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ORIGINAL PAPER

Genetic dissection of grain yield and physical grain quality in bread wheat (*Triticum aestivum* L.) under water-limited environments

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Abstract In the water-limited bread wheat production environment of southern Australia, large advances in grain yield have previously been achieved through the introduction and improved understanding of agronomic traits controlled by major genes, such as the semi-dwarf plant stature and photoperiod insensitivity. However, more recent yield increases have been achieved through incremental genetic advances, of which, breeders and researchers do not fully understand the underlying mechanism(s). A doubled haploid population was utilised, derived from a cross between RAC875, a relatively drought-tolerant breeders' line and Kukri, a locally adapted variety more intolerant of drought. Experiments were performed in 16 environments over four seasons in southern Australia, to physiologically dissect grain yield and to detect quantitative trait loci (QTL) for these traits. Two stage multi-environment trial analysis identified three main clusters of experiments (forming distinctive environments, ENVs), each with a distinctive growing season rainfall patterns. Kernels per square metre were positively correlated with grain yield and influenced by kernels per spikelet, a measure of fertility. QTL analysis detected nine loci for grain yield across these ENVs, individually accounting for between 3 and 18% of genetic variance within their respective ENVs, with the RAC875 allele conferring increased grain yield at seven of these loci. These loci were partially dissected by the detection of co-located QTL for other traits, namely kernels per square metre. While most loci for grain yield have previously been reported, their deployment and effect within local germplasm are now better understood. A number of novel loci can be further exploited to aid breeders' efforts in improving grain yield in the southern Australian environment.

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Introduction

In southern Australia, water availability presents one of the most common limitations to bread wheat production. Here, crops are sown in late autumn (April/May) and rely on winter rainfall through into spring, when increasingly infrequent rainfall creates cyclical and ultimately terminal drought. Shallow soil profiles, often with hostile subsoils, preclude significant soil moisture being stored. Subsequent

to the large advances in grain yield achieved through the introduction of major genes controlling key agronomic characteristics, particularly the semi-dwarf plant stature and photoperiod insensitivity, incremental genetic advances have driven most improvements in grain yield in this environment.

Breeders often release superior varieties without knowledge of the genetic/physiological basis of improved grain yield. If the genetic/physiological basis was better understood, it could lead to targeted breeding efforts to more rapidly improve traits driving grain yield in target environments. A wide range of traits that support grain yield and its components have been identified in a variety of different environments, with yield commonly viewed as a function of grain number, grain size, the efficiency of the use of available water and traits affecting these components (Passioura 1977). In water-limited environments, these traits have included water soluble carbohydrates (WSC) (Blum et al. 1994; Rattey et al. 2009), leaf glaucousness (Richards et al. 1986), transpiration efficiency (Condon and Hall 1997) and spikelet fertility (Briggs et al. 1999). However, the extent of variation for these traits within locally adapted germplasm has not been studied extensively in many cases, hence the value of each trait for grain yield within these target environments is poorly understood.

QTL analysis has been used previously to identify chromosomal regions in wheat associated with traits of relatively simple genetic control—rust resistance, nutritional toxicities and deficiencies and ear emergence time (EET). However, it is now becoming an increasingly popular method to genetically dissect more complex traits, such as yield under water-limited and/or heat-stressed conditions (Kuchel et al. 2007; Kumar et al. 2007; Mason et al. 2010; McIntyre et al. 2010; Pinto et al. 2010). Although many studies have previously identified QTL for bread wheat grain yield and yield components under drought conditions, few have been under conditions similar to that experienced by crop production in the southern Australian Mediterranean-type environment. Further to this, the deployment of any previously identified genetic loci within locally adapted germplasm is currently unknown. The detection of genetic regions associated with grain yield, physical grain quality and traits supporting these would form the basis of future investigations to identify genetic markers linked to these loci, which could be deployed to breeding programs targeting southern Australia, for marker assisted selection (MAS).

This study therefore aimed to investigate the trait and genetic basis of grain yield and physical grain quality within two locally adapted lines, Kukri and RAC875 and a doubled haploid (DH) population derived from a cross between the two. More specifically, the aims were (1) to identify key genetic relationships between grain yield and yield components and also the influence of these traits on various physical grain quality characteristics and (2) to identify chromosomal regions associated with grain yield, physical grain quality and any related traits, independent of EET, within this population.

Materials and methods

Plant material

A doubled haploid population, derived from a cross between RAC875 and Kukri was sown at 16 sites over four seasons. The population contained 368 individuals but in 2007, 2008 and 2010, a subset of 260 (or in 2010, 180) lines was sown to minimise the confounding impact of phenology and reduce resources required for phenotyping (Bennett et al. 2011). The parents of the population have been described and physiologically dissected by Izanloo et al. (2008). Briefly, RAC875 is a breeders' line that has previously shown a relatively stable yield in water-limited conditions, while Kukri is a locally adapted variety that has significantly reduced grain yield under the same conditions.

Field experiments and phenotypic measurements

Each field experiment was arranged in two complete randomised blocks with appropriate contrasting check lines. Experiments sown in 2010 used partially replicated (20%) designs. Seed was sown aiming for an average 200 seeds per square metre. Grain yield field plots constituted of either five or six rows and were sown 1.25 m wide and 5 m long and reduced to 3.2 m in length prior to anthesis by herbicide application in all environments. In the MIN06, MIN07, NUN08, PIE07, PIE08 and STR08 experiments, field plots were sown as 1.8 m wide and 6 m long, reduced to 5 m long by the method above. Fertiliser application and management regime for each site followed best local practice.

Early vigour was scored by visual rating when the trial was at approximately Zadoks growth stage 25 (Zadoks et al. 1974), with a score of one assigned to the least vigorous and a score of nine assigned to the most vigorous plant growth. Plant counts were conducted on two-one metre rows in each plot just after seedling emergence and tiller counts were recorded at an approximate trial average of Zadoks growth stage 50 on the same two-one metre rows. In the RAC08 trial, the number of tillers producing fertile spikes was also counted. Anthesis biomass cuts (BIO), WSC and associated measurements were sampled and measured following the methodology of Rebetzke et al. (2008b). Plant height was measured at physiological

maturity, as the distance between the ground and the tip of the spike, excluding awns, using a ruler.

Samples for harvest index (HI) were taken after physiological maturity from the two-one metre rows within the plot, avoiding outside rows and the end of the rows of the plot. These were tied in a bundle with string for later weighing and threshing. Threshed grain was weighed and expressed as a proportion of total biomass of the bundle. Five tillers were also sampled from the plot prior to harvest for measurement of peduncle length (PED), flag leaf length (FLL), flag leaf width (FLW), spikelets per spike and kernels per spike (KPS), which allowed kernels per spikelet (KPSL) to be derived. Grain was machine harvested, total plot weight recorded and converted to kg ha^{-1}. Screenings (SCR) were expressed as a percentage of a 100 g subsample of grain that passed through a 2.2-mm sieve. A Contador seed counter (Pfueffer GmBH, Germany) was used to count 500 kernels to estimate 1,000 kernel weight (TKW). Test weight (TWT) was measured on a sample of grain from each plot. Kernels per square metre (KPM^2) were calculated by dividing the harvested plot grain weight by the average kernel weight (derived from TKW). Not all traits were measured at all sites (Table 1).

