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ORIGINAL PAPER

Multi-environment analysis and improved mapping of a yield-related QTL on chromosome 3B of wheat

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Abstract Improved mapping, multi-environment quantitative trait loci (QTL) analysis and dissection of allelic effects were used to define a QTL associated with grain yield, thousand grain weight and early vigour on chromosome 3BL of bread wheat (*Triticum aestivum* L.) under abiotic stresses. The QTL had pleiotropic effects and showed QTL x environment interactions across 21 diverse environments in Australia and Mexico. The occurrence and the severity of water deficit combined with high temperatures during the growing season affected the responsiveness of this QTL, resulting in a reversal in the direction of allelic effects. The influence of this QTL can be substantial, with the allele from one parent (RAC875) increasing grain yield by up to 12.5 % (particularly in environments where both heat and drought stress occurred) and the allele from the other parent (Kukri) increasing grain yield by up to 9 % in favourable environments. With the application of additional markers and the genotyping of additional recombinant inbred lines, the genetic map in the QTL region was refined to provide a basis for future positional cloning.

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Introduction

Grain yield is a complex trait that depends on multiple genes and/or gene-network cascades interacting with each other and with the environment (Holland 2007; Shi et al. 2009; Wu et al. 2012). In wheat (*Triticum aestivum* L.), improvements in grain yield have been achieved through the deployment of major genes such as the *Rht* plant height genes (dwarf and semi-dwarf) (Rebetzke et al. 2012), the *Ppd* photoperiod-sensitivity genes (Beales et al. 2007; Wilhelm et al. 2009) and the *Vrn* vernalisation-response genes (Zhang et al. 2008; Yoshida et al. 2010). However, the grain yield of wheat can be dramatically affected by abiotic stresses such as timing and severity of water deficit and/or high temperature during the crop cycle (Kosina et al. 2007) and by the interaction of these stresses with plant phenology and morphology. The confounding effects of loci affecting plant phenology and morphology can interfere with the detection of other genetic regions that may individually have minor effects but could collectively account for a large proportion of phenotypic variation (Reynolds et al. 2009a). The detection of such loci may be possible with appropriate statistical tools.

Quantitative trait locus analysis has been used to dissect the genetic component of grain yield of wheat grown in

favourable environmental conditions (Maccaferri et al. 2008; McIntyre et al. 2010) and with exposure to drought and/or heat (Diab et al. 2008; Mason et al. 2010; Pinto et al. 2010). This enabled the detection of some of the major genes mentioned above, but also the discovery of other genetic regions with minor effects on wheat grain yield. These minor QTL are of great interest but are rarely consistent and reliable across environments. Alleles that confer improvement of grain yield in one set of environments may be irrelevant in other environments (Malosetti et al. 2008). The molecular basis for the minor genes affecting grain yield is also poorly understood. Although positional cloning has been used to identify genes underlying QTL (Krattinger et al. 2009), no QTL for grain yield under heat and drought conditions have been cloned from wheat.

The research reported here focussed on a QTL on chromosome 3BL that had been previously detected in a population of doubled haploid (DH) lines derived from a cross between the drought and heat stress-tolerant wheat breeding line RAC875 and the wheat cultivar Kukri. This QTL was associated with grain yield, thousand grain weight and early vigour in field experiments grown in north-western Mexico (Bennett et al. 2012a). When the same population was evaluated under apparently similar environmental conditions in Australia, the QTL was detected only for the proportion of small grains (*Q.Scr.aww-3B-2*) (Bennett et al. 2012b). The aim of the research reported here was to further investigate the region of interest by improving the genetic map of that region and by evaluating QTL effects across a diverse range of heat- and drought-related conditions using multi-environment linear mixed model analyses.

Materials and methods

Plant material

The material used in this project includes a set of 368 DH lines derived from the F_1 generation of a cross between the drought tolerant wheat breeding line RAC875 (RAC655/3/Sr21/4*Lance//4*Bayonet) and the cultivar Kukri (76ECN44/76ECN36//Madden/6*RAC177), and a set of 768 recombinant inbred (RI) lines developed from the same cross (Fleury et al. 2010).

Marker genotyping

Genetic map of the RAC875/Kukri DH population

The genetic map of the target region on 3BL was built using the RAC875/Kukri genetic map and genotypic data published by Bennett et al. (2012a) as a basis. Linkage analysis was performed with simple sequence repeat (SSR) and DArT (Diversity Arrays Technology Pty Ltd, Canberra, Australia) markers assayed on the 368 DH lines. The genetic map was generated using MapManager Version QTXb20 (Manly et al. 2001), with the marker order refined using RECORD (Van Os et al. 2005). Before any further genetic and statistical analysis, a curation of the genotypic data was conducted and 14 lines were excluded due to inconsistencies that may have been caused by contamination of DNA samples.

Polymorphic markers on chromosome 3B

Based on comparison of the linkage map of chromosome 3B with a neighbour genetic map of the same chromosome (Paux et al. 2008) that was developed from 13 genetic maps using the approach described by Cone et al. (2002), 56 additional markers (38 SSR and 18 insertion site-based polymorphism (ISBP; Paux et al. 2010; Paux et al. 2011) were selected for parental screening. For 46 of these markers, primer sequences are publicly available (Online Resource 1). For the other ten markers, primer sequences were obtained from Institut fuer Pflanzengenetik und Kulturpflanzenforschung, Gatersleben and TraitGenetics Ltd (http://www.traitgenetics.com) (markers with the prefix "gwm") and from Genoplante, INRA France (markers with the prefix "gpw"). All of the polymorphic markers were then assayed on each of 368 DH lines using Multiplex-Ready technology (Hayden et al. 2008). The polymorphic markers were added to the map using MapManager Version QTXb20 (Manly et al. 2001). Genetic distances between marker loci were then re-calculated using the hidden Markov algorithm of Lander and Green (1987) available in the R/qtl package (Broman et al. 2010). This package is available from the Comprehensive R Archive Network (CRAN) at http://CRAN.R-project.org/package=qtl. The marker segregation distortion was tested using a Chi-squared (χ^2) test ($p < 0.01$). Prior to further statistical analysis, the genotype of each DH line for each marker was coded as 1 (homozygous for the RAC875 allele) or −1 (homozygous for the Kukri allele). Missing values were imputed using the flanking marker algorithm of Martinez and Curnow (1992).

Selection of recombinant inbred lines for phenotyping

Two marker loci (*barc77* and *gwm114*) that flank the target QTL region were selected based on the results reported by Bennett et al. (2012a). These two markers were assessed on 768 RI lines using Multiplex-Ready technology (Hayden et al. 2008). Lines exhibiting recombination between these two markers (RAC875 genotype at one locus and Kukri genotype at the other one) were screened with a marker for *Ppd-D1* (Beales et al. 2007), using the SSR marker *barc13* that was reported as being closely linked with a QTL for

flowering time in the RAC875/Kukri population (*QEet.aww-2B*) (Bennett et al. 2012c) and with other markers that had been mapped between the two SSR loci *barc77* and *gwm114* using the DH population. A total of 109 RI lines were used in this research based on their genotypes at these four markers. The genotype of each RI line for each marker was coded as 1 (homozygous for the RAC875 allele), −1 (homozygous for the Kukri allele) or 0 (heterozygous) prior to analysis.

Phenotypic evaluation

Phenotypic data from 21 field experiments (Table 1) were used for multi-environment analysis of grain yield, thousand grain weight, and early vigour. Sixteen of the 21 experiments have been described previously by Reynolds et al. (2009a) and Bennett et al. (2012a, b, c). In four of these experiments, the entire population of 368 DH lines was phenotyped. In the other 12 experiments, a subset of 260 DH lines was evaluated in experiments conducted in Australia and of these 255 DH were evaluated in experiments conducted in Mexico. These lines had been selected based on ear emergence time under field conditions, in order to limit confounding effects of phenological variation (Table 1).

Five additional experiments were conducted in this work: three in 2009 in Australia under a 4.5 m × 26 m polyurethane tunnel (polytunnel) with removable sides on the Waite Campus of the University of Adelaide (Urrbrae, South Australia, 34°52′S 138°30′E, 48 m above sea level) and two in 2011 in north-western Mexico at a managed-environment field site (CIMMYT, Ciudad de Obregon, 27°25′N 109°54′W, 38 m above sea level).

In the polytunnel experiments, the entries were the parents RAC875 and Kukri, three other wheat cultivars (Gladius, Excalibur and Drysdale) and 46 DH lines that had been selected based on flowering time and on evidence of recombination between *barc77* and *gwm114*. These experiments were sown in early September, which is much later than the normal commercial sowing time (April/May) for wheat in South Australia. The late sowing ensured the plants would be exposed to high temperatures and drought stress during flowering and grain filling. Within each experiment, there were two plots of each entry, arranged in a completely randomised design. Each plot consisted of two rows of eight plants (0.6 m × 0.2 m), with data collected on 12 of these plants. The experiments were irrigated every second day from sowing to flowering, using sprinklers. One experiment (designated AusUrr09_SI-S_LS, for Australia-Urrbrae (location) 2009—sprinkler irrigation—saturated—late sowing) was supplied with sufficient water to maintain the leaf water potential close to 0 MPa. The second experiment (AusUrr09_2_SI-W_LS, where W denotes well-irrigated) received sufficient water that the leaf water potential was −0.2 MPa at flowering time. In the third experiment (AusUrr09_SI-D_Ls, where D denotes moderate drought), water supply was withheld after anthesis, decreasing water potential to −0.6 MPa. The soil was shallow (40–60 cm deep) and rich in clay.

In Mexico, one experiment (MexObr11_DI_CS for Mexico—2011—drip irrigation—conventional sowing) was sown in December 2010. During the first 2 months, this experiment received around 150 mm of water from a drip irrigation system. This experiment included 114 entries: 34 DH lines (20 of which were common with the three experiments conducted in Australia in 2009), 77 RI lines, RAC875, Kukri, and one control (cultivar Sokoll). A second experiment in Mexico (designated MexObr11_FI_LS where FI denotes flood irrigation and LS late sowing) was sown in March so that it would be exposed to very high temperatures at flowering time (average $T_{max} > 32$ °C). This experiment received around 1,050 mm of water by flood irrigation. This experiment included 146 entries: the same 34 DH lines as in MexObr11_DI_CS, 109 RI lines (including the 77 RI lines that were in MexObr11_DI_CS), RAC 875, Kukri and other cultivars (Sokoll and Weebil 1).

All of the DH and RI lines included in these two experiments were selected based on their genotypes for the SSR locus *barc13* (representing the photoperiod-sensitivity locus *Ppd-B1* that underlies the QTL *QEt.aww-2B*), the *Ppd-D1* locus for the photoperiod-sensitivity and the SSR loci *barc77* and *gwm114*. Each of the selected lines carried one of the parental allelic combinations at *barc13* and *Ppd-D1* (i.e., the photoperiod insensitivity allele *Ppd-B1a* and the photoperiod-sensitivity allele *Ppd-D1b* as in RAC875, or the photoperiod-sensitivity allele *Ppd-B1b* and the photoperiod insensitivity allele *Ppd-D1a* as in Kukri) and a non-parental (recombinant) allelic combination for the markers *barc77* and *gwm114*. In each of these experiments, a two-replicate alpha-lattice design was used, with each plot consisting of a raised bed with 2 m rows separated from each other by 30 cm. The soil type at the experimental station has been described by Olivares-Villegas et al. (2007) and Deckers et al. (2009).

Early vigour was scored on a scale from 1 (lowest vigour) to 9 (highest vigour) in each of eight experiments AusRos08_NI_CS, AusPie07_NI_CS, AusBoo07_NI_CS, AusMin07_NI_CS, AusRob07_NI_CS, MexObr07_FI_CS, MexObr08_FI_CS, MexObr08_FI_LS. In Mexico, grain was machine harvested, whereas in Australia spikes were manually harvested and threshed. For five experiments (AusUrr09_SI-S_LS, AusUrr09_SI-W_LS, AusUrr09_SI-D_LS, MexObr11_DI_CS and MexObr11_FI_LS), a seed counter (Pfueffer GmBH, Germany) was used to count out dry samples (10 % moisture content) of between 250 and 500 grains, which were then weighed to estimate thousand grain weight. Mid-day leaf water potential (MPa) was

Table 1 Descriptions of 21 environments in which experiments were conducted; showing locations, water supply from rainfall and/or irrigation, criteria used to select (RAC875/Kukri) lines for inclusion, numbers and types included, sowing densities, mean temperatures around flowering time, and statistical analyses performed

Environment	Location	Latitude	Longitude	Altitude (M)	Selection criterion[a]	Lines tested	Sowing density seed m^{-2}	Rainfall and/or irrigation mm	Mean temperature around flowering time		Analysis performed[b]	
											MET	Allele effect
									Sept	Oct		
AusRos07_NI_CS	Roseworthy (SA)	34°57′S	138°36′E	68	All	368 DH	200	153	21.8	25.4	✔	✔
AusRos08_NI_CS	Roseworthy (SA)	34°57′S	138°36′E	68	All	368 DH	200	223	20.8	25.2	✔	✔
AusPie07_NI_CS	Piednippie (SA)	32°68′S	134°31′E	35	Flowering time	260 DH	200	113	22.4	24.1	✔	✔
AusPie08_NI_CS	Piednippie (SA)	32°80′S	135°15′E	35	Flowering time	260 DH	200	212	22	25.6	✔	✔
AusHor08_NI_CS	Horsham (VIC)	35°21′S	138°74′E	132	Flowering time	260 DH	200	187	18.5	23.7	✔	✔
AusBoo07_NI_CS	Booleroo (SA)	32°88′S	138°35′E	342	Flowering time	260 DH	200	159	20.8	24.2	✔	✔
AusMin07_NI_CS	Minnipa (SA)	32°80′S	135°15′E	165	Flowering time	260 DH	200	86	23.9	26.1	✔	✔
AusStr08_NI_CS	Streaky Bay (SA)	32°80′S	134°22′E	27	Flowering time	260 DH	200	95	22	25.6	✔	✔
AusRob07_NI_CS	Robinvale (VIC)	34°61′S	142°81′E	89	Flowering time	260 DH	200	99	21.4	25.2	✔	✔
AusNun08_NI_CS	Nunjikompita (SA)	32°27′S	134°31′E	73	Flowering time	260 DH	200	96	22	25.6	✔	✔
									Feb	March		
MexObr07_DI_CS	Ciudad de Obregon	27°28′N	109°56′W	38	All	368 DH	200	150	25.2	28.6	✔	✔
MexObr07_FI_CS	Ciudad de Obregon	27°28′N	109°56′W	38	All	368 DH	200	750	25.2	28.6	✔	✔
MexObr08_FI_CS	Ciudad de Obregon	27°28′N	109°56′W	38	Flowering time	255 DH	200	750	24.4	27	✔	✔
MexObr09_DI_CS	Ciudad de Obregon	27°28′N	109°56′W	38	Flowering time	255 DH	200	50	25.6	26.9	✔	✔
MexObr11_DI_CS	Ciudad de Obregon	27°28′N	109°56′W	38	Marker genotypes	34 DH/77 RI	200	150	24.5	29.3	●	✔
									April	May		
MexObr08_FI_LS	Ciudad de Obregon	27°28′N	109°56′W	38	Flowering time	255 DH	200	900	30.7	35	✔	✔
MexObr09_FI_LS	Ciudad de Obregon	27°28′N	109°56′W	38	Flowering time	255 DH	200	1,050	30.5	35.6	✔	✔
MexObr11_FI_LS	Ciudad de Obregon	27°28′N	109°56′W	38	Marker genotypes	34 DH/109 RI	200	1,050	30.7	34.8	●	✔
									Oct	Nov		
AusUrr09_SI-S_LS	Urrbrae (SA)	34°57′S	138°36′E	225	Recombinant	46 DH	133	N/A	21.8	27.5	✔	✔
AusUrr09_SI-W_LS	Urrbrae (SA)	34°57′S	138°36′E	225	Recombinant	46 DH	133	N/A	21.7	27.5	✔	✔
AusUrr09_SI-D_LS	Urrbrae (SA)	34°57′S	138°36′E	225	Recombinant	46 DH	133	N/A	21.7	27.5	✔	✔

Aus Australia, *Mex* Mexico, *07-11* 2007 to 2011, *DI* drip irrigation, *NI* not irrigated but rainfed, *FI* flooding irrigation, *SI* sprinkler irrigation, *D* drought, *S* saturated, *W* well watered, *LS* late sowing, *CS* conventional sowing, *DH* doubled haploid lines, *RI* recombinant inbred lines

[a] Marker genotypes: recombinant lines between the two loci *barc77* and *gwm114* and genotyped for *PPd-D1* and *barc13*

[b] Black dot indicates that phenotypes from only the doubled haploid lines were included in the analysis

measured using a Scholander pressure chamber (Scholander et al. 1964).

Statistical analysis

Single environment single trait analysis

Initially for each environment, the traits grain yield, thousand grain weight, and early vigour were analysed using a linear mixed model that partitioned and accounted for genetic and non-genetic or extraneous variation. Where $\boldsymbol{y} = (y_1, \ldots, y_n)$ is a vector for trait observations, the single environment linear mixed model was defined as

$$\boldsymbol{y} = \boldsymbol{X\tau} + \boldsymbol{Zu} + \boldsymbol{Z}_g\boldsymbol{g} + \boldsymbol{e} \quad (1)$$

where $\boldsymbol{X\tau}$ is the fixed component of the model containing an indicator term that differentiates the DH lines from control lines (parents and cultivars) as well as marker information for *Ppd-D1* and *barc13* to control for differences in phenology. This term was also used to capture possible covariate information or linear trends that might exist across rows or columns of the field experiment. The term $\boldsymbol{Zu}$ is a random component that captures extraneous non-genetic variation possibly existing in the environment. Most importantly, $\boldsymbol{Z}_g\boldsymbol{g}$ is a random component that models the genetic effects among DH lines grown in the environment. The genetic effects were assumed to have a distribution $\boldsymbol{g} \sim N(\boldsymbol{0}, \sigma_g^2\boldsymbol{I}_g)$ where σ_g^2 is the genetic variance and $\boldsymbol{I}_g$ is the identity matrix. The residual error, $\boldsymbol{e}$, was assumed to be distributed $\boldsymbol{e} \sim N(\boldsymbol{0}, \sigma^2\boldsymbol{R})$ where σ^2 is the residual variance and $\boldsymbol{R}$ is a matrix that typically contains a parameterization for a separable AR1 × AR1 process (AR1 = auto-regressive process of order 1) to capture the correlation between observations due to the proximity of neighbouring plots in the environment. The effects $\boldsymbol{u}$, $\boldsymbol{g}$, and $\boldsymbol{e}$ were considered to be independent. The terms of this model varied according to the experimental layout and the numbers of DH and control lines sown at each location.

The best linear unbiased predictions (BLUPs) of the genetic effects for each line were extracted from each model and a generalized heritability (h_g^2) was calculated using the formula developed by Cullis et al. (2006) and Oakey et al. (2006), namely

$$h_g^2 = 1 - \frac{\text{PEV}}{2\sigma_g^2},$$

where PEV is the average pairwise prediction error variance of the BLUPs and σ_g^2 is the genetic variance.

Multi-environment single trait analysis

The multi-environment trial (MET) model for a single trait can be considered as an extension of the single environment single trait model defined in (1). In this extended model, the vector of trait observations is $\boldsymbol{y} = (\boldsymbol{y}_1, \ldots, \boldsymbol{y}_t)$ where t is the number of environments involved in the analysis. The terms $\boldsymbol{X\tau}$ and $\boldsymbol{Zu}$ were partitioned according to the fixed effects and non-genetic random effects at each location. The vector of residual errors was $\boldsymbol{e} = (\boldsymbol{e}_1, \ldots, \boldsymbol{e}_t)$ which at the jth site, was assumed to have distribution $\boldsymbol{e}_j \sim N(\boldsymbol{0}, \sigma_j^2\boldsymbol{R}_j)$; $j = 1, \ldots, t$. In this model, $\boldsymbol{e}_i$ and $\boldsymbol{e}_j$ for all $i = j$ were considered independent. Thus, phenotypically, the environments were deemed to be unrelated.

An important aspect of the MET model is the parsimonious modelling of the variance–covariance structure for the genotype by environment interaction. Typically, the genetic effects are assumed to have a distribution $\boldsymbol{g} \sim N(\boldsymbol{0}, \Sigma \otimes \boldsymbol{I}_g)$, where $\boldsymbol{\Sigma}$ is an unstructured $(t \times t)$ variance–covariance matrix that reflects the relationships of the DH and control genetic effects between environments and $\otimes$ represents the direct product or Kronecker operator. Computationally, the estimation of $\boldsymbol{\Sigma}$ is difficult and an approximation was sought that models the genetic effects using the factor analytic (FA) approach of Smith et al. (2001, 2005). Under this approximation, the variance of the genetic effects becomes

$$\text{var}(\boldsymbol{g}) = (\boldsymbol{\Gamma\Gamma}^{\text{T}} + \boldsymbol{\Psi}) \otimes \boldsymbol{I}_g,$$

where $\boldsymbol{\Gamma}$ is a $(t \times k)$ matrix of factor loadings, k is the number of factors involved in the approximation and $\boldsymbol{\Psi}$ is a diagonal matrix of environment specific variances. This FA approximation reduces the number of parameters required for estimation from $t(t + 1)/2$ in the unstructured case, to tk.

If $\boldsymbol{D}$ represents $(t \times t)$ a diagonal matrix of genetic variances extracted from the diagonal values of $\boldsymbol{\Gamma\Gamma}^{\text{T}} + \boldsymbol{\Psi}$ then the correlation matrix for the genetic effects can be calculated using:

$$\text{cor}(\boldsymbol{g}) = \boldsymbol{D}^{-1/2}(\boldsymbol{\Gamma\Gamma}^{T} + \boldsymbol{\Psi})\boldsymbol{D}^{-1/2} \otimes \boldsymbol{I}_g \quad (2)$$

The expression on the left hand side of the Kronecker operator was used to summarize the genetic correlation of the trait across the environments used in the analysis.

Multi-environment QTL analysis

For multi-environment QTL analyses, the MET model for each trait was extended by partitioning the multi-environment genetic effects:

$$\boldsymbol{g} = (\boldsymbol{I}_t \otimes \boldsymbol{m}_i)\boldsymbol{a}_i + \boldsymbol{p}, \quad (3)$$

where $\boldsymbol{a}_i = (a_{i1}, \ldots, a_{it})$ is a vector for the fixed QTL by environment marker effects for the ith marker, $\boldsymbol{m}_i$. In this expression, $\boldsymbol{p}$ represents the residual component of the genetic model not captured by the marker. Analogous to the MET linear mixed model, these become residual genotype by environment interaction effects and are

modelled using the FA approach of Smith et al. (2001, 2005). Each marker of chromosome 3B was then considered in turn and a separate MET QTL model was fitted for each marker in the linkage group.

To detect putative multi-environment QTL on chromosome 3B, an appropriate hypothesis test was used. From each MET QTL model, the single marker multi-environment QTL effects were tested simultaneously under the null hypothesis $H_0 : a_{i1} = a_{i2} = \cdots = a_{it} = 0$ and a Wald statistic was calculated (Kenward and Roger 1997). Using the Wald statistics, a multi-environment QTL profile, analogous to a LOD (logarithm base 10 of odds) QTL profile, spanning the linkage group 3B was calculated for each trait. Significant QTL regions were identified as peaks exceeding a Wald statistic threshold that was calculated through back transformation of an adjusted Bonferroni corrected p-value with significance level $\alpha = 0.05$ (Li and Ji 2005). This threshold differed for each trait due to the number of sites involved in the multi-environment QTL analysis.

Multi-environment allele effects with DH and RI

Using the QTL profiles of all three traits from the multi-environment QTL analysis, a set of four markers on chromosome 3B were chosen as representing a specific QTL region for further investigation. For this analysis, genetic marker information for each of the four markers was combined across DH lines and RI lines, with missing marker scores set to zero. The MET QTL model for each trait was then refitted for each of the four markers. For each trait by marker combination, the marker effect was estimated and its significance was tested using a Wald statistic.

Computations

All single and multivariate site analyses were performed using the linear mixed modelling package ASReml-R (Butler et al. 2009; software at http://www.vsni.co.uk) available in the R Statistical Computing Environment. ASReml-R uses a residual maximum likelihood (REML) approach of Patterson and Thompson (1971) for estimation of the model parameters. It also provides useful diagnostic tools such as variograms and residual plots to help determine environmental trends possibly existing in the field.

Results

Grain yield means and trait heritability

The mean grain yield for the RAC875/Kukri DH population varied considerably across environments, ranging from 0.32 to 6.53 t/ha (Table 2). Across experiments, the mean grain yield of RAC875 was significantly ($p = 0.03$) higher than that of Kukri. Heritability of grain yield (Table 2) was moderate to high (ranging from 0.58 to 0.87) for most experiments, but low (0.23 and 0.30) for two of the polytunnel experiments. The heritability of thousand grain weight was moderate to high (0.60–0.92) for all experiments except for Mex08_FI_LS (0.41). The heritability of early vigour was between 0.43 and 0.65 for seven of the eight experiments in which this trait was scored, but was only 0.29 for MexObr07_FI_CS.

Multi-environment analysis

For each of the traits, a sequential set of FA models was fitted for the genotype by environment interaction component of the MET model (Table 3). The most parsimonious model for each trait was then chosen by minimizing the model selection criterion, the Bayesian Information Criterion (BIC) developed by Schwarz (1978). For early vigour, the FA2 (factor analytic model of order 2) was found to be the most appropriate (Table 3). Thousand grain weight and grain yield required FA3 and FA4 models, respectively (Table 3). For these traits, the need for more factors was due to the diverse range and number of environments used in the MET analysis. For each trait, the estimated multi-environment genetic variance–covariance matrix was extracted from the MET analysis and converted to a multi-environment genetic correlation matrix using Eq. (2). An empirical threshold of $r = 0.4$ was used to help discriminate environments. For grain yield, five groups of experiments Gya to Gye were identified (Fig. 1). The group Gya consists of the three polytunnel experiments (Table 1), which were poorly correlated ($r < 0.4$) with the 18 other experiments. The experiments conducted in Mexico formed three overlapping groups (Gyb, Gyc and Gyd in Fig. 1). The groups Gyb and Gyc included all experiments conducted in Mexico that received flood irrigation except for MexObr09_DI_CS. The group Gyd consisted of four experiments conducted in Mexico (including an environment in which drought occurred early in the growth cycle) and the three lowest yielding experiments conducted in 2007 in Australia. Two of these environments had high temperatures at flowering time (Table 1). All of the experiments conducted under rainfed conditions in Australia grouped together (group Gye). For thousand grain weight, the multi-environment genetic correlation plot identified four groups of experiments (Online Resource 2) one of which consisted of just one experiment from Mexico and three of which each contained experiments from both Australia and Mexico. For early vigour, the multi-environment genetic correlation exhibited two distinct groups: Gva (three experiments in Mexico) and Gvb (five experiments in Australia) (Online Resource 2).

Table 2 Mean grain yields of RAC875, Kukri and the RAC875/Kukri lines, and heritability estimates for grain yield, early vigour and thousand grain weight for each environment in which data were collected

Environment	Lines evaluated	Mean grain yield t/ha			Heritability		
		Population	RAC875	Kukri	Grain yield	Early vigour	Thousand grain weight
AusRos07_NI_CS	368 DH	2.36	2.94	2.59	0.87	–	0.81
AusRos08_NI_CS	368 DH	2.18	2.86	2.17	0.86	0.65	–
AusPie07_NI_CS	260 DH	0.32	0.45	0.24	0.79	0.52	0.92
AusPie08_NI_CS	260 DH	1.43	1.47	1.36	0.67	–	0.92
AusHor08_NI_CS	260 DH	0.96	0.94	0.89	0.77	–	–
AusBoo07_NI_CS	260 DH	1.57	1.60	1.64	0.68	0.43	0.89
AusMin07_NI_CS	260 DH	0.41	0.45	0.35	0.77	0.67	0.89
AusStr08_NI_CS	260 DH	0.65	0.67	0.55	0.75	–	0.84
AusRob07_NI_CS	260 DH	0.55	0.60	0.53	0.65	0.48	0.79
AusNun08_NI_CS	260 DH	0.53	0.60	0.50	0.83	–	0.92
MexObr07_DI_CS	368 DH	1.46	1.39	0.87	0.62	–	0.6
MexObr07_FI_CS	368 DH	4.40	5.57	4.86	0.58	0.29	0.83
MexObr08_FI_CS	255 DH	5.14	5.40	5.50	0.67	0.63	0.83
MexObr09_DI_CS	255 DH	2.23	2.75	2.04	0.71	–	0.85
MexObr11_DI_CS	34 DH/77 RI	1.11	1.33	1.10	0.74	–	0.84
MexObr08_FI_LS	255 DH	1.54	2.04	1.56	0.84	0.69	0.42
MexObr09_FI_LS	255 DH	2.27	2.41	2.68	0.75	–	0.77
MexObr11_FI_LS	34 DH/109 RI	2.79	3.84	3.05	0.75	–	0.87
AusUrr09_SI-S_LS	46 DH	6.53	7.08	7.09	0.61	–	0.71
AusUrr09_SI-W_LS	46 DH	5.88	5.46	4.68	0.23	–	0.61
AusUrr09_SI-D_LS	46 DH	2.00	1.84	2.22	0.3	–	0.81

Aus Australia, *Mex* Mexico, *07-11* 2007 to 2011, *DI* drip irrigation, *NI* not irrigated but rainfed, *FI* flooding irrigation, *SI* sprinkler irrigation, *D* drought, *S* saturated, *W* well watered, *LS* late sowing, *CS* conventional sowing, *DH* doubled haploid lines, *RI* recombinant inbred lines

Map of chromosome 3B

Twenty molecular markers (Fig. 2) were added to the QTL region on chromosome 3BL in the RAC875/Kukri genetic map, including 9 markers (5 SSR and 4 ISBP) between *wPt-4401* and *gwm114*, the markers reported previously by Bennett et al. (2012a) as delimiting the 95 % confidence interval for the QTL. Comparison of the resulting genetic map with a neighbour genetic map of chromosome 3B (Paux et al. 2008) indicated a similar order of markers. None of the markers mapped on chromosome 3B exhibited significant segregation distortion.

Multi-environment QTL analysis

Extension of the MET model for each trait to individually incorporate each of the 47 markers that had been mapped on chromosome 3B resulted in Wald statistic profiles for each trait (Fig. 3). For each trait, there was a highly significant peak in the 5.2 cM chromosome region that includes the loci *gwm1266*, *cfb43*, *gwm299*, and *wmc236* and several minor peaks elsewhere on the chromosome.

Allele effects of single marker across all environments

When the MET QTL model for grain yield and thousand grain weight was refitted with the combined genetic information from the DH and the RI lines (Table 2), it was found that the magnitudes and directions of allelic effects at the four marker loci in the QTL peak region varied among environments (Fig. 4). For grain yield, there were statistically significant effects in 10 of 21 experiments, with the favourable allele coming from RAC875 in eight experiments (all of group Gyc, most of group Gyb and Gyd and part of group Gye) and a favourable effect from Kukri (at marker locus *wmc236* only) in two of the three experiments in group Gya (polytunnel). For thousand grain weight, there were statistically significant effects in 7 of 19 environments, with the favourable allele coming from RAC875 in four experiments (group Gyb, Gyc and Gyd) and from Kukri in three experiments (groups Gya and Gyb). For six of the eight experiments in which early vigour was rated, there were significant positive allelic effects ($p < 0.05$), indicating that RAC875 carries an allele improving early vigour (Online Resource 3).

Table 3 Genotype by environment interaction models used in multi-environment analysis

Trait (number of environments)	Model	Number of parameters	Log-likelihood	BIC	Efficiency %
Yield (21)	FA5	119	9,990.13	−18878.72	78.4
	FA4	100	9,957.11	−18988.55	78.0
	FA3	81	9,866.99	−18984.19	50.7
	FA2	62	9,740.44	−18906.97	40.5
	FA1	42	9,543.25	−18697.71	22.9
Thousand grain weight (19)	FA4	91	8,057.50	−15285.93	84.2
	FA3	73	8,012.04	−15359.01	80.3
	FA2	56	7,867.67	−15225.13	73.3
	FA1	38	7,717.46	−15088.71	59.2
Early vigour (8)	FA3	29	−336.76	919.12	73.6
	FA2	23	−342.67	880.13	71.7
	FA1	16	−409.14	953.79	40.6

FA5 factor analytic model of order $k = 5$, *BIC* Bayesian information criterion

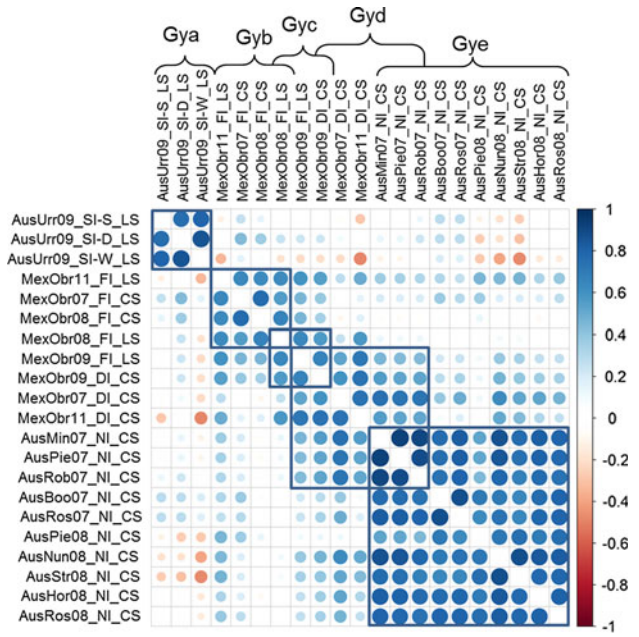

Fig. 1 Multi-environment genetic correlation for grain yield extracted from the associated FA4 model. Each circle represents a pairwise correlation coefficient between environments of 0.4 or more, with the diameter of the circle proportional to the absolute value of the correlation coefficient and the colour of the circle indicating whether the correlation is positive (*blue*) or negative (*red*). Groups of genetically correlated environments are outlined by *squares* and labelled at the *top* of the figure (color figure online)

Allele effects using recombinant inbred lines

To further investigate allele effects in the chromosomal region of interest, a simplified bi-environment model was carried out on the two experiments containing 109 RI lines (MexObr11-DI-CS and MexObr11-FI-LS). In this analysis, the complete phenotypic information for the DH and RI lines was used to ensure environmental and design effects were appropriately modelled but the genetic marker information of the DH lines was ignored. The results show the RAC875 allele was favourable in MexObr11_FI_LS ($p < 0.05$) at three loci (*gwm1266*, *cfb43* and *cfp49*), increasing grain yield by 4 % and for thousand grain weight in MexObr11_DI_CS with a maximum of 6.2 % increase at *cfp1556* locus (Fig. 5).

Discussion

Crop yield under stress is determined by complex interactions between the genetic make-up of the plant and the nature and timing of the environmental stress with respect to the plant's development. In a previous study using the RAC875/Kukri population of wheat, a region on chromosome 3B was found to be associated with yield under drought and heat stress (Bennett et al. 2012a). The key objectives of the current study were to define the yield response across different environments and provide a basis for fine mapping and ultimately cloning the gene(s) underlying the QTL. The first component of the study involved an assessment of the performance across environments of the DH lines that were used for the initial mapping. This provided an improved map of the target region and a basis for identifying additional molecular markers. A second larger population of RI lines was then used to confirm the location of the QTL.

Most of the environments in which experiments were grown experienced moderate or severe stress. The severity of stress can be assessed by comparing the average grain yields across the experiments. Under the most favourable growing conditions (AusUrr09_SI-S_LS), some lines yielded more than 10 t/ha. In the experiments used in the present study, the average site yields ranged from 0.32 to 6.53 t/ha indicating that the stress levels were reducing yields by up to 95 % of the potential. Yields of less than 1 t/ha are generally regarded as non-commercial, however,

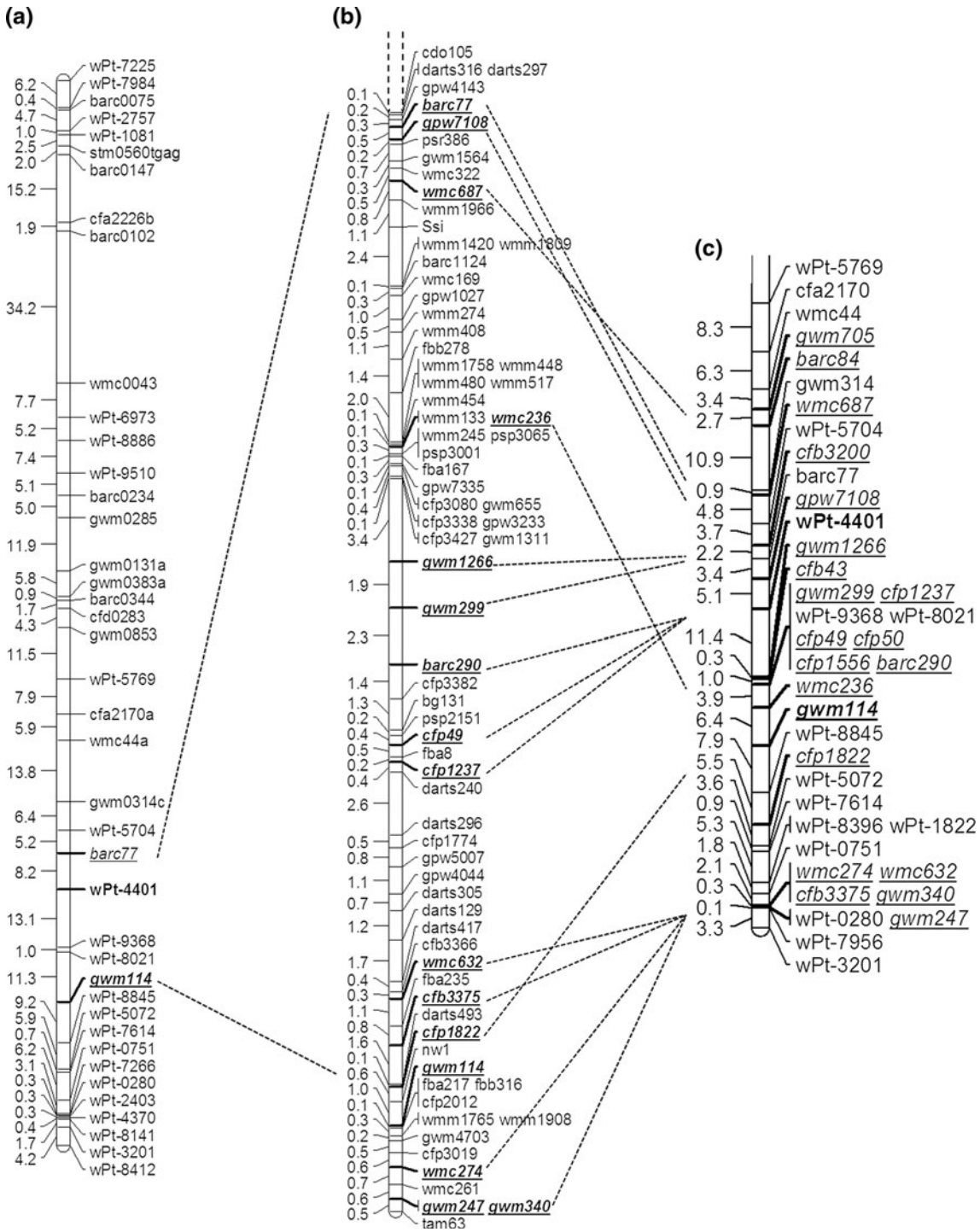

Fig. 2 Relationships between three genetic maps of chromosome 3BL: **a** the map of chromosome 3B (RAC875/Kukri) published by Bennett et al. (2012a), **b** part of a neighbour map of chromosome 3B adapted from Paux et al. (2008), **c** the region of interest of chromosome 3BL (RAC875/Kukri). Common markers between the three maps are indicated with lines. The new markers mapped here, are shown in *italic font* and *underlined* (**b**, **c**). The two markers that flank the interval found by Bennett et al. (2012a) are shown in *bold font* (**a**, **c**)

in certain parts of the world such as South Australia, a grain yield lower that 1 t/ha is still considered useful (Hunt and Kirkegaard 2011).

The grouping of environments used in this study follows a pattern that reflects the importance of the nature and pattern of drought exposure. The experiments conducted in

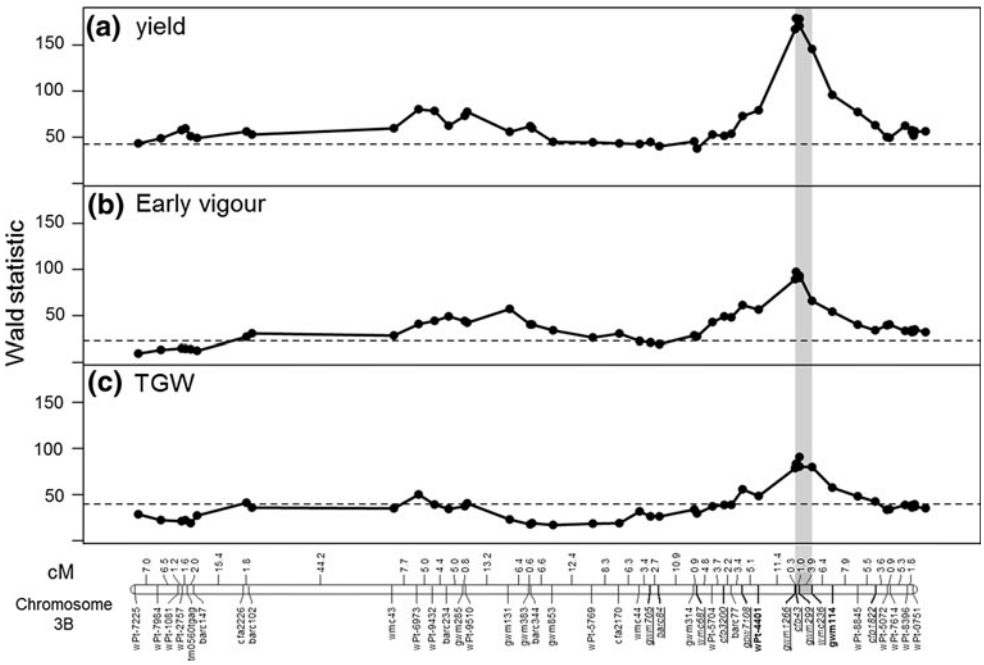

Fig. 3 Wald-statistic profiles on chromosome 3B based on a multi-environment QTL scan. The profiles correspond to three traits: **a** grain yield (21 environments), **b** early vigour (eight environments), and **c** thousand grain weight (19 environments). The two markers that flank the interval found by Bennett et al. (2012a) are shown in *bold font*. Co-located markers have been omitted from the map displayed below the profiles

Mexico separated into those where flood irrigation was used as opposed to drip irrigation. Given the deep soils at the Obregon site, flood irrigation followed by drought is expected to provide an advantage to lines that are able to track moisture as it retreats down the soil profile. In contrast, the drip irrigation system would impose a drought stress more similar to the sites in Australia, where intermittent and declining rainfall provides adequate moisture during earlier stages of the crop cycle, but can result in strong terminal drought stress. Consistent with this, the drip-irrigated experiments conducted in Mexico showed considerable genetic correlation with the most severely droughted experiments in Australia. The differences in performance of lines between flood-irrigated experiments and the drip-irrigated and rainfed experiments may well be related to the genetic control of root architecture, an aspect worthy of further experimentation. The high temperatures experienced in all of the experiments conducted in Mexico may have added significantly to the stress severity, yet these experiments still showed reasonable grain yield (Table 1).

The three experiments conducted in a polytunnel (AusUrr09) extended the range of environmental conditions under which the RAC875/Kukri materials were evaluated. The polytunnel experiments all received adequate moisture throughout vegetative growth. This was in contrast to the conditions experienced in field environments in both Mexico and Australia, where plants would have encountered moisture limitations early in their development. The very high grain yields achieved in the saturated (AusUrr09_SI-S_LS) and well-watered (AusUrr09_SI-W_LS) polytunnel experiments were likely attributable to the pre-anthesis establishment of a strong source of photosynthates prior to anthesis, combined with continuation of an adequate water supply throughout grain filling. These high yields were achieved despite exposure to very high temperatures during grain filling; mean temperatures of 27 °C in November, with 5 (AusUrr09_SI-W_LS) to 10 (AusUrr09_SI-S_LS) days with maximum temperatures exceeding 35 °C). With imposition of post-anthesis drought (AusUrr09_SI-D_LS), grain yield was substantially reduced.

Malosetti et al. (2008) reported that the directions of allelic effects for grain yield, ear number, and anthesis-silking interval in maize (*Zea mays* L.) depended on the environmental conditions (drought and/or nitrogen supply) under which a mapping population was investigated. In our study in wheat, one parent (RAC875) contributed the favourable QTL allele for grain yield in almost all of the experiments conducted in Mexico and in two low-yielding experiments in Australia (AusPie07_NI_CS and AusMin07_NI_CS), whereas the other parent (Kukri) contributed the favourable allele for grain yield under the more favourable environmental conditions in Australia (Fig. 4). In seven out of ten environments in which significant effects were detected for grain yield, there were also effects on thousand grain weight. In six of these environments, the allele that increased grain yield also increased thousand grain weight. The one exception was MexObr07_FI_CS, where the RAC875 allele increased grain yield but decreased thousand grain weight. That allele must have had a favourable effect on one or both of the other two yield components, the number of tillers per m^2 and/or the

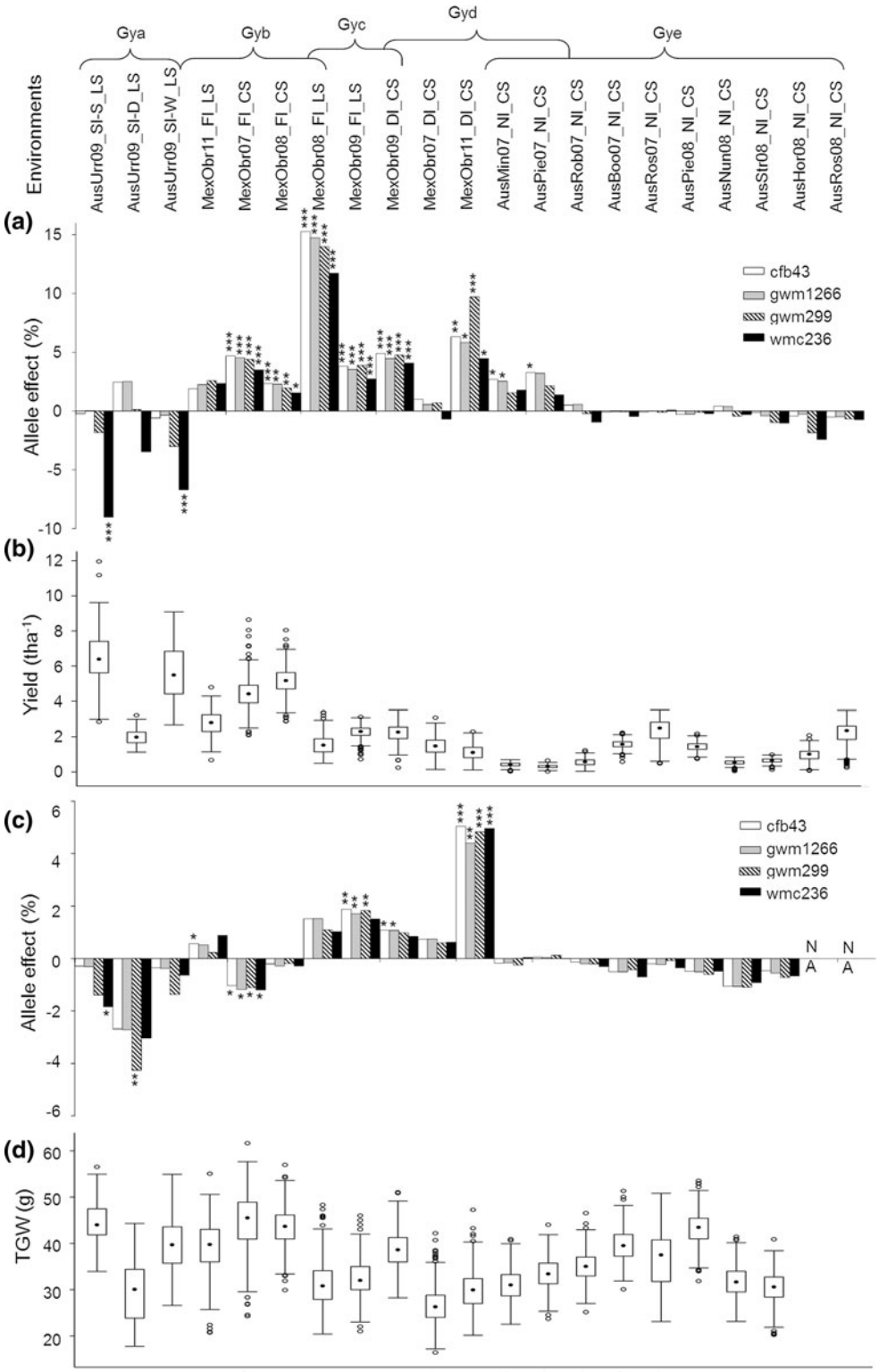

Fig. 4 Allele effects (**a**, **c**) of four loci (*cfb43*, *gwm1266*, *gwm299* and *wmc236*) on chromosome 3B and trait means (**b**, **d**) for: grain yield (**a**, **b**) and thousand grain weight (**c**, **d**) in each environment. Allele effects are expressed as percentage relative to the trait mean. A positive effect indicates that the RAC875 allele increased the trait value while a negative effect indicates that the Kukri allele increased the trait value. Levels of significance are represented by: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. In the boxplots (**b**, **d**), the *black dot* indicates the median value, the box encloses the second and third quartiles, the whiskers extend to $\pm$ 1.5 times the inter-quartile range, and the *empty dots* indicate outliers. The groups Gya (Group yield a) to Gye (Group yield e) were selected based on multi-environment correlation analysis with an empirical threshold of $r = 0.4$ (Fig. 1)

number of grains per tiller. Lines with the RAC875 allele were apparently better able to exploit the favourable conditions of this irrigated environment to establish and retain more tillers and/or grains, increasing yield without affecting grain size.

In addition to heat and drought stress, several crop management factors could explain the opposite allelic effects including: (1) sowing density (200/m^2 in Australia vs. 133/m^2 in Mexico) which could affect competition among plants, (2) water supply (sprinkler vs. flooding

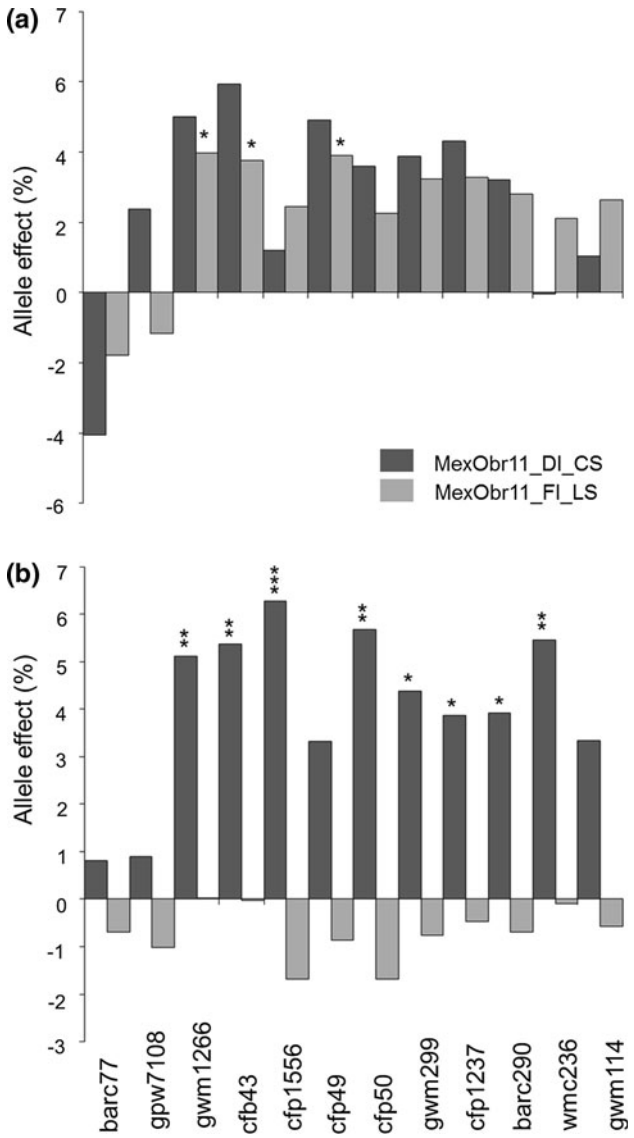

Fig. 5 Allele effects at 12 loci on chromosome 3B using a bi-environmental analysis of: **a** grain yield and **b** thousand grain weight of for 77 recombinant inbred lines evaluated in the drought-stressed Mex11_DI_CS experiment (*dark gray*) and for 109 recombinant inbred lines in the late-sown Mex11_FI_LS experiment in which high temperatures were experienced during grain filling (*light gray*). Allele effects are based on genetic marker information from recombinant inbred lines only. A positive effect indicates that the RAC875 allele increased the trait value while a negative effect indicates that the Kukri allele increased the trait value. Levels of significance are represented by: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

irrigation), (3) soil composition, with a light fertile soil in Australia (McCord and Payne 2004) and a deep fertile soil in Mexico (Northwest of Mexico, Valle del Yaqui, CIMMYT) (Deckers et al. 2009), (4) field management (raised beds to avoid anoxia in Mexico vs. flat plots in Australia) (5) biotic stresses (pathogens, pests or weed competition), or abiotic stresses other than drought and heat (e.g. salt or heavy metals) and (6) differences in photoperiod due to differences in latitude and/or sowing date. Some of these differences could have provided contrasting advantages for different types of root systems (Palta et al. 2011). For example, the deep soil and flood irrigation in Mexico could have benefited plants with deep roots whereas this trait was unlikely to provide significant advantages on the shallow soils and with intermittent rainfall in Australia.

Both water deficit and high temperature can affect the water flow through the plant. These stresses (individually or combined) are reflected in the water status of the plants (Tardieu and Davies 1993). Canopy temperature is also a good indicator for water status; differences in canopy temperature have been associated with the same genetic region on 3BL in RAC875/Kukri population by Bennett et al. (2012a) and also in another wheat population (Pinto et al. 2010). Other measurements such as stomatal conductance or leaf water potential can also represent plant water status. In other crops, such as rice (*Oryza sativa* L.), QTL associated with both leaf water potential and grain yield have been identified under different water regimes (Qu et al. 2008). Jongdee et al. (2002) proposed leaf water potential as a selection criterion for improving drought tolerance. In the present study, based on measurement of daytime leaf water potential taken at flowering time in five experiments (AusUrr09_SI-S_LS, AusUrr09_SI-W_LS, AusUrr09_SI-D_LS, MexObr11_DI_CS and MexObr11_FI_LS), the grain yield advantage provided by the RAC875 allele was greatest in environments with severe water stress (−2 to −3.8 MPa in Mexico vs. −1.4 to −0.6 MPa in Australia) (Online Resource 4).

In the RAC875/Kukri population, the early vigour QTL was co-located with the grain yield QTL in stressed and non-stressed environments. Early vigour has been reported to be a beneficial adaptive trait for warm and dry climates (Ludwig and Asseng 2010), leading to strong plants throughout the growing season. Early vigour in shoots has been found to increase root growth (Watt et al. 2005; Richards et al. 2007) and nitrogen uptake (Liao et al. 2006). However, early vigour does not necessarily lead to high yield. It is generally associated with high biomass, which can be unfavourable if drought occurs during grain filling (terminal drought) since large leaf area can lead to high rate of water loss under high temperatures.

The multi-environment QTL analysis methods used here combine a multiplicative FA approach to model the genotype by environment interaction of grain yield and yield-related traits whilst simultaneously estimating individual marker effects across environments. By accurately capturing the genetic correlation of the DH and RI lines across the environments this approach becomes a powerful tool for estimation of QTL, such as the QTL studied here,

as well as assisting in determining the correct parental allele for target environments.

From the Wald statistic profile along chromosome 3B, it was clear that a narrow genetic region had pleiotropic effects on grain yield, thousand grain weight and early vigour (Fig. 3). The effects of each marker within this region were then evaluated across 21 experiments (environments) using data from both DH lines and RI lines (Figs. 4, 5). The analysis showed that this region plays an important role in determining grain yield in a range of environments and is likely to be a useful target for selection in breeding programs. The MET analysis incorporated new loci (*gwm1266*, *cfb43*, *gwm299*, and *wmc236*) that were not available for the analysis conducted by Bennett et al. (2012a). These new markers revealed positive effects of the RAC875 allele on grain yield in two experiments in Australia (AusPie07_NI_CS and AusMin07_NI_CS) and a negative effect of the RAC875 allele on thousand grain weight in Mexico (MexObr07_FI_CS) (Fig. 4).

After initial genetic analysis using the mapping population of DH lines, selected RI lines were phenotyped for validation of allele effects, to narrow down the genetic region and to permit the identification of candidate genes for functional analysis. The starting point of this analysis was a region of over 20 cM (Bennett et al. 2012a). The inclusion of additional markers in the multi-environment analysis allowed the region to be narrowed down to around 5 cM. The analyses of stress responses of RI lines confirm the significance of the region. While the nature and the function of the gene underlying this QTL remain unknown, addition of new markers to the linkage map has been useful to select the associated genetic region and to track it with reliable molecular markers (Figs. 3, 4).

Despite evidence for substantial genetic variation in response to water deficit (Reynolds et al. 2009b), it is difficult to attribute this variation to specific genes. Many studies have attempted to define genomic regions associated with drought and/or heat responses in Triticeae crops and there is now a substantial body of literature describing regions of potential relevance; in bread wheat (Mathews et al. 2008; Mason et al. 2010; Alexander et al. 2012), in durum wheat (Peleg et al. 2009) and in barley (von Korff et al. 2008; Chen et al. 2010). The region of chromosome 3BL that contains the marker locus *gwm299* has often been reported as associated with quantitative traits related to tolerance to abiotic stress (heat and/or drought) or biotic stress (pathogenic fungi or nematodes) (Online Resource 5). This co-location of QTL may be due to multiple important genes in this region or to gene(s) with pleiotropic effects. Improvements in marker technologies and in methods for analysis of genetic and environmental data have allowed us to revisit this chromosome region.

These results, coupled with ongoing wheat whole-genome sequencing (http://www.wheatgenome.org/), provide a promising foundation for positional cloning of the QTL affecting grain yield under moderate to extreme drought and heat stress. Isolation of putative candidate gene(s) underlying this QTL will require a better understanding of gene content and order in this region using the ongoing gene annotation of chromosome 3B sequence (http://www.wheatgenome.org/content/view/full/407). Further, dissection of the genotype by environment interactions and the effects of alleles might be obtained through additional field experiments with precise quantification of the environmental characteristics including soil factors (moisture, composition and depth), drought severity and air temperatures. Careful genetic characterisation of the impact of this chromosome region on grain yield across a range of environments will permit assessment of the usefulness of this locus for wheat improvement through marker-assisted selection.

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