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Use of a large multiparent wheat mapping population in genomic dissection of coleoptile and seedling growth

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Abstract

Identification of alleles towards the selection for improved seedling vigour is a key objective of many wheat breeding programmes. A multiparent advanced generation intercross (MAGIC) population developed from four commercial spring wheat cultivars (cvv. Baxter, Chara, Westonia and Yitpi) and containing ca. 1000 F2-derived, F6:7 RILs was assessed at two contrasting soil temperatures (12 and 20 °C) for shoot length and coleoptile characteristics length and thickness. Narrow-sense heritabilities were high for coleoptile and shoot length (h2 = 0.68-0.70), indicating a strong genetic basis for the differences among progeny. Genotypic variation was large, and distributions of genotype means were approximately Gaussian with evidence for transgressive segregation for all traits. A number of significant QTL were identified for all early growth traits, and these were commonly repeatable across the different soil temperatures. The largest negative effects on coleoptile lengths were associated with Rht-B1b (-8.2%) and Rht-D1b (-10.9%) dwarfing genes varying in the population. Reduction in coleoptile length with either gene was particularly large at the warmer soil temperature. Other large QTL for coleoptile length were identified on chromosomes 1A, 2B, 4A, 5A and 6B, but these were relatively smaller than allelic effects at the Rht-B1 and Rht-D1 loci. A large coleoptile length effect allele (a = 5.3 mm at 12 °C) was identified on chromosome 1AS despite the relatively shorter coleoptile length of the donor Yitpi. Strong, positive genetic correlations for coleoptile and shoot lengths (rg = 0.85-0.90) support the co-location of QTL for these traits and suggest a common physiological basis for both. The multiparent population has enabled the identification of promising shoot and coleoptile QTL despite the potential for the confounding of large effect dwarfing gene alleles present in the commercial parents. The incidence of these alleles in commercial wheat breeding programmes should facilitate their ready implementation in selection of varieties with improved establishment and early growth.

Use of a large multiparent wheat mapping population in genomic dissection of coleoptile and seedling growth

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Summary

Identification of alleles towards the selection for improved seedling vigour is a key objective of many wheat breeding programmes. A multiparent advanced generation intercross (MAGIC) population developed from four commercial spring wheat cultivars (cvv. Baxter, Chara, Westonia and Yitpi) and containing ca. 1000 F_2-derived, $F_{6:7}$ RILs was assessed at two contrasting soil temperatures (12 and 20 °C) for shoot length and coleoptile characteristics length and thickness. Narrow-sense heritabilities were high for coleoptile and shoot length ($h^2 = 0.68$–0.70), indicating a strong genetic basis for the differences among progeny. Genotypic variation was large, and distributions of genotype means were approximately Gaussian with evidence for transgressive segregation for all traits. A number of significant QTL were identified for all early growth traits, and these were commonly repeatable across the different soil temperatures. The largest negative effects on coleoptile lengths were associated with Rht-B1b (-8.2%) and Rht-D1b (-10.9%) dwarfing genes varying in the population. Reduction in coleoptile length with either gene was particularly large at the warmer soil temperature. Other large QTL for coleoptile length were identified on chromosomes 1A, 2B, 4A, 5A and 6B, but these were relatively smaller than allelic effects at the Rht-B1 and Rht-D1 loci. A large coleoptile length effect allele ($a = 5.3$ mm at 12 °C) was identified on chromosome 1AS despite the relatively shorter coleoptile length of the donor Yitpi. Strong, positive genetic correlations for coleoptile and shoot lengths ($r_g = 0.85$–0.90) support the co-location of QTL for these traits and suggest a common physiological basis for both. The multiparent population has enabled the identification of promising shoot and coleoptile QTL despite the potential for the confounding of large effect dwarfing gene alleles present in the commercial parents. The incidence of these alleles in commercial wheat breeding programmes should facilitate their ready implementation in selection of varieties with improved establishment and early growth.

Introduction

Establishment is a key phase in the development of high-yielding cereal crops. Optimal plant stands and early leaf area development rely on a seedling's ability to elongate and emerge through soil before commencing photosynthesis and the accumulation of biomass. Yet, conditions for early growth and emergence are commonly challenging with growers sometimes re-sowing following the establishment failures. Moisture necessary for germination and growth is often deep in the soil profile while some soils may crust to restrict emergence. Crops are commonly sown at depth to access moisture deep in the soil profile, to avoid predators, or accidently where sowing is rushed or equipment is not calibrated correctly (Rebetzke et al., 2007a).

The length of the coleoptile (a sheathlike structure that permits the delivery of the elongating stem and first seedling leaves to the soil surface) determines the maximum depth at which seed can be sown. Sowing depths exceeding the length of the coleoptile limit the seedling's ability to promote the first leaf through the soil surface. Short coleoptiles are a problem in many cereals including barley, rice and wheat and commonly result in poor establishment

and/or late emergence and slow early leaf area development (Cairns et al., 2009; Huang and Taylor, 1993; Rebetzke et al., 2007a; Takahashi et al., 2001; Whan, 1976). Long coleoptiles would enable growers to sow into moisture deep in the soil profile (Rebetzke et al., 2007a; Schillinger et al., 1998), improve establishment when sowing into stubble (Rebetzke et al., 2005), or deep sown to avoid seed predation (Brown et al., 2003). Deep sowing also benefits where high soil temperatures can dry the soil surface to increase seedling mortality (Mahdi et al., 1998) or reduce coleoptile extension to lower seedling establishment (Glover et al., 2008).

Large genotypic differences in seedling emergence indicate the potential for genetic improvement of wheat establishment through selection for greater coleoptile length (Rebetzke et al., 2007a; Schillinger et al., 1998; Whan, 1976). Inheritance studies have reported coleoptile length to be under the complex control of many genes (Murphy et al., 2008; Rebetzke et al., 1999, 2007b). Mapping studies have confirmed the earlier reports (Allan, 1989) of shorter coleoptile and subcrown internode lengths associated with the presence of the Rht-B1b (Rht1) and Rht-D1b (Rht2) dwarfing genes (Rebetzke et al., 2007b; Yu and Bai, 2010). The Rht-B1b and Rht-D1b genes are associated with

reduced cell elongation to decrease cell size and shoot lengths (Botwright *et al.*, 2001, 2005).

Rapid leaf area development is an essential requisite in achieving a good stand and large canopy early in the season. Greater leaf size (or early vigour) affects performance in many ways. Firstly, shading the soil surface reduces water loss through soil evaporation to increase water-use efficiency (López-Castañeda and Richards, 1994); a more rapid crop growth increases a crop's capacity to compete with weeds (Coleman *et al.*, 2001); and early nutrient uptake is closely linked to more rapid seedling growth (Liao *et al.*, 2004). Genetic variation for the early vigour in wheat is large and repeatable (Rebetzke and Richards, 1999), and under genetic control of many alleles (e.g. Landjeva *et al.*, 2008; Rebetzke *et al.*, 2001; ter Steege *et al.*, 2005). The gibberellic acid-insensitive (GAI) *Rht-B1b* and *Rht-D1b* dwarfing genes decrease cell elongation to reduce leaf length and shoot biomass (Botwright *et al.*, 2005; Ellis *et al.*, 2004).

Complex, polygenic inheritance reported for coleoptile length and seedling leaf area indicates variation across multiple loci in phenotyped populations. Indeed, separate mapping studies have identified multiple independent alleles with the potential for marker-aided selection of increased coleoptile length (e.g. Rebetzke *et al.*, 2001, 2007b; Spielmeyer *et al.*, 2007; Yu and Bai, 2010). However, these studies were based on populations commonly developed for genetic and QTL mapping studies, and the genomic regions and alleles not directly relevant for use in commercial breeding programmes. Further, population sizes were small (<200 lines), and both numbers of loci and estimates of genetic effects biased to reduce confidence in detection (Vales *et al.*, 2005). Multiparent advanced generation intercross (MAGIC) populations were proposed to increase the number of alleles being sampled within the same population and to provide higher resolution for genetic mapping due to increased recombination and a larger population size (Huang *et al.*, 2012).

