tables 6 and ESM Table 2. Epistatic QTL showing QTL × QTL (QQ) and QTL × QTL × environment (QQE) interaction were also studied to gain a better understanding of the genetic basis of GY and related traits in wheat. The results of QQ and QQE under WDS are shown in Table 7. Under WDS, three QQ interactions were detected for GY, TGW and DTF. Although some of the important genomic regions identified in the present study were involved in QE, QQ and QQE interactions, their effects were much less prominent than the main additive effects (*a*), thereby suggesting that the additive effects were more important than epistatic effects in determining the DT traits studied here.

Comparative mapping and identification of candidate genes

To demarcate rice genomic regions corresponding with the QTLs for GY and other agronomic traits present on wheat chromosome arm 3BS, DNA sequences of 3 markers i.e. *cfp56*, *gpw7774*, *cfb3530* were compared with the rice genomic DNA sequences using ‘BLAST’ functions. The wheat chromosome arm 3BS showed maximum homology to rice chromosome 1, as 6 sequences identified their orthologs on rice chromosome 1 (ESM Table 6; Supplementary Fig. 3). The collinear region of 3BS in wheat on the rice chromosome 1 spans 3 candidate genes including *CKX2* and *SPP1* encoding cytokinin oxidase and indole acetic acid synthase respectively and transcription factors which may play an important role in GN formation in rice.

Discussion

Enhancing grain yield under drought stress is a major aim of most wheat breeding programs for the drought prone areas. The present study was conducted to identify consistent QTLs for GY and associated traits under WDS at three locations in the north-western and central India. We identified several consistent QTLs for GY and related traits under WDS in WL711/C306 RIL population. Alleles from C306 contributed nine major QTLs with relatively large effects for GY and its component traits under WDS (Table 6). Involvement of major QTLs with relatively large effect acting together with a number of minor QTLs and large environmental effects has emerged as a common feature of quantitative traits such as GY and DT in crop plants (Cattivelli et al. 2008; Fleury et al. 2010; Mir et al. 2012). In our study, major QTLs located on chromosomes 3BS, 4AS, 6AS and 7BL were identified for GY and related traits under WDS in WL711/C306 RIL population. QTLs on 3BS and 6AS identified in the present study are novel. In bread wheat, QTLs for GY and associated traits under WDS have been reported on chromosomes 2BS, 2DS, 4AS, 4AL, 4BS, 6AS, 6BL, 7AL and 7BL (Quarrie et al. 2005; Kirigwi et al. 2007; McIntyre et al. 2010; Wu et al. 2012; Kumar et al. 2012; Kadam et al. 2012).

The RIL population showed transgressive segregation for all the traits investigated which enabled us to identify some RILs combining DT with GY higher than C306 under WDS (Table 2; Khanna-Chopra et al. 2013). C306 contributed allele at all the QTLs for TGW, TN, SPN, DTF and PHT and at some QTLs for GY, BM and GN and WL711 contributed allele for all the QTLs for HI and DSI of GY under WDS (Table 6). In our study, alleles were more frequently identified from the parent with the larger trait value and hence many QTLs associated with the DS parent WL711 were also detected. In wheat, Pinto et al. (2010) reported a QTL for GY under drought on chromosome 4A associated with the susceptible parent Serim82 in the Seri/Babax RIL population. Bennett et al. (2012a, b) detected a GY QTL on chromosome 3D under drought condition with allele coming from the susceptible parent Kukri. Also in case of rice, a major QTL for DT *qDTY12.1* has been reported in the Vandana/Way Rarem cross where the allele was contributed by the susceptible parent Way Rarem (Bernier et al. 2007).

GY is a complex quantitative trait controlled by multiple genes with low heritability. Dissection of GY into its component traits can assist breeders to identify desirable traits with high heritability and linked with stable molecular markers for more reliable selection criteria as compared to selection for yield *per se*. In our study, broad sense heritability was high for TGW and DTF while GY and GN showed moderate heritability under WDS (Table 3; McIntyre et al. 2010; Pinto et al. 2010).

