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ORIGINAL PAPER

Genetic control of grain yield and grain physical characteristics in a bread wheat population grown under a range of environmental conditions

Lancelot Maphosa · Peter Langridge · Helen Taylor · Boris Parent · Livinus C. Emebiri · Haydn Kuchel · Matthew P. Reynolds · Ken J. Chalmers · Anzu Okada · James Edwards · Diane E. Mather

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Abstract
Key message **Genetic analysis of the yield and physical quality of wheat revealed complex genetic control, including strong effects of photoperiod-sensitivity loci.**
Abstract Environmental conditions such as moisture deficit and high temperatures during the growing period affect the grain yield and grain characteristics of bread wheat (*Triticum aestivum* L.). The aim of this study was to map quantitative trait loci (QTL) for grain yield and grain quality traits using a Drysdale/Gladius bread wheat mapping population grown under a range of environmental conditions in Australia and Mexico. In general, yield and grain quality were reduced in environments exposed to drought and/or heat stress. Despite large effects of known photoperiod-sensitivity loci (*Ppd-B1* and *Ppd-D1*) on crop development, grain yield and grain quality traits, it was possible to detect QTL elsewhere in the genome. Some of these QTL were detected consistently across environments. A locus on chromosome 6A (*TaGW2*) that is known to be associated with grain development was associated with grain width, thickness and roundness. The grain hardness (*Ha*) locus on chromosome 5D was associated with particle size index and flour extraction and a region on chromosome 3B was associated with grain width, thickness, thousand grain weight and yield. The genetic control of grain length

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L. Maphosa · P. Langridge · B. Parent · K. J. Chalmers · A. Okada · D. E. Mather (✉)
Australian Centre for Plant Functional Genomics and School of Agriculture, Food and Wine, Waite Research Institute, The University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia
e-mail: diane.mather@adelaide.edu.au

Present Address:
L. Maphosa
Department of Environment and Primary Industries, 110 Natimuk Road, Horsham, VIC 3400, Australia

H. Taylor
New South Wales Department of Primary Industries, Wagga Wagga, NSW 2650, Australia

Present Address:
B. Parent
Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux, Institut National de Recherches Agronomiques (INRA), Place Viala, F-34060 Montpellier, France

L. C. Emebiri
E.H. Graham Centre for Agricultural Innovation, New South Wales Department of Primary Industries and Charles Sturt University, Wagga Wagga, NSW 2650, Australia

H. Kuchel
Australian Grain Technologies and School of Agriculture, Food and Wine, Waite Research Institute, The University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia

M. P. Reynolds
Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), Int. Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico

J. Edwards
Australian Grain Technologies, PMB 1, Glen Osmond, SA 5064, Australia

appeared to be largely independent of the genetic control of the other grain dimensions. As expected, effects on grain yield were detected at loci that also affected yield components. Some QTL displayed QTL-by-environment interactions, with some having effects only in environments subject to water limitation and/or heat stress.

Introduction

Physical characteristics of wheat grain, including grain weight, dimensions, shape, uniformity, density and texture, can affect the storage, transportation, grading, conditioning, milling and market value of bread wheat (*Triticum aestivum* L.). Grain texture (hardness or softness) is important in determining the milling behaviour and end use of wheat. Hard grain resists mechanical crushing and is more difficult to mill into fine particles than soft grain. Grain dimensions (length, width and thickness) and shape (often described in terms of aspect ratio and/or roundness) affect the amount of flour that can be extracted in milling (Berman et al. 1996; Marshall et al. 1984, 1986), with large, round and plump grain yielding more flour than small, thin and shrivelled grain. The uniformity of grain size can be important in determining the market value of grain, with small thin grains ('screenings') having to be removed prior to milling. Grain weight is also an important component of grain yield and is often negatively associated with grain number (Miralles and Slafer 2007).

Hexaploid wheat is broadly categorised into hard and soft market classes based on grain texture. These classes differ at a locus (*Ha*, for hardness) on the short arm of chromosome 5D (Morris 2002; Gale 2005), at which there are two genes (*Pina-D1* and *Pinb-D1*) that encode puroindoline proteins. The common haplotypes are *Pina-D1a* and *Pinb-D1a*, (soft texture), *Pina-D1a* and *Pinb-D1b* (intermediate hardness) and *Pina-D1b* and *Pinb-D1a* (hard texture) (Gale 2005; Békés 2012). The *Ha* locus also contains a gene encoding a 'grain softness' protein, *GSP-1*, but its role is not clearly defined (Bhave and Morris 2008; Mohler et al. 2012). Although the *Ha* locus is the major determinant of grain hardness, quantitative trait loci (QTL) affecting grain hardness have also been detected elsewhere in the wheat genome (Wilkinson et al. 2008; Wang et al. 2012), including at positions on group-7 chromosomes at which there are members of a recently discovered family of puroindoline genes (*Pinb-2*) (Chen et al. 2010; Geng et al. 2012).

Various QTL studies have shown that the different grain dimensions are largely under different genetic control mechanisms and exhibit QTL × QTL and genotype × environment interactions (Bergman 2000; Breseghello and Sorrells 2007; Campbell et al. 1999; Dholakia et al. 2003; Gegas et al. 2010; Prashant et al. 2012; Sun et al. 2009; Xiao et al. 2011). Though genes controlling the grain shape dimensions have not been identified, candidate genes have been proposed, including *TaGW2* on chromosome 6A (Su et al. 2011) and a sucrose synthase 2 (*TaSus2*) gene on chromosome 2B (Jiang et al. 2011).

Grain yield and grain weight are influenced by genetic factors, by environmental variation and by genotype-by-environment interaction. With genetic mapping, it is possible to attribute some genetic variation to specific quantitative trait loci (QTL) and to dissect genotype-by-environment interaction into component QTL-by-environment interactions. In bread wheat, QTL have been detected for grain yield (Groos et al. 2003; Marza et al. 2006; Kirigwi et al. 2007; Kuchel et al. 2007; McIntyre et al. 2010; Bennett et al. 2012a,b) and for thousand grain weight (Varshney et al. 2000; Groos et al. 2003; Huang et al. 2006; Xiang-Zheng et al. 2008; Sun et al. 2009; Tsilo et al. 2010; Bennett et al. 2012a; Maphosa et al. 2013).

Both grain yield and weight can be severely affected by environmental stresses such as moisture deficit and high temperature (Guttieri et al. 2001; Gooding et al. 2003; Weightman et al. 2008; Labuschagne et al. 2009). These stresses, which occur in many wheat-growing regions, often coincide with the sensitive grain-filling phase of wheat development. The effects of environmental stress are influenced by the phenological stage at which the crop experiences the stress. This makes it important to take into account the genetic determinants of plant phenology in any genetic analysis of grain yield or grain quality traits. In wheat, the transition from the vegetative to the reproductive stage is particularly important. This transition is controlled by three groups of loci: those affecting photoperiod responses (Worland et al. 1994; Beales et al. 2007; Eagles et al. 2009), vernalisation requirements (Fu et al. 2005; Trevaskis et al. 2007; Eagles et al. 2009) and earliness *per se*. Photoperiod-response (*Ppd*) genes are located on group 2 chromosomes (Worland et al. 1998; Beales et al. 2007) and vernalisation (*Vrn*) genes are predominantly found on group 5 chromosomes (Law and Worland 1997; Snape et al. 2001; Yan et al. 2004). Earliness *per se* loci, which affect phenological development when all photoperiod and vernalisation requirements have been satisfied, have been mapped in numerous regions of the wheat genome (Hanocq et al. 2004, 2007; Kuchel et al. 2006; Griffiths et al. 2009; Kamran et al. 2013).

To investigate the genetic control of grain yield and grain physical characteristics in wheat germplasm adapted to dry and hot environments, we developed a mapping population from a cross between two Australian wheat cultivars and evaluated it in a range of environments, including some that experienced severe drought stress and/or heat stress.

Materials and methods

Plant materials

The Australian cultivars Drysdale (http://pbr.ipaustralia.plantbreeders.gov.au) and Gladius (http://pbr.ipaustralia.plantbreeders.gov.au) were chosen as parents based on their performance under dry and hot conditions (Fleury et al. 2010). Drysdale (pedigree Hartog*3/Quarrion) was chosen based on carbon isotope discrimination, an indicator of water use efficiency (Fleury et al. 2010), while Gladius, which was derived from a complex cross involving derivatives of RAC875, Krichauff, Excalibur and Kukri, is considered to be tolerant to heat and drought. Approximately 5,000 recombinant inbred lines (RILs) were derived from a cross between Drysdale and Gladius. A random subset of 205 of these RILs was used to construct a linkage map and was evaluated in experiments conducted in several environments.

DNA extraction and genotyping

DNA was extracted from 2.0 g of bulked leaf tissue from three young plants of each parent or RIL, using a mini-prep ball-bearing extraction method (Rogowsky et al. 1991) with some modifications (Pallotta et al. 2000). The parents and each of 205 RILs were genotyped with DArT markers (Wenzl et al. 2004; Akbari et al. 2006), simple sequence repeat (SSR) markers, and markers for the *Vrn-A1*, *Vrn-D1*, *Ppd-B1*, *Ppd-D1*, *Pina-D1*, *Pinb-D1, Glu-D3* and *TaGW2* loci.

For each line to be genotyped with DArT markers, about 100 ng/µl DNA (30 µl) was sent to Triticarte Pty Ltd (Yarralumla, Australian Capital Territory, Australia). DArT markers were scored based on the presence or absence of hybridisation. For SSR markers, polymerase chain reaction (PCR) amplification, marker analysis and scoring were carried out using Multiplex-Ready technology (Hayden et al. 2008).

Variation in the promoter region of *Vrn-A1* was detected using the primer pair VRN1AF/VRN1R (Yan et al. 2004), with the PCR reaction mixture and programme described by Eagles et al. (2009). Variation in the first intron of *Vrn-D1* was detected using a mixture of the primers Intr1/D/F, Intr1/D/R3 and Intr1/D/R4 (Fu et al. 2005), with the PCR reaction mixture and programme described by Eagles et al. (2009). For *Ppd-B1*, the method described by Díaz et al. (2012) was used to detect the *a* allele carried by Gladius. Alleles of the pseudo-response regulator *Ppd-D1* on chromosome 2D were detected using the primers Ppd-D1_F, Ppd-D1_R1 and Ppd-D1_R2 (Beales et al. 2007), and the PCR protocol described by Eagles et al. (2009). The puroindoline genes *Pina-D1* and *Pinb-D1* were assayed as described by Cane et al. (2004) using the primers described by Gautier et al. (1994) and Giroux and Morris (1997). For the low-molecular-weight glutenin gene *Glu-D3*, a multiplex PCR was carried out as described by Appelbee et al. (2009) using the primer pair M2F12/M2R12 (Zhao et al. 2007a) to amplify the *a* allele carried by Gladius and the primer pair M4F3/M4R3 (Zhao et al. 2007b) to amplify the *g* allele carried by Drysdale. For *TaGW2*, a gene that has been reported to affect grain size, the PCR primer pair and reaction mix were those described by Su et al. (2011) and amplicons were run on a LightCycler 480 instrument (Roche Diagnostics Australia Pty Limited) with RIL genotypes scored based on differential high-resolution melting curves.

Linkage map construction

For each marker, distortion from the expected segregation ratio of 1:1 was tested using a Chi square test ($\alpha = 0.01$). DArT and SSR markers showing significant segregation distortion were excluded from map construction. Marker data were also visually inspected to identify lines with large numbers of missing data or heterozygous scores and these lines were excluded. Markers were grouped into linkage groups using the program Multipoint (http://www.multiqtl.com/). A LOD score of 3.0 was set as the minimum threshold to indicate linkage between markers. Linkage groups were assigned to chromosomes and oriented by comparing with published maps available on GrainGenes (http://wheat.pw.usda.gov/) and Triticarte (www.triticarte.com.au/) web sites and with a wheat consensus map (Somers et al. 2004). Final marker order was obtained using the program RECORD (Isidore et al. 2003). Map distances were calculated using R/qtl (Broman et al. 2003). Maps were drawn using MapChart (Voorrips 2002).

Field experiments

Drysdale, Gladius, the 205 Drysdale/Gladius RILs and 11 control cultivars (Diamondbird, EGA Gregory, Espada, Excalibur, Janz, Krichauff, Kukri, Mace, Sokoll, Ventura and Waagan) were grown in eight field experiments: two at Roseworthy, South Australia, Australia (34°S 138°E), one at Booleroo, South Australia, Australia (33°S 138°E), four at Yanco, New South Wales, Australia (35°S 146°E) and one at Ciudad Obregon, Sonora State, Mexico (27°S 109°E).

The experiment conducted in 2008 at Roseworthy (SAR08) was principally a seed multiplication experiment with one replicate of each line, parent and control cultivar. The experiments conducted in 2009 at Roseworthy (SAR09) and Booleroo (SAB09) were randomised using nearest neighbour designs with two replicates of each entry.

In all three of these experiments, the size of each plot was 3.9 m^2. In the SAR09 experiment, plots were scored once before anthesis using the Zadoks cereal development scale (Zadoks et al. 1974). The total rainfall recorded during the growing season was 449 mm in SAR08, 347 mm in SAR09 and 355 mm in SAB09. During the months in which flowering and grain filling took place (August, September and October) the mean maximum temperature was 21.0 °C in SAR08, 20.5 °C in SAR09 and 21.9 °C in SAB09. The highest monthly mean temperatures were in December (26.4°) in SAR08 and in November in SAR09 and SAB09 (32.3 °C in both locations).

At Yanco, two experiments were conducted in 2009 and two in 2010, each arranged as a spatially optimised incomplete block design, with two replicates of each entry. The area of each experimental unit (plot) was 7.5 m^2. These experiments were irrigated to eliminate confounding effects of drought stress and moisture sensors were fitted in the soil to monitor soil moisture and schedule irrigation. In the experiments conducted in 2010, the data of ear emergence was recorded for each plot. Two experiments (NSW09 in 2009 and NSW10 in 2010) were sown at the conventional time (June) and two (NSW09L in 2009 and NSW10L in 2010) were sown very late (August). The purpose of the late sowing was to make it likely that the experiments would experience high temperatures during grain filling. In 2009, the late-sown experiment was exposed to high temperatures, as expected. In 2010, however, conditions were unusually cool and wet, and the late-sown experiment was flooded and suffered severe weather damage. The mean maximum temperature during flowering and grain filling was 21.6 °C in NSW09 but only 17.7 °C in NSW10 (both for August, September and October) and 26.6 °C in NSW09L but only 22.1 °C in NSW10L (both September/October/November). The highest monthly mean temperatures were in November 2009 (33.2 °C) and December 2010 (29.7 °C).

The experiment at Ciudad Obregon (Mex11) was arranged in two-replicate alpha-lattice design. It was sown in December 2010. The area of each plot was 1.6 m^2. Plots had a total of 150 mm of water available down to 1.2 m depth that was applied at sowing. No further irrigation was applied and there was no rainfall. The date of ear emergence was recorded for each plot. Plots were mechanically harvested and cleaned. The mean maximum and minimum temperatures for the cycle were 26.1 and 6.9 °C, respectively and the highest mean monthly temperature occurred in March during grain filling (29.2 °C).

Managed-environment experiments

In 2010, four additional experiments were conducted at Urrbrae, South Australia, Australia (35°S 139°E). Two of these experiments were grown under a polyurethane tunnel (polytunnel) and the other two were grown under netting (to exclude birds). In each of these experiments, each experimental unit consisted of a single row of 12 plants, with 10 cm between rows and between plants within rows. The two experiments conducted in the polytunnel (SAU10H and SAU10HD) were arranged in completely randomised designs, with two replicates of each 60 RIL (60 selected as being of intermediate maturity based on assessment of growth stages in the previous year (SAR09)), four replicates of each parent (Drysdale and Gladius) and four replicates of each of the 11 control cultivars. The two experiments conducted under netting (SAU10 and SAU10D) were arranged in partially replicated designs, with two replicates of each of the 60 RILs used in the polytunnel experiment, one replicate of each of the remaining 145 RILs and four replicates of each parent or control cultivar.

From sowing until booting (Zadoks growth stage 45), all four experiments experienced ambient temperatures (mean maximum temperature of 15.6 °C) and were irrigated daily to full soil moisture capacity. When about 10 % of the plots in the polytunnel experiments had reached Zadoks growth stage 45, the walls of the polytunnel were lowered to elevate the temperature. The mean maximum temperature during flowering and grain filling (August, September and October) was 17.8 °C and the highest monthly mean was 26.7 °C in December. With the walls lowered, the temperature inside the polytunnel was roughly 10 °C higher than ambient temperature. At the same time, one of the two experiments under netting was covered with a polyurethane sheet to exclude rain. In that experiment (SAU10D) and in one of the polytunnel experiments (SAU10HD), cyclic drought was imposed by withholding irrigation until soil moisture levels decreased to a critical level (-9 bar), after which the plots were re-watered to full capacity. Cyclic drought was imposed because it is the common form of drought in the environment (South Australia) in which the experiments were conducted. The other two experiments (SAU10 under netting and SAU10H in the polytunnel) were irrigated daily. The date of ear emergence was recorded for each plot in all four experiments. Spikes were harvested by hand and were threshed with a Kimseed Multi-Thresher CW08 (Kimseed Engineering, Australia).

Grain weight, grain number, percentage screenings and test weight

Grain weight was measured using a Contador grain counter (Pfueffer GmBH, Germany) to count out 250 grains. Grain number per m^2 was estimated based from the thousand grain weight and dry weight harvested from the plot. A Graintech™ grain screener (Engineering Service Providers, Australia) was used to measure percentage screenings.

For each sample, a 300-g subsample was placed on a 2-mm slotted agitator screen and shaken 40 times to separate small grains (screenings) from plump grains. The weight of the sample passing through the screen was reported as a percentage of the initial sample weight. A sliding test weight instrument (Wagga Wagga Agricultural Institute, in-house design) was used to measure hectolitre weight ($kghl^{-1}$).

Grain dimensions and shape

A SeedCount digital imaging analysis scanner version 2.0 (Weiss Enterprises) was used to measure grain length (the longest line through the grain, which almost always runs from the embryo to the distal end), width (the longest line through the grain at a 90° angle to the length), thickness (the longest line through the grain that is perpendicular to both the length and the width) and area (the area of a polygon defining the outline of the grain) for individual grains in 30 g samples of cleaned grain from each plot in each of six experiments (NSW09, NSW10, SAU10, SAU10D, SAU10H and SAU10HD). Aspect ratio was calculated by dividing grain length by grain width, and roundness was calculated as

$$\left(\frac{\text{grain length}}{\text{grain width}} + \frac{\text{grain length}}{\text{grain thickness}} + \frac{\text{grain width}}{\text{grain thickness}}\right) \Big/ 3$$

For each of the grain dimensions and grain shape parameters, a mean value was calculated across all of the individual grains measured in each sample.

Particle size index and flour extraction

Percentage moisture content and particle size index (PSI) were estimated by NIR (RACI-CCD 2010) using a Foss 6500 NIR instrument (FOSS NIR Systems, Inc., Laurel, MD) with calibration to the AACC (1999) methods for air oven moisture determination and PSI. High PSI units correspond to low hardness (soft grain).

For the grain harvested from each experimental unit at Yanco, approximately 1 kg of grain was conditioned overnight to 15 % moisture content (AACC 1999) and milled on a Bühler MLU-202 laboratory test mill (Bühler AG, Uzwil, Switzerland). Recovered flour was weighed and flour extraction was expressed as a percentage of the initial grain weight. For grain harvested from each experimental unit of the experiments at Urrbrae, samples were allowed to equilibrate for three weeks in a conditioning cabinet and an equal amount of water was added to each sample before milling on a Quadrumat Junior test mill (Brabender, Germany). Room temperature, humidity and mill temperature were controlled by using an air-conditioned room and allowing the mill to cool down periodically (after about every 15 samples) during the course of milling. Percentage flour extraction was calculated as a fraction of total products (100 × flour/(flour + bran)).

Statistical and genetic analysis

Statistical analyses, including broad-sense heritability estimates and QTL analysis, were conducted using GenStat 14 (Payne et al. 2009). QTL analysis was conducted for each of the experiments in which 205 RILs were included, but not for the polytunnel experiments, which included only 60 RILs. For each trait to be subjected to QTL analysis, best linear unbiased estimates (BLUEs) were generated by incorporating block, row and range effects into the analysis (Gilmour et al. 1997). The BLUEs were used as the trait values in QTL analysis. The best variance–covariance model for QTL multi-environment trial analysis was automatically selected based on the Schwarz information criterion. Candidate QTL were selected based on an initial simple interval mapping scan. The candidate QTL were then used as co-factors in composite interval mapping with a minimum co-factor proximity of 30 cM, and a final QTL model was selected. The maximum step size along the genome was set at 10 cM and the genome-wide significance level was $\alpha = 0.05$. Epistatic interactions among loci were tested using QTLNetwork 2.0 (http://ibi.zju.edu.cn/software/qtlnetwork/) with a genome-wide significance level of $\alpha = 0.05$ established based on 1,000 random permutations, with a walking distance of 1 cM.

Results

Linkage map

The genetic linkage map generated consisted of 28 linkage groups (Online Resource 1), with a total of 10 known genes, 618 DArT marker loci and 92 SSR marker loci. The genes *Pina-D1* and *Pinb-D1* co-segregated with each other but were not found to be linked with any other markers. All linkage groups were assigned to specific wheat chromosomes, with at least one linkage group assigned to each chromosome except chromosomes 4D and 6D.

Heritability estimates

For the polytunnel experiments (SAU10H and SAU10HD), the broad-sense heritability estimates for grain yield and all grain traits were low (between 12 % and 28 %, Table 1). The heritability of crop development traits in these experiments was somewhat higher (e.g. 64 % in SA10UH and 55 % in SAU10HD for time from sowing until ear emergence) but not nearly as high as for the same traits in the

Table 1 Estimates of broad-sense heritability (%) for traits measured on Drysdale/Gladius recombinant inbred lines in up to 12 experiments

	Experiment[a]											
	SAR08	SAR09	SAB09	NSW09	NSW09L	NSW10	NSW10L	MEX11	SAU10	SAU10D	SAU10H	SAU10HD
Zadoks score	–[b]	98	–	86	–	–	–	–	–	–	–	–
Time from sowing to ear emergence	–	–	–	–	–	94	95	93	87	92	64	55
Grain yield	72	77	47	75	45	66	–	51	73	45	16	16
Thousand grain weight	–	–	–	84	81	84	–	85	73	82	15	19
Grain number	–	–	–	70	39	69	–	49	69	44	13	12
Test weight	–	–	–	78	80	84	–	–	–	–	–	–
Screenings	–	–	–	87	79	38	–	–	–	–	–	–
Grain length	–	–	–	96	–	93	–	–	87	85	26	25
Grain width	–	–	–	91	–	85	–	–	73	79	12	16
Grain thickness	–	–	–	81	–	85	–	–	72	89	17	17
Grain area	–	–	–	93	–	89	–	–	76	81	18	19
Grain aspect ratio	–	–	–	92	–	86	–	–	83	81	20	28
Grain roundness	–	–	–	88	–	83	–	–	82	84	22	28
Particle size index	–	–	–	77	52	60	–	–	58	60	20	16
Flour extraction	–	–	–	65	56	54	–	–	85	91	22	22

NSW: experiment conducted at Yanco in New South Wales, Australia; *MEX*: experiment conducted at Ciudad Obregon, Mexico; 09: experiment conducted in 2009; 10: experiment conducted in 2010; *L*: late-sown experiment; *D*: cyclical drought treatment; *H*: heat treatment

[a] SAR, SAB and SAU: experiments conducted in South Australia, Australia (at Roseworthy, Booleroo and Urrbrae, respectively)

[b] Trait not assessed in this experiment

outdoor experiments (between 86 and 98 % for Zadoks score). In all outdoor experiments, heritability was high for thousand grain weight (between 73 and 85 %), test weight (between 78 and 84 %) and all grain dimensions and shape parameters (between 72 and 96 %).

Differences between Drysdale and Gladius

Across all the experiments in which crop development was assessed, the mean values for the two parental lines did not differ greatly from each other (Table S1 in Online Resource 2). The two parents exhibited genotype × environment interaction for grain yield, thousand grain weight, grains per m^2, screenings, test weight and many grain dimensions and shape parameters (Tables S2 to S6 in Online Resource 2). Drysdale yielded more than Gladius in SAU10, SAU10D and SAU10H, but less than Gladius in NSW09 and SAR09. For particle size index, there were no significant differences between the parents, while for flour extraction, the only significant difference was in SAU10HD, where Gladius had higher flour extraction than Drysdale (Table S7 in Online Resource 2).

Correlation among traits

There were significant phenotypic correlations among some traits (Table 2). For multi-environment means, there were strong correlations between grain number and yield, between grain area and thousand grain weight, between grain width and grain thickness and between thousand grain weight and both grain width and thickness. Aspect ratio and roundness were positively correlated with each other and with grain length. Grain length was positively correlated with grain width but not with grain thickness. Grain area was positively correlated with its component traits and with grain thickness. Flour extraction was most correlated with particle size index. Grain yield exhibited moderate positive correlation with test weight, thousand grain weight, grain width, grain thickness and grain area. Thousand grain weight and test weight were positively correlated with each other and negatively correlated with percentage screenings. Some specific within-environment correlations were also observed (Table S8 in Online Resource 2). Grain width and grain number were positively correlated in SAU10 but negatively correlated in NSW10. Grain area and roundness were positively correlated in NSW10 but negatively correlated in SAU10D. Particle size index and percentage screenings were negatively correlated in the early-sown NSW09 experiment but positively correlated in the late-sown NSW09L experiment. Percentage screenings was negatively correlated with yield only in the late-sown NSW09L experiment.

Table 2 Significant ($p < 0.001$) phenotypic correlation coefficients among the multi-environment means for grain yields and grain characteristics measured on a set Drysdale/Gladius recombinant inbred lines

	Grain length	Grain width	Grain thickness	Grain aspect ratio	Grain roundness	Grain area	Grain number	Grain yield	Thousand grain weight	Test weight	Percentage screenings	Particle size index
Grain width	0.28											
Grain thickness	ns[a]	0.89										
Grain aspect ratio	0.53	−0.67	−0.66									
Grain roundness	0.61	−0.54	−0.67	0.95								
Grain area	0.80	0.78	0.63	ns	ns							
Grain number	ns	ns	ns	ns	ns	ns						
Grain yield	ns	0.41	0.30	ns	ns	0.38	0.90					
Thousand grain weight	0.61	0.84	0.81	ns	ns	0.90	ns	0.43				
Test weight	ns	0.33	0.37	−0.32	−0.33	ns	0.36	0.47	0.43			
Percentage screenings	ns	−0.42	−0.59	ns	0.29	−0.35	ns	ns	−0.58	−0.31		
Particle size index	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
Flour extraction	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.45

[a] Correlation not statistically significant

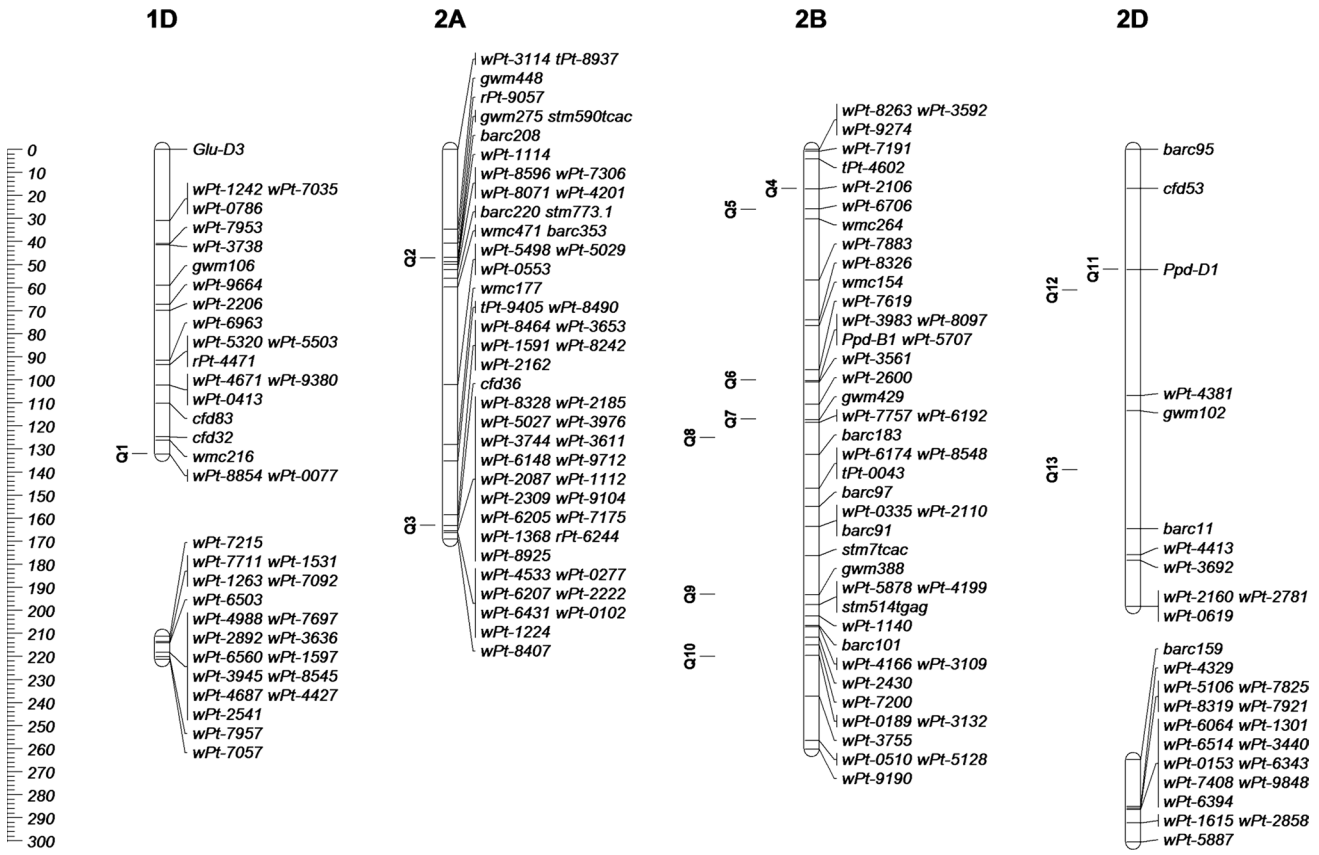

Fig. 1 Locations (Q1 to Q13) on chromosomes of homoeologous groups 1 and 2 of wheat at which QTL were detected for one or more traits, as listed in Tables 2, 3, 4, 5, 6. The *scale on the left* indicates genetic distances in cM

Quantitative trait loci

For all traits and experiments, the range of values observed for the RILs exceeded that observed for the parents. For each trait, some of this variation was attributed to the regions of chromosomes 2B and 2D that contain the photoperiod-sensitivity loci *Ppd-B1* and *Ppd-D1* (positions Q6 and Q11 in Fig. 1). For some traits, effects were also detected at or near the vernalisation-response loci *Vrn-A1* and *Vrn -D1* (positions Q30 and Q32 in Fig. 3), at the marker *TaGW2* (position Q33 in Fig. 3), at the puroindoline locus *Pina-D1* (Q31 in Fig. 3) and at other positions in the genome.

The *Ppd-D1* locus was particularly important for crop development traits, with the photoperiod-sensitivity allele from Gladius delaying development in all environments in which Zadoks score or time to ear emergence was evaluated (Table 3). Similarly, at *Ppd-B1* on chromosome 2B, the allele for photoperiod sensitivity (from Drysdale) was associated with delayed development, as reflected by QTL detected directly at *Ppd-B1* (position Q6 in Fig. 1) or 17 cM from *Ppd-B1* (position Q7 in Fig. 1). At the vernalisation-response loci *Vrn-A1* and *Vrn-D1*, winter (vernalisation-responsive) alleles were associated with small delays in ear emergence. At *Vrn-A1*, the winter allele from Drysdale delayed ear emergence in two field experiments that were exposed to high temperatures (NSW10L and MEX11). At *Vrn-D1,* the winter allele from Gladius consistently delayed ear emergence in five field experiments.

For grain yield, grain weight and grain number, the QTL with the largest effects were at *Ppd-B1* and *Ppd-D1* (Table 4). The alleles for photoperiod-sensitivity (from Drysdale at *Ppd-B1* and from Gladius at *Ppd-D1*) were usually associated with lower yield and smaller grain. The SAB09 experiment was a notable exception; in that experiment, the photoperiod-sensitivity alleles at both loci were associated with yield advantages.

QTL on chromosomes 3B and 6A (positions Q23 in Fig. 2 and Q33 (*TaGw2*) in Fig. 3) consistently affected thousand grain weight across all experiments. At position Q23, the allele from Drysdale increased thousand grain weight. At position Q24 (12 cM from Q23, the allele from Drysdale increased grain yield. At *TaGW2*, the allele from Gladius increased thousand grain weight. At position Q34 (15 cM from *TaGW2*), the allele from Gladius increased

Table 3 Genetic map positions, LOD scores and estimated additive effects of the Gladius allele for QTL detected for Zadoks score in two experiments and for the number of days from sowing to ear emergence in five experiments

Trait and QTL	QTL position[a]	Position (cM)	Closest marker	LOD	Experiments[b] and estimated additive effects						
					SAR09	NSW09	NSW10	NSW10L	MEX11	SAU10	SAU10D
Zadoks score											
QZad.aww-2B	Q6	100	*Ppd-B1*	33.5	5.3	2.0	–[c]	–	–	–	–
QZad.aww-2D	Q11	52	*Ppd-D1*	31.1	−7.5	−5.7	–	–	–	–	–
Time from sowing to ear emergence (d)											
QEet.aww-2B	Q7	117	*gwm429*	22.4	–	–	−1.9	−1.4	−3.1	−3.7	−4.3
QEet.aww-2D	Q11	52	*Ppd-D1*	65.0	–	–	3.1	2.7	3.9	6.9	6.2
QEet.aww-5A	Q30	260	*Vrn-A1*	8.2	–	–	ns[d]	−0.7	−0.7	ns	ns
QEet.aww-5D	Q32	0[e]	*Vrn-D1*	5.4	–	–	1.1[f]				

[a] Position as marked in Figs. 1 or 3 and in Supplementary Figure 1

[b] SAR and SAU: experiments conducted in South Australia, Australia (at Roseworthy and Urrbrae, respectively); NSW: experiment conducted at Yanco in New South Wales, Australia; MEX: experiment conducted at Ciudad Obregon, Mexico; 09, 10 and 11: experiments conducted in 2009, 2010 and 2011, respectively; L: late-sown experiment; D: cyclical drought treatment

[c] Trait not assessed in this experiment

[d] No significant effect detected

[e] On linkage group 5D1

[f] No significant QTL-by-experiment interaction

grain yield in SAR09, NSW09, NSW09L and MEX11, but reduced grain yield in SAU10. QTL on chromosomes 3A (position Q16 in Fig. 2) also affected grain yield in SAB09, NSW09 and SAU10D. A region close to *Vrn-A1* (position Q29) was associated with differences in grain number in SAR08, SAR09, NSW09 and MEX11.

With test weight, the QTL with the largest effects were at *Ppd-B1* and *Ppd-D1* (Table 5). At both of these loci, the alleles for photoperiod sensitivity (from Drysdale at *Ppd-B1* and from Gladius at *Ppd-D1*) were associated with higher test weight in the NSW09L and NSW10 experiments. In the NSW09 experiment, the *Ppd-D1* allele for photoperiod sensitivity was associated with lower test weight. At both of these loci, the allele for photoperiod sensitivity was also associated with reduced percentage screenings. For percentage screenings, the QTL with the largest effects was on chromosome 3A (position Q17 in Fig. 2), at which the allele from Gladius reduced screenings.

The QTL with the largest effects for all grain dimensions and shape parameters were at *Ppd-B1* and *Ppd-D1* (Table 6). At those loci, the alleles for photoperiod sensitivity were usually associated with smaller trait values, except that the *Ppd-D1* allele for photoperiod sensitivity was associated with a higher trait value for grain roundness and had no effect on grain length. Effects were also detected at or near the marker *TaGW2* (Q33 and Q34 in Fig. 3), with the allele from Gladius increasing grain width, thickness and area and decreasing grain roundness.

For particle size index and flour extraction, the QTL with the largest effects was at the puroindoline locus *Pina-D1* (Table 7). The Gladius allele was associated with higher values of both traits (softer grain and greater flour extraction). These traits were also affected by QTL at or near *Ppd-D1* (Q11 and Q12 in Fig. 1), at which the Gladius allele for photoperiod sensitivity was associated with lower values of both traits in most experiments. There were also some environments in which the *Ha* locus had no significant effect on particle size index (NSW10) or flour extraction (NSW09 and NSW10). In some environments, photoperiod sensitivity seemed to be more important than the *Ha* locus, with the *Ppd-D1* locus affecting particle size index (NSW09L and NSW10) and/or flour extraction (NSW09 and NSW10).

There were several genomic regions that affected yield and grain characteristics but that did not affect plant phenology. These included some for which effects were detected in most or all environments: Q13 on chromosome 2D (thousand grain weight; grain width, thickness and aspect ratio), Q19 on chromosome 3B and Q27 on chromosome 4A (thousand grain weight), Q25 on chromosome 4A and Q28 on chromosome 4B (grain thickness), Q26 on chromosome 4A (percentage screenings), Q4 and Q5 on chromosome 2B (grain aspect ratio and roundness, respectively) and Q14 on chromosome 3A (grain roundness).

For Zadoks score, days from sowing to ear emergence, grain yield, thousand grain weight, grain width, grain thickness and particle size index, the variance

Table 4 Genetic map positions, LOD scores and estimated additive effects of the Gladius allele for QTL detected for grain yield, thousand grain weight and the number of grains per m^2 in six −experiments

Trait and QTL	QTL position[a]	Position (cM)	Closest marker(s)	LOD	Experiments[b] and estimated additive effects								
					SAR08	SAR09	SAB09	NSW09	NSW09L	NSW10	MEX11	SAU10	SAU10D
Grain yield (kg ha^{-1})													
QYld.aww-2A	Q3	163	*cfd36*	5.3	ns[c]	ns	ns	179	ns	73	ns	ns	−377
QYld.aww-2B-1	Q6	100	*Ppd-B1*	12.6	110	233	−54	ns	ns	70	160	ns	215
QYld.aww-2B-2	Q10	220	*wPt-3132*	3.6	−78	ns	46	−250	ns	ns	ns	ns	−296
QYld.aww-2D	Q11	52	*Ppd-D1*	15.5	−135	−183	53	74	ns	−130	−138	ns	ns
QYld.aww-3A	Q16	229	*barc324*	4.1	ns	ns	−40	−224	ns	ns	ns	ns	−272
QYld.aww-3B	Q24	301	*wPt-1870 - wPt2685*	12.5	−69	ns	−86	−345	−126	ns	−258	ns	ns
QYld.aww-5A	Q29	233	*wPt-4262 - Vrn-A1*	6.7	−108	−219	ns	196	ns	ns	−207	ns	ns
QYld.aww-6A	Q34	17	*wPt-0902 - wPt5834*	7.5	ns	98	ns	179	115	ns	202	−394	ns
Thousand grain weight (g)													
QTgw.aww-2B	Q6	100	*Ppd-B1*	7.5	–[d]	–	–	0.6	ns	0.5	ns	ns	2.1
QTgw.aww-2D-1	Q11	52	*Ppd-D1*	21.4	–	–	–	−1.2	0.4	−0.4	ns	−0.8	−3.1
QTgw.aww-2D-2	Q13	139	*gwm102 - barc11*	7.2	–	–	–	−0.4	ns	−1.3	−0.4	−2	−2.6
QTgw.aww-3B-1	Q19	24	*wPt-7961 - wPt-9066*	4.5	–	–	–	0.4	1.4	1	ns	0.5	1
QTgw.aww-3B-2	Q23	289	*wPt-2391*	5.7	–	–	–	−0.7[e]					
QTgw.aww-4A	Q27	145	*wPt-0023*	3.6	–	–	–	−0.7	−1	ns	ns	−0.6	−1.1
QTgw.aww-6A	Q33	2	*wPt-0902*	4.3	–	–	–	0.6[e]					
Grains per m^2													
QGrn.aww-2B-1	Q4	17	*wPt-2106*	4.4	–	–	–	−306	−171	ns	−202	−1,140	−716
QGrn.aww-2B-2	Q6	100	*Ppd-B1*	3	–	–	–	299	−142	ns	278	−415	ns
QGrn.aww-3A	Q16	232	*barc306*	6.3	–	–	–	ns	ns	−302	ns	−686	−901
QGrn.aww-5A	Q29	242	*wPt-4262 - Vrn-A1*	7.3	–	–	–	−753	ns	ns	ns	955	992

For QTL for which the position estimate coincides exactly with the position of a marker locus, the name of that marker is shown. For QTL for which the position estimate falls within an interval between marker loci, the names of the flanking markers are shown

[a] Position as marked in Figs. 1, 2 or 3 and in Supplementary Figure 1

[b] SAR, SAB and SAU: experiments conducted in South Australia, Australia (at Roseworthy, Booleroo and Urrbrae, respectively); NSW: experiment conducted at Yanco in New South Wales, Australia; MEX: experiment conducted at Ciudad Obregon, Mexico; 08, 09 and 10: experiments conducted in 2008, 2009 and 2010, respectively; L: late-sown experiment; D: cyclical drought treatment

[c] No significant effect detected

[d] Trait not assessed in this experiment

[e] No significant QTL-by-experiment interaction

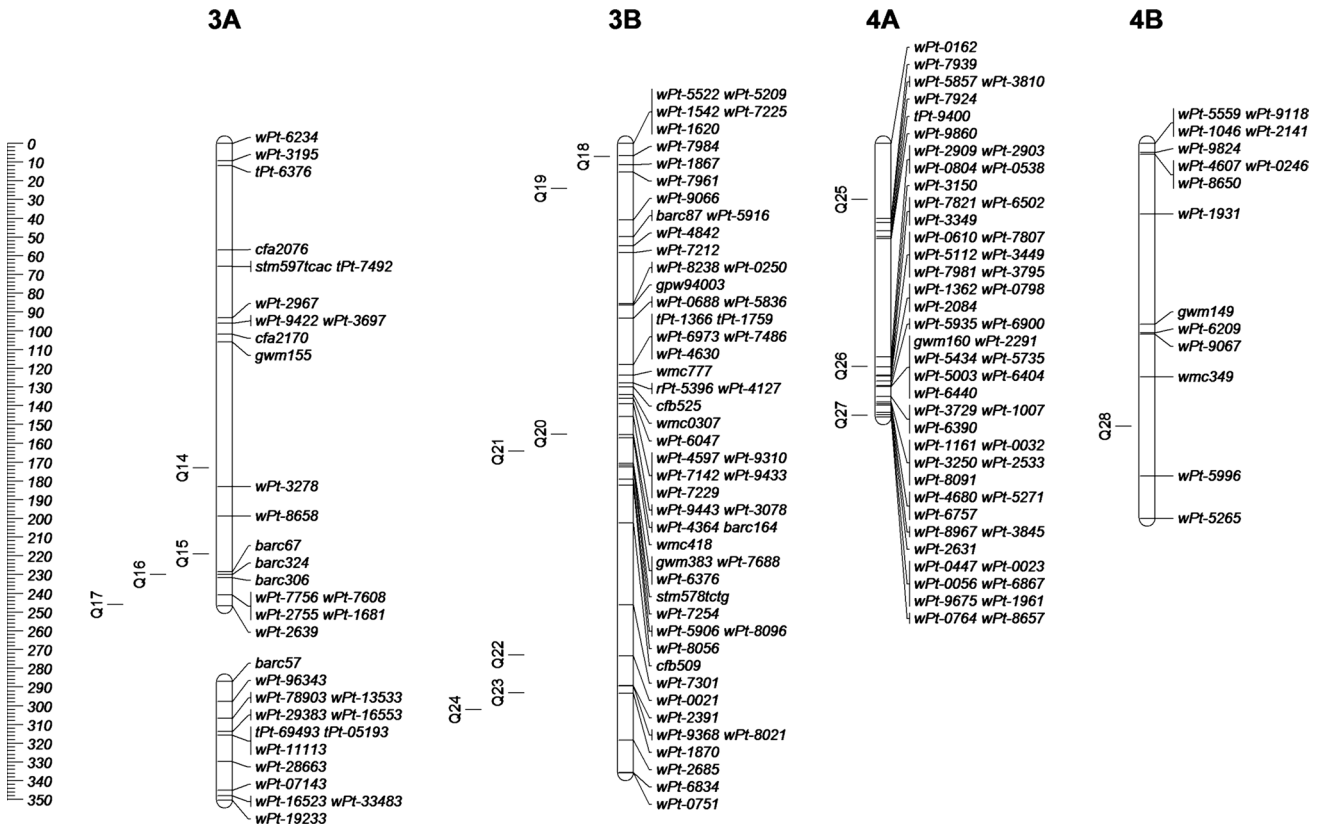

Fig. 2 Locations (Q14 to Q28) on chromosomes of homoeologous groups 3 and 4 of wheat at which QTL were detected for one or more traits, as listed in Tables 2, 3, 4, 5, 6. The *scale on the left* indicates genetic distances in cM

attributable to additive × additive epistasis exceeded that attributable to additive effects. For the first six traits, the most significant additive × additive epistatic interaction was between *Ppd-B1* and *Ppd-D1*. As expected, the genotypic class with both alleles for photoperiod sensitivity (*Ppd-B1b* and *Ppd-D1b*) had the latest ear emergence (Fig. 4). Presence of the photoperiod insensitivity (*a*) allele at either locus hastened ear emergence, and there was little further effect from having *a* alleles at both loci. In most environments, the genotypic classes with one or both alleles for photoperiod insensitivity outyielded the class with neither of these alleles. For particle size index, *Ppd-B1* interacted with the *Ha* (*Pin-A1* and *Pin-B1*) locus, with the *Ppd-B1a* (insensitivity) allele increasing the difference in particle size index between the two *Ha* haplotypes in SAU10D (Fig. 5).

Discussion

As is often the case in linkage mapping of wheat, the number of linkage groups in the genetic map (28) exceeded the number of chromosomes ($n = 21$). Non-polymorphic 'gaps' in this map could be due to: (1) the shortage of available markers for some genomic regions, especially in the D genome; (2) exclusion of markers exhibiting segregation distortion; and/or (3) genomic regions in which the two parents are identical by descent. Relatively few markers were mapped to D-genome chromosomes. On chromosome 5D, where *Pina-D1* and *Pinb-D1* are located on the short arm and *Vrn-D1* on the long arm, no DArT or SSR markers were found to be linked with the puroindoline genes and only two markers were found to be linked with *Vrn-D1*. Nevertheless, the linkage map provided an opportunity for QTL mapping of important traits.

The two parental lines stem from different breeding programmes and were developed for different ecogeographic regions. They were chosen as parents based on their performance under drought and heat stress conditions (Fleury et al. 2010). Gladius was considered to be more tolerant of drought and heat, while Drysdale was chosen based on carbon isotope discrimination (CID), an indicator of water use efficiency (WUE). While both may tolerate moisture limitations, they differ in the mechanism of tolerance. This cross therefore had the potential to combine the broad adaptation of Gladius to the harsh growing conditions that are often experienced in South Australia with the higher WUE of Drysdale. Although neither CID nor WUE were measured

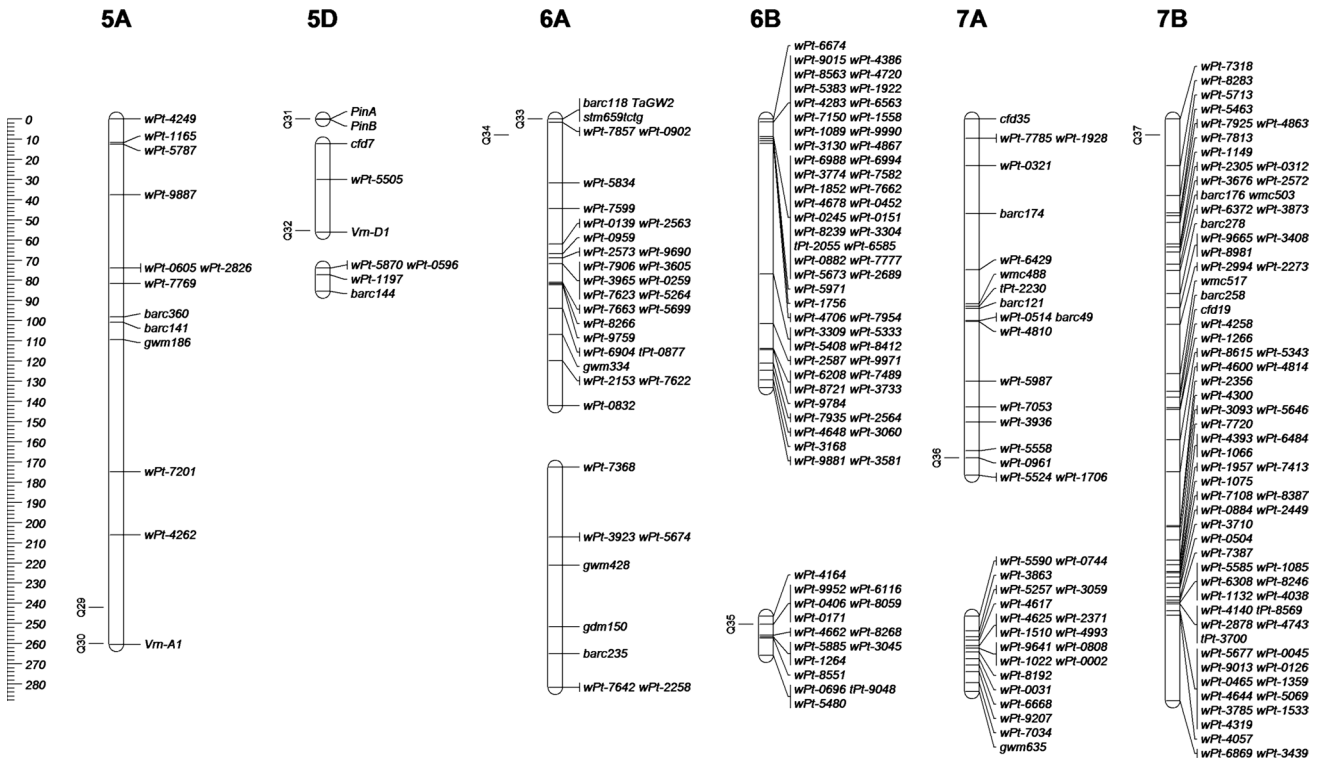

Fig. 3 Locations (Q29 to Q37) on chromosomes of homoeologous groups 5, 6 and 7 of wheat at which QTL were detected for one or more traits, as listed in Tables 2, 3, 4, 5, 6. The *scale on the left* indicates genetic distances in cM

Table 5 Genetic map positions, LOD scores and estimated additive effects of the Gladius allele for QTL detected for test weight and percentage screenings in three experiments

Trait and QTL	QTL position[a]	Position (cM)	Closest marker	LOD	Experiments[b] and estimated additive effects		
					NSW09	NSW09L	NSW10
Test weight (kg hl^{-1})							
QTwg.aww-1D	Q1	132	*wPt-0077*	4.4	ns[c]	−0.4	−0.3
QTwg.aww-2B	Q6	100	*Ppd-B1*	15.0	ns	−0.4	−0.5
QTwg.aww-2D	Q11	52	*Ppd-D1*	32.0	−0.4	0.7	0.6
QTwg.aww-3B	Q20	155	*barc164*	4.1	ns	0.3	ns
QTwg.aww-7B	Q37	8	*wPt-7318 – wPt-8283*	6.6	ns	−0.2	−0.6
Screenings (%)							
QPsc.aww-2B-1	Q6	100	*Ppd-B1*	9.6	ns	0.3	ns
QPsc.aww-2B-2	Q9	193	*gwm388*	5.1	1.0	0.4	ns
QPsc.aww-2D	Q11	52	*Ppd-D1*	8.8	0.6	−0.6	ns
QPsc.aww-3A	Q17	247	*wPt-2639*	10.2	−1.1	−0.7	ns
QPsc.aww-3B-1	Q18	7	*wPt-7984*	3.1	−0.4	−0.9	ns
QPsc.aww-3B-2	Q22	273	*wPt-0021*	4.3	0.5	0.5	0.3
QPsc.aww-4A	Q26	119	*wPt-3150*	4.0	0.4	0.8	−0.1

[a] Position as marked in Figs. 1, 2 or 3 and in Supplementary Figure 1

[b] SAR, SAB and SAU: experiments conducted in South Australia, Australia (at Roseworthy, Booleroo and Urrbrae, respectively); NSW: experiment conducted at Yanco in New South Wales, Australia; *MEX*: experiment conducted at Ciudad Obregon, Mexico; 08, 09 and 10: experiments conducted in 2008, 2009 and 2010, respectively; L: late-sown experiment; *D*: cyclical drought treatment

[c] No significant effect detected

Table 6 Genetic map positions, LOD scores and estimated additive effects of the Gladius allele for QTL detected for grain length, grain width, grain thickness, grain area, grain aspect ratio and grain roundness in four experiments

Trait and QTL	QTL position[a]	Position (cM)	Closest marker(s)	LOD	Experiments[b] and estimated additive effects			
					NSW09	NSW10	SAU10	SAU10D
Grain length (mm)								
QGle.aww-2B	Q6	100	*Ppd-B1*	13.7	0.02	0.06	ns[c]	0.09
QGle.aww-6B	Q35	4	*wPt-0171*	4.0	0.06[d]			
Grain width (mm)								
QGwi.aww-2B	Q6	100	*Ppd-B1*	6.9	0.03	0.02	ns	0.07
QGwi.aww-2D-1	Q11	52	*Ppd-D1*	15.3	−0.05	−0.03	−0.03	−0.09
QGwi.aww-2D-2	Q13	148	*gwm102 - barc11*	6.2	−0.02	−0.05	−0.06	−0.08
QGwi.aww-3B	Q24	302	*wPt-1870 - wPt-2685*	4.2	−0.03	−0.03	−0.03	−0.03
QGwi.aww-6A	Q33	0	*TaGW2*	8.0	0.03	0.03	0.03	0.03
Grain thickness (mm)								
QGth.aww-2B	Q6	100	*Ppd-B1*	6.3	ns	ns	ns	0.04
QGth.aww-2D-1	Q11	52	*Ppd-D1*	17.6	−0.03	ns	−0.03	−0.08
QGth.aww-2D-2	Q13	139	*gwm102 – barc11*	4.5	−0.01	−0.04	−0.05	−0.06
QGth.aww-3B	Q23	293	*wPt-1870*	6.0	−0.02[d]			
QGth.aww-4A	Q25	30	*wPt-0162 – wPt-7939*	5.9	−0.03[d]			
QGth.aww-4B	Q28	151	*wmc349 – wPt-5996*	6.6	0.04[d]			
QGth.aww-6A	Q33	0	*TaGW2*	11.1	0.03[d]			
Grain area (mm^2)								
QGar.aww-2B	Q6	100	*Ppd-B1*	9.5	0.22	0.31	0.14	0.59
QGar.aww-2D	Q11	52	*Ppd-D1*	12.3	−0.28	−0.28	−0.20	−0.69
QGar.aww-6A	Q34	8	*TaGW2 – wPt-5834*	3.8	0.22	0.26	0.15	-ns
Grain aspect ratio								
QGra.aww-2B-1	Q4	17	*wPt-2106*	4.7	0.02[d]			
QGra.aww-2B-2	Q6	100	*Ppd-B1*	5.4	−0.02	ns	ns	−0.02
QGra.aww-2D-1	Q11	52	*Ppd-D1*	11.6	0.03	ns	0.02	0.03
QGra.aww-2D-2	Q13	139	*gwm102 - barc11*	4.8	ns	0.02	0.03	0.03
Grain roundness								
QGro.aww-2B-1	Q5	26	*wPt-6706*	5.9	0.02[d]			
QGro.aww-2B-2	Q6	100	*Ppd-B1*	7.9	ns	0.01	ns	ns
QGro.aww-2D	Q11	52	*Ppd-D1*	17.1	0.01	ns	0.01	0.02
QGro.aww-3A	Q14	173	*gwm155 – wPt-3278*	3.7	−0.02[d]			
QGro.aww-6A	Q33	0	*TaGW2*	4.1	−0.01[d]			

For QTL for which the position estimate coincides exactly with the position of a marker locus, the name of that marker is shown. For QTL for which the position estimate falls within an interval between marker loci, the names of the flanking markers are shown

[a] Position as marked in Figs. 1, 2 or 3 and in Supplementary Figure 1

[b] SAU: experiments conducted at Urrbrae, South Australia, Australia; NSW: experiment conducted at Yanco in New South Wales, Australia; 09 and 10: experiments conducted in 2009 and 2010, respectively; D: cyclical drought treatment

[c] No significant effect detected

[d] No significant QTL-by-experiment interaction

in these experiments, the positions of QTL detected here can be compared with those that have been reported for those traits in other populations (Rebetzke et al. 2008; Wu et al. 2011). Of the QTL detected here, only those in the *Ppd-B1* and *Ppd-D1* regions seem to collocate with previously reported QTL for CID and WUE (Rebetzke et al. 2008).

Although the parents, Drysdale and Gladius, had similar ear emergence times, differing significantly in only one environment, the Drysdale/Gladius progeny lines displayed considerable variation in developmental traits. At the photoperiod-response loci *Ppd-B1* and *Ppd-D1*, alleles for photoperiod-sensitivity delayed ear emergence, with larger effects at *Ppd-D1* than *Ppd-B1* in all environments. This is

Table 7 Genetic map positions, LOD scores and estimated additive effects of the Gladius allele for QTL detected for particle size index and flour extraction in seven experiments

Trait and chromosome	Position (cM)	QT position[a]	Closest marker(s)	QTL	LOD	Experiments[b] and estimated additive effects				
						NSW09	NSW09L	NSW10	SAU10	SAU10D
Particle size index										
2A	47	Q2	*gwm275*	*QPsi.aww-2A*	7.6	0.5	0.5	0.5	0.5	0.5
2D	52	Q11	*Ppd-D1*	*QPsi.aww-2D*	9.4	−0.3	−0.6	0.7	−1.7	–[c]
3B	164	Q21	*wmc418/gwm383*	*QPsi.aww-3B*	4.4	0.5	0.4	–	–	−0.5
5D	0	Q31	*Pina-D1-Pinb-D1*	*QPsi.aww-5D*	13.1	1.2	0.4	–	0.4	0.8
Flour extraction (%)										
2B	125	Q8	*wPt-6192/barc183*	*QFex.aww-2B*	5.9	0.4	–	–	0.7	0.6
2D	61	Q12	*Ppd-D1/wPt-4381*	*QFex.aww-2D*	10.5	−0.6	–	−0.2	−1.1	−0.7
3A	219	Q15	*wPt-8658/barc67*	*QFex.aww-3A*	6.2	−0.6	–	−0.3	−0.5	−0.8
5D	0	Q31	*Pina-D1-Pinb-D1*	*QFex.aww-5D*	31.9	0.3	–	–	1.1	1.4
7A	168	Q36	*wPt-0961*	*QFex.aww-7A*	4.5	–	–	–	0.5	0.3

For QTL for which the position estimate coincides exactly with the position of a marker locus, the name of that marker is shown. For QTL for which the position estimate falls within an interval between marker loci, the names of the flanking markers are shown

[a] Position as marked in Figs. 1, 2 or 3 and in Supplementary Figure 1

[b] NSW: experiment conducted at Yanco in New South Wales, Australia; MEX: experiment conducted at Ciudad Obregon, Mexico; SAU: experiment conducted at Urrbrae in South Australia, Australia; 09: experiment conducted in 2009; 10: experiment conducted in 2010; L: late-sown experiment; D: cyclical drought treatment

[c] No significant effect detected

consistent with the fact that all of the experiments in which the population was evaluated were conducted under short-photoperiod conditions, in which photoperiod sensitivity is expected to delay flowering. The magnitudes of the QTL effects for ear emergence varied among environments, with the smallest effects in the late-sown NSW10L experiment, in which photoperiod would have been less limiting than in the other experiments. In contrast to other wheat mapping experiments (Hanocq et al. 2004, 2007; Kuchel et al. 2006; Griffiths et al. 2009; Kamran et al. 2013), no earliness *per se* loci were detected.

Under most conditions, the alleles conferring photoperiod insensitivity were associated with higher thousand grain weight, lower percentage screenings and higher grain yield. The early flowering of the photoperiod-insensitive lines not only enabled them to escape the effects of late-season abiotic stresses, but also extended the duration of the grain-filling period, permitting grain dimensions to develop to maximum capacity. The effects of photoperiod sensitivity observed here are consistent with those from previous reports. For example, the *Ppd-B1* region has previously been reported to be associated with thousand grain weight (Groos et al. 2003; Huang et al. 2006) and the *Ppd-D1* region has been reported to be associated with test weight and thousand grain weight (Huang et al. 2006). Further, *Ppd-D1* and regions near *Ppd-B1* have been reported to be associated with CID/WUE in three Australian populations (Rebetzke et al. 2008).

At the *Vrn-D1* locus, the Gladius-derived winter allele consistently delayed ear emergence. Similarly, at the *Vrn-A1* locus, the Drysdale-derived winter allele delayed ear emergence in a late-sown experiment in which plants developed under relatively warm conditions. In the early-sown experiments, *Vrn-A1* had no significant association with time to ear emergence. The associations observed here for *Vrn-D1* and *Vrn-A1* are consistent with previous observations that *Vrn-D1* has a larger vernalisation requirement than *Vrn-A1* (Yoshida et al. 2010) and that *Vrn-D1* consequently delays ear emergence more than *Vrn-A1* (Eagles et al. 2010).

Differences in grain hardness were associated with the *Ha* locus, at which the puroindoline genes *Pina-D1* and *Pinb-D1* are located. It is well known that presence of both functional alleles (*Pina-D1a* and *Pinb-D1b*, encoding PINA and PINB proteins, respectively) results in the soft endosperm texture found in soft (pastry) wheat, and that deletion of the *Pina-D1* gene (allele *Pina-D1b*) and/or presence of various alternative alleles of *Pinb-D1* confer harder grain (Bhave and Morris 2008). Polymorphism at the *Ha* locus has also been reported to be associated with differences in flour extraction (Cane et al. 2004; Tsilo et al. 2011; Wang et al. 2012). Although both Drysdale and Gladius are hard wheats, Drysdale carries the allele combination *Pina-D1b/Pinb-D1a* (absence of PINA in combination with the wild-type form of PINB), while Gladius carries the allele combination *Pina-D1a/Pinb-D1b* (wild-type form of

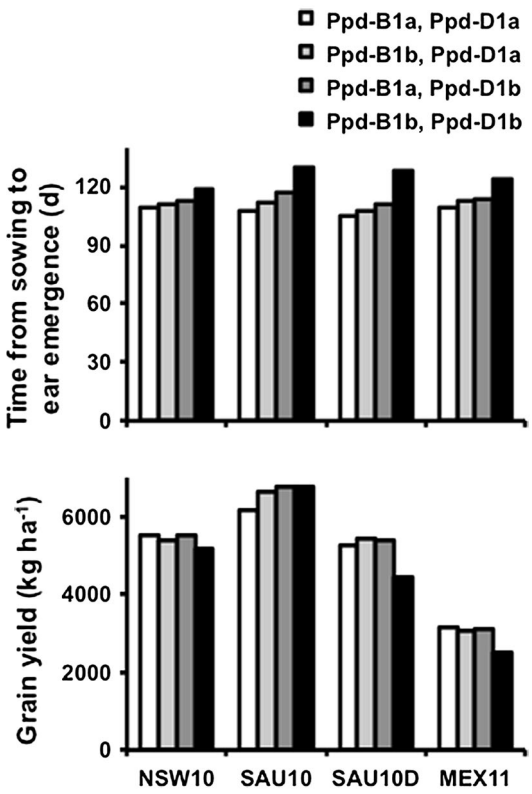

Fig. 4 Mean time from sowing to ear emergence and grain yield for each of four classes of lines defined by their genotypes at the *Ppd-B1* and *Ppd-D1* loci, in each of four environments. *SAU*: experiments conducted at Urrbrae in South Australia, Australia; *NSW*: experiment conducted at Yanco in New South Wales, Australia; *MEX*: experiment conducted at Ciudad Obregon, Mexico; 10 and 11: experiments conducted in 2010 and 2011, respectively; *D*: cyclical drought treatment

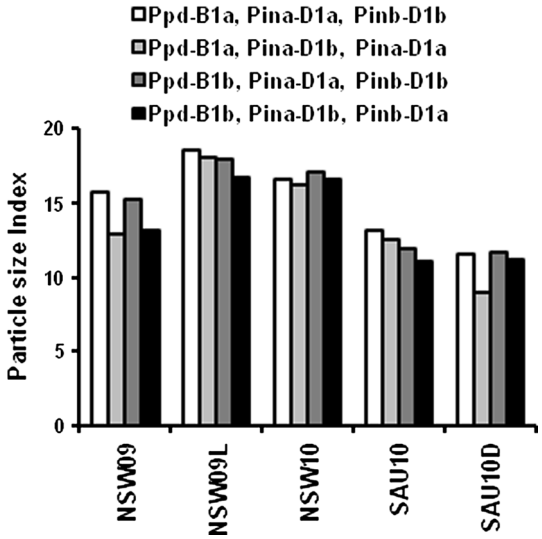

Fig. 5 Mean particle size index for each of four classes of lines defined by their genotypes at the *Ppd-B1* and *Ha* (*Pina-D1*/*Pinb-D1*) loci, in each of five environments in Australia. SAU: experiments conducted at Urrbrae in South Australia; *NSW*: experiment conducted at Yanco in New South Wales; 09 and 10: experiments conducted in 2009 and 2010, respectively; *L*: late-sown experiment; *D*: cyclical drought treatment

PINA in combination with a variant form of PINB). The Gladius haplotype was associated with a somewhat higher particle size index (softer grain) and greater flour extraction than the Drysdale haplotype. A significant epistatic interaction between the *Ha* and *Ppd-B1* loci reflected interaction in an environment (SAU10D) in which a cyclic drought stress treatment was imposed. In SAU10D, the *Ha* effect was amplified in the presence of the photoperiod insensitivity allele (*a*) at *Ppd-B1,* i.e., in lines that flowered late and would have experienced drought stress at a critical times during grain filling. This interaction is consistent with the idea (Lesage et al. 2012) that kernel hardness is related to the amplification of a stress response during endosperm development. Another chromosome region that was associated with flour extraction (position Q36 on chromosome 7A, Fig. 3) may coincide with a region previously shown to be associated with grain hardness and to contain members of the recently discovered *Pin-2* family of puroindoline genes (Wilkinson et al. 2008; Chen et al. 2010; Geng et al. 2012).

Consistent with the suggestion of Su et al. (2011) that *TaGW2* can be used as a diagnostic marker for grain weight, an effect on grain weight was detected near *TaGW2* (at position Q33 in Fig. 3). This region was also associated with thickness and roundness, reflecting the contributions of grain dimensions to grain weight. Grain yield QTL were also detected close to this marker in this study (position Q34 in Fig. 3) and in other studies (Snape et al. 2007; Sun et al. 2009).

Not surprisingly, correlated traits such as grain thickness, thousand grain weight and yield were associated with collocating QTL. Correlations among these traits could be partially due to gene linkage or pleiotropy. On chromosome 3B, there are QTL for grain thickness, thousand grain weight and yield at the same or adjacent positions (Q23 or Q24, Fig. 2), all with positive effects from Drysdale alleles. Based on map comparisons using the common markers *wPt-8021* and *wPt-0751*, this genomic region seems to be the same one that has been shown to be associated with yield in the RAC875/Kukri mapping population (Bennett et al. 2012b; Bonneau et al. 2013). On chromosome 2D, a potentially novel grain thickness QTL is collocated with a thousand grain weight QTL (position Q13 in Fig. 1). Both the 3B and 2D QTL present opportunities to increase grain dimension and weight that are independent of loci that affect photoperiod sensitivity. A position on chromosome 3A (Q16) that is associated with yield and grain number probably corresponds to one reported by Bennett et al. (2012b).

Even though phenology loci were the major determinants of grain yield and physical grain characteristics in the Drysdale/Gladius population, it was possible to detect other genomic regions that were associated specifically with those traits. Grain width and grain thickness were found to be under similar genetic control and to be strongly associated with thousand grain weight and grain yield. Grain length was also associated with grain yield but its genetic control seems to differ from that of grain width.

Some QTL detected in this study were consistent over environments, while others showed QTL-by-environment interaction. The *Ppd-B1* and *Ppd-D1* loci both exhibited QTL-by-environment interaction, with large effects in most experiments, but smaller and fewer effects in the late-sown NSW09L field experiment, which developed under longer days. The yield QTL on chromosome 3A (Q16 in Fig. 2) also interacted with environments. At this locus, the effect of the Drysdale allele was positive in two experiments conducted in South Australia but negative in one experiment conducted in New South Wales, and had no detectable effects in the other experiments. Similarly, for the yield QTL detected on chromosome 2A (Q3 in Fig. 1), the Drysdale allele had increasing effect in SAU10D, decreasing effect in NSW10 and no effect in other environments. This seems to be due to environmental conditions other than moisture deficit and high temperature.

Genotype-by-environment interaction was also evident for particle size index and flour extraction. In some environments, the *Ha* locus had the largest effects on these traits, while in others (in New South Wales), photoperiod sensitivity effects were more important. In the environments in which phenology had a greater effect than the *Ha* locus (or where there were epistatic interactions between phenology loci and *Ha*) the allele for photoperiod insensitivity was associated with early flowering, larger grains, softer grain and higher milling yield. This is consistent with the expectation that large and/or soft grain will yield more flour than small and/or hard grain. In contrast, photoperiod insensitivity was associated with later flowering, resulting in exposure of the plants to higher temperatures during grain filling. This would lead to smaller, harder grain with higher protein concentration. In the late-sown experiment (NSW09L) in which plants were exposed to extreme heat, no QTL were detected for flour extraction, perhaps because most lines had small grain and there was little variation in flour extraction.

A key objective of the study was to investigate the relationship between quality traits and heat and drought stress on the mapping population. Across 12 environments that differed in the duration, time and severity of stress, the most significant genetic effects were associated with the control of plant development, particularly the photoperiod response. Although breeders have generally optimised maturity and photoperiod response for their target production environments, there may still be opportunities to fine tune these phenological traits to protect against adverse conditions during grain filling by modifying the vegetative *versus* reproductive phases of growth. For many of the quality traits, QTL with small genetic effects were detected. In appropriate combinations, the alleles at these QTL could also offer significant opportunities for development of wheat cultivars that will tolerate heat and drought and produce high-quality grain despite adverse conditions.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The experiments reported here comply with the current laws of the countries in which they were performed.

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