The identification of genomic regions contributing to greater establishment and early growth is of considerable value to breeding programmes. This study aims to identify QTL for seedling shoot and coleoptile growth and investigate their genetic relationship at multiple soil temperatures in a large MAGIC population developed from commercially relevant wheat parents.

Results

The influence of soil temperature was large on all aspects of coleoptile and shoot growth (Table 1). Mean progeny dimensions at 12 and 20 °C were as follows: coleoptile length—105 and 87 mm; shoot length—280 and 257 mm; coleoptile width—1.95 and 2.03; and coleoptile thickness—1.09 and 1.14 mm. All mean differences were statistically significant ($P < 0.01$). Mean coleoptile and shoot length were significantly greater (c. 20 and 10%, respectively) at the cooler 12 °C temperature while coleoptiles were typically thicker and had greater width with increased soil temperature.

Parents (founders) varied for all characters both within and across temperatures (Table 1). Among-parent differences in coleoptile length were statistically significant ($P < 0.05$) but not large (e.g. 11% change at 12 °C) compared with differences in shoot length (e.g. 27% at 12 °C). Variation in coleoptile width (e.g. 15% at 12 °C) and thickness (e.g. 6% at 12 °C) was also not large across parent means. By contrast, progeny varied widely for all coleoptile growth parameters including coleoptile width (squash) (Table 1 and Figures 1 and 2). The range for coleoptile length was from 32 to 174 mm and from 25 to 170 mm at 12 and 20 °C, respectively. The large range (and variance) was not consistent with the small differences observed in parental means. Distributions of progeny means were continuous and approximately normal for coleoptile and shoot lengths (Figures 1 and 3) and for coleoptile size characters' width (Figure 2) and thickness (data not shown). The large range among progeny for all traits exceeded the variation encompassed by the parents (Table 1), indicative of transgressive segregation. Together, these data suggest that more than one gene is likely affecting the genic expression of different coleoptile and shoot growth traits. Heritabilities were largest for length-associated parameters, coleoptile and shoot lengths (Table 1), and were similar in size for each of the two soil temperatures. Of the coleoptile size-related parameters, coleoptile width was the most repeatable, particularly at the warmer soil temperature. The coleoptile width (squash) parameter was only assessed at the cooler soil temperature and was small in heritability (18%).

Genotypic relationships were obtained between shoot and coleoptile characteristics measured in the MAGIC population

Table 1 Parent means and ranges of progeny for coleoptile and shoot growth characteristics at two soil temperatures

Character	Temperature	Parents				Progeny				
		Baxter	Chara	Westonia	Yitpi	Mean	Range	SD*	$h^{2\dagger}$	SED‡
Coleoptile length (mm)	12	95.0	98.8	104.3	107.4	105	32–174	20.5	70	18.8
	20	75.3	63.8	79.7	80.1	87	25–170	21.1	68	11.5
Coleoptile width (mm)	12	1.80	2.11	2.09	2.07	1.95	0.90–2.90	0.21	28	0.21
	20	1.94	2.19	1.98	2.20	2.03	0.21–2.82	0.22	48	0.17
Coleoptile thickness (mm)	12	1.03	1.09	1.04	1.06	1.09	0.60–1.73	0.16	7	0.23
	20	1.07	1.20	1.25	1.20	1.14	0.60–2.00	0.17	17	0.20
Coleoptile width (squash) (mm)	12	0.63	0.59	0.52	0.58	0.65	0.20–1.24	0.19	18	0.28
Shoot length (mm)	12	236.2	264.2	287.0	320.0	280	66–415	45.2	68	27.3
	20	215.3	210.6	247.2	277.9	257	70–415	56.8	68	34.2

*Standard deviation.

†Narrow-sense heritability.

‡Average standard error of a difference for comparisons between progeny and parent means.
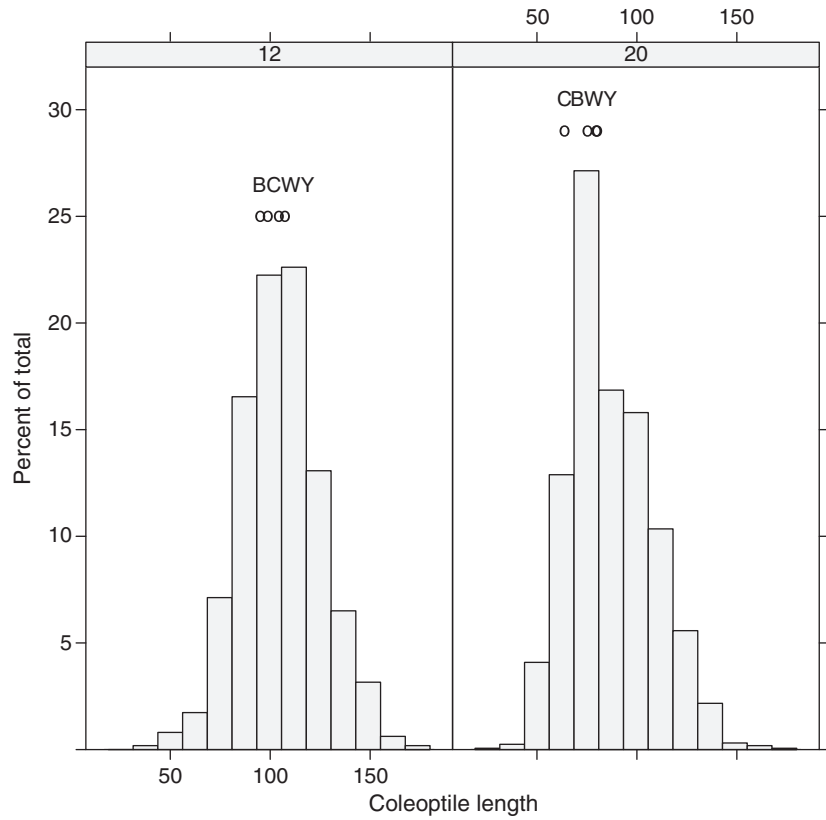
Figure 1 Frequency distributions for coleoptile length, in mm, measured on random inbred lines evaluated at 12 and 20 °C. Founder (parent) means are indicated with arrows (B = Baxter, C = Chara, W = Westonia and Y = Yitpi).
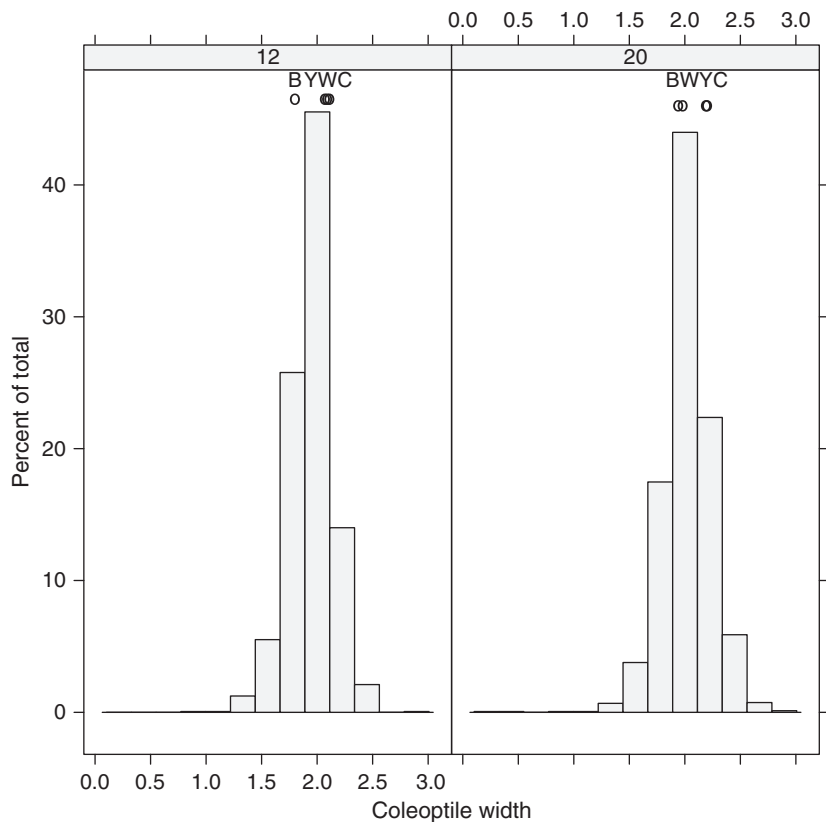
Figure 2 Frequency distributions for coleoptile width, in mm, measured on random inbred lines evaluated at 12 and 20 °C. Founder (parent) means are indicated with arrows (B = Baxter, C = Chara, W = Westonia and Y = Yitpi).