The QTL, *qGYWD.3B.1* reported here was a major novel QTL for GY under WDS on the short arm of chromosome 3B accounting for 18.7 % of PV. The trait enhancing allele at this locus was contributed by the DT parent C306. QTL *qGYWD.3B.1* at 92.3 cM appears to be a novel one, as major GY QTLs under WDS have been reported at ~11 cM on 3BS (Maccaferri et al. 2008) and on 3BL at ~120 and 190 cM respectively (Pinto et al. 2010; Bennett et al. 2012b). Hence, *qGYWD.3B.1* is distant from the QTLs reported in the literature as per the wheat consensus map given by Somers et al. (2004). Another major novel and consistent QTL for GY *qGYWD.3B.2* was detected on chromosome arm 3BS in an interval adjacent to the QTL *qGYWD.3B.1* at 103.6 cM with positive allele coming from the high yielding parent WL711. In order to utilize the positive effect of both of these QTLs, we need to recombine these two which

are closely linked with an interval of 11.3 cM. Among the 206 RILs, 33 RILs were identified that combined the positive alleles from both the parents. Of which 50 % RILs showed yield higher than C306 under WDS. The genomic region, *qGYWD.3B.1* was co-located with QTLs for GN, HI, BM, TGW, SPN, TN, DTF and CT during grain filling (Table 6; Fig. 1), while *qGYWD.3B.2* was co-located with QTLs for GN, TN and SPN under WDS. GN showed highest correlation with GY followed by BM, SPN and TN under WDS in this population across locations. In wheat, GN is reported to be highly correlated with GY as compared to TGW under WDS (Miralles and Slafer 2007; Olivares-Villegas et al. 2007; Rattey et al. 2009; Pinto et al. 2010, Bennett et al. 2012b). HI of the RIL population was highly correlated with GY followed by GN and TGW under WDS across locations.

It is interesting to note that QTL *qGYWD.3B.1* was associated with both increased GN and TGW, the traits showing negative correlation in this population across locations with positive allele for GN coming from WL711 while C306 contributed positive allele for TGW at this locus (Table 6). To the best of our knowledge, this is the first report on co-location of GN and TGW QTL in wheat under WDS environment. In earlier studies GY QTLs co-located either with TGW (Snape et al. 2007) or GN under WDS environment (Quarrie et al. 2005; Kirigwi et al. 2007; McIntyre

Table 7 Epistatic QTLs and QTL × QTL × environment interaction for grain yield and related traits identified by two locus analysis using QTL Network software in 206 RILs

Traits ^a	QTL _i ^b	Interval _i	QTL _j	Interval _j	QQ ^c	QQE ^d	R ² %	
							QQ ^e	QQE ^f
GY	<i>qGYWD.3B.1</i>	cfp56-gpw7774	<i>qGYWD.3B.2</i>	gpw7774-cfp3530	-10.345	-1.5674	0.51**	0.35**
TGW	<i>qTGWWD.1D.1</i>	cfp61-cfd72	<i>qTGWWD.3B.1</i>	cfp56-gpw7774	-0.6543	-0.2135	0.64**	0.51**
DTF	<i>qDTFWD.2D.1</i>	barc168-gwm484	<i>qDTFWD.3B.1</i>	cfp56-gpw7774	-0.2456	-0.0012	0.27**	0.02**

A positive value means that the parent-type effect is greater than the recombinant-type effect

A negative value means that the parent-type effect is less than the recombinant-type effect

^a GY-grain yield, TGW-1,000-grain weight, DTF-days to flowering

^b QTL_i and QTL_j are a pair of QTL involved in epistasis

^c QQ, the epistatic main effect

^d QQE, the epistasis × environment interaction effects

^e R² (QQ) %, Phenotypic variation explained by QQ effects

^f R² (QQE) %, Phenotypic variation explained by QQE effects

* P < 0.05; ** P < 0.01 respectively

derived from WL711/C306 across Delhi, Karnal and Indore trials under water deficit stress treatment

et al. 2010; Pinto et al. 2010). However, recently a GY QTL co-locating with QTLs of both GN and TGW has been reported on chromosome 3BS under irrigated environment (Bennett et al. 2012b).

The sequence of chromosome 3B is now available and its gene annotation is under process (<http://www.wheatgenome.org/content/view/full/407>). The information on genes located on chromosome 3B is scanty. In WL711/C306 RIL population, Kadam et al. (2012) studied differentially expressed genes under drought stress between parents and the DT and DS RIL bulks through transcriptome profiling (ESM Table 7). On 3BS, a differentially expressed gene encoding *glutathione S transferase* was identified which was down-regulated 2.36-fold in the DT parent C306 and the tolerant RIL bulk. Glutathione is an important antioxidant, redox buffer and detoxifier (Noctor and Foyer 1998).