Statistical analysis

The methods of Gilmour et al. (1997) were followed to minimise or remove spatial effects of field variation. For each trait in each experiment, linear mixed model analysis using the method of residual maximum likelihood (REML) was performed in GenStat release 8.2 (Payne et al. 2005). Genotype was firstly fitted as a random effect to calculate broad sense heritability, and then the data were re-analysed with Genotype as a fixed effect, to produce the best linear unbiased estimates (BLUEs), which were used for QTL mapping. EET was also fitted as a covariate in the analysis for each trait in each experiment. When the effect was not significant, it was excluded from the model. For grain yield, genetic correlations were generated during multi-environment analysis following the methods of Mathews et al. (2008) and a heat map generated using R (R Development Core Team (2005) to identify clusters of sites performing most similarly with respect to genotype ranking. Genotype performance was averaged across sites within each cluster to form three main environment (ENVs) cluster means, with two sub-ENVs for two of these. All traits (except for tillers per plant, tillers per square metre, final tiller number and WSC measurements) were then averaged across the same ENV clusters as grain yield to enable direct comparisons between grain yield and all other traits. Where split clusters (i.e. ENV2, ENV2-cool and ENV2-hot) detected the same QTL, only the first ENV result was reported. A MET across all experiments was also included to identify loci imparting a robust and repeatable grain yield effect. Where a trait illustrated high genetic correlations between all experiments, average performance across all sites was used (ALL EXPTs).

QTL mapping

QTL analysis was performed to the same standards as Bennett et al. (2011). Trait abbreviations and QTL designations were defined adopting the nomenclature suggested by the wheat catalogue of gene symbols (McIntosh et al. 2003). However, a number of traits had not previously been assigned a symbol and we propose the following: 'QTpa.' tillers per square metre; and 'QKpsl.' KPSL.

Results

Climatic dissection of ENVs for grain yield

The three main ENV clusters (ENV1, ENV2 and ENV3) were initially assigned based on genetic correlation between experiments for grain yield (Fig. 1; Table 2). ENV1 received a higher proportion of its growing season rainfall early in the vegetative growth stage (Fig. 2) and was the warmest environment in most climatic variables (Table 3). In-season rainfall for ENV2 was spread across the vegetative growth stage but like ENV1, had received around 95% of the seasons' rainfall before reaching anthesis (Zadoks growth stage 65, approximately 1,300° days (based on field observations, data not shown; Fig. 2). ENV3 received slightly lower early season rainfall but during grain fill, still received approximately 20% of that environments' rainfall. ENV1 and ENV2 received similar rainfall patterns to the 'Environment Type (ET) 4' drought characterised by Chenu et al. (2011). Meanwhile, ENV3 was similar to the 'ET 2' pattern of water availability identified by the same authors. Sub-ENVs were formed within ENV2 and ENV3, with ENV2-hot experiencing more extremely hot minimum and maximum temperatures than ENV2-cool and ENV3-hot experienced more hot days during reproductive development than ENV3-cool (Table 3).

Phenotypic summary

Mean grain yield ranged from 314 kg ha^{-1} at PIE07 to 5,275 kg ha^{-1} at RAC10 (Table 1) in the 16 site by year combinations. 2006, 2007 and 2008 were 3 years of severe drought across much of southern Australia and the level of yield relative to other sites was generally indicative of rainfall received (Table 1). RAC875 was significantly higher yielding than Kukri in 14 out of 16 trials ($P < 0.05$;

Table 1 The environments and which traits were measured in each experiment in the Kukri/RAC875 doubled haploid population

CODE	Location (Year)	Rainfall (mm)	Traits measured	Yield (kg ha^{-1})				
				Kukri	RAC875	Population average	Range	h^2
BOO07	Booleroo, SA (2007)	159	YLD, TKW, KPM^2, SCR, TWT, EV, FLL, FLW, PED, HT, KPS	1,496	1,541	1,569	1,219–1,784	0.70
BOO10	Booleroo, SA (2010)	216	YLD	1,108	1,550	1,379	678–1,800	0.70
HOR08	Horsham, VIC (2008)	187	YLD, TKW, KPM^2, SCR, TWT, FLL, FLW, HT, KPSL	844	1,033	954	272–1,417	0.75
MIN06	Minnipa, SA (2006)	68	YLD, TKW, KPM^2, SCR, EV, HT, KPSL	418	557	453	356–557	0.41
MIN07	Minnipa, SA (2007)	86	YLD, TKW, KPM^2, SCR, EV, FLL, FLW, TPA, TPP, PED, HT, KPS	408	511	409	258–520	0.54
NER10	Neridup, WA (2010)	222	YLD	1,123	1,332	1,059	229–2,031	0.58
NUN08	Nunjikompita, SA (2008)	96	YLD, TKW, KPM^2, SCR, TWT, TPA, HT	462	650	530	119–690	0.63
PIE07	Piednippie, SA (2007)	113	YLD, TKW, KPM^2, SCR, EV, TPA, PED, HT	299	441	314	174–465	0.66
PIE08	Piednippie, SA (2008)	212	YLD, TKW, KPM^2, SCR, TWT, HI, FLL, FLW, TPP, BIO, HT, KPS, KPSL	1,394	1,488	1,424	1,045–1,667	0.67
RAC06	Roseworthy, SA (2006)	131	YLD, TKW, KPM^2, SCR, EV, PED, HT, KPS, KPSL	2,128	2,359	2,201	1,711–2,554	0.73
RAC07	Roseworthy, SA (2007)	153	YLD, TKW, KPM^2, SCR, TWT, HI, EV, FLL, FLW, BIO, WSCC, WSCT, WSCA, PED, HT	2,493	2,646	2,395	1,365–3,137	0.67
RAC08	Roseworthy, SA (2008)	223	YLD, TKW, KPM^2, SCR, TWT, HI, EV, TPP, EN, BIO, WSCC, HT	2,150	2,874	2,459	1,587–3,028	0.69
RAC10	Roseworthy, SA (2010)	327	YLD, TKW, KPM^2	5,934	6,371	5,275	3,778–6,371	0.84
ROB07	Robinvale, VIC (2007)	99	YLD, TKW, KPM^2, SCR, TWT, FLL, FLW, HT, KPS	578	580	549	415–714	0.42
SHE10	Sheep Hills, VIC (2010)	309	YLD	2,373	2,372	2,327	1,071–3,064	0.74
STR08	Streaky Bay, SA (2008)	95	YLD, TKW, KPM^2, SCR, TWT, HI, BIO, HT, KPSL	607	702	648	373–803	0.68

Experiment code is the first three letters of the site plus the last two digits of the year in which the trial was sown, rainfall at that site (or nearest available Bureau of Meteorology station) and the list of traits uses the abbreviation assigned to QTL for that trait. Each site is further characterised in terms of yield performance of the parents and population average yield, range within the DH population and broad sense heritability of yield at that site

YLD grain yield, *TKW* 1,000 kernel weight, *KPM*^{2} grains per square metre, *KPS* kernels per spike, *KPSL* kernels per spikelet, *SCR* screenings, *TWT* test weight, *PED* peduncle length, *HT* plant height, *EV* early vigour, *FLL* flag leaf length, *FLW* flag leaf width, *TPA* tillers per square metre, *TPP* tillers per plant, *BIO* biomass at maturity, *WSCC* water soluble carbohydrate content (mg g^{-1}), *WSCT* water soluble carbohydrate per tiller, *WSCA* water soluble carbohydrate per square metre, *EN* final tiller number per plant, *HI* harvest index

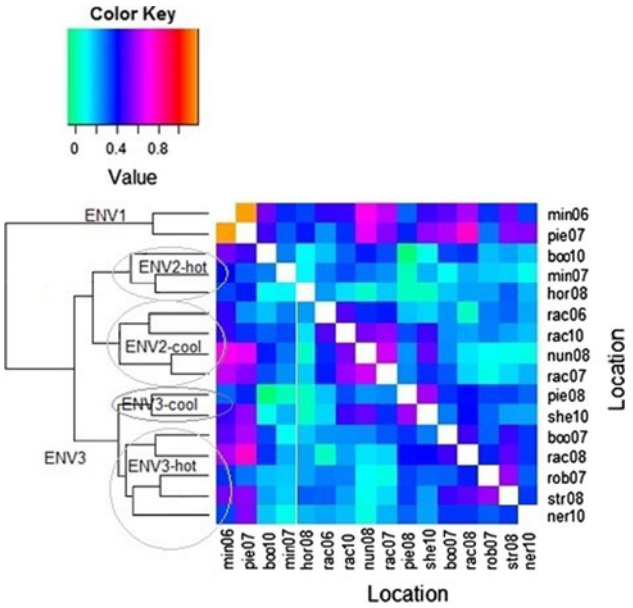

Fig. 1 Heat map generated using genetic correlations between field experiments where the Kukri/RAC875 doubled haploid population was grown. The dendrogram on the *left hand side* was used to identify clusters of environments (ENVs)

Table 1) and not significantly different at the remaining two (ROB07, SHE10). Heritability for grain yield ranged from moderate (0.41) to high (0.84), with a lower heritability generally the result of adjustment for maturity at some sites (data not shown). RAC875 exhibited greater early vigour than Kukri but during the growing season, had fewer tillers, greater plant height, shorter peduncle and as a result, lower biomass and consequently greater harvest index, also bolstered by a greater grain size (Table 2; Izanloo et al. 2008). In the KPS ENV-ALL and three KPSL ENVs, RAC875 had significantly more kernels than Kukri. In all but one SCR ENVs, RAC875 had a significantly lower percentage than Kukri, while in the four TWT ENVs, RAC875 had a lower value than Kukri (Table 2). There was significant transgressive segregation in both directions within the DH population for all traits measured.

KPM^2 was the trait being mostly correlated with grain yield, with KPS and KPSL significantly correlated with KPM^2 and grain yield (Table 4). KPSL was negatively correlated with TKW, and peduncle length negatively correlated with WSC. There was a significant negative correlation between FLW and four out of seven SCR ENVs (Table 4), which were generally the ENVs with the highest average SCR percentage (Table 2). This was also reflected through a significant positive correlation between FLW and TGW and a negative correlation between TGW and SCR (Table 4). WSCC was significantly correlated with plant height and also TGW in most ENVs (Table 4).

Chromosomal regions associated with QTLs for yield and yield related traits

A total of 163 QTL were detected for 20 traits, with at least one significant ($P < 0.05$) QTL detected for each ENV for each trait; and QTL were detected on all linkage groups except for 5D (Supplementary Table 1). A total of nine genetic loci were associated with grain yield (Fig. 3), with the RAC875 allele at seven of these contributing between 18.1 and 34.1 kg ha^{-1} greater grain yield over the Kukri allele. However, it was the Kukri allele at the locus of largest effect (QYld.aww-2D-2, located approximately 42 cM proximal to the *Ppd-D1* locus), that increased grain yield. Seven of the nine yield QTL for grain yield were detected in more than one ENV, with QYld.aww-1A only detected in the ALL ENV cluster and QYld.aww-2A and QYld.aww-2D-1 only detected in ENV3-cool. Yield QTL detected on chromosomes 1A, 4D and 6D were also associated with increases in KPM^2, where the RAC875 allele was associated with increases in both traits (Supplementary Table 1). The RAC875 allele at QTL detected on chromosomes 1B, 2B, 7AS and 7AL increased grain yield also, but were not associated with an increase in SCR and the RAC875 allele either had a positive or neutral effect on TKW. The RAC875 allele at 7AL was also associated with an increase in KPSL and harvest index and accounted for more than 10% of genetic variance for KPSL.

The RAC875 allele at QTL detected on chromosomes 1D, 3D, 5B, 7A and 7D contributed to greater TKW and accounted for between 2 and 11% of genetic variance for that trait. A QTL on chromosome 6A was detected for TKW, with the RAC875 allele also associated with an increase in FLW and a decrease in SCR, accounting for up to 11% of the variance for TKW and 25% of FLW. The QTkw.aww-6A locus was also located close to a QTL for WSC per unit area and per tiller. Meanwhile, the RAC875 allele on 4A was not associated with the expression of TKW or SCR.

The RAC875 allele at two loci increased test weight (on chromosomes 3A and 6A); and at the remaining four loci resulted in a lower value relative to the Kukri allele. No other QTL were detected at the QTwt.aww-1D and QTwt.aww-2A loci, while QTwt.aww-6A was associated with a corresponding increase in TWT and TKW, by the presence of the RAC875 allele. A lower test weight at QTwt.aww-4A was also associated with a shorter flag leaf and increased final tiller number relative to the effect of the Kukri allele.

Comparison of chromosome regions detected for EET, yield and yield components within the Kukri/RAC875 DH population

Despite the adjustment of data for relative maturity were significant, a number of regions previously reported to be

Table 2 Summary of traits and the experiments forming the environment clusters (ENVs) used for QTL detection in the RAC875/Kukri doubled haploid population

Trait	Cluster name	Experiments	Kukri	RAC875	Population average	Range	Single experiment h^2 range
Yield (kg ha^{-1})	ENV1	MIN06, PIE07	378.0	442.0	382.5	312.5–440.0	0.41–0.65
	ENV2	RAC06, MIN07, RAC07, HOR08, NUN08, BOO10, RAC10	1,984.1	2,156.8	1,952.2	1,477.1–2,190.6	0.55–0.84
	ENV2–1	MIN07, HOR08, BOO10	1,571.6	1,728.7	1,580.1	1,192.3–1,821.4	0.55–0.67
	ENV2–2	RAC06, RAC07, NUN08, RAC10	1,277.3	1,420.3	1,306.7	762.5–1,548.3	0.62–0.84
	ENV3	BOO07, ROB07, PIE08, RAC08, STR08, NER10, SHE10	2,690.9	2,893.3	2,597.7	2,098.4–2,861.0	0.57–0.70
	ENV3–1	PIE08, SHE10	1,865.8	1,932.0	1,870.2	1,214.0–2,271.5	0.67–0.69
	ENV3–2	BOO07, ROB07, RAC08, STR08, NER10	1,487.5	1,670.6	1,497.2	1,148.1–1,758.7	0.57–0.70
Early vigour (visual)	ENV1	MIN06, PIE07	4.4	4.8	4.7	3.3–6.5	0.41–0.64
	ENV2	RAC06, MIN07	4.9	4.5	4.9	4.0–6.0	0.51–0.52
	ENV3	BOL07, RAC08	6.1	6.2	6.1	4.8–7.2	0.50–0.78
Tillers							
Tillers per square metre		MIN07	76.5	65.3	73.9	67.3–80.4	0.27
		PIE07	75.8	60.6	70.1	62.6–81.8	0.43
		NUN08	57.9	43.3	46.5	40.4–57.1	0.48
Tillers per plant		MIN07	1.6	1.5	1.6	1.5–1.6	0.13
		PIE08	1.5	1.2	1.3	0.9–2.4	0.45
		RAC08	4.4	4.3	4.3	3.9–4.9	0.32
Final tiller number		RAC08	2.33	2.41	2.38	2.2–2.5	0.24
Flag leaf length (mm)	ALL EXPTs[a]	BOO07, HOR08, PIE08, MIN07, RAC07, ROB07	111.3	101.3	116.4	70.2–155.2	0.61–0.78
Flag leaf width (mm)	ALL EXPTs[a]	PID07, BOL07, RAC07, HOR08, ROB07, MIN07	10.1	11.5	10.7	8.0–13.1	0.68–0.85
Biomass (g)	ALL EXPTs[a]	PIE08, RAC08, RAC07, STR08	85.7	79.2	82.9	56.9–103.3	0.13–0.40
Water soluble carbohydrates							
WSC-content (mg g^{-1})	ALL EXPTs[a]	RAC07, RAC08	171.6	177.3	170.2	147.3–194.6	0.27–0.57
WSC-area (g m^{-2})		RAC07	110.9	127.3	118.9	99.1–152.7	0.45
WSC-tiller (mg)		RAC07	0.23	0.24	0.26	0.11–0.51	0.46
Peduncle length (mm)	ALL EXPTs[a]	BOL07, BOL06, RAC07, PID07, RAC06, MIN07	174.6	156.7	166.2	149.0–185.5	0.27–0.78
Height (cm)	ALL EXPTs[a]	ALL EXPERIMENTS	53.4	51.4	51.9	44.7–59.4	0.31–0.87
Harvest index	ALL EXPTs[a]	RAC06, RAC07, PIE08, RAC08, STR08	0.33	0.37	0.36	0.26–0.41	0.34–0.84
Kernels per spike	ALL EXPTs[a]	RAC06, BOL07, MIN07, ROB07, PID08	27.3	27.8	27.0	20.7–34.6	0.47–0.63
Kernels per spikelet	ENV1	MIN06	1.16	1.28	1.27	0.91–1.68	0.87
	ENV2	RAC06, HOR08	1.82	2.03	1.93	1.56–2.23	0.79–0.89
	ENV3	PID08, STR08	2.42	2.57	2.45	2.16–2.80	0.70–0.82

Table 2 continued

Trait	Cluster name	Experiments	Kukri	RAC875	Population average	Range	Single experiment h^2 range
Kernels per square metre	ENV1	MIN06, PIE07	1,058.5	1,203.5	4,397.8	784.0–1,481.2	0.81–0.89
	ENV2	RAC06, MIN07, RAC07, HOR08, NUN08	3,746.5	3,385.7	3,484.0	1,976.9–4,490.1	0.33–0.86
	ENV2–1	MIN07, HOR08	2,458.4	2,641.5	2,659.5	1,582.5–3,742.5	0.84–0.86
	ENV2–2	RAC06, RAC07, NUN08	5,854.1	5,010.4	5,195.1	2,898.7–6,992.8	0.33–0.85
	ENV3	BOL07, ROB07, PIE08, RAC08, STR08	2,284.2	2,143.5	2,216.5	1,502.6–2,765.6	0.25–0.85
	ENV3–1	PID08	3,104.1	3,024.0	2,967.5	1,961.1–3,766.5	0.75
	ENV3–2	BOL07, ROB07, RAC08, STR08	2,772.3	2,564.4	2,704.2	1,657.7–3,425.6	0.25–0.85
1,000 kernel weight (g)	ALL EXPTs[a]	ALL EXPERIMENTS	32.8	38.4	35.3	26.5–42.1	0.58–0.92
Screenings (%)	ENV1	PIE07	2.2	1.2	2.0	0.4–5.1	0.77
	ENV2	MIN07, RAC07, HOR08, NUN08	4.5	2.6	3.9	1.7–11.8	0.82–0.90
	ENV2–1	MIN07, HOR08	3.6	3.0	3.6	1.6–11.6	0.84–0.90
	ENV2–2	RAC07, NUN08	5.3	2.3	4.1	1.5–12.0	0.82–0.89
	ENV3	BOL07, ROB07, PIE08, STR08	11.8	2.8	5.4	1.9–19.1	0.69–0.88
	ENV3–1	PID08	1.9	2.1	2.0	0.5–4.3	0.88
	ENV3–2	BOL07, ROB07, RAC08, STR08	14.2	3.0	6.2	2.1–23.3	0.69–0.85
Test weight (kg 100 l^{-1})	ENV2	RAC07, NUN08	79.3	77.9	78.2	73.6–81.4	0.83–0.87
	ENV3	BOL07, PIE08, STR08	79.1	77.6	78.3	75.1–81.7	0.79–0.82
	ENV3–1	PID08	81.7	79.0	80.1	74.3–83.4	0.79
	ENV3–2	BOL07, RAC08, STR08	78.5	77.2	77.8	74.4–81.5	0.79–0.82

The mean value for each of the parents, the population, the range in populations and the range in single experiment heritabilities within each ENV

[a] All sites indicate all experiments that trait was measured in

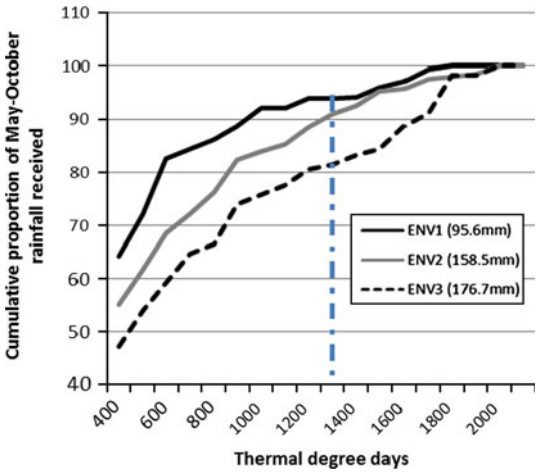

Fig. 2 The average cumulative percentage of growing season rainfall (including opening rains) received by experiments in the three distinct yield MET groupings (where daily rainfall data were available, ENV1, $n = 2$; ENV2, $n = 4$; ENV3, $n = 3$). Average total rainfall for each ENV is given in *parentheses*. Anthesis was assumed to occur at approximately 1,300° days (*vertical line*) and physiological maturity around 2,100, based on field experiment observations

influencing EET in the RAC875/Kukri DH population were detected for yield and yield components in the present study. These included QEet.aww-2B (*Ppd-B1*), QEet.aww-2D (*Ppd-D1*), QEet.aww-4A, QEet.aww-4B, QEet.aww-5B and QEet.aww-7A-1 and QEet.aww-7A-2 (Bennett et al. 2011). In general, the later flowering allele at each locus had a negative relationship with the allele increasing a given trait, although this was not always the case. At the QEet.aww-2D locus, QEn.aww-2D, QEv.aww-2D and QFll.aww-2D were detected with the RAC875 allele resulting in a greater value. At the QEet.aww-4B locus, QKpsl.aww-4B was detected with the Kukri allele resulting in a greater value.

Comparison of QTL for glaucousness, yield and yield components within the RAC875/Kukri DH population

In the present study, QTL were detected on chromosome 3A, around 60 cM on the linkage group, with the RAC875 allele increasing TWT, plant height and WSC (expressed on a per tiller, content and per square metre basis) and resulting in a reduced peduncle length, KPS and KPM^2 relative to the Kukri allele. To test the independence of agronomic and the glaucousness QTL previously detected on chromosome 3A in this population (Bennett et al. 2011), the DH population was split into two subpopulations, based on those individuals with the RAC875 allele and those individuals with the Kukri allele at marker locus wmc0264, the closest marker to the glaucousness QTL (Bennett et al. 2011; Fig. 3). Within these two subpopulations, segregation for marker locus gwm0002 was used to

Table 3 Total growing season rainfall and different temperature characteristics of each ENV cluster over the grain fill period (September and October)

Environment cluster	Rainfall (mm)			September						October					
	Total	Pre-anthesis	Post-anthesis	Average daily (°C)			Monthly extreme (°C)			Average daily (°C)			Monthly extreme (°C)		
				Max.	Min.	Difference	Max.	Min	Days > 30	Max.	Min.	Difference	Max.	Min	Days > 30
ENV1	95.6	87.5	8.1	25.25	7.50	17.75	34.50	0.15	7.00	28.00	9.40	18.60	40.15	2.05	10.50
ENV2	158.5	124.5	21.6	21.43	5.80	15.63	31.03	−0.43	2.29	25.63	7.26	18.37	37.21	0.64	7.14
ENV3	176.7	143.5	33.2	21.07	6.60	14.47	30.66	1.99	1.57	25.36	8.97	16.39	36.89	1.84	7.71
ENV2-cool	136.1	119.2	16.9	20.13	5.47	14.67	31.37	0.47	0.67	24.63	7.33	17.30	35.73	0.77	5.67
ENV2-hot	145.2	129.2	25.0	22.40	6.05	16.35	30.78	−1.10	3.50	26.38	7.21	19.17	38.33	0.55	8.25
ENV3-cool	154.5	121.4	33.4	20.98	6.40	14.58	31.06	1.40	0.80	25.02	8.98	16.04	36.88	1.78	7.20
ENV3-hot	221.3	187.9	33.1	21.30	6.50	14.80	29.65	1.55	3.50	26.20	8.95	17.25	36.90	2.00	9.00
Significance	<0.001	<0.001	0.009	0.03	ns	0.01	0.04	ns	ns	0.002	ns	ns	0.03	ns	ns
Effect	11.8	13.2	23.3	−116.9		−210.60	−117.5			−187.1			−187.9		

Average daily minimum and maximum and the difference between the two, as well as the most extreme daily maximum and minimum temperature and the total number of days greater than 30°C for both months are shown. The difference between these measures between the 2 months, as well as which characteristics that were having a significant effect on grain yield (on a single site basis) and the effect (in kg ha^{-1} deg^{-1} for all characteristics, except for rainfall, which was in kg ha^{-1} mm^{-1}). Data are the average of all sites within that ENV cluster, with climatic data from Australian Bureau of Meteorology

ns not significant at $P < 0.05$

Table 4 Phenotypic correlations between yield and yield components in different ENV clusters in the Kukri/RAC875 doubled haploid population

		1,000 kernel weight	Yield	ENV1	ENV2	ENV2-cool	ENV2-hot	ENV3	ENV3-hot	ENV3-cool	Kernels per spike	Kernels per spikelet		
		ALL ENV	ALL ENV								ALL ENV	ENV1	ENV2	ENV3
1,000 kernel wt	ALL ENV	1.00												
Yield	ALL ENV		1.00											
	ENV1		**0.76**	1.00										
	ENV2		***0.89***	*0.65*	1.00									
	ENV2-cool		***0.92***	**0.72**	**0.65**	1.00								
	ENV2-hot		**0.76**	*0.50*	***0.85***	*0.56*	1.00							
	ENV3		**0.80**	*0.62*	***0.89***	*0.58*	*0.52*	1.00						
	ENV3-hot		**0.72**	*0.61*	*0.50*	**0.79**	*0.40*	*0.46*	1.00					
	ENV3-cool		***0.87***	*0.65*	*0.62*	***0.94***	*0.55*	*0.54*	*0.53*	1.00				
Kernels per spike	ALL ENV	*−0.25*	*0.31*	*0.34*	*0.26*	*0.29*	*0.28*	*0.19*	*0.17*	*0.30*	1.00			
Kernels per spikelet	ENV1	*−0.31*	*0.25*	*0.24*	*0.27*		*0.28*	*0.19*		*0.21*	*0.41*	1.00		
	ENV2	*−0.35*	*0.32*	*0.23*	*0.28*	*0.30*	*0.28*	*0.22*		*0.32*	*0.49*	*0.53*	1.00	
	ENV3		*0.32*	*0.24*	*0.28*	*0.30*	*0.29*	*0.19*		*0.32*	*0.59*	*0.53*	*0.59*	1.00
Kernels per square metre	ENV1	*−0.43*	*0.53*	**0.70**	*0.46*	*0.48*	*0.39*	*0.41*	*0.40*	*0.44*	*0.41*	*0.50*	*0.43*	*0.40*
	ENV2	*−0.59*	*0.55*	*0.35*	*0.55*	*0.46*	*0.51*	*0.46*	*0.34*	*0.45*	*0.34*	*0.36*	*0.41*	*0.31*
	ENV2-cool	*−0.38*	*0.55*	*0.44*	*0.57*	*0.45*	*0.63*	*0.38*	*0.29*	*0.46*	*0.36*	*0.36*	*0.47*	*0.34*
	ENV2-hot	*−0.58*	*0.46*	*0.25*	*0.45*	*0.39*	*0.37*	*0.41*	*0.30*	*0.37*	*0.27*	*0.30*	*0.32*	*0.24*
	ENV3	−0.56	0.50	0.47	0.49	0.43	0.47	0.39	0.31	0.42	0.53	0.43	0.41	0.43
	ENV3-hot	*−0.58*	*0.34*	*0.24*	*0.26*	*0.36*	*0.22*	*0.23*	*0.41*	*0.27*	*0.18*		*0.27*	*0.21*
	ENV3-cool	*−0.48*	*0.48*	*0.47*	*0.49*	*0.39*	*0.48*	*0.38*	*0.24*	*0.40*	*0.56*	*0.45*	*0.39*	*0.43*
Flag leaf width	ALL ENV	*0.52*									0.33			
Peduncle length	ALL ENV													
Water sol carbos	ALL ENV	*0.16*												*−0.20*
Flag leaf width	ALL ENV			*−0.18*		*−0.18*	*0.26*		*−0.32*		*0.29*			*0.19*
Plant height	ALL ENV	*0.19*				*−0.17*		*−0.21*						
Screenings	ENV1	*−0.30*										*0.24*	*0.17*	
	ENV2	*−0.57*									*0.23*	*0.20*	*0.20*	
	ENV2-cool	*−0.40*			*0.17*		*0.22*			*0.20*	*0.20*	*0.21*	*0.23*	
	ENV2-hot	*−0.59*									*0.20*			
	ENV3	*−0.63*												
	ENV3-hot		*0.22*		*0.18*	*0.22*	*0.20*			*0.22*	*0.23*			
	ENV3-cool	*−0.64*												
Ear emergence time	RAC MET						*−0.45*							

Table 4 continued

		Kernels per square metre							Flag leaf width	Ped. length	Water sol carbos	Flag leaf length	Plant height
		ENV1	ENV2	ENV2-cool	ENV2-hot	ENV3	ENV3-hot	ENV3-cool	ALL ENV	ALL ENV	ALL ENV	ALL ENV	ALL ENV
Kernels per square metre	ENV1	1.00											
	ENV2	*0.56*	1.00										
	ENV2-cool	*0.56*	**0.73**	1.00									
	ENV2-hot	*0.46*	***0.95***	*0.48*	1.00								
	ENV3	**0.66**	**0.67**	*0.63*	*0.57*	1.00							
	ENV3-hot	*0.43*	*0.54*	*0.36*	*0.53*	*0.59*	1.00						
	ENV3-cool	*0.63*	*0.61*	*0.62*	*0.50*	***0.98***	*0.39*	1.00					
Flag leaf width	ALL ENV		*−0.18*		*−0.21*	*−0.20*	*−0.37*		1.00				
Peduncle length	ALL ENV						*−0.15*		*0.18*	1.00			
Water sol. carbs	ALL ENV					*−0.18*		*−0.18*			1.00		
Flag leaf width	ALL ENV					*0.17*		*0.23*	*0.20*	*0.28*	*−0.22*	1.00	
Plant height	ALL ENV	*−0.20*	*−0.23*	*−0.17*	*−0.21*	*−0.17*	*−0.26*			*0.38*	*0.31*	*0.23*	1.00
Screenings	ENV1					*0.18*		*0.18*					
	ENV2	*0.29*	*0.45*	*0.35*	*0.42*	*0.43*	*0.33*	*0.39*	*−0.26*		*−0.17*		*−0.24*
	ENV2-cool	*0.28*	*0.45*	*0.43*	*0.37*	*0.41*	*0.23*	*0.41*			*−0.17*	*0.20*	*−0.17*
	ENV2-hot	*0.25*	*0.37*	*0.21*	*0.37*	*0.35*	*0.34*	*0.30*	*−0.31*				*−0.25*
	ENV3		*0.39*	*0.22*	*0.40*	*0.34*	*0.32*	*0.30*	*−0.37*		*−0.15*		
	ENV3-hot							*0.17*	*0.20*				
	ENV3-cool	*0.18*	*0.39*	*0.22*	*0.40*	*0.34*	*0.33*	*0.29*	*−0.39*				
Ear emerg. time	RAC MET							*−0.38*				**−0.64**	

		Screenings							Ear emerg time
		ENV1	ENV2	ENV2-cool	ENV2-hot	ENV3	ENV3-hot	ENV3-cool	RAC MET
Kernels per square metre	ENV1								
	ENV2								
	ENV2-cool								
	ENV2-hot								
	ENV3								
	ENV3-hot								
	ENV3-cool								
Flag leaf width	ALL ENV								
Peduncle length	ALL ENV								

Table 4 continued

		Screenings							Ear emerg time
		ENV1	ENV2	ENV2-cool	ENV2-hot	ENV3	ENV3-hot	ENV3-cool	RAC MET
Water sol. carbs	ALL ENV								
Flag leaf width	ALL ENV								
Plant height	ALL ENV								
Screenings	ENV1	1.00							
	ENV2	*0.59*	1.00						
	ENV2-cool	*0.48*	***0.86***	1.00					
	ENV2-hot	*0.55*	***0.91***	*0.56*	1.00				
	ENV3	*0.49*	**0.71**	*0.56*	**0.68**	1.00			
	ENV3-hot	*0.43*	*0.40*	*0.38*	*0.33*	*0.30*	1.00		
	ENV3-cool	*0.47*	**0.69**	*0.54*	**0.67**	***1.00***	*0.24*	1.00	
Ear emerg. time	RAC MET								1.00

Italicised correlations indicate significance at $P < 0.05$, bold at $P < 0.01$, and bold and italicised at $P < 0.001$. Correlation not shown if not significant

detect recombinants between the two loci and the phenotypic performance of the RAC875 allele in these backgrounds compared. Significant phenotypic differences ($P < 0.05$) between the traits listed above were identified (Fig. 4), confirming the independence of two different loci. No QTL were detected at QW.aww-3A, or a number of other glaucousness loci that were independent of EET (QW.aww-3D, QW.aww-5BS and QW.aww-5BL).

QW.aww-1D was detected in a similar region to QTkw.aww-1D and QTpa.aww-1D, where the RAC875 allele contributed a lower flag leaf glaucousness score, TPA and greater TKW, relative to the Kukri allele, which was also the case at QW.aww-2B. At QW.aww-4D, the RAC875 allele also resulted in lower flag leaf glaucousness relative to the Kukri allele but was associated with an increase in grain yield and KPM^2. The RAC875 allele at QW.aww-7D resulted in greater glaucousness but decreased KPM^2 and FLL.

Discussion

While almost half of the experiments used for this study could be considered very low yielding (<1,000 kg ha^{-1} average), we would argue that these remain highly relevant for the target environment. The average bread wheat grain yields for South Australia during this study were 660, 1,080, 1,130 and 2,690 kg ha^{-1} for 2006, 2007, 2008 and 2010, respectively, compared to the 10-year average of 1,470 kg ha^{-1} (Australian Crop Forecasters, Melbourne, VIC, Australia). This highlights the prevalence of drought across southern Australia and the relevance of these extremely dry environments is further bolstered by numerous examples in the present study of the relative performance of lines in very low yielding experiments having a strong genetic correlation with higher yielding environments, even after removing any EET effect. In addition, the pattern of rainfall over the three main ENV clusters appears to be similar to the two predominant ET's in southern Australia identified by Chenu et al. (2011).

Many traits have been proposed as being beneficial for bread wheat production in water-limited environments. However, only few studies have considered the southern Australian climate to identify those traits that are driving grain yield production in this challenging climate. The present study has taken the approach of identifying those traits imparting an effect on grain yield, particularly in drier environments and then identifying QTL underlying these traits. As identified by Kuchel et al. (2007), KPM^2 was found to be a large driver of grain yield in many of the experiments the population was grown in. This was further explained by KPSL and KPS, two measures of spike and floret fertility, indicating these are key traits for improving yield in the target environment.

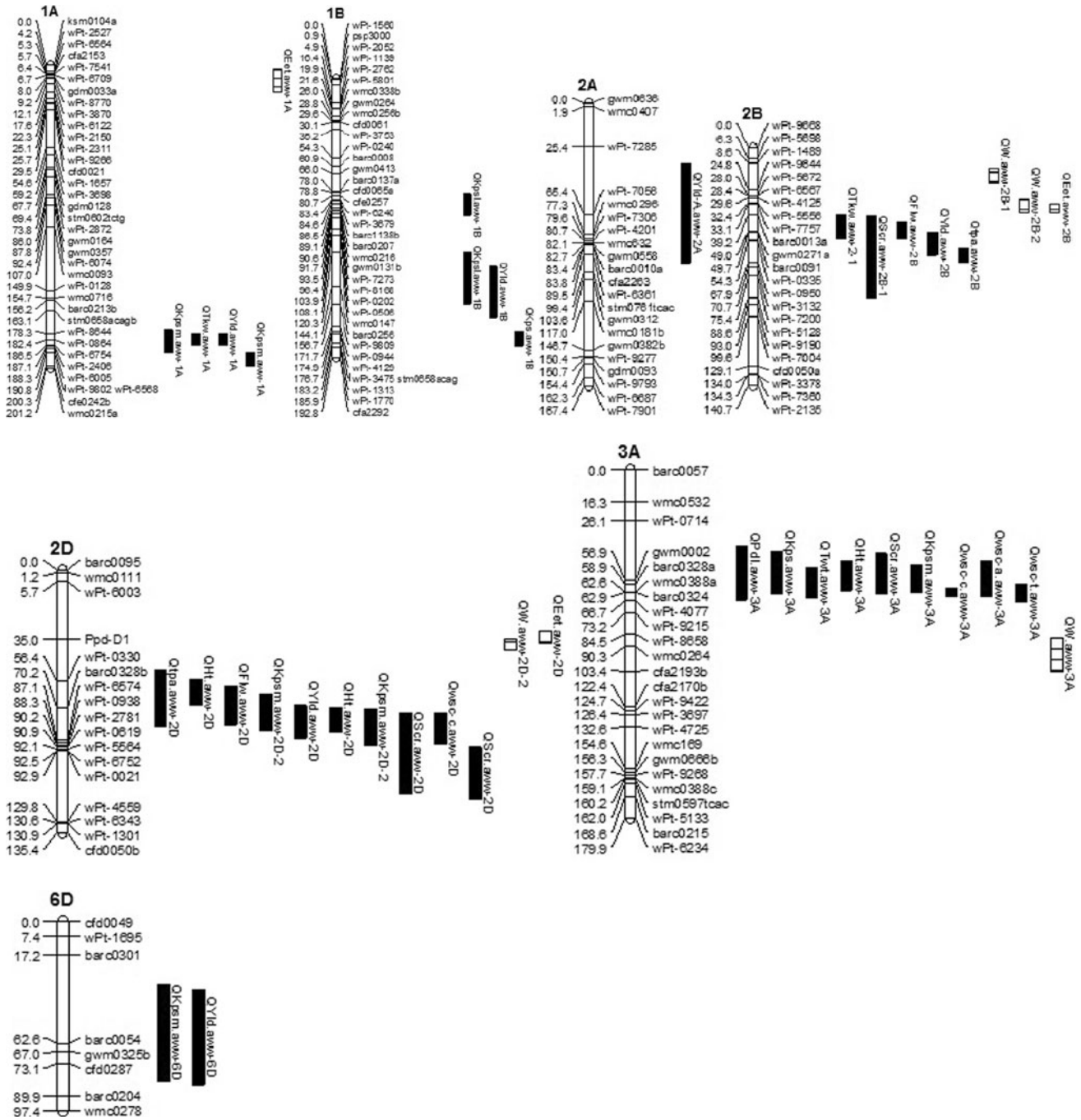

Fig. 3 Quantitative trait loci (QTL) detected for grain yield and co-located QTL for associated yield components in the Kukri/RAC875 doubled haploid population. QTL positions for ear emergence time (*Eet*) and flag leaf glaucousness (*W*) have been included and are discussed in Bennett et al. (2011)

Novel chromosomal regions for grain yield and grain yield components, not associated with EET or flag leaf glaucousness

As with previous QTL studies on bread wheat grain yield and associated yield components under water-limited environments (Kuchel et al. 2007; Mathews et al. 2008; McIntyre et al. 2010; Pinto et al. 2010), the present study has detected numerous genetic loci influencing grain yield production. Of these nine loci, five were identified in more than one distinct ENV cluster, all with the higher yielding allele from RAC875, suggesting a robust, repeatable effect on grain yield. Four other loci were detected in single ENVs consisting of at least two moderate to highly genetically correlated experiments. This indicates that these QTL were still reliable and impart a repeatable effect across the experiments.

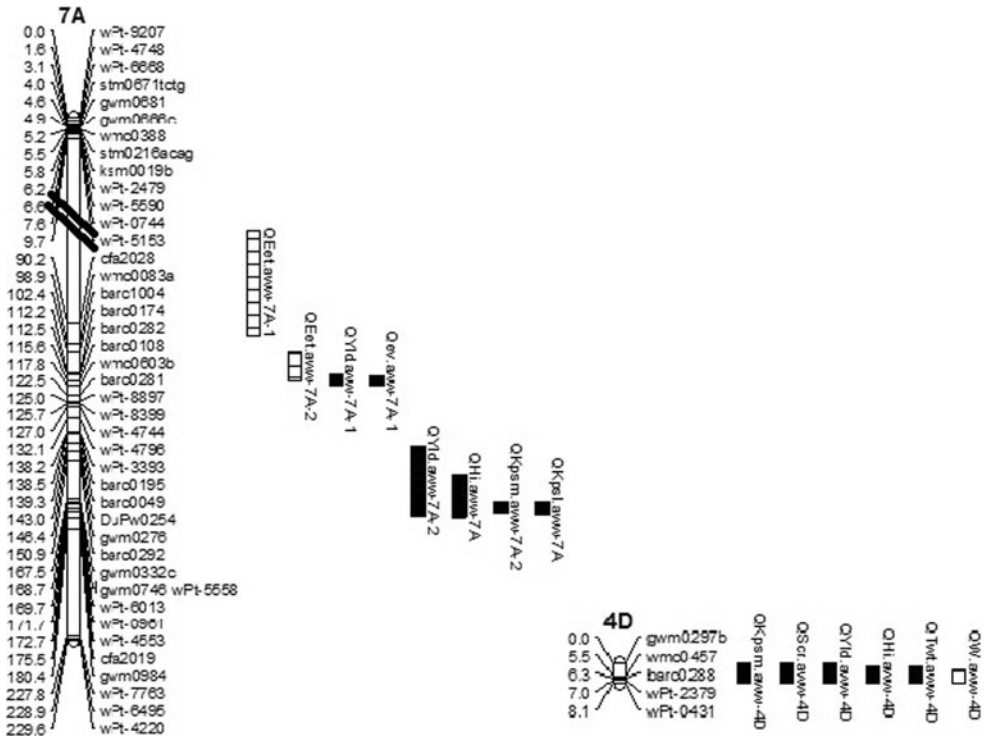

Fig. 3 continued

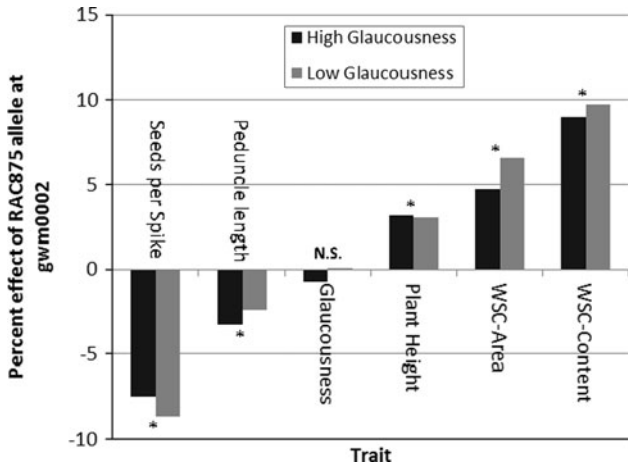

Fig. 4 Relative effect of RAC875 allele at marker locus gwm0002 on chromosome 3A in subpopulations fixed for flag leaf glaucousness (marker locus wmc264) within the doubled haploid population derived from a cross between RAC875 and Kukri. *Asterisks* indicate significant differences ($P < 0.05$) between alleles, where the Kukri allele effect at marker locus gwm0002 is fixed at 0. The difference between high and low glaucousness subpopulations was not significant for any trait

There were a number of loci where the RAC875 allele increased grain yield and KPM^2 without the detrimental increase in screenings and had either a neutral or positive effect on grain size (*QYld.aww-2B*, *QYld.aww-7A-1* and *QYld.aww-7A-2*). These represent the most interesting options for exploitation in breeding through MAS. In particular, the RAC875 allele at *QYld.aww-7A-2* offers improvements in yield without the pleiotropic effect on EET. *QYld.aww-7A-2* was also co-located with a QTL accounting for a relatively large percentage of genetic variance for spikelet fertility (KPSL) and therefore associated with an increase in not only yield in a hotter environment (ENV2-hot), but also KPS, KPM^2 and harvest index. Previous studies have identified QTL in this region influencing these traits (Kumar et al. 2007; McIntyre et al. 2010) and one suggestive MET QTL for yield (Zhang et al. 2010) but this is otherwise the first report of a significant yield QTL in this region. One further locus on chromosome 1B was distinct from a yield and yield-related QTL previously reported by Kuchel et al. (2007), Marza et al. (2006) and Mason et al. (2010). This locus, as well as *QYld.aww-4D* were detected in the same ENVs, where heat stress was a differentiating factor, indicating that these may be useful loci for improving heat stress tolerance in the southern Australian environment and this warrants further validation.

The *QYld.aww-2D-2* loci were detected independent of the near by *Ppd-D1* locus, although the allele from RAC875 resulted in a lower grain yield relative to the Kukri allele and at the *Ppd-D1* locus, a later EET. However, the separation of these two loci by approximately 40 cM indicates the presence of two independent loci, most likely the same locus previously detected for grain yield by

Kumar et al. (2007) and Verma et al. (2004), as well as Marza et al. (2006) for KPS and the yield MET QTL detected by Zhang et al. (2010). While genetic loci influencing grain yield have been reported in similar regions on chromosome 1A (Kumar et al. 2007) and 4D (Huang et al. 2006; Kuchel et al. 2007), there have only been reports of loci for grain fill duration under heat stress (Mason et al. 2010), TKW (McCartney et al. 2005), canopy temperature (G. Rebetzke, personal communication) and possibly carbon isotope discrimination (Rebetzke et al. 2008a) in a similar region to *QYld.aww-6D*. It is possible that the environments used in the former two studies were not water-limited and as such, the locus did not have a significant effect on grain yield. Referring to the consensus map for wheat (Somers et al. 2004), the yield QTL in the present study was determined to be different to a number of other loci previously identified on chromosome 6D (Kuchel et al. 2007; McIntyre et al. 2010) and therefore appears to be a novel yield QTL.

The detection of numerous QTL for TKW within the RAC875/Kukri DH population was expected given the high heritability of the trait and the results of previous authors (Cuthbert et al. 2008; Groos et al. 2003; Huang et al. 2006; Pinto et al. 2010). The first of two loci being detected with large effect, *QTkw.aww-6A*, appears to have been detected for TKW in previous studies (Groos et al. 2003; McIntyre et al. 2010; Sun et al. 2010) and there have been previous reports of QTL for plant height (Maccaferri et al. 2008; Marza et al. 2006) and EET (Maccaferri et al. 2008; Peleg et al. 2009) located on the long arm of chromosome 2B, the region of the second QTL. However, in the present study, no QTL were detected in this region for these traits and there did not appear to be any previous reports of grain size QTL here. Interestingly, the RAC875 allele at *QTkw.aww-2B* was also associated with fewer tillers square metre, which was also observed at *QTkw.aww-1D*. Dreccer et al. (2009) identified higher levels of WSC in lines with fewer tillers, as was the case for a QTL detected on chromosome 6A in the present study and this may partially explain this observation.

Consistent with previous studies, genetic loci associated with TWT were largely independent of yield and yield components (Groos et al. 2003). While these studies have detected QTL for TWT, none have detected QTL in a similar region to *QTwt.aww-1D* or *QTwt.aww-3A*. Loci on 2A, 4A and 6A appear to have been previously reported (Sun et al. 2009, 2010). As identified by numerous authors (Huang et al. 2006; McCartney et al. 2005; Sun et al. 2009), a number of loci influencing TWT also increased TKW. In the present study, the same allele increased both TWT and TKW, and the locus conferring this (*QTwt.aww-6A*) represents an opportunity to improve both traits through MAS without any negative side effects.

WSC has previously been shown to be a useful source of assimilates, particularly for grain fill in water-limited and heat-stressed environments (Rattey et al. 2009; Reynolds and Condon 2007; Yang et al. 2007) and this was the case in the present study. The RAC875 allele at *QWsc-t.aww-3A* (as well as *QWsc-c.aww-3A* and *QWsc-a.aww-3A*) increased the level of WSC and TWT and led to lower levels of screenings in ENV clusters of more water-limited sites. However, this locus also affected plant height and it is possible that this pleiotropic effect accounts for the increased WSC levels, as observed by Rebetzke et al. (2008b) and Yang et al. (2007). Interestingly, the RAC875 allele at this locus resulted in a greater plant height overall, but a shorter peduncle length, which was unexpected given a greater peduncle length has been demonstrated to supply a greater relative proportion of WSC (Ehdaie et al. 2006; Wardlaw and Willenbrink 2000).

Influence of EET loci and effect of adjustment of data for its pleiotropic effects

EET controls a large proportion of adaptation in southern Australia (Richards 1991) and the major genes deployed in locally adapted germplasm are well understood by breeders. This study therefore aimed to identify genomic regions associated with greater relative yield in water-limited environments, independent of loci for EET. As such, a combination of approaches were utilised to minimise the effect of EET and increase the ability to detect novel loci for grain yield and grain yield components. These included reducing the size of the population by removing phenologically extreme individuals from experiments and adjustment of data for phenology (through either days to ear emergence or Zadoks growth score), both of which have previously been discussed and shown to be successful strategies for minimising the impact of EET on QTL detection in this population (Bennett et al. 2011; Reynolds et al. 2009). The adjustment of data in such a manner assumes that the influence of all genetic loci for ear emergence has the same effect on a given trait, i.e. later flowering has a negative effect on, say, grain yield or kernel weight. While this was the case at most loci, some EET loci had the reverse effect to what was observed for other EET loci and further investigation is required to establish whether this is a pleiotropic effect or caused by a closely linked gene.

It must also be acknowledged that the adjustment of data for EET cannot account for the fact that later flowering lines experienced stronger drought at a different growth stage than earlier flowering lines. The result of this may be that we failed to detect QTL of more minor effect or located close to these loci but the high number of experiments that the population was grown in should have permitted an environment where such QTL could have been

detected. We would also expect that treating experiments of relatively high genetic correlation as ENV clusters improves the ability to identify any loci of minor effect, as identified by Schon et al. (2004), due to increased phenotypic repetition at any given allele.

The influence of loci previously identified for flag leaf glaucousness

Previous reports on the effects of flag leaf glaucousness on cereal grain yield (Gonzalez and Ayerbe 2010; Johnson et al. 1983; Richards et al. 1986) and the large level of variation for this trait in the DH population (Bennett et al. 2011) suggested that leaf glaucousness could be having a large effect on grain yield in the present study. *QW.aww-3A* accounted for a large percentage of variation for glaucousness, but in the present study, failed to exert influence on any other traits to a level where even a suggestive QTL could be detected. Further to this, where the numerous other flag leaf glaucousness loci were segregating within the population and found to co-locate with other grain yield-related traits, any advantage that the glaucousness allele offered was uncertain. The two exceptions to this were *QW.aww-3B* and *QW.aww-6A*, where the RAC875 allele increased TKW and glaucousness and resulted in a lower level of SCR. However, given the 6A locus accounted for a large percentage of genotypic and phenotypic variation for FLW, it is possible that the increased leaf area unintentionally resulted in lines with greater leaf area being assigned a greater relative glaucousness score.

One possible explanation for the lack of relationship between glaucousness and grain yield and yield components is that expression of this trait may have exceeded the advantageous levels identified by Johnson et al. (1983) and Richards et al. (1986). Indeed, the highest level of glaucousness identified by these authors was described as covering a majority of the abaxial flag leaf surface, whereas Kukri often expressed a level of glaucousness extending to a small part of the adaxial flag leaf surface and RAC875 an even larger proportion of the adaxial surface. Clarke et al. (1993) found that in a number of segregating populations, lines with a low glaucousness score had significantly lower epicuticular wax content than those in mid and high glaucousness categories. However, the latter two categories did not display a significant difference in physical epicuticular wax content (i.e. higher glaucousness lines did not necessarily have greater physical wax content), which suggests that above a certain level, greater leaf glaucousness no longer correlates with physical wax content. This means that lines having assigned a greater glaucousness score may actually not have greater wax content; future investigations should aim to measure the level of epicuticular wax present to test this hypothesis.

Conclusion

The present study investigated the genetic basis of bread wheat production in southern Australia, using a cross between two locally adapted lines, with RAC875 displaying superior grain yield in drier climates. Across the 16 site-by-year combinations, three distinct seasonal conditions were apparent and temperatures during grain fill had a significant effect on grain yield. KPM^2 was correlated with grain yield, KPSL and KPS, while TKW was negatively associated with these but showed a neutral effect on yield, clearly indicating increases in KPM^2 may improve grain yield in the target environment. While many loci for these traits identified in the present study had previously been reported, their occurrences in the local germplasm pool is now better understood and this knowledge could be utilised by breeders for maintaining or improving these traits through strategic cross design. Three novel genomic regions associated with grain yield (*QYld.aww-1B*, *QYld.aww-6D* and *QYld.aww-7A-2*) were partially dissected, which will improve breeders' confidence in their value, potentially aiding in more rapid adoption for MAS in breeding programs targeting southern Australia and other similar climates. Further to this, a novel locus for test weight (*QTwt.aww-6A*) was identified, where the RAC875 allele also increased TKW and this could be exploited to improve both traits concurrently.

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