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ORIGINAL PAPER

Multi-environment QTL mixed models for drought stress adaptation in wheat

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Abstract Many quantitative trait loci (QTL) detection methods ignore QTL-by-environment interaction (QEI) and are limited in accommodation of error and environment-specific variance. This paper outlines a mixed model approach using a recombinant inbred spring wheat population grown in six drought stress trials. Genotype estimates for yield, anthesis date and height were calculated using the best design and spatial effects model for each trial. Parsimonious factor analytic models best captured the variance–covariance structure, including genetic correlations, among environments. The 1RS.1BL rye chromosome translocation (from one parent) which decreased progeny yield by 13.8 g m^{-2} was explicitly included in the QTL model. Simple interval mapping (SIM) was used in a genome-wide scan for significant QTL, where QTL effects were fitted as fixed environment-specific effects. All significant environment-specific QTL were subsequently included in a multi-QTL model and evaluated for main and QEI effects with non-significant QEI effects being dropped. QTL effects (either consistent or environment-specific) included eight yield, four anthesis, and six height QTL. One yield QTL co-located (or was linked) to an anthesis QTL, while another co-located with a height QTL. In the final multi-QTL model, only one QTL for yield (6 g m^{-2}) was consistent across environments (no QEI), while the remaining QTL had significant QEI effects (average size per environment of 5.1 g m^{-2}). Compared to single trial analyses, the described framework allowed explicit modelling and detection of QEI effects and incorporation of additional classification information about genotypes.

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Introduction

Multi-environment trials (METs) are commonly used to assess the performance of genotypes across a range of sites and years to sample the target population of production environments (Comstock 1977). Increasingly, METs that include populations of genetically related individuals are being studied to understand the genetic control of adaptive traits by identifying associations with quantitative trait loci (QTL). A characteristic of METs is large genotype-by-environment interaction (GEI), i.e. relative changes in performance of genotypes conditional on the environment, especially for complex traits, such as yield. Complex traits are the outcome of physiological processes and environmental influences over the crop cycle and result from the interactions of many gene pathways. Detecting QTL for these types of traits in environments with varying degrees of stress is potentially difficult as the QTL effects tend to be small and controlled by more than one gene pathway and by interactions among genes, traits and environments.

In the dryland wheat environments of northern Australia, GEI is large, and often causes rank changes of tested lines across environments (Brennan and Byth 1979; Cooper and Woodruff 1993). The size of the GEI component tends to increase where METs encounter a range of environmental stresses, for example, drought through irrigated conditions (Mathews et al. 2002). The typical assumptions of common trial variance and a common correlation among environments rarely hold in across-trial analyses of these trials. Improved modelling of the genetic variance–covariance matrix can accommodate the commonly ignored occurrence of trial heterogeneity and variation in among trial correlation. In recent years, this has become the basis for modelling the genetic variance–covariance matrix using multiplicative and/or mixed models (Piepho 1997; Smith et al. 2001b; Crossa et al. 2004), which results in more precise estimates of line performance, and facilitates better selection decisions by plant breeders.

Freely available QTL analysis software packages (e.g. QTL Cartographer (Wang et al. 2005), QTLNetwork (Yang et al. 2005), PlabQTL (Utz and Melchinger 1996) are limited in flexibility. The software does not account for experimental error effects (i.e. they use means as input, with no adjustment of residual error) and does not allow parsimonious modelling of the genetic variance–covariance matrix of MET datasets. For multi-trait analyses, either an unstructured (i.e. heterogeneous trial variances and heterogeneous between-trial correlations) or diagonal model is assumed to model the variance–covariance matrix. The former is theoretically an ideal structure for modelling the genetic variance–covariance matrix. However, estimating the components of this matrix quickly becomes computationally difficult with increasing numbers of trials and/or traits. Importantly, the modelling process for variance–covariance matrices is not transparent in these packages, and frequently MET QTL analyses simply compare the QTL detected across a series of single trial analyses. In many studies, the detection of QTL for yield and other traits rarely utilise more than a small number of trials or traits, e.g. 2 to 4 (Kuchel et al. 2006; Kumar et al. 2007; Rebetzke et al. 2007), with arbitrary emphasis placed on QTL that are detected in two or more year–site combinations. Whilst this may be appropriate for detecting reliable QTL in highly heritable traits with minimal GEI, this approach is less optimal for complex traits with low heritability and large GEI. As QTL analyses become further integrated into plant breeding programs, there is an increasing need to accommodate the larger numbers of environments that are studied, and to predict how different genomic regions contribute to adaptation to environmental variables (e.g. Boer et al. 2007). High throughput genotyping is allowing plant breeders to screen the progeny of multiple crosses, with phenotyping undertaken as part of standard plant breeding trials. Analysis and software methods are required for routine analyses of such unbalanced data during the plant breeding process, so that identified QTL can be applied in future generations using marker-assisted selection.

In recent years, mixed model frameworks have been used to detect QTL-by-environment (QEI) effects while modelling the variance–covariance matrix (Piepho 2000; Verbyla et al. 2003; Malosetti et al. 2004; van Eeuwijk et al. 2005; Boer et al. 2007). Verbyla et al. (2003) fitted QEI effects as random, while others considered these effects as fixed. A simulation study showed that modelling the variance–covariance matrix within a mixed model framework was more powerful in detecting fixed QTL and QEI effects than when fixed models were used (Piepho 2005). The methodology presented here extends the models described by Malosetti et al. (2004) to incorporate individual plot variability and illustrates the flexibility of the mixed model approach to accommodate additional genotype and experiment factors. It is easily implemented in any statistical software that accommodates mixed models.

Herein, the objective was to develop a mixed model approach that uses molecular marker data to explain the underlying GEI patterns of complex traits, such as yield in dryland environments. A spring bread wheat (*Triticum aestivum*) population of a cross between elite drought adapted parents was investigated for yield QTL in drought-stressed environments in northern Australia. The QTL detection results were compared to those obtained from the common practice of detecting QTL on a single trial basis using the same dataset. The results demonstrate the flexibility of the mixed model framework to incorporate factors to account for field variability, and additional genotypic factors, such as chromosome translocations. Finally, results for QTL analyses of anthesis and height were used to interpret the QTL detected for yield.

Methods

Dataset

Population description

A recombinant inbred line (RIL) population from a reciprocal cross between two elite spring bread wheats, Seri M 82 (a released line from the Veery cross, KVZ/BUHO//KAL/BB) and Babax (a line derived from a cross known as Babax, BOW/NAC//VEE/3/BJY/COC) was developed at CIMMYT (International Maize and Wheat Improvement Center), specifically to study physiological traits in a population with a small range in phenology (Olivares-Villegas et al. 2007). Using pedigree information back five

generations within a set of fixed lines representing a large sample of CIMMYT and Australian germplasm (Mathews et al. 2007), the coefficient of parentage (COP) between the parents was 0.3316 (McLaren et al. 2005), which conveys the strong relatedness in this cross (a COP = 0 indicates no relationship; 1 = identical lines). Seri M 82 contains the rye translocation (Rajaram et al. 1983) on the short arm of chromosome 1B, and the notation is 1RS.1BL (also known as 1B.1R or 1B/1R in the literature). As the cross Babax was known to segregate for 1RS.1BL, the Babax line selected as a parent was selected to have 1BS.1BL, i.e. without the rye translocation. The population was selfed whilst in quarantine in Australia, and was field increased twice before use in northern Australia in 2002, i.e. producing F7 seed. In successive years, the seed source was from harvested seed in previous trials. Leaves from the 194 F4-derived, then single seed descent, F7:8 progeny were used to produce a molecular map (McIntyre et al. 2006).

Map construction

The molecular markers were a combination of 74 SSRs, 249 AFLPs and 264 DArT markers (Wenzl et al. 2004). Two lines were removed from the analysis; one contained incorrect markers and was most likely a rogue (data not shown) while the second line contained a high number of missing DArT values (40%) (data not shown). JoinMap® 3.0 (van Ooijen and Voorrips 2001) was used for marker diagnostics and to determine the linkage groups. The marker order was refined using RECORD (Isidore et al. 2003). Some regions of the map were quite dense for DArT markers, and these were thinned in an iterative process of eliminating markers within 2 cM and recalculating the distances for the remaining markers using the R statistical software (R Development Core Team 2008) and R/qtl package (Broman et al. 2003). Linkage groups of length less than 15 cM or with less than three markers were not included in the QTL analysis described below. The map used in the QTL mapping procedure described here contained 29 linkage groups with 401 markers for 192 individuals, with only chromosomes 3D and 7D missing.

The markers on linkage group 1B-a showed a segregation ratio of 3:1 (Babax-type: Seri-type). In addition, markers on linkage group 1B-a and 4A-b showed a reduced rate of recombination resulting in a high proportion of largely parental chromosomes. Neither of these observations were associated with the reciprocal cross or with each other: χ^2(1B-a, reciprocal cross) = 0.0353, χ^2(4A-b, reciprocal cross) = 0, χ^2(1B-a, 4A-b) = 0.0065 ($\chi^2 = 3.841$ at $\alpha = 0.05$). Progeny were also screened with two rye-specific markers to identify progeny carrying the 1RS.1BL translocation from Seri M 82. PCR was undertaken using rye 5S rDNA primers (Koebner 1995) and primers to the rye-specific repeated DNA sequence, Iag95 (Mohler et al. 2001; Mago et al. 2002), following the protocols described therein.

Phenotypic data

As part of a research program into drought adaptation, the population was grown in eight environments in the northern wheat region of Australia from 2002 to 2006. The majority of trials were grown under dryland conditions, with sowing into moist soil profiles after a rainfall event, with no further water input except for rainfall. From 2002 to 2004, a subset of the population, 189 RILs, was grown under dryland conditions in two-replicate alpha-lattice designs at the CSIRO Gatton Research station (27.54°S; 152.34°E) in south east Queensland. All 194 progeny were grown in 2005 at four sites in the north-eastern wheat region of Australia in single-replicate augmented-check designs, with 20% checks. Of these trials two were grown at the Gatton site, one under dryland conditions and one irrigated. The other trials were grown at Biloela (24.38°S, 150.52°E) in Central Queensland (supplementary irrigated) and Lundavra (28.99°S, 150.02°E) in south-western Queensland. Hailstorms just prior to harvest at Gatton in 2003 and 2005 (irrigated trial only), resulted in loss of plot yield data. In 2006, the 194 RILs were grown under irrigation at the Gatton site in a single-replicate augmented-check design trial with 20% of plots as parents or checks. The fully irrigated trials at Gatton in 2005 and 2006 were irrigated throughout the crop cycle sufficiently to eliminate water stress. Yield (g m^{-2}), anthesis (days) and height (cm) were measured in most trials. A small-plot harvester was used to harvest plots that were 1.8 m wide (6 or 8 rows) and between 3 and 5.5 m in length. Grain was cleaned and left to air-dry for several days prior to weighing, with all grain yields being expressed as oven-dry values following determination of the grain moisture content of samples from each plot. Anthesis was measured as the number of days from sowing to when 50% of the plot had plants with anthers extruding and plant height (cm) was an average of two measurements per plot, taken during grain-filling. There was some imbalance across traits and trials. In both the phenotypic and QTL analyses described below, all available data were used for each trait.

Genotypic factor

The 1RS.1BL rye translocation has been reported to enhance grain yield in optimum and drought-stressed environments (Villareal et al. 1998). To detect yield QTL which were independent of this effect, a genotypic factor, *rye*, was coded. It was based on two rye-specific markers, rye 5S rDNA primers and the rye-specific repeated DNA

sequence primers, Iag95; and confirmed by the segregation distortion groups on 1B-a from the molecular map. A line that was scored with both these rye-specific markers was assigned a value of 1 (1RS.1BL), and otherwise a value of 0 (1BS.1BL). Fifty-one progeny contained the rye 1RS.1BL translocation. When included as a fixed effect, there was a significant main effect for all traits at the phenotype level and so the environment-specific *rye* effect was included in the QTL analyses to account for the variation expected from the presence of the *rye* translocation.

Statistical analysis

Phenotypic analysis

For each trait, a two-stage process was used to perform the phenotypic analysis. In the first stage, each of the t trials was modelled separately and the m genotype means produced for input into the second stage. In the second stage, the two-way table of genotype-by-trial means (of size $m \times t$) was used to model the genetic variance–covariance matrix.

In the first stage, best spatial models for yield, anthesis and height were determined for each trial (environment) following Gilmour et al. (1997) using Residual Maximum Likelihood (REML) in GenStat Release 9.0 (Payne et al. 2006) and assuming random genotype effects. Following the notation of Welham et al. (2006), for an individual trial, j ($j = 1,\ldots, t$), the mixed model in vector notation can be written as:

$$\boldsymbol{y}_j = \boldsymbol{M}_j\boldsymbol{\eta}_j + \boldsymbol{\varepsilon}_j \qquad (1)$$

where $\boldsymbol{y}_j$ is the ($n_j \times 1$) vector of observations for the jth trial, with n_j plots; $\boldsymbol{\eta}_j$ is the ($m \times 1$) vector of m random genotype effects; $\boldsymbol{M}_j$ is the ($n_j \times m$) design matrix of genotype effects at each trial j; $\boldsymbol{\varepsilon}_j$ is the vector of residual errors at trial j. The within-trial residuals $\boldsymbol{\varepsilon}_j$ were modelled such that experimental design parameters (such as replicate and sub-block within replicate for the two-replicate alpha-lattice designs) were fitted as random; and the checks were fitted as fixed effects in the single-replicate augmented-check designs. An autoregressive process in each of the row and column directions (separable AR × AR model) modelled the spatial trend while further global effects (broad trends such as gradient or fertility trends in the column and/or row direction) and extraneous spatial effects (trial management practices such as irrigation pipe placement or harvest order) were fitted as fixed and random effects, respectively (Gilmour et al. 1997).

It is usual to report an estimate of line mean heritability on a single trial basis. However, when the $\boldsymbol{\varepsilon}_j$ vector is modelled using spatial effects, there is no longer a single estimate for the trial residual variance, σ^2. To overcome this Cullis et al. (2006) and Oakey et al. (2006) formulated the generalised heritability as:

$$h^2_g = 1 - \frac{\text{PEV}}{2\sigma^2_g}, \qquad (2)$$

where PEV is the predicted error variance, or average variance of the difference (VPREDICT statement in Genstat 9th edition), and σ^2_g is the genotypic variance. The generalised heritability can be interpreted as the proportion of total phenotypic variance explained by the genotypic component and can be used to calculate the expected genetic gain (Cullis et al. 2006; Piepho and Möhring 2007). The above formulation can be extended to calculate the heritability for an across-site analysis and to include the additive relationship matrix to calculate the narrow sense heritability (Oakey et al. 2006) as breeders would generally select on performance across sites (Hanson 1963). The trial mean and generalised heritability on a single trial basis for each trait were calculated from the best spatial models where the genotypes were fitted as random effects (Table 1).

While it is appropriate to assume genotypes as random during the process of modelling spatial and residual effects, the Best Linear Unbiased Predictors (BLUPs) produced are not appropriate for a two-stage MET analysis as they are scaled by their individual trial heritability and hence, are based on different variance estimates. Smith et al. (2001a) identified this problem and recommended obtaining the Best Linear Unbiased Estimates (BLUEs) from the best spatial model before proceeding with the MET analysis. That is, determine the best spatial model with the genotypes as random, then refit the genotypes as fixed effects keeping all other random effects constant (fixed to the values determined in the random model). According to Smith et al. (2001a), the resulting BLUEs, collected in the ($m \times 1$) vector $\boldsymbol{z}_j$, are based on more efficient estimates than if the spatial and design parameters had been initially determined from a model with fixed genotypes. The genotype BLUEs are now considered to be on the same scale, and hence comparable across trials. To accommodate known trial variance heterogeneity, the inverse of the variance matrix of means from each trial is used to generate weights for use in the MET analysis (Smith et al. 2001a). That is, if the variance matrix of the BLUEs, $\boldsymbol{z}_j$, is $\text{var}(\boldsymbol{z}_j) = \tilde{\Sigma}_j$; the weights, as defined by Smith et al. (2001a), are the diagonal of the inverse of this matrix, $\boldsymbol{\pi}_j = \boldsymbol{diag}(\tilde{\Sigma}_j^{-1})$.