Four differentially expressed genes identified in this region of chromosome 3BL included *β -glucosidase* while the remaining genes coded for a hypothetical protein with unknown function. *β -glucosidase* was up-regulated 2.5-fold in the DT parent C306 and the tolerant RIL bulk as compared to the DS parent WL711 and the sensitive RIL bulk (Kadam et al. 2012). *β -glucosidases* have been implicated in the release of sugars for remobilization and completion of energy dependent senescence program under drought induced senescence in *Arabidopsis* (Mohapatra et al. 2010). The *qGYWD.3B.1* and *qGYWD.3B.2* genomic regions of wheat chromosome 3B show synteny with 0.01 to 21.61 Mb region of rice chromosome 1 (Sorrells et al. 2003; Singh et al. 2007). This syntenic region of rice chromosome 1 has been associated with several DT related traits such as root shoot ratio, deep root mass, root thickness, RWC, CT, cell membrane stability (CMS), osmotic adjustment (OA), transpiration rate, stomatal conductance and GY related traits i.e. BM, GN, TGW and TN under drought stress (Diab et al. 2007; Babu 2010; Vikram et al. 2011).

On comparisons with the wheat-rice genomic DNA sequences 3 putative genes and transcription factors have been identified in the region of interest namely GA insensitive dwarf 2 (*GID2like*), cytokinin oxidase (*CKX2*), spikelet per panicle (*SPP1*), Bowman-Birk type bran trypsin inhibitor precursor, NAD dependent epimerase dehydratase family and MYB like DNA binding domain containing protein (ESM Table 6; Supplementary Fig. 3). Gene *CKX2* is involved in

accumulation of phytohormone cytokinin (CK) in shoot apical meristem, which is a major parameter determining seed production (Ashikari et al. 2005). The whole genome sequence of barley was recently published (IBGSC 2012). In barley, cytokinin oxidase gene *CKX3* has been mapped in the region of interest (Zalewski et al. 2010). Gene *SPP1* encoded for indole acetic acid (IAA) synthetase in rice inflorescence. It is well known that IAA is the most universal auxin in plants and promotes cell division and growth. IAA may play an important role in promoting reproductive growth and the development of inflorescence meristem in rice (Zhao 2010).

A consistent novel QTL for TGW, *qTGWWD.3B.5* was detected on chromosome 3BL under WDS with positive allele coming from C306 (Table 6). QTLs for processes involved in grain filling like WSC remobilization efficiency, grain filling rate and duration have also been mapped in wheat in a region close to *qTGWWD.3B.5* (Yang et al. 2007, Rebetzke et al. 2008). QTL *qTGWWD.3B.5* is located in a similar region of chromosome 3BL of wheat where cell wall invertase gene *IVR1-3B.51* has been mapped (Webster et al. 2012). Cell wall invertase catalyses the hydrolysis of sucrose to glucose and fructose, the essential energy substrates that support grain filling. The *qTGWWD.3B.5* genomic region of wheat chromosome 3B shows synteny with 31.94–41.88 Mb region of rice chromosome 1 (Sorrells et al. 2003, Singh et al. 2007). The syntenic region of rice chromosome 1 has been associated with several DT related traits such as root shoot ratio, deep root mass, root thickness, RWC, CT, CMS, OA, transpiration rate, stomatal conductance and GY related traits i.e. TGW and GN under drought stress (Babu 2010, Vikram et al. 2011). Cell wall invertase gene *CIN4* was found in this syntenic region of rice chromosome 1 (www.gramene.org, Hirose et al. 2002, Cho et al. 2005). On chromosome arm 3HL of barley in a region syntenic to QTL *qTGWWD.3B.5*, QTLs for water soluble carbohydrates (WSC) and RWC under drought conditions have been reported (Diab et al. 2004). Also, *WRKY* DNA binding domain has been mapped in the syntenic region of barley (Tondelli et al. 2006; Mayer et al. 2011). A gene BM816303 coding for amino methyl transferase in the syntenic region of barley chromosome 3HL was reported to be cross matched to the sequence for RZ409 on rice chromosome 1 (Diab et al. 2007). Amino methyl transferase is involved in the

breakdown of glycine, which when present in the form of glycine betaine is involved in OA during drought stress in plants (Chen and Murata 2008). Hence, it is likely that the *qTGWWD.3B.5* region of wheat chromosome 3B may be important in grain filling process under WDS in wheat.

Genes affecting plant morphology (*Rht*) and phenology (*Ppd*) have been the focus of wheat breeding for many years as a means to reduce its height and to fit better to the environments by selecting for appropriate phenology (Quarrie et al. 2005). Co-location of QTL for GY with PHT and DTF has been reported in earlier studies also (Quarrie et al. 2005; Kirigwi et al. 2007; Maccaferri et al. 2008; Peleg et al. 2009; Kadam et al. 2012). The pleiotropic effects of *Rht* and *Ppd* genes can decrease the probability of detection of QTL/genes of relatively minor effects for complex traits such as GY and its component traits (Reynolds et al. 2009; Pinto et al. 2010). QTL *qGYWD.3B.1* was apparently not related to PHT as major QTL for PHT was detected on chromosome 4BS at ~35 cM under both water regimes. A QTL for PHT on chromosome 4BS with one of the flanking markers (*gwm368*) has been reported earlier in this cross by Kadam et al. (2012) and in Seri/Babax population by McIntyre et al. (2010). The non-association between QTL *qGYWD.3B.1* and PHT QTL/gene makes it a major genomic region without the confounding effects of *Rht* genes. A minor QTL for DTF associated with C306, the late flowering parent on chromosome 3BS also co-located with QTL *qGYWD.3B.1*. In this study, a major QTL for DTF *qDTFWD.2D.1* was detected on chromosome 2DS under WDS with allele coming from C306 at 80.2 cM (Table 6). This region is close to the *Ppd-D1* gene reported by Hanocq et al. (2004). This confirms the association of QTL *qDTFWD.2D.1* on chromosome 2DS with flowering time in wheat.

Another major, highly consistent QTL for GY under WDS, *qGYWD.7B.1*, was detected on 7BL (Fig. 1). QTLs for GY on chromosome 7B have been reported under irrigated/drought stress (Quarrie et al. 2005, Kadam et al. 2012; Alexander et al. 2012). The corresponding syntenic region in rice on chromosomes 6 and 8 carries QTLs for traits related to GY, root and physiological parameters such as OA (Zeng et al. 2006; Babu 2010). In barley, QTLs for RWC, WSC and gene *bss1B* coding for sucrose synthase have been mapped in a region syntenic to *qGYWD.7B.1* on chromosome arm 7HL (Diab et al. 2004). The enzyme

sucrose synthase is involved in carbohydrate metabolism that may help plant to cope with WDS. Hence, this region on chromosome 7BL may be important for influencing GY under WDS in wheat.

There are few reports on QTLs for BM and HI under WDS in wheat. QTLs for BM are reported on chromosomes 1DS, 4AS, 4BS, 7AL under WDS (Quarrie et al. 2006; Kirigwi et al. 2007; McIntyre et al. 2010; Kadam et al. 2012). A novel QTL for BM, *qBMWD.6A.1* associated with C306 was detected on chromosome 6AS under WDS in this study which finds support from syntenic region of rice. QTLs for physiological traits like CT, WSC content and photosynthetic activity at grain filling have also been reported on chromosome 6AS under drought stress in wheat (Yang et al. 2007; Peleg et al. 2009; Liang et al. 2010, Kumar et al. 2012). QTLs for BM, root traits and OA have been reported in rice on chromosome 2 in a region syntenic to *qBMWD.6A.1* (Kamoshita et al. 2002; Robin et al. 2003; Xu et al. 2004; Lian et al. 2005; Nguyen et al. 2004).

A major and consistent QTL for DSI (yield), *qDSIGY.4A.1* was detected on chromosome 4AL with drought susceptibility allele coming from WL711 in this population. Earlier, Kirigwi et al. (2007) have reported a major QTL for DSI (yield) on chromosome 4AS in the Dharwar Dry/Sitta RIL population in wheat, while Kadam et al. (2012) have detected a major QTL for DSI (yield) on chromosome 4BS in the present population. Hence, Group 4 chromosomes appear to be influencing DSI (yield) in this population. The most consistent QTL *qDSI.4B.1* for GY per plant reported by Kadam et al. (2012) in the pipe experiments was not detected in the field experiments reported here.

We report here major QTLs for grain yield and related traits on chromosomes 3B in the WL711/C306 RIL population under water deficit stress condition. The QTL *qGYWD.3B.1* was located on chromosome 3BS with positive allele being contributed by C306 accounting for a large proportion of phenotypic variation. This QTL was co-located with QTLs for yield components, canopy temperature, days to flowering and was apparently independent of plant height. The additive effect at this QTL was higher than the QE, QQ and QQE effects. Another major novel and consistent QTL for grain yield, *qGYWD.3B.2* was associated with WL711. Other important QTLs for

grain yield, biomass, 1,000-grain weight and drought susceptibility index for grain yield mapped on chromosome arms 7BL, 6AS, 3BL and 4AL are in consonance with the QTLs reported earlier for yield, yield components and drought tolerance related traits and/or genes either in rice or barley (Cho et al. 2005; Babu 2010). Particularly, a QTL for drought susceptibility index for grain yield (*qDSIGY.4A.1*) was consistently detected in six of the seven environments. These genomic regions need to be further validated, fine mapped for marker development and gene discovery, which will help in marker assisted breeding for yield improvement under water limited environments in wheat.

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