(Table 2). Across all lines, coleoptile (r_g = 0.986) and shoot (r_g = 0.973) lengths, coleoptile width (r_g = 0.886) and thickness (r_g = 0.919) were strongly, positively correlated across the two

soil temperatures. Genotypic relationships were also given for pairs of traits (Table 2). Among traits, coleoptile width and thickness were moderately correlated (r_g = 0.691 at 20 °C to

Figure 3 Frequency distributions for shoot length, in mm, measured on random inbred lines evaluated at 12 and 20 °C. Founder (parent) means are indicated with arrows (B = Baxter, C = Chara, W = Westonia and Y = Yitpi).
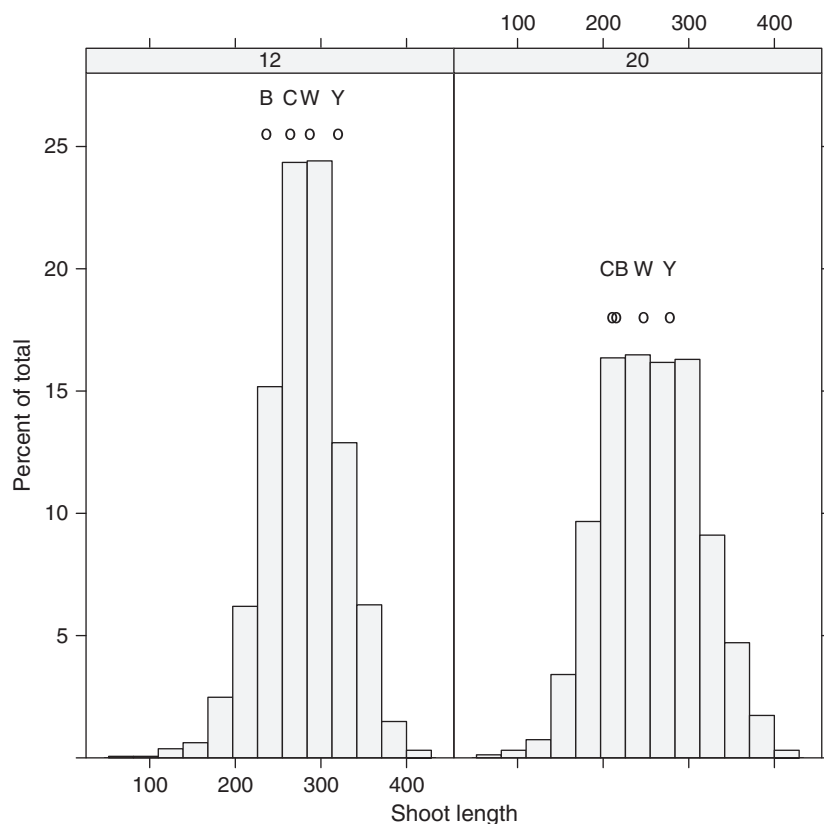
Table 2 Table of correlations (genetic upper diagonal (shaded), residual lower diagonal) for coleoptile and shoot characters measured at two soil temperatures on progeny in the MAGIC four-way population. Unless indicated as 'ns' all genetic correlations were significantly different from zero

Character	Coleoptile length 12 °C	Coleoptile length 20 °C	Coleoptile thickness 12 °C	Coleoptile thickness 20 °C	Coleoptile width 12 °C	Coleoptile width 20 °C	Coleoptile width (squash) 12 °C	Shoot length 12 °C	Shoot length 20 °C
Coleoptile length 12 °C	–	0.986	−0.131	−0.466	−0.020 ns	−0.212	−0.161	0.849	0.947
Coleoptile length 20 °C	0.084	–	−0.080	−0.407	−0.006 ns	−0.211	−0.071 ns	0.772	0.899
Coleoptile thick 12 °C	0.188	−0.039	–	0.886	0.904	0.721	0.965	0.094	0.030 ns
Coleoptile thick 20 °C	0.022	−0.060	0.018	–	0.766	0.691	0.882	−0.258	−0.332
Coleoptile width 12 °C	0.194	−0.025	0.140	0.006	–	0.919	0.796	0.256	0.169
Coleoptile width 20 °C	0.158	0.050	−0.044	0.139	−0.106	–	0.605	0.046 ns	−0.050 ns
Col. width (squash) 12 °C	0.112	0.001	0.373	−0.009	0.043	−0.030	–	0.053 ns	−0.009 ns
Shoot length 12 °C	0.679	0.138	0.068	0.001	0.130	0.003	−0.059	–	0.973
Shoot length 20 °C	0.017	0.754	−0.063	−0.100	−0.036	−0.027	−0.072	0.095	–

0.904 at 12 °C) and closely related to coleoptile width with squashing (r_g = 0.605 to 0.965). Coleoptile and shoot lengths were strongly correlated across temperatures (r_g = 0.849 at 12 °C to 0.899 at 20 °C), and coleoptile length weakly correlated with coleoptile thickness at 12 °C (−0.131) and 20 °C (−0.407) and coleoptile width at 12 °C (−0.020) and 20 °C (−0.211). Similarly, genotypic variation in shoot length was weakly correlated with coleoptile width and thickness (Table 2). Importantly, associations between traits were robust across temperatures. For example, coleoptile length was correlated with reduced coleoptile width and increased shoot length at both soil temperatures (Table 2). Table 2 shows that most of the correlations of residuals

across traits are close to zero, with the exception of coleoptile length and shoot length for the two temperatures.

A comprehensive, SNP-based molecular map utilizing multiple founders was developed and is reported for the four-way MAGIC population in Huang *et al.* (2012). Using this map (including known gene markers as described in the materials and methods section) together with the R package, WGAIM which detects and models multiple QTL simultaneously, numerous QTL were identified for the different coleoptile growth characteristics (Tables 3–6). The largest genetic effects on coleoptile length were associated with QTL on chromosomes 4BS and 4DS. Marker resolution was sufficient to establish the location of the chromosome 4BS

QTL in proximity to the dwarfing gene *Rht-B1b* (*wsnp_Ex_c14026_21924297*), whereas the chromosome 4DS QTL mapped directly to the *Rht-D1* SNP. Genetic effects within each dwarfing locus were not consistent with the reduction in coleoptile length being greater at *Rht-B1* for Baxter (cf. −5.49 and −7.96 mm) and *Rht-D1* for Yitpi (cf. −7.48 and −9.47 mm) at 12 °C soil temperature (Table 3). The relative difference at the *Rht-B1* locus was maintained at the warmer temperature, whereas the effect of the Westonia *Rht-B1b* allele was stronger at 20 °C. Across the founders, the Yitpi and Westonia *Rht-D1b* alleles reduced coleoptile length by 8.1 and 13.8% at 12 and 20 °C, respectively, whereas the Baxter and Chara *Rht-B1b* alleles reduced coleoptile length by 6.4 and 10.0% for the same temperatures. The *Rht-D1b* dwarfing allele appears to have a stronger effect in reducing coleoptile length particularly at warmer soil temperatures. Together, lines containing both *Rht-B1b* and *Rht-D1b* dwarfing genes reduce the potential coleoptile length by an average of 15 (12 °C) and 24 (20 °C) percentage when compared with tall, nondwarfing gene containing lines.

In addition to the known effects of the *Rht-B1* and *Rht-D1* dwarfing genes, up to 20 other significant QTL were identified for coleoptile length (Table 3). Many were repeatable at both temperatures and included QTL of large genetic effect. For example, at 12 °C, the Yitpi allele on chromosome 1AS has an effect of 6.1 mm (Table 3). Other moderate-to-large effect coleoptile length QTL (with founders) were identified on chromosomes 1AS (Baxter), 2BS (Baxter), 2DL (Chara), 3AS (Baxter), 3D_ (Chara), 4AS (Chara), 5BL (Baxter and Chara), 6AS (Baxter)

Table 3 QTL location and genetic effects for coleoptile length (in mm) at 12 and 20 °C soil temperatures (shaded cells are 12 °C coleoptile length QTL also significant at 20 °C). The indicated chromosome-arm designations are hypothetical, based on the available evidence at the time of writing this article, and some may be subject to revision in the future

Chromosome	Molecular marker	Distance (cM)	LOD	Founder effects (mm)			
				Yitpi	Chara	Baxter	Westonia
12 °C soil temperature							
1AS*	*wsnp_Ex_c3253_5995011*	106.9	7.13	6.06	−0.71	−3.47	−1.89
1BS	*wsnp_Ku_c207_407862*	66.2	1.78	−1.39	−1.17	2.43	0.13
1BL	*wsnp_CAP11_c2596_1325540*	133.9	1.67	−0.76	1.03	1.78	−2.05
2BS*	*wsnp_Ex_c14760_22866930*	102.2	3.71	2.04	0.04	−2.82	0.75
2DL	*wsnp_Ra_c17636_26538543*	249.1	2.62	1.07	3.01	−1.39	−2.70
3AS	*wsnp_Ku_c38911_47455924*	60.2	2.36	0.05	−1.04	2.77	−1.78
3D2	*wsnp_Ra_c17636_26538543*	19.8	2.62	1.07	3.01	−1.39	−2.70
4AS	*wsnp_Ex_c13615_21393511*	78.6	4.87	0.32	−3.94	1.35	2.27
4BS*	*wsnp_Ex_c14026_21924297*	47.9	41.66	6.88	−5.49	−7.96	6.57
4DS*	Rht-D1 (Rht2)	9.8	38.92	−9.47	8.71	8.24	−7.48
4DS*	*wsnp_Ex_c683_1341113*	27.7	3.69	0.14	1.11	0.36	−1.61
5BL*	*wsnp_Ku_c12562_20256747*	132.6	3.16	1.79	1.24	−3.21	0.18
5BL	*wsnp_Ex_c214_421541*	191.1	2.32	−2.14	2.79	−1.13	0.47
5DS	*wsnp_Ku_c55961_59662821*	8.2	1.94	−0.35	2.23	0.53	−2.41
6AS*	*wsnp_RFL_Contig2182_1514692*	89.7	2.67	0.88	1.84	−3.59	0.88
6BL	*wsnp_Ex_rep_c70767_69655253*	143.7	4.91	2.94	0.37	0.44	−3.75
7AS	*wsnp_Ex_c61603_61581218*	28.0	1.97	0.34	−1.35	−1.24	2.24
20 °C soil temperature							
1AS*	*wsnp_Ex_c12117_19381493*	109.9	5.83	5.47	−2.14	−2.57	−0.76
1BS	*wsnp_Ex_c13310_20984763*	50.6	1.68	−0.11	0.35	1.31	−1.55
1DL	*wsnp_Ex_c8188_13842273*	189.4	1.85	2.39	−0.37	−1.06	−0.96
2BS	*wsnp_Ex_c30447_39360584*	88.5	3.62	4.33	−1.54	−0.96	−1.83
2DS	*wsnp_CAP7_c2782_1329707*	68.7	1.86	−2.90	−0.80	4.23	−0.53
3AS	*wsnp_Ku_c38911_47455924*	60.2	3.78	0.29	−1.71	2.45	−1.03
3AL	*wsnp_Ex_rep_c102478_87635370*	108.6	2.23	0.08	0.56	1.53	−2.17
3BL*	*wsnp_Ex_rep_c66380_64573939*	192.3	2.43	−0.25	−2.86	−0.58	3.68
4AS	*wsnp_Ex_rep_c69093_68002098*	73.1	2.24	−0.64	−2.01	0.16	2.49
4BS*	*wsnp_Ex_c14026_21924297*	47.9	72.73	9.65	−8.16	−9.31	7.82
4DS*	Rht-D1 (Rht2)	9.8	76.97	−11.20	12.10	11.94	−12.84
4DS*	*wsnp_Ex_c683_1341113*	27.7	11.73	−1.17	0.50	1.18	−0.52
5BL*	*wsnp_Ku_c6464_11320381*	157.7	2.57	0.45	3.52	−3.21	−0.76
6AS*	*wsnp_Ex_rep_c70951_69806211*	96.8	2.93	2.24	0.88	−3.30	0.17
6BL	*wsnp_Ex_rep_c71537_70252046*	141.1	2.33	2.32	2.51	−2.09	−2.74
7AL*	*wsnp_Ex_c42653_49180485*	132.2	1.94	0.20	2.31	−2.34	−0.18
7AL*	*wsnp_Ex_c43009_49439922*	194.9	1.65	1.90	−0.84	−1.81	0.75
7BS	*wsnp_Ex_c323_629581*	34.5	3.07	1.41	1.38	0.90	−3.69

*Significant QTL for shoot length at 20 °C.

Table 4 QTL location and genetic effects for total shoot length (in mm) at 12 and 20 °C soil temperatures (shaded cells are 12 °C shoot length QTL also significant at 20 °C). The indicated chromosome-arm designations are hypothetical, based on the available evidence at the time of writing this article, and some may be subject to revision in the future

Chromosome	Molecular marker	Distance (cM)	LOD	Founder effects (mm)			
				Yitpi	Chara	Baxter	Westonia
12 °C soil temperature							
1AS	wsnp_Ex_c3253_5995011	106.9	4.12	10.11	−2.61	−4.78	−2.73
2BS	wsnp_Ex_c14760_22866930	102.2	1.39	0.72	−2.49	−5.62	7.38
2BL	stm773	116.6	7.60	12.27	−2.27	−11.27	1.28
2BL	wsnp_CAP11_rep_c9018_3888047	134.3	1.94	−6.77	8.59	−5.42	3.60
2DS	wsnp_Ex_c2251_4218338	46.5	1.98	−6.49	5.50	0.56	0.42
4BS	wsnp_Ex_c14026_21924297	47.9	22.72	11.06	−11.27	−11.95	12.17
4DS	Rht-D1 (Rht2)	9.8	38.98	−22.00	15.70	23.63	−17.34
4DS	wsnp_Ku_c9140_15390166	28.2	5.38	0.14	3.41	−0.24	−3.31
5AS	wsnp_Ex_rep_c109532_92292121	67.3	2.49	8.33	0.88	−7.35	−1.86
5AL	wsnp_Ex_rep_c68829_67704044	149.7	2.09	−1.13	6.08	−3.20	−1.75
5BL	wsnp_Ku_c10296_17072695	131.6	1.74	3.73	−0.35	−3.23	−0.16
5BL	wsnp_JD_c11594_12033647	145.2	2.10	8.51	1.93	−5.08	−5.35
5BL	wsnp_Ex_rep_c67471_66073729	189.6	2.04	−5.55	6.38	−2.81	1.98
6AS	wsnp_Ku_c38451_47086066	78.5	2.43	7.13	−1.22	−7.26	1.35
6AS	wsnp_Ku_c37942_46693718	89.2	3.81	0.80	4.54	−6.86	1.52
6AL	wsnp_Ex_c965_1846161	105.0	1.63	−0.29	2.41	0.49	−2.60
7DL	wsnp_Ra_c8297_14095831	113.8	2.44	−5.08	3.49	2.84	−1.25
20 °C soil temperature							
1AS	wsnp_Ex_c2749_5091813	105.9	3.33	10.52	−3.13	−3.51	−3.88
2BL	stm773	116.6	3.67	10.95	−7.57	−7.39	4.01
2BL	wsnp_JD_c1472_2090800	127.3	9.04	2.91	5.82	−14.54	5.82
3BL	wsnp_BE444579B_Ta_2_2	188.7	10.64	4.15	−15.98	4.37	7.46
4BS	wsnp_Ex_c14026_21924297	47.9	46.83	22.46	−19.17	−19.48	16.19
4DS	Rht-D1 (Rht2)	9.8	94.57	−32.41	32.22	38.10	−37.90
4DS	wsnp_Ex_c683_1341113	27.7	11.01	−2.26	1.31	2.54	−1.60
5BL	wsnp_JD_c11594_12033647	145.2	5.06	12.32	−11.03	−1.22	−0.07
5BL	wsnp_Ex_c6100_10676217	158.7	2.19	−4.10	14.83	−6.98	−3.76
5BL	wsnp_BF201102B_Ta_2_5	214.4	3.30	−3.73	4.16	3.58	−4.01
6AL	wsnp_Ex_rep_c70951_69806211	96.8	2.83	6.79	3.04	−8.02	−1.81
7AL	wsnp_Ra_rep_c105976_89839782	111.8	2.39	−3.77	2.53	−5.42	6.66
7AL	wsnp_Ex_c43009_49439922	194.9	4.31	5.55	−8.12	−3.30	5.87

and 6BL (Westonia). Similar-sized founder effects were observed at 20 °C with the exception of a larger Yitpi allele effect on chromosome 2BS and a large effect Westonia allele on chromosome 7BS (Table 3). Despite the modest-sized effects estimated at many loci, when combined, these QTL can together potentially produce superior genotypes, with predictably up to 70 mm greater coleoptile length. The linked *Rht-D1b* 4DS coleoptile length QTL (e.g. *wsnp_Ex_c683_1341113*) is likely to be a residual *Rht-D1b* allelic effect. Figure 4a summarizes the relationship for the genetic effects at 12 coleoptile length QTL (and their four founder effects) common at both 12 and 20 °C soil temperatures. The figure shows that the ranking and relative sizes of the effects are strongly maintained ($r = 0.96$, $P < 0.01$) across these two contrasting temperatures consistent with the large genetic correlation reported for coleoptile length measured on all genotypes at 12 and 20 °C (Table 2).

Up to 17 significant shoot length QTL were identified across the two soil temperatures (Table 4). Among the largest genetic effects were associated with genomic regions on the short arms of chromosomes 4B and 4D, the locations of the *Rht-B1b* and

Rht-D1b dwarfing genes. The Baxter and Chara 4BS alleles were associated with a reduction in shoot length of 4.3 and 4.1, and 7.6 and 7.5% at 12 and 20 °C, respectively. Similarly, Westonia and Yitpi 4DS alleles were associated with a 6.2 and 7.9, and 14.8 and 12.6% reduction in coleoptile length at 12 and 20 °C, respectively. Average reductions associated with *Rht-D1b* appear larger than for *Rht-B1b*, consistent with genetic effects observed for coleoptile length. Genetic effects at the 4BS QTL were relatively similar for Baxter and Chara dwarfing alleles, whereas the Westonia 4BS 'tall' allele was markedly reduced in shoot length compared with the Yitpi allele at 20 °C (Table 4). Similarly, the Baxter *Rht-D1a* tall allele was associated with greater shoot length than for the Chara tall allele at both soil temperatures. The Yitpi *Rht-D1b* dwarfing allele was associated with greater shoot length reduction at 12 °C, whereas the Westonia allele contributed to greater shoot reduction at 20 °C. Large effect shoot length alleles independent of *Rht-B1b* and *Rht-D1b* were observed on chromosomes 1AS (Yitpi), 2B (Westonia and Yitpi), 5A (Yitpi), 5A (Baxter and Yitpi), 5B (Chara and Yitpi) and 6A (Baxter and Yitpi). An additional large effect Chara allele was

Table 5 QTL location and genetic effects for coleoptile width (in mm) at 12 and 20 °C soil temperatures (shaded cells are width QTL significant at 20 °C). The indicated chromosome-arm designations are hypothetical, based on the available evidence at the time of writing this article, and some may be subject to revision in the future

Chromosome	Molecular marker	Distance (cM)	LOD	Founder effects (mm)			
				Yitpi	Chara	Baxter	Westonia
12 °C soil temperature							
1AS	wsnp_Ra_c11877_19161832	73.9	3.29	−0.01	0.02	0.02	−0.03
2BS*	wsnp_Ex_c14760_22866930	102.2	2.63	0.01	0.00	−0.03	0.03
2BS*	stm773	116.6	10.66	0.02	0.02	−0.06	0.02
2DS	wsnp_Ex_c2251_4218338	46.5	3.24	−0.03	0.03	0.00	0.00
4BS	wsnp_Ex_c18318_27140346	12.9	1.98	0.00	0.00	−0.02	0.03
5AL	wsnp_Ex_c3369_6192815	172.3	1.99	−0.01	−0.02	0.03	0.00
5BS	wsnp_Ex_c29304_38355434	75.9	3.08	0.00	0.02	0.00	−0.02
5BL	wsnp_Ku_c5308_9450093	218.5	2.32	−0.02	−0.01	0.03	0.00
6BL*	wsnp_Ex_rep_c71537_70252046	141.1	2.19	−0.01	−0.01	0.00	0.03
20 °C soil temperature							
2AS	wsnp_Ex_rep_c101866_87158671	114.3	2.32	−0.02	−0.01	0.01	0.02
2BS[†]	wsnp_Ex_c7285_12506938	66.5	1.58	−0.03	0.02	−0.02	0.02
2BS[†]	wsnp_Ex_c15301_23528979	102.7	1.69	0.01	0.02	−0.03	0.01
2BS	stm773	116.6	5.27	0.02	0.01	−0.04	0.02
3BS	wsnp_CAP11_c232_211960	21.2	3.03	0.01	−0.03	0.01	0.02
4AS[†]	wsnp_Ex_c7528_12868250	77.1	2.18	0.00	0.03	−0.01	−0.02
4BS[†]	wsnp_Ex_c14026_21924297	47.9	1.78	−0.01	0.03	0.01	−0.02
4DS[†]	Rht-D1 (Rht2)	9.8	5.56	0.02	−0.04	−0.02	0.03
5BL	vrnB	72.8	2.22	−0.01	0.03	−0.01	−0.01
5BL	wsnp_Ku_c10296_17072695	131.6	3.08	−0.01	−0.02	0.03	0.00
6AS	wsnp_Ku_rep_c68790_67934209	37.5	2.46	0.01	0.01	−0.03	0.01
7BS[†]	wsnp_Ex_c8400_14157060	39.6	2.81	0.03	−0.01	0.00	−0.02

*Significant QTL for coleoptile length at 12 °C.
[†]Significant QTL for coleoptile length at 20 °C.

Table 6 QTL location and genetic effects for coleoptile thickness and coleoptile width with squashing (in mm) at 12 °C soil temperature. The indicated chromosome-arm designations are hypothetical, based on the available evidence at the time of writing this article, and some may be subject to revision in the future

Chromosome	Molecular marker	Distance (cM)	LOD	Founder effects (mm)			
				Yitpi	Chara	Baxter	Westonia
Coleoptile thickness							
1BS*	wsnp_Ex_c2004_3770146	42.0	3.13	−0.02	0.02	0.02	−0.02
2AS	wsnp_Ra_c11564_18736249	110.3	2.65	−0.02	0.01	0.00	0.01
2BS*	wsnp_Ex_c27867_37030229	83.9	5.18	0.02	−0.01	−0.04	0.03
5AS	wsnp_Ex_c18107_26909127	39.9	3.54	0.00	−0.01	−0.01	0.03
6AS*	wsnp_BF483993A_Ta_2_1	69.9	4.90	0.03	−0.02	0.00	−0.01
Coleoptile width (squash)							
2BL*	wsnp_BE399688B_Ta_2_1	123.2	3.76	0.01	0.00	−0.02	0.01
5BL	wsnp_Ex_c9301_15450818	238.5	3.08	0.01	−0.02	−0.01	0.01
6AS*	wsnp_BF483993A_Ta_2_1	69.9	4.89	0.02	−0.01	−0.01	−0.01
7AS*	wsnp_Ex_c61603_61581218	28.0	2.61	0.00	−0.01	0.00	0.02

*Significant QTL for coleoptile length at 12 °C.

identified on chromosome 3BL at 20 °C (Table 4). As for coleoptile length, there was a strong association ($r^2 = 0.74$) for shoot length QTL effects across the two soil temperatures (Figure 4b).

Many of the shoot length QTL co-located with the coleoptile length QTL reported earlier (cf. Tables 3 and 4). For example, the size and magnitude of the alleles at the 1AS QTL for shoot length were consistent with those for coleoptile length including that of the large effect Yitpi allele. Of the 17 QTL for shoot length at 12 °C, nine mapped close to, or directly at QTL for coleoptile length, and of the 14 QTL for shoot length at 20 °C, nine mapped to coleoptile length QTL. The relationship for shoot and coleoptile
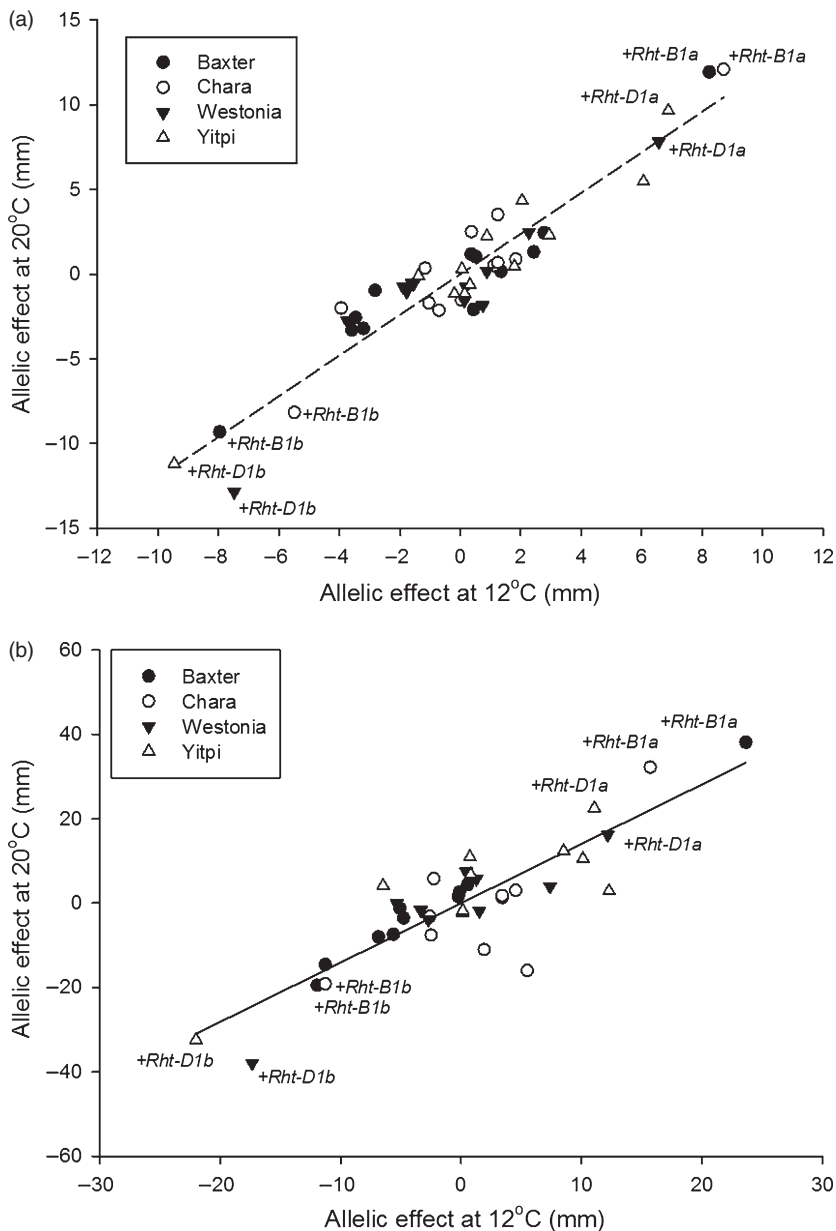
Figure 4 Relationship of (a) coleoptile length and (b) shoot length allelic effects for the four founder parents at 12 and 20 °C. Effects are obtained for 12 and 10 QTL common to the two soil temperatures for coleoptiles and shoot lengths, respectively. The dashed line represents the line of best fit (a: $y = 8.81 + 1.24 \ x$, $r^2 = 0.89$, $P < 0.01$; b: $y = 0.06 + 1.41 \ x$, $r^2 = 0.75$, $P < 0.01$).

genetic effects for 10 QTL common to both characteristics is demonstrated in Figure 5. Across all alleles, increases in shoot length at 12 °C were positively correlated ($r = 0.96$, $P < 0.01$) with increases in coleoptile length. The correlation was equally strong ($r = 0.95$, $P < 0.01$) at 20 °C. Both correlations were consistent with the strong, positive genotypic correlations reported for shoot and coleoptile lengths measured on all genotypes at 12 and 20 °C (Table 2). One interesting exception to the strong relationship of QTL for coleoptiles and shoot length was the QTL on 2B (near the *Sr36* introgression). At this QTL, the shoot length QTL contributed by Yitpi was large (~16 mm) while the coleoptiles length was moderate (2 mm) in size.

As for the previous traits, genetic influence of the coleoptile characteristics width and thickness was under control of many loci (Tables 5 and 6). Between 9 and 12 loci were identified for coleoptile width including two (chromosomes 2B and 5B) that were repeatable across soil temperatures. A particularly large chromosome 2B effect consistent with the Sr36 locus (Baxter) reduced the

coleoptile width at 12 and 20 °C and reduced coleoptile thickness and width with squashing (Table 6). Dwarfing gene alleles on chromosomes 4BS and 4DS had no effect on coleoptile width or thickness at 12 °C but increased coleoptile width at 20 °C (cf. Tables 5 and 6). The QTL for coleoptile thickness and to a lesser extent width with squashing were fewer in number and largely independent of QTL for coleoptile width (cf. Tables 5 and 6). The one exception was the co-location of width and thickness with width squashing QTL on chromosome 2B, where Baxter consistently transmitted an allele for reduced coleoptile size.

Discussion

Wheat is grown under a diverse range of agronomic and environmental conditions. Target environments will continue to challenge growers as the potential for increased climate variability places greater demand on the successful sowing, establishment and early growth of rainfed wheat crops (Glover et al., 2008).
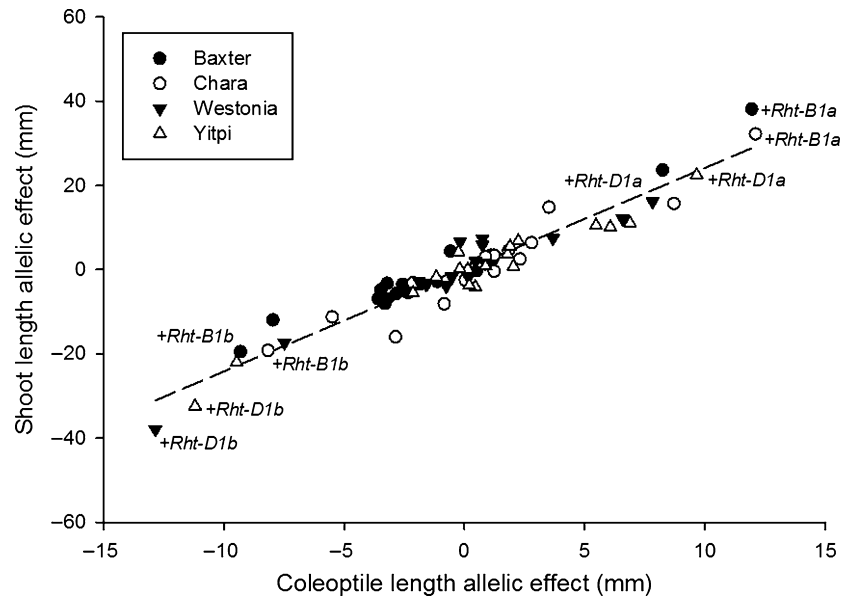
Figure 5 Relationship of shoot length and coleoptile length allelic effects for the four founder parents measured at 12 and 20 °C. Effects are obtained for nine QTL common to both characters. The dashed line represents the line of best fit (y = 1.06 + 2.42 x, r^2 = 0.92, P < 0.01).

Accordingly, the genetic potential for high and stable yields will be determined much earlier in the crop cycle with the capacity for robust early growth. The coleoptile limits the potential for wheat establishment and final plant number of the wheat crop. Both the length and width of the coleoptile affect the capacity to emerge whether seed is sown deep (e.g. where there is deep moisture or stubble) or the soil surface is hard or crusted.

The development of semi-dwarf stature wheat varieties producing longer coleoptiles is a well-established objective of many rainfed breeding programmes (e.g. Murphy et al., 2008; Rebetzke et al., 1999). Genotypic variation has been reported for coleoptile length and width in wheat (Ellis et al., 2004; Marais and Botma, 1987; Rebetzke et al., 1999, 2004, 2007b). The study reported herein demonstrated considerable phenotypic variation for coleoptile characteristics in a large wheat mapping population. Parental lines ranked as expected (Greg Rebetzke unpublished data) while variation for all coleoptile parameters was repeatable as evidenced by the moderate-to-high narrow-sense heritabilities and strong, positive genetic correlations across the two soil temperatures for the individual traits.

The considerable population size, large phenotypic variation and high narrow-sense heritabilities enabled good resolution of QTL associated with coleoptile length and width and shoot length. A number of the coleoptile QTL reported herein were repeatable across the contrasting soil temperatures. The *Rht-B1b* and/or *Rht-D1b* dwarfing genes were contributed from across the four founder parents and were associated with large reductions in coleoptile length. The co-location of QTL for reduced coleoptile length and the GA-insensitive *Rht-B1b* and *Rht-D1b* dwarfing genes confirmed the previous studies (e.g. Allan, 1989; Ellis et al., 2004; Rebetzke et al., 2001, 2007b; Whan, 1976) reporting pleiotropic effects of these dwarfing genes on reductions in wheat seedling growth. Further, as reported in Rebetzke et al. (2007a,b), there was good evidence that reduction in coleoptile length was greater for lines containing *Rht-D1b* than for *Rht-B1b*. Allan (1989) and Rebetzke et al. (2007a) demonstrated that wheat varieties containing GAI *Rht-B1b* and/or *Rht-D1b* produced shorter coleoptiles to slow the time to emergence and reduce final plant establishment with deep sowing when compared to

tall or gibberellic acid-sensitive *Rht8*-containing genotypes. The reduction in coleoptile length reflects reduced coleoptile elongation and elongation rates associated with reduced gibberellic acid sensitivity of the *Rht-B1b* and *Rht-D1* alleles, particularly at warmer soil temperatures (Botwright et al., 2001). Indeed, the greater reduction in coleoptile length at warmer temperatures highlights the potential danger with climate change and poor establishment of GA-insensitive semi-dwarf wheat varieties.

A few studies have been published reporting the genetic control for coleoptiles length in wheat. Coleoptile length and thickness is under strong additive genetic control (e.g. Rebetzke et al., 2004, 2007b), indicating the potential for selection of longer and thicker coleoptiles with better emergence characteristics. Mapping studies indicate numbers of significant QTL ranging from one (Mohammadi et al., 2006) or two (Liu et al., 2011; Yu and Bai, 2010) to five (Landjeva et al., 2008) and seven (Rebetzke et al., 2007b), reflecting the commonly smaller population sizes, reduced opportunity for recombination, and restricted number of parental alleles varying in tested biparental populations. The consistent identification of coleoptile QTL mapping to *Rht-B1b* and *Rht-D1b* confirms the robustness of the coleoptile phenotyping and data used in the mapping of QTL herein, thereby increasing confidence in those QTL identified independent of *Rht-B1b* and *Rht-D1b*.

A large number of QTL for coleoptile length were identified across the genome of small to moderate size. The largest of these were associated with the GA-insensitive *Rht-B1b* and *Rht-D1b* dwarfing genes. The next largest genetic effects were associated with the Yitpi allele on chromosome 1AS. This allele mapped in proximity to a long coleoptile Halberd allele reported previously (Rebetzke et al., 2007b). Yitpi and Halberd are closely related through the long coleoptile parent Insignia (Paull et al., 1998), highlighting the likely value of this allele through the selection in existing breeding efforts. Other larger effect QTL (additive (a) effects greater than 3.0 mm) were identified on chromosomes 2B, 2D, 3B, 5B, 6A, 6B and 7B QTL. These were commonly distributed across different parents so that positive effect alleles were countered by negative effect alleles at other loci to reduce any overall benefit on coleoptile length. Indeed, when summing

across all non-*Rht-B1* and *Rht-D1* alleles, the aggregate genotype (i.e. Σ2a) was large with predicted coleoptile lengths of 80 and 83 mm at 12 and 20 °C, respectively. Along with the 1AS QTL, a number of the chromosomal regions for coleoptile length were reported previously across four different populations in Rebetzke *et al.* (2007b) while the 1BS QTL reported herein was located near to a 1BS QTL for increased coleoptile length in Landjeva *et al.* (2008) and Yu and Bai (2010). In addition to previous reports, the MAGIC population identified a number of new QTL not previously reported including genetic effects on chromosome 1D, 3A, 5B, 6A and 7B.

Poor wheat establishment can sometimes reflect resistance to emergence through hard and crusted soils and crop stubble. Genotypic increases in coleoptile thickness were associated with greater shoot strength and the ability of sorghum seedlings to push through hard and crusted soils (Mason *et al.*, 1994). Repeatable genotypic variation exists for coleoptile diameter in wheat (Marais and Botma, 1987), and this variation reflects largely additive gene action (Rebetzke *et al.*, 2004). The current study confirms the heritable basis for the differences in coleoptile width and thickness and their strong genetic association. The physiological basis for genotypic differences in coleoptile thickness is unclear although we reported shorter coleoptile genotypes, including those containing the GAI *Rht-B1b* and *Rht-D1b* dwarfing alleles that tended to produce thicker coleoptiles with a larger cross-sectional area (Rebetzke *et al.*, 2007b). The present study confirmed a weak albeit significant negative genetic association for coleoptile length and thickness. The presence of the *Rht-B1b* and *Rht-D1b* dwarfing alleles were both associated with increased coleoptile thickness but only at warmer soil temperatures. Also, their effects were small, indicating the potential in developing longer, thicker coleoptiles wheat varieties with the potential for improved establishment. By squashing coleoptiles, we aimed to restrict coleoptile cross-section to a single plane. However, despite the range in progeny coleoptile width and thickness, the genetic association with width after squashing appeared strong and repeatable at both soil temperatures.

Many of the QTL for coleoptile length mapped close to QTL for shoot length while the genetic correlation for the two lengths was very strong and positive at each of the two soil temperatures. The coleoptile is a modified true leaf, which acts as a protective covering for the plumule allowing it to grow and emerge from the soil. The leaf is a major component of total shoot length; thus, many shoot and coleoptile QTL should be common. As for coleoptile length, the GAI dwarfing genes *Rht-B1b* and *Rht-D1b* have large negative effects on shoot length. These effects were particularly strong at warmer temperatures with reduction being greater for *Rht-D1b*. The effects of the GAI dwarfing alleles on reduction in shoot (especially leaf) size are well understood (e.g. Ellis *et al.*, 2004). Of the few studies reporting shoot length QTL, Landjeva *et al.* (2008) indicated large effect shoot length QTL on chromosomes 2BS, 2DS and 5BL.

In conclusion, this study has identified genomic regions associated with genotypic variation in a range of shoot and coleoptile growth characteristics in wheat. A number of these have been reported previously and confirm their value in subsequent genetic studies and/or use in breeding. A common genetic basis for coleoptile and shoot length suggests a common underpinning physiological mechanism and potential in selection of coleoptile length through changes in shoot size. A greater reduction in coleoptile and shoot size with the GA-insensitive *Rht-B1b* and *Rht-D1b* dwarfing genes with increasing soil tempera-ture highlights a real concern with the use of these dwarfing genes in environments predicted to be warmer and more variable with climate change.

Materials and methods

Population

A four-parent MAGIC population was used in this study as described by Huang *et al.* (2012). Briefly, the four commercial Australian bread wheat varieties—Baxter, Chara, Westonia and Yitpi—were intercrossed by crossing F_1 plants of pairs of the founders to generate 850 unique individuals all containing contributions from the four founders; subsequently, two seeds from each of these were descended to F_6 for genotyping and subsequent phenotyping in the following generation. In total, 1458 $F_{6:7}$ recombinant inbred lines (RILs) and parental varieties were utilized for phenotyping in this study. The genetic map utilized for this study was that reported by Huang *et al.* (2012) with the inclusion of the gene markers for *PPD-D1, VRN1-5A, VRN1-5B, VRN-5D, RHT-B1, RHT-D1* and the SSR marker *STM773* (linked to the *Sr36* introgression inherited from Baxter). The founders Baxter and Chara contained the GA-insensitive dwarfing gene *Rht-B1b*, and the founders Westonia and Yitpi contained dwarfing gene *Rht-D1b*, respectively (Ellis *et al.*, 2002).

Coleoptile and shoot growth

All seeds were sampled from plants grown and harvested from the same glasshouse sowing. Good-quality seed free of any visible damage were sourced for all lines. Each seed was restricted to a size range of 40 to 50 mg and avoiding any obvious shrivelling. Nonetheless, individual seeds of all lines were weighed. Seeds of all parental and progeny lines were sown in deep, wooden seedling trays (dimensions 600(L) × 300(W) × 120(D)mm) containing a fertile, compost-based potting mix and at a sowing depth of *ca.* 2 cm below the soil surface. The experimental design was a row–column design constraining genotypes from being sown in the same position across replicates or temperatures. A partial-replicate (*p-rep*) design (Cullis *et al.*, 2006) was used containing replication of random lines and parental checks. Approximately 35% of lines were replicated twice at each temperature. Each tray was watered thoroughly before covering with a lid and securing with a large opaque plastic bag to exclude light. Trays were placed into separate darkened growth cabinets set at constant soil temperatures of 12 and 20 °C. Temperatures were chosen to represent soil temperatures commonly encountered throughout Australian and other global cropping zones at sowing (e.g. Rebetzke *et al.*, 1999). Trays were left until 200 °C Cd (assuming a base temperature of 0 °C) whereupon they were removed for assessment.

Coleoptile lengths were determined with a ruler as the distance from the scutellum to the tip of the coleoptile. Coleoptile width and thickness were determined midway along the coleoptile for all lines after Rebetzke *et al.* (2004). Briefly, coleoptile width was assessed as the distance across the coleoptile at the widest axis and then thickness perpendicular to this axis. A digital micrometre was used for all coleoptile diameter measurements. Shoot length was determined on the same seedlings as the distance from the seed to the tip of the fully elongated leaf 1. After completing all measurements, the seedling was laid horizontal on the bench and a moderate, downward force applied midway along the coleoptile. Coleoptile width was then measured on the squashed coleoptile.

Statistical and genetic analyses

Coleoptile growth data were analysed after first checking for normality and error variance homogeneity at each temperature. Each temperature was analysed separately with the best spatial models being determined after first fitting the experimental design and then modelling the residual variation with autoregressive row and column terms using ASReml in the programming language R (R Development Core Team, 2012). Significant spatial effects were then identified, and residuals assessed before determinations made to the need for fitting of other (e.g. linear) effects (Gilmour *et al.*, 1997). Variance components, their standard errors and best linear unbiased predictors were then obtained following the analysis by the method of restricted maximum likelihood. Progeny were considered random for the analyses of all data. Statistical significance of variance components was ascertained from log-likelihood ratio tests for full and reduced models.

The method for QTL mapping is an extension of whole genome average interval mapping (WGAIM) as proposed by Verbyla *et al.* (2007). WGAIM uses all markers simultaneously in the analysis. These markers enter the analysis as random effects, and a forward selection approach is used to select putative QTL from the full set of markers. This method was shown to outperform and is much quicker than the standard composite interval mapping approach. For the analysis of MAGIC, WGAIM was extended to allow for multi-allelic markers. In addition, the false discovery rate (FDR) approach of Storey (2002) and Storey and Tibshirani (2003) were used to determine the final set of putative QTL.

The analysis was conducted using markers as proposed in the study by Verbyla *et al.* (2007). Thus, the analysis has similarities to an association study but uses the information that markers are on specific chromosomes or linkage groups. It is therefore possible to find association of two adjacent markers to the trait being analysed because both markers are in linkage disequilibrium with the underlying QTL.

The extension of WGAIM for MAGIC involves a postprocessing to determine the size of QTL for each founder allele. This requires probabilities that the marker allele for each line is identical by descent to each founder allele as presented in Huang *et al.* (2012), where map construction for MAGIC is outlined.

Acknowledgements

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