In the second stage of the analysis, the two-way genotype-by-trial table of BLUEs from the single trial analyses was modelled together with the weights described above. The models fitted here included environment as a fixed main effect, and a random environment-specific genotypic effect, i.e. there was no explicit partitioning of random genotype main effect and random GEI effects, resulting in

Table 1 Sowing dates, trial mean, range and heritability (h_g^2) for yield (g m^{-2}), anthesis (days) and height (cm); phenotypic correlations between yield and anthesis and yield and height (r_p), and the environment-specific *rye* effects for yield and height

Trial[a]	Sowing date	Yield (g m^{-2})		Anthesis (days)		Height (cm)		r_p	r_p	Environment-specific rye effect[b]	
		Mean (range)	h_g^2	Mean (range)	h_g^2	Mean (range)	h_g^2	Yield-anthesis	Yield-height	Yield[c]	Height[c]
BILO05	9/06/2005	473 (325–597)	0.89	82.8 (75–91)	0.95	92.2 (78–105)	0.79	−0.75	0.18	12.5	0.81
LUND05	25/05/2005	352 (244–441)	0.86	–	–	86.5 (70–101)	0.91	–	0.06	19.8	2.60
GATD02	4/06/2002	317 (200–393)	0.71	94.2 (85–101)	0.88	76.7 (65–89)	0.70	−0.26	0.08	14.9	0.13
GATD03	11/06/2003	–	–	88.2 (82–97)	0.93	69.6 (55–89)	0.65	–	–		1.10
GATD04	16/06/2004	416 (296–492)	0.53	90.0 (84–98)	0.91	82.6 (71–94)	0.83	−0.02	0.24	3.2	0.88
GATD05	27/05/2005	222 (63–391)	0.59	92.7 (87–104)	0.94	61.6 (45–80)	0.77	−0.18	0.34	12.7	1.50
GATI05	14/06/2005	–	–	89.8 (84–98)	0.87	–	–	–	–	–	–
GATI06	23/05/2006	631 (447–803)	0.44	94.4 (85–103)	0.93	91.8	0.66	0.15	0.10	19.7	−0.13

[a] *BILO05* Biloela 2005, *LUND05* Lundavra 2005, *GATD* Gatton Dryland, *GATI* Gatton Irrigated, 02-06: 2002 to 2006

[b] +ve represents absence of rye segment on yield and height for the Seri/Babax population grown in the eight trials in the northern region of Australia, 2002–2006

[c] Average standard error of difference for yield = 8.4; height = 0.92

a so-called GGE model (Crossa and Cornelius 1997; Yan et al. 2000). The GGE model can be interpreted as a type of principal components model for the two-way genotype-by-environment table, with the genotypes as objects and the environments as variables, and with the environmental columns centred, i.e. with correction for the environmental main effects. The mixed model in vector notation can be written as.

$$z = \boldsymbol{\eta} + \boldsymbol{v} \tag{3}$$

where $\boldsymbol{z}$ is the ($mt \times 1$) stacked vector of m genotype BLUEs, $\boldsymbol{z}_j$, for the $j = 1,\ldots, t$ environments; $\boldsymbol{\eta}$ is the ($mt \times 1$) vector of GGE effects for all m by t combinations (the modelling of which is described in more detail below); $\boldsymbol{v}$ is the ($mt \times 1$) vector of errors associated with the estimation of $\boldsymbol{z}$, (i.e. BLUEs), and is normally distributed with variance matrix $\text{var}(\boldsymbol{v}) = \boldsymbol{\Pi}^{-1}$ and $\boldsymbol{\Pi} = \boldsymbol{diag}(\boldsymbol{\pi}_1', \ldots, \boldsymbol{\pi}_t')$, the weights from the single trial analyses. The model for the GGE effects, $\boldsymbol{\eta}$, can now be written as.

$$\boldsymbol{\eta} = \boldsymbol{X}_{\boldsymbol{\eta}}\boldsymbol{\tau}_{\boldsymbol{\eta}} + \boldsymbol{Z}_{\boldsymbol{\eta}}\boldsymbol{u}_{\boldsymbol{\eta}} \tag{4}$$

where $\boldsymbol{\tau}_{\boldsymbol{\eta}}$ is a ($t \times 1$) vector of fixed effects, for example, including an intercept term and ($t - 1$) differences with the intercept, representing environment main effects; $\boldsymbol{X}_{\boldsymbol{\eta}}$ is the ($mt \times t$) design matrix associated with $\boldsymbol{\tau}_{\boldsymbol{\eta}}$, $\boldsymbol{u}_{\boldsymbol{\eta}}$ is the ($mt \times 1$) vector of random GGE effects and $\boldsymbol{Z}_{\boldsymbol{\eta}}$ is its associated ($mt \times mt$) design matrix. For a completely balanced dataset $\boldsymbol{Z}_{\boldsymbol{\eta}}$ will be an identity matrix of size mt, $\boldsymbol{I}_{mt}$; for unbalanced data the size of the matrix will be the same, however, it will have a column of zeros, $\boldsymbol{0}_{ij}$, where genotype i is missing in environment j, so that unbalanced data are accommodated. The variance–covariance matrix of $\boldsymbol{u}_{\boldsymbol{\eta}}$, $\text{var}(\boldsymbol{u}_{\boldsymbol{\eta}})$, can be represented by the separable variance structure $\text{var}(\boldsymbol{u}_{\boldsymbol{\eta}}) = \boldsymbol{G}_{\boldsymbol{e}} \otimes \boldsymbol{G}_{\boldsymbol{v}}$. $\boldsymbol{G}_{\boldsymbol{e}}$ is the $t \times t$ environment component matrix, or genetic variance matrix; $\boldsymbol{G}_{\boldsymbol{v}}$ is the $m \times m$ genotype component matrix and in the present analyses $\boldsymbol{G}_{\boldsymbol{v}} = \boldsymbol{I}_{\boldsymbol{m}}$. Four variance structures for $\boldsymbol{G}_{\boldsymbol{e}}$ from a GGE model were investigated to determine the best fit to the data (1) a diagonal structure allowing heterogeneous environment variances; (2) factor analytic ($k = 1$), FA1; (3) factor analytic ($k = 2$), FA2, and (4) unstructured which models all environment variances and correlations. For comparison, the commonly used 'compound symmetry' model was included with one variance component for the genotypic main effect and another component for the GEI interaction. To compare these models both the Akaike Information Criteria (AIC, (Akaike 1974)) and Bayesian Information Criteria (BIC, (Raftery 1986)) were calculated. If the best models had similarly small AICs (i.e. differed by <2), then the most parsimonious (low BIC) model was selected as the variance–covariance model to use in the QTL mixed model. The genetic correlations between environments were calculated from this model.

Multi-environment QTL mixed model

For each of the three traits, the strategy for modelling multi-environment QTL combined mixed model methodology with regression using the two-way table of means and comprises variations of Malosetti et al. (2004) and Boer et al. (2007). A genome-wide scan for significant QTL expression was performed using a SIM procedure. Each marker (or between-marker) position was fitted as a fixed environment-specific QTL effect while retaining the best variance–covariance structure that had been previously determined in the GGE model. Each fixed marker or

between-marker QTL effect thus combines the QTL main effect and the QEI effects (QQE model). Positions that represented significant QTL in the SIM scan were selected for inclusion in a multi-QTL model. At each of the selected positions, QTL effects were decomposed and tested for QTL main effects (consistent effect across environments) and QEI effects (environment-specific QTL effects). For those positions where QEI effects were not significant, only the QTL main effect term was retained in the model.

SIM was performed at a grid of evaluation positions along the genome. The derivation of corresponding genetic predictors is described below. At a single position, q, the model is.

$$\eta = X_q\tau_q + X_\eta\tau_\eta + Z_\eta \boldsymbol{u}_\eta^q \quad (5)$$

where $\boldsymbol{X_q}$ is the ($mt \times t$) design matrix of genetic predictors, i.e. markers and virtual markers (see below), at the evaluation positions for all m genotypes at the qth position. This can be expressed as $\boldsymbol{X_q} = \boldsymbol{p_q} \otimes \boldsymbol{I_t}$; where $\boldsymbol{p_q}$ is the ($m \times 1$) genetic predictor at the qth evaluation position, $\boldsymbol{p_q} = (x_1, x_2, \ldots, x_m)'$ and $\boldsymbol{I_t}$ is the identity matrix of dimension t. $\boldsymbol{\tau_q}$ is the ($t \times 1$) vector of fixed additive QTL effects at the qth evaluation position, with the effects for each of the t environments. Genetic background effects were assumed to be normally distributed, such that $\boldsymbol{u_\eta^q} \sim N(0, \text{var}(\boldsymbol{u_\eta^q}))$. Note that var($\boldsymbol{u_\eta^q}$) represents the genetic (co)variance that is not explained by the genetic predictor at the evaluation position, q. The presence of the 1RS.1BL translocation in the wheat RIL population used here was shown to decrease yield, on average, by 13.8 g m^{-2}. Hence, the *rye* factor, determined using rye markers, was included in the SIM scan, to correct for the 1RS.1BL translocation and potentially improve the detection of yield QTL on the same or other linkage groups. In terms of matrix notation, this simply means that the vector of fixed effects, $\boldsymbol{\tau_\eta}$ (defined in Eq. 4), is now [$2t \times 1$] long to incorporate t environment-specific *rye* effects and the associated design matrix, $\boldsymbol{X_\eta}$, increases by t columns to [$mt \times 2t$]. This notation illustrates the flexibility of the mixed model platform to accommodate known genotypic (or environmental) factors.

Estimation of genetic predictors and testing

For QTL detection and estimation it is recommended to have good marker coverage along the genome, although the definition of 'good' is dependent on the population size and gene effect (Darvasi et al. 1993). To accommodate uneven coverage that is typical of most maps, Lander and Green (1987) developed the SIM method based on Bayes theorem and Markov chain methodology which allows the QTL effects and positions to be estimated, based on flanking markers. This was generalised to calculate the probability of obtaining a particular locus genotype based on all observed marker phenotypes in the region with non-missing information from an individual (Jiang and Zeng 1997). For the current RIL population, additive genetic predictors (evaluation positions) were constructed such that the maximum distance between consecutive predictors was 5 cM, which resulted in 614 positions to be tested. In the mapping of this RIL population, co-dominant markers were scored as −1, 0 or 1, referring to marker genotype aa, Aa or AA, respectively (and interpreted as qq, Qq and QQ for QTL genotypes). Additive genetic predictors at evaluation positions in between markers were calculated as the difference between the conditional probabilities for QTL genotypes QQ and qq: Pr(QQ|flanking markers)-Pr(qq|flanking markers). Each of these genetic predictors was then used as an explanatory variable in a mixed model to test for a significant association with the trait of interest.

For each genetic predictor (evaluation position), q, the null hypothesis of no QTL effect in any environment j (H_0: $\boldsymbol{\tau_q} = 0$, for all j) was compared to the alternative (H_0: $\boldsymbol{\tau_q} \neq 0$, for at least one j). As this is a fixed effect in a mixed model, the null hypothesis was tested using the Wald test statistic (Searle et al. 1992; Verbeke and Molenberghs 2000), which is asymptotically distributed as χ^2_r, where r equals the number of parameters being estimated, i.e. t for each $\boldsymbol{\tau_q}$. Since the test was repeated for each genetic predictor, it was necessary to correct for the inflation of the Type I error (false discovery). A Bonferroni-type correction adjusts the experiment-wide error rate for the number of tests performed but, since it assumes that all tests are independent (which they are not) this correction results in a too conservative threshold for QTL detection. The problem can be alleviated by approaches which determine the effective number of tests based on a principal component decomposition of the full set of explanatory variables (Cheverud 2001; Li and Ji 2005). In this study, the Li and Ji (2005) adjustment was calculated at a significance level of $\alpha = 0.10$. The Wald statistic P values were transformed to a $-\log_{10}$ scale to produce QTL profiles for each linkage group. These profiles are analogous to the logarithm of odds ratio (LOD) profiles produced in standard QTL packages. Profiles revealed QTL by peaks in the $-\log_{10}(P)$ values above the significance threshold ($\alpha = 0.10$). A confidence interval for the QTL position was determined by reading off the positions that corresponded to a 1.5 drop-off in the $-\log_{10}(P)$ profile at either side of the maximum in the profile that coincides with the point estimate for the QTL (Keurentjes et al. 2006).

Multi-environment multi-QTL mixed model

All positions identified as QTL from the SIM analyses were included in a multi-QTL model. To determine which QTL were significant in the multi-QTL model, the Wald

statistic was calculated after dropping each individual QTL separately from the full model. Non-significant QTL in this multi-QTL model were then excluded. Each of the remaining QTL were tested to determine significance of QEI, and if not significant, only a QTL main effect was fitted at the QTL position.

The final multi-QTL model can be represented as

$$\boldsymbol{\eta} = \boldsymbol{X_M}\boldsymbol{\tau_M} + \boldsymbol{X_Q}\boldsymbol{\tau_Q} + \boldsymbol{X_\eta}\boldsymbol{\tau_\eta} + \boldsymbol{Z_\eta}\boldsymbol{u_\eta^Q} \quad (6)$$

where M is the number of main effect QTL (i.e. QTL with a consistent effect across the environments) and Q is the number of QTL with inconsistent effects across environments (i.e. QTL with significant QEI) determined from the SIM analysis. $\boldsymbol{X_M}$ is the $(mt \times M)$ design matrix and can be expressed as $\boldsymbol{X_M} = \boldsymbol{p_M} \otimes \mathbf{1}_t$, where $\boldsymbol{p_M}$ is the $(m \times M)$ matrix of genetic predictors for the M QTL and $\boldsymbol{\tau_M}$ is a $(M \times 1)$ vector of fixed main effect QTL. $\mathbf{1}_t$ is a $t \times 1$ vector of 1's. Similarly, $\boldsymbol{X_Q}$ is the $(mt \times tQ)$ design matrix of additive genetic predictors for the Q QTL. It can be expressed as $\boldsymbol{X_Q} = \boldsymbol{p_Q} \otimes \boldsymbol{I_t}$, where $\boldsymbol{p_Q}$ is the $(m \times \mathrm{Q})$ matrix of genetic predictors for the Q QTL; each q column of $\boldsymbol{p_Q}$ is equivalent to $\boldsymbol{p_q} = (x_1, x_2, \ldots, x_m)'$ as before; $\boldsymbol{I_t}$ is the identity matrix of dimension t. $\boldsymbol{\tau_Q}$ is the $(tQ \times 1)$ vector of fixed additive effects for all Q putative QTL across the t environments. All other terms in the model are as defined as for the single QTL model (Eq. 5) except for the random effect $\boldsymbol{u_\eta^Q}$ whose variance, var($\boldsymbol{u_\eta^Q}$), now represents the genetic (co)variance that is not explained by the M and Q QTL. For simplicity in the following discussion the number of QTL are defined as, $Q_N = M + Q$.

From this final model, the QTL effects and the genetic variance explained by each QTL were determined. For plant breeders, the value of a QTL depends on the extent to which the alleles affect the trait of interest (e.g. increase yield). The amount of variation explained by each QTL can also be useful, for example, to define a weight in a multi-QTL selection index. In regression analyses, the amount of variation explained by a term can be expressed as the percentage difference between the residual variance of a model with and without the term. Analogously, as an informal strategy to determine the amount of variance explained by the multi-environment multi-QTL model, genetic variances were estimated for each of the t environments obtained from a model with and without the QTL (Eqs. 6 and 4, respectively). The explained genetic variance (as percentage of the total genetic variance) was calculated as: % explained genetic variance by all QTL = 100 × [1 − (genetic variance in model with all QTL/genetic variance in model without QTL)]. A similar rationale was followed to estimate the contribution of individual QTL. We compared genetic variances of models without QTL (Eq. 4) with variances of models with only a particular QTL included (Eq. 5); % explained genetic variance by a QTL = 100 × [1 − (genetic variance in model with single QTL/genetic variance in model without QTL)]. This estimate for the contribution of an individual QTL can be loosely interpreted as representing an upper bound for the percentage of explained genetic variation. An alternative, lower bound, estimate can be obtained by comparison of the genetic variance of a model with all the QTL included (Eq. 6) with the variance of a model in which all QTL are included except the specific QTL under evaluation; % explained genetic variance by a QTL = 100 × [(genetic variance in model with all QTL − genetic variance in model with all but one QTL)/genetic variance in model without QTL]. For some QTL, the percentage of explained variance was very small ($<10^{-2}$), in which case it was set to 0.

Single trial QTL analyses

Currently, the common practice for QTL analyses is to perform analyses on a single trial basis using dedicated, yet inflexible software. For the purposes of comparison with the methodology presented here, the map and BLUEs from the single trial analyses were analysed using QTL Cartographer for Windows Version 2.0 (Wang et al. 2005). QTL were identified via composite interval mapping (CIM) (Zeng 1993, 1994) using the program's default values, namely Forward Regression with five background markers, a window size of 10.0 cM and a walk speed of 2 cM. Other parameters were investigated, including backward and forward regression and 3–7 background markers, but as these had little effect on the detection of QTL (data not shown) default values were retained. The output from QTL Cartographer is a table of the genetic predictor positions where the logarithm of odds ratio (LOD) is greater than 1. The nearest left flanking marker of the reported genetic predictor is also reported. QTL were defined as two or more markers that were closely linked (<10 cM or adjacent markers at >10 cM) and significantly associated in one trial (single trial QTL) or one marker significantly associated in more than one trial (multi-trial QTL). To facilitate the comparison of methods, QTL at $2 < \mathrm{LOD} < 3$ were differentiated from those with LOD > 3.

Results

Multi-environment mixed model and genetic correlations

For each trait and trial, the trial mean and generalised heritability are summarised for the best spatial models where the genotypes were fitted as random (Table 1). For yield, the generalised heritability h_g^2 was lowest in two

Gatton trials (Table 1) that experienced severe drought at flowering, an event that tends to increase variability for this trait (Cooper et al. 1997). In the compound symmetry model, the ratio of the variance components G to GEI for yield was low (0.482) but typical for these environments (Cooper et al. 1997). The AIC suggested the unstructured model as the best model. However, the factor analytic model of order $k = 2$ (FA2) is more parsimonious (lower value of BIC) and was selected for modelling the residual genetic variance–covariance matrix for yield (Table 2). There was a low genetic correlation (<0.45) between the southern Queensland (Gatton) environments and trials at the two other locations, Lundavra 2005 in western Queensland and Biloela 2005 in Central Queensland (Table 3).

The G to GEI ratio for anthesis (2.628) was much greater than for yield confirming the high across-environment repeatability for anthesis. The lowest individual trial h_g^2 was 0.87 (Table 1). Mean anthesis for the whole population was earliest in Biloela 2005 (83 days) and latest at Gatton 2002 and 2006 (94 days). The range in anthesis among RILs within trials was 14–18 days across environments, although the majority of RILs in each environment flowered within 7 days of one another (data not shown). An FA1 model had the lowest AIC and BIC and was selected for modelling the residual genetic variance–covariance matrix for anthesis. (Table 2). Phenotypic correlations, r_p, between yield and anthesis were generally small, although they were strongly negative in Biloela 2005 (Table 1).

The G to GEI ratio for height (1.667) was lower than for anthesis (2.628) but greater than for yield (0.482). Individual trial h_g^2 for height ranged from 0.65 to 0.91. An FA2 model fitted the residual genetic variance–covariance matrix best, having the lowest AIC of the FA*k* models (Table 2) and a lower BIC than the unstructured model. The phenotypic correlation between yield and height was greatest in the Gatton Dryland 2005 trial (Table 1).

Table 2 Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) for residual genetic variance-covariance matrix models, for yield (g m^{-2}), anthesis (days) and height (cm)

Model[a]	Yield (g m^{-2})			Anthesis (days)			Height (cm)		
	q[b]	AIC	BIC	q	AIC	BIC	q	AIC	BIC
Compound symmetry	1	9,794	9,799	1	4,747	4,751	1	5,860	5,864
Diagonal	6	9,689	9,714	7	4,642	4,671	7	5,807	5,836
FA1	12	9,581	9,631	14	3,482	3,541	14	5,252	5,311
FA2	18	9,558	9,632	21	3,483	3,571	21	5,224	5,312
Unstructured	21	9,556	9,642	28	3,492	3,610	28	5,220	5,338

[a] FAk = factor analytic of order $k = 1$ or $k = 2$

[b] q is the number of parameters in each model

Table 3 Genetic correlations of yield from the multi-environment model, using FA2 to model the genetic variance–covariance matrix

Trial[a]	BILO05	LUND05	GATD02	GATD04	GATD05
LUND05	0.78				
GATD02	0.29	0.42			
GATD04	0.19	0.26	0.55		
GATD05	0.24	0.30	0.55	0.33	
GATI06	0.06	0.16	0.52	0.31	0.30

[a] *BILO05* Biloela 2005, *LUND05* Lundavra 2005, *GATD* Gatton Dryland, *GATI* Gatton Irrigated, 02–06: 2002 to 2006

Multi-environment QTL mixed model

Rye factor

The *rye* factor, either with consistent or environment-specific effects, was included in the model at all stages to account for the 1RS.1BL translocation known to be present in the population (Seri). Its interaction with environment was significant for both yield and height (Table 1); 1RS.1BL lines yielded on average 13.8 g m^{-2} less than 1BS.1BL lines and in all but Gatton Irrigated 2006 they were, on average, 1.2 cm shorter. The *rye* factor was also significant for anthesis as a main effect (0.83-day delay when 1RS.1BL was present), but was not environment-specific. The *rye* factor was highly correlated with genetic predictors on the 1B-a linkage group. The most significant effect for the *rye* factor co-located with a QTL in a model without the *rye* factor (data not shown). The residual variation was expected to decrease with the inclusion of this factor and therefore the power to detect putative QTL on other linkage groups should increase. Indeed there were substantial shifts in the profiles for linkage groups 1B-a (decrease) and 5A-a (increase) when *rye* was included in the yield model (Fig. 1). Several other profiles, such as 7A-a, were relatively unchanged by the inclusion of environment-specific *rye* effects (Fig. 1).

Multi-environment mixed model genome scan (SIM model)

Sixteen genetic predictors were identified from the anthesis, height and yield SIM analyses which related to regions where QTL might be present. The position (cM) of the genetic predictor identified as a putative QTL, its significance level ($\alpha = 0.10$ or 0.05), region (cM), defined at LOD drop of 1.5, and nearest left flanking marker are reported in Table 4.

Eight QTL for yield were on linkage groups 1D-a, 1D-b, 4B-b, 5A-a, 6B-a, 6D-a, 7A-a and 7B-a (Table 4). For anthesis, two QTL were detected at a genome-wide threshold significance level of $\alpha = 0.10$ and a QTL on 2B-a was significant at $\alpha = 0.05$. For height, five QTL

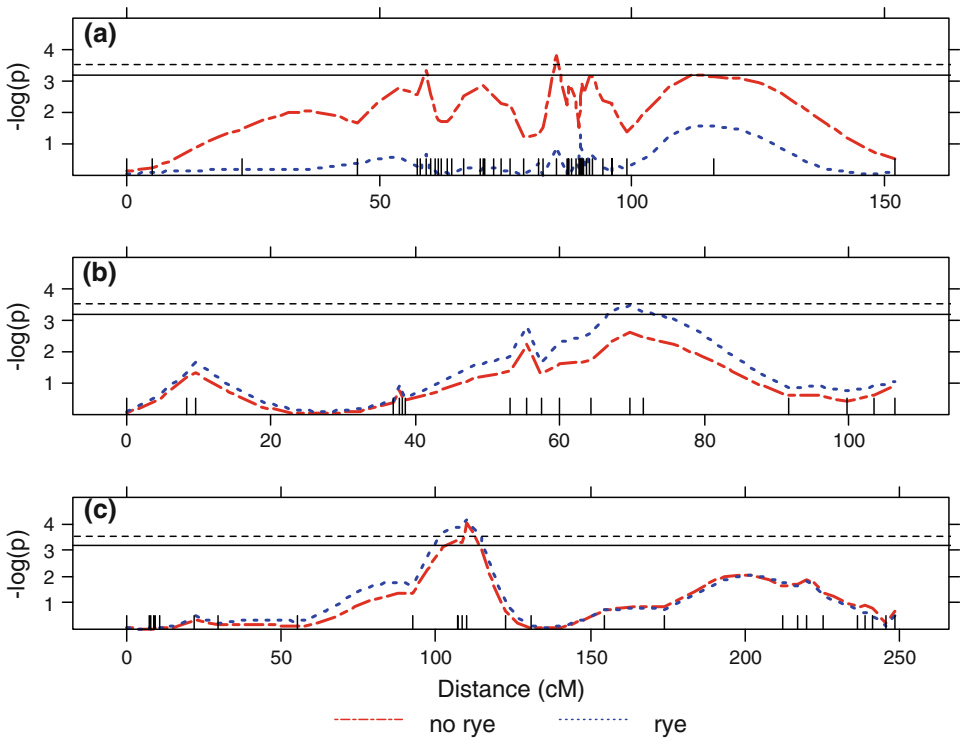

Fig. 1 $-\log_{10}(P)$ profile for (**a**) 1B-a, (**b**) 5A-a, (**c**) 7A-a, illustrating the effect of the *rye* factor on yield. The *horizontal lines* represent different significance levels; solid line is $\alpha = 0.10$, *dashed* is $\alpha = 0.05$. The *vertical lines* indicate marker positions

were detected on different linkage groups ($\alpha = 0.10$) of which three were significant at $\alpha = 0.05$. Two of the yield QTL co-located with QTL for either anthesis or height. None of the anthesis and height QTL co-located (Table 4). Since genetic predictors were calculated at a maximum distance of 5 cM, it is possible that co-located QTL (the same genetic predictor) for the different traits may be a result of genetic linkage rather than pleiotropy.

Multi-environment multi-QTL model for yield

The genetic predictor with the highest $-\log_{10}(P)$ in a QTL region identified by SIM was selected for inclusion in the multi-QTL model. The final yield multi-QTL model thus included all QTL identified in the SIM analysis. That is, yield QTL were detected on linkage groups 1B-a (*rye*), 1D-a, 1D-b, 4B-a, 5A-a, 6B-a, 6D-a, 7A-a and 7B-a. The yield QTL on 1D-b was shown to co-locate with an anthesis QTL. All the yield QTL, except for the one on 6D-a (a main effect), were determined to have significant QEI effects (Table 5). A positive effect indicates the Babax allele contributed to an increase in the trait value and a negative one indicates the Seri allele contributed to an increase in the trait value. On average, the Babax parent reached anthesis 2–3 days earlier, was 5–10 cm taller and yielded up to 50 g m^{-2} less than Seri M 82 in the environments studied here.

The largest yield QTL detected was on linkage group 7A-a (Tables 4, 5). The QTL effects detected on 7A-a were all positive, i.e. can be attributed to the Babax marker alleles and the largest percentage of genetic variance explained was in the environments Lundavra 2005 (4.5–10.0%), Gatton Dry 2002 (8.0–21.9%) and Gatton Irrigated 2006 (4.6–8.5%) (Table 6; Fig. 2b). The superimposed $-\log(P)$ profiles for 7A-a from the SIM analysis for all three traits suggests that anthesis and height QTL may also be present on this linkage group (Fig. 2a). However, these were not significant in this analysis although QTL for these traits on this linkage group have been reported for other populations (Kuchel et al. 2006).

The most consistent yield effects for Seri marker alleles were observed on linkage groups 5A-a and 6B-a, with the largest Seri effect occurring in Biloela 2005 on chromosome 1D-b. QEI effects can be a result of contrasting effects of the parent alleles across environments (Fig. 3). For example, the yield QTL on 1D-b resulted from a cross-over of the Seri marker alleles in Biloela 2005, Lundavra 2005 and Gatton Dryland 2004 (an average increase in yield of 10 g m^{-2}, shown in blue) with the Babax marker alleles in Gatton Irrigated 2006 (an increase in yield of 7 g m^{-2}, shown in red/yellow) (Fig. 3b). In Gatton Irrigated 2006 the difference in yield between a line homogeneous for Seri alleles and one homogeneous for Babax alleles on 1D-b would be 2×6.8 g m^{-2}, in favour of the Babax-type line (Table 5). In contrast, in Biloela 2005 a line homogeneous for Babax alleles would yield 14.7 g m^{-2} less than one homogeneous for Seri alleles. This yield QTL co-located with an anthesis QTL (Table 4;

Table 4 Linkage groups where QTL were detected for yield, anthesis and height from SIM analyses ($\alpha = 0.1$).

Linkage group	Marker[a]	Yield	Anthesis	Height
1B-a	gwm413	(59–83)^{**m}	(66–92)^{**m}	(46–71)^{**m}
1D-a	wPt-9380	60** (58–64)		(38–64)^{**m}
1D-b	act/ctc-4	4.5** (1–9) (0–4) ^{*s}	4* (0–6) (0–8)^{**m}	
2B-a	aag/ctg-12, gwm388		53** (38–60)	40** (31–88) (34–88)^{**m}
3A-b	aac/cta-4			13* (0–15) (3–14)^{**s}
4A-a	act/cag-3			12** (9–15) (13–18)^{**m}
4A-b			(25–46)^{**m}	
4B-b	wmc048, aag/cta-5a	38** (28–44) (19–33)^{*m}		42^{c} (38–44) (18–54)^{**m}
4D-a			(0–2)^{**s}	
5A-a	aag/ctg-10, gwm617a, barc040	69** (60–80) (60–70) ^{**s}	9* (8–23)	106* (96–106) (100–103)^{**m}
5B-a		(18–21)^{*s}		(17–26)^{*m}
6A-a		(0–6)^{*s}		
6B-a	wPt-4764, aca/cac-3	111**(107–113) (20–24)^{*s}(99–111)^{**s}		75** (75–77) (67–84)^{**m}
6D-a	gdm132	0** (0–13) (6)^{*m}		
7A-a	barc121	110** (97–114) (107–123)^{**m}	(92–107)^{*m}	
7B-a	acc/ctc-7	9** (5–9) (0–2)^{**s}	(0–9)^{**m}	
UA-a[b]			(14)^{*m}	

The position (cM) and confidence region (in brackets) are presented. The QTL regions identified from the single trial analyses (QTLCartographer – see Supplementary Table 1) are in bold italics, in brackets. The markers are the left flanking marker from the mixed model QTL analyses

[a] The order of the reported markers is for anthesis, height then yield where a QTL effect was reported

[b] UA-a = unassigned linkage group

[c] This QTL is only slightly below the significance level of $\alpha = 0.10$

* Significant at $\alpha = 0.10$ (QQE model) or $2 < \text{LOD} < 3$ (single trial analyses); ** Significant at $\alpha = 0.05$ or LOD > 3; *m* = multiple sites, *s* = single sites for the single trial analyses

Table 5 Environment-specific QTL effects (or main effect) for each QTL in the yield (g m^{-2}) multi-QTL model and their average standard error of difference or standard error

Trial[a]	Rye.Env[b]	1D-a	1D-b	4B-b	5A-a	6B-a	6D-a	7A-a	7B-a
Main							−6.1		
BILO05	14.8	11.7	−14.7	6.5	−5.2	−12.2		6.2	7.7
LUND05	22.7	−3.0	−2.9	5.6	−9.1	−4.4		8.9	7.1
GATD02	14.2	−1.0	−2.0	−2.4	−1.0	−7.5		8.9	−3.0
GATD04	1.4	−0.7	−4.7	−1.0	2.6	−2.3		4.0	−3.9
GATD05	13.3	0.3	−1.0	6.8	−0.5	−2.1		2.2	3.8
GATI06	20.7	1.3	6.8	13.8	−0.4	0.9		18.9	−7.0
(avsed)	(8.0)	(4.7)	(4.4)	(4.4)	(4.7)	(4.4)	(1.5)[c]	(4.3)	(4.3)

[a] *BILO05* Biloela 2005, *LUND05* Lundavra 2005, *GATD* Gatton Dryland, *GATI* Gatton Irrigated, 02-06: 2002 to 2006

[b] Rye.Env: is the *rye* factor by environment effect fitted in the model, on linkage group 1B-a. Note that the positive effect indicates presence of 1BS.1BL (Babax) and absence of 1RS.1BL segment (Seri)

[c] Standard error

Fig. 3a), indicating a potential for pleiotropic or linkage effects. The anthesis QTL on this linkage group was a main effect, with the Babax allele (shown as red) contributing to an average delay in anthesis of 0.9 days in all environments (Fig. 3c). Note that, overall, this anthesis QTL has a small effect, as in general Babax-type lines take less time to mature than Seri-type lines in these environments.

Greater genetic variance was explained by the final multi-QTL model for the three environments with the highest generalised heritability: Biloela 2005 and Lundavra 2005 and Gatton Dryland 2002 (Tables 1, 6). The proportion of genetic variance explained by the full QTL model in each environment varied between 0% (Gatton Dry 2005) and 40% (Gatton Dryland 2002). Thus, on

Table 6 For yield, trial genetic variance for the multi-environment model and the percentage of variance explained by the multi-QTL model

Trial[a]	Genetic variance, multi-env model	% genetic variance explained by full QTL model	Rye.Env[b]		1D-a		1D-b		4B-b		5A-a		6B-a		6D-a		7A-a		7B-a	
BILO05	2,090	28	0.5	2.1	3.3	6.6	6.9	13.6	0.0	2.0	1.4	0.6	5.7	9.0	0	0	0.8	1.7	1.2	3.2
LUND05	1,065	32	4.4	13.4	0.2	0.4	0.9	1.0	1.3	3.8	5.1	9.6	0.4	2.2	0	0.5	4.5	10.0	4.4	6.3
GATD02	560	40	3.9	10.1	0	0	0.2	0	2.6	0.1	0	0	6.5	15.9	6.7	13.2	8.0	21.9	0.7	1.3
GATD04	446	24	0	0	0	0	4.7	5.8	0.8	0	0.7	0.7	0.4	0.1	12.6	15.7	1.4	4.0	2.7	2.5
GATD05	987	0	2.0	2.2	0	0	0	0	3.0	3.6	0	0	0	0	0	0	0	0	0	0
GATI06	3,623	11	1.3	1.9	0	0	0.8	1.0	1.6	4.6	0	0	0	0	0.5	1.6	4.6	8.5	0	0.6

The percentage of variance explained for each QTL (including *Rye.Env* effect) in each environment, expressed as a function of (1) the evaluated QTL only and (2) the full QTL model and a model where the evaluated QTL was excluded

[a] *BILO05* Biloela 2005, *LUND05* Lundavra 2005, *GATD* Gatton Dryland, *GATI* Gatton Irrigated, 02-06: 2002 to 2006

[b] *Rye.Env* is the *rye* factor by environment effect fitted in the model

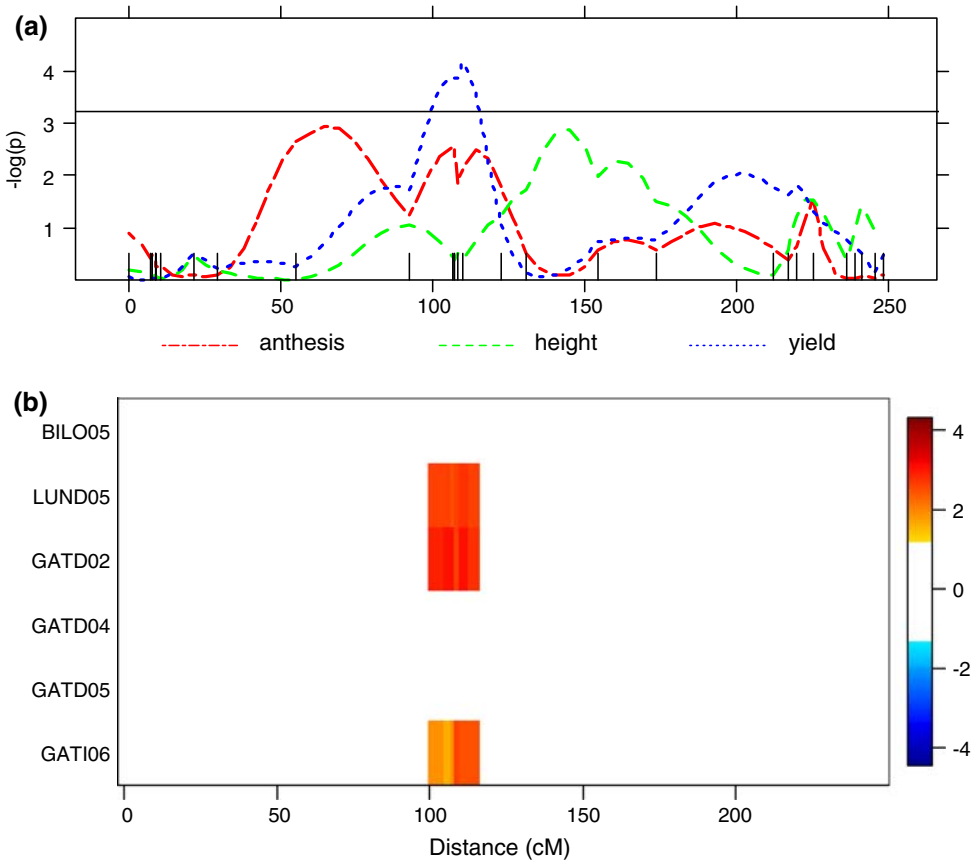

Fig. 2 $-\log_{10}(P)$ profile for yield, anthesis and height for linkage group 7A-a, and in **b** specific environment yield effects for linkage group 7A-a. In **a** the *horizontal line* represents the $\alpha = 0.10$ significance level. The *vertical lines* indicate the marker positions. In **b** *red* = Babax marker allele, *blue* = Seri marker allele, the darker the larger the effect on yield. The legend in **b** represents the approximate *t* statistic of the allele effects, *t* = effect/standard error. Only significant effects where the QTL profile is greater than $\alpha = 0.10$ are shown

average, approximately 75% of the genetic variance was unexplained, highlighting the large variability and subsequent difficulty in detecting QTL in drought-stressed environments. The two methods of estimating the percentage of genetic variance explained for individual QTL returned similar values. For example, in Biloela 2005 the genetic variance explained by the full QTL model (including all 9 QTL) was 28%. The QTL on 1D-a in this environment explained 3.3% of the genetic variance when modelled alone. When all other QTL, except the one on 1D-a, were modelled, the genetic variance explained was 21.4% which equates to 6.6% of the genetic variance

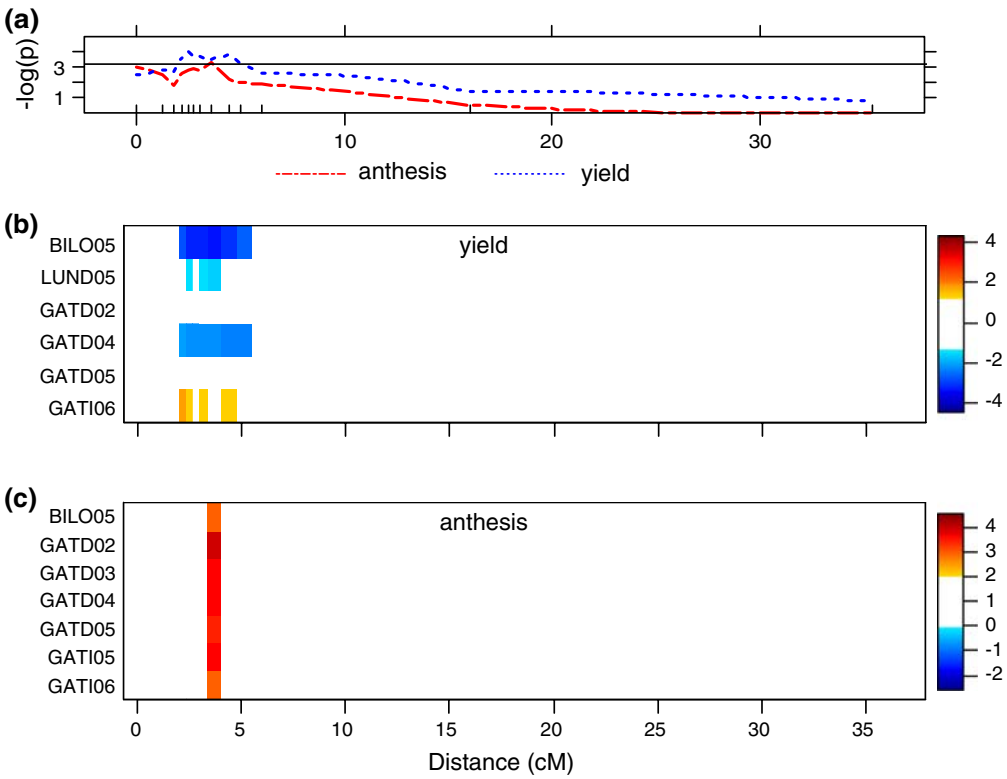

Fig. 3 Co-incidence of yield and anthesis QTL and environment-specific effects for linkage group 1D-b. In **a** the *horizontal line* represents the $\alpha = 0.10$ significance level. The *vertical lines* indicate the marker positions. In **b** and **c** *red* = Babax marker allele, *blue* = Seri marker allele, the darker the larger the effect. The legend in **b** and **c** represents the approximate *t*-statistic of the allele effects, t = effect/standard error. Only significant effects where the QTL profile is greater than $\alpha = 0.10$ are shown

explained by the 1D-a QTL (Table 6). None of the QTL, explained more than 4% variance explained in the Gatton Dry 2005 environment. In this environment crop establishment was poor, and there was substantial residual error which highlights the need for good quality phenotypic data for QTL analyses.

Comparison of results from mixed model QTL and single trial analyses

The mixed model methodology presented here was compared with a single trial, single trait CIM analysis performed in QTL Cartographer for the same dataset (Supplementary Table 1). QTL that were detected at LOD > 2 in both single and multiple trials using QTL Cartographer are included in Table 4 for the 29 linkage groups analysed using the mixed model approach. The complete output from QTL Cartographer for yield, anthesis and height is available in Supplementary Table 1. In most cases, the genomic regions determined by the single trial analysis coincided with the QTL region reported for the mixed model analyses (Table 4; Supplementary Table 1).

For the eight yield QTL detected from the mixed model, seven were also detected by the single trial analyses (Table 4; Supplementary Table 1). One of the seven QTL was detected in multiple environments at LOD > 3. Two of the seven QTL were detected in multiple environments at 2 < LOD < 3, three more QTL were detected in single environments at LOD > 3 and the seventh QTL was detected in a single environment at 2 < LOD < 3. In the single trial analysis, a strong multi-environment QTL at LOD > 3 was detected on 1B-a (Table 4; Supplementary Table 1). In the mixed model approach, this effect was deliberately accommodated as the *rye* factor.

For anthesis, two of the three QTL detected from the SIM model were also detected by the single trial analysis in multiple trials at LOD > 3 (Table 4; Supplementary Table 1). The single trial analyses identified four additional QTL (LOD > 2), two of which were detected across multiple trials, that were not detected by the mixed QTL model (Table 4; Supplementary Table 1). However, one of these (the one on 1B-a) was already accounted for by the *rye* factor in the mixed model.

For height, all five QTL detected from the mixed model QTL analysis were also detected in the single trial analyses, four of which were detected in multiple trials at LOD > 3; the remaining QTL was detected in a single site only at LOD > 3 (Table 4; Supplementary Table 1). The single trial analyses identified four additional QTL that were not detected by the mixed model approach (Table 4; Supplementary Table 1). Again, one of these was on linkage group 1B-a and, in the mixed model approach had already been represented by the *rye* factor.

Discussion

This paper illustrates a mixed model methodology to detect QTL, using yield, anthesis and height data for wheat from drought-stressed environments in north-eastern Australia. A compound symmetry model is typically used for these types of datasets, but this assumes a common variance and a common between-trial correlation. However, for all three traits, the AIC statistic for the compound symmetry model was consistently greater (i.e. indicating poorer fit) than for any of the models which accounted for the genetic variance–covariance structure (Table 2). A factor analytic model, either order 1 or 2, provided a good (low AIC) and parsimonious (low BIC) fit to the genetic variance–covariance matrix for all three traits. Thus testing and detection of QTL effects should be more precise and reliable when these improved across-trial models are used, rather than the compound symmetry model (Piepho 2005).

Spring bread wheats with a Veery pedigree generally contain the 1RS.1BL translocation (Merker 1982) which has conferred adaptation to marginal environments in Veery by pre-Veery crosses (Cooper et al. 1994; Villareal et al. 1998; Peake 2003). In the population studied here, the rye translocation on chromosome 1B was present in ~30% of lines when genotypes were classified using rye markers. Including the environment-specific *rye* factor increased the detection of other QTL. The rye translocation in this Veery by Veery cross significantly decreased yield by 13.8 g m^{-2} averaged across environments (Table 1). This was in contrast to the findings of Villareal et al. (1998) regarding the positive effect of this alien chromosome segment. Our results confirm those found by Peake (2003) in this region, who found a similar negative effect on yield associated with the *rye* translocation in other crosses involving the Seri parent.

The QTL mapping model was based on a two-stage analysis using BLUEs and weights determined from single trial analyses where the genotype factor was fitted as a fixed effect (Smith et al. 2001a). Others have proposed that to prevent the loss of individual plot information, a single-stage analysis using raw plot data from all trials would provide better estimates of the genotype-by-trial means (Cullis et al. 1998; Smith et al. 2001b). However, a simulation study has shown that there was no difference in the mean square error of predictions when comparing the single-stage method with a two-stage method using BLUEs with weights (Welham et al. 2006). Both the two-stage with BLUEs and weights and the single-stage analyses require the raw plot data. Unfortunately this is not always available. Since many MET datasets consist only of means (BLUPs or BLUEs) without weights, a simulation study comparing the power of QTL detection using means with and without weights would be useful. This would be analogous to the work of Welham et al. (2006) on detection of genotype differences.

In performing multiple tests, such as in QTL detection, errors in the rate of null hypothesis rejection become an issue. In this paper, a Wald test was performed at 5-cM intervals along the genome; equivalent to 614 different tests for each trait. A modified Bonferroni correction was applied to take into account the correlations between tests (Li and Ji 2005). An alternative approach to reduce the number of multiple tests performed is to simultaneously fit all genetic predictors across the genome as random effects, with either a variance component per chromosome or a variance component for the whole genome, and use an outlier detection method to detect QTL in a nested iterative approach (Gilmour 2007; Verbyla et al. 2007).

In single trial analyses of this dataset (C.L. McIntyre, personal communication; Supplementary Table 1)), no cofactor was included to control for the rye translocation on chromosome 1B. Many of the QTL detected using the mixed model method were also detected in the single trial analyses. However, applying a common convention of focusing on QTL with LOD > 3 and presence in multiple trials, some of these single trial QTL would not have been reported. The mixed model methodology identified environment-specific QTL, e.g. the yield QTL on 1D-a (not detected in single trial analysis) and 1D-b (only detected in a single trial at LOD < 3). It also detected several QTL not detected in the single trial analyses, e.g. the yield QTL on 1D-a and the anthesis QTL on 5A-a. Yield and anthesis QTL on these chromosomes have been reported previously (Kato et al. 2000; Huang et al. 2003; Li et al. 2007). The mixed model methodology should allow more reliable detection of QTL by accounting for the genetic variance–covariance matrix; thereby, producing more appropriate tests than the single trial approach.

After allowing for the rye translocation, eight yield QTL were identified using the mixed model methodology. Superimposing the $-\log_{10}(P)$ profiles of yield, anthesis and height showed that two of the yield QTL co-locate with anthesis or height QTL. This may be genetic linkage or pleiotropy (anthesis on 1D-b and height on 4B-b). For example, the delayed anthesis due to the Babax alleles on the 1D-b QTL had a positive effect on yield in the Gatton Irrigated 2006 environment. Later flowering genotypes growing in this irrigated environment had a longer time to intercept radiation, generate biomass and have a greater final yield. These same 'delayed flowering' alleles were likely to be a disadvantage in Biloela 2005 and Gatton Dryland 2004 which both experienced terminal drought conditions, i.e. slightly earlier flowering was associated with higher yield because earlier lines partially escaped this late season drought. Figure 3 illustrates how a QEI effect for yield in drought environments that differ in timing of

drought can be explained by a main effect for a component trait such as anthesis.

An alternative analysis was to introduce the genotype values for anthesis and height as fixed co-variates into the mixed model. This was not done as yield is known not to be independent of either anthesis or height (Cooper et al. 1997). Further, genotype responses are non-linear across environments where the timing and intensity of drought differs. For example, taller lines may have stored more water-soluble carbohydrate available for re-translocation and so have a potential advantage in environments with post-anthesis drought (Loss and Siddique 1994). To deal with trait interactions, Malosetti et al. (2008) have proposed an extension of the methodology presented here to accommodate multi-trait multi-environment correlations.

For this dataset, similar results were found between the mixed model approach and the less flexible QTL software available. The flexible framework of the mixed model approach allowed appropriate modelling of among trial correlations and individual trial variance heterogeneity using readily available mixed model software. In addition, it accommodated the fitting of a known genotypic factor, *rye*, which might otherwise have masked the detection of other QTL. Furthermore, the preferred method presented here was able to test whether the QTL were consistent across environments or were specific to particular environments.

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