Theor Appl Genet (2007) 114:1173–1183
DOI 10.1007/s00122-007-0509-1

ORIGINAL PAPER

Molecular mapping of genes for Coleoptile growth in bread wheat (*Triticum aestivum* L.)

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Received: 6 July 2006 / Accepted: 13 January 2007 / Published online: 9 February 2007

Abstract Successful plant establishment is critical to the development of high-yielding crops. Short coleoptiles can reduce seedling emergence particularly when seed is sown deep as occurs when moisture necessary for germination is deep in the subsoil. Detailed molecular maps for a range of wheat doubled-haploid populations (Cranbrook/Halberd, Sunco/Tasman, CD87/Katepwa and Kukri/Janz) were used to identify genomic regions affecting coleoptile characteristics length, cross-sectional area and degree of spiralling across contrasting soil temperatures. Genotypic variation was large and distributions of genotype means were approximately normal with evidence for transgressive segregation. Narrow-sense heritabilities were high for coleoptile length and cross-sectional area indicating a strong genetic basis for differences among progeny. In contrast, heritabilities for coleoptile spiralling were small. Molecular marker analyses identified a number of significant quantitative trait loci (QTL) for coleoptile growth. Many of the coleoptile growth QTL mapped directly to the *Rht-B1* or *Rht-D1* dwarfing gene loci conferring reduced cell size through insensitivity to endogenous gibberellins. Other QTL for coleoptile growth were identified throughout the genome. Epistatic interactions were small or non-existent, and there was little evidence for any QTL × temperature interaction. Gene effects at significant QTL were approximately one-half to one-quarter the size of effects at the *Rht-B1* and *Rht-D1* regions. However, selection at these QTL could together alter coleoptile length by up to 50 mm. In addition to *Rht-B1b* and *Rht-D1b*, genomic regions on chromosomes 2B, 2D, 4A, 5D and 6B were repeatable across two or more populations suggesting their potential value for use in breeding and marker-aided selection for greater coleoptile length and improved establishment.

Communicated by C. Hackett.

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Introduction

In cereals, the coleoptile is a sheath-like structure that permits the delivery of the elongating stem and first seedling leaves from the embryo to the soil surface. The length of the coleoptile determines the depth at which seed can be sown as sowing depths exceeding this length limit the ability of the first leaf to push through to the soil surface. Short coleoptiles can result in poor establishment particularly when crops are sown deeper than around 5 cm (Whan 1976; Takahashi et al. 2001; Rebetzke et al. 2007). Occasionally, deep-sown seedlings do emerge but much later and lack good early vigour (Hadjichristodoulou et al. 1977; Rebetzke et al. 2007). Genotypic differences in seedling establishment and subsequent leaf area development were also associated with improved weed competitiveness among wheat and barley genotypes (O'Donovan et al. 2005). Wheat varieties containing longer coleoptiles would provide many benefits. Longer coleoptiles would enable growers to sow into moisture deep in the soil profile (Mahdi et al. 1998; Schillinger et al. 1998), improve establishment when sowing into stubble (Rebetzke et al. 2005), or deep-sown to avoid animal

predation of seed (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).

Varietal differences have been widely reported for coleoptile length in wheat (Whan 1976; Schillinger et al. 1998). Access to genotypic variation indicates potential for genetic improvement of establishment through selection for greater coleoptile length. Indeed, breeding for greater coleoptile length has been of considerable interest to wheat breeders worldwide (e.g. Whan 1976; Schillinger et al. 1998). Inheritance studies have reported coleoptile length to be under polygenic control (Singhal et al. 1985; Rebetzke et al. 1999). Further, there is evidence of strong additive gene action for coleoptile length indicating the potential ease with early- or late-generation selection for greater coleoptile length (Fick and Qualset 1976; Rebetzke et al. 2004). Shorter coleoptile and sub-crown internode lengths have been reported as pleiotropic effects associated with presence of the *Rht-B1b* (*Rht1*) and *Rht-D1b* (*Rht2*) dwarfing genes in wheat (Fick and Qualset 1976; Allan 1989; Richards 1992). Indeed, two large QTL for reduced coleoptile length were reported in close proximity to *Rht-B1b* in a wheat mapping population varying for coleoptile length (Rebetzke et al. 2001). The *Rht-B1b* and *Rht-D1b* genes are associated with reduced cell elongation to decrease cell size (Hoogendoorn et al. 1990). The greater coleoptile lengths of the standard height wheat genotypes Halberd and its *Rht8*, biparental-derivative, HM14bS, were associated with a faster coleoptile elongation rate compared to slower elongation rates of *Rht-D1b* semi-dwarf wheats (Botwright et al. 2001).

A number of studies (e.g. Takeda and Takahashi 1999; Rebetzke et al. 2007) have demonstrated that factors in addition to coleoptile length can impact seedling establishment. For example, genotypic increases in coleoptile diameter were associated with greater shoot strength and the ability of pasture grass (Andrews et al. 1997) and sorghum (Mason et al. 1994) seedlings to push through hard and crusted soils. Genotypic variation exists for coleoptile diameter or coleoptile cross-sectional area (CCSA) in wheat (Marais and Botma 1987; Matsui et al. 2002) while large genotypic differences among F_1 progeny for CCSA in wheat was attributable to both additive and non-additive gene action (Rebetzke et al. 2004). Similarly, Chen et al. (2003) suggested that the spiralling coleoptile growth characteristic of some wheat varieties assisted seedling emergence in soils affording high mechanical resistance.

There have been few studies reporting genomic regions associated with genetic differences in coleoptile length in cereals. Two independent QTL accounting for *ca.* 40% of the phenotypic variance was reported for coleoptile length in rice (Redona and Mackill 1996), and a single QTL for coleoptile length (mapping to a location for emergence with deep-sowing) was identified in a barley population widely-varying for coleoptile length (Takahashi et al. 2001). In Rebetzke et al. (2001), we reported three QTL for coleoptile length mapping to chromosomes 4BS, 4BL and 5AL in a single population varying for coleoptile length in wheat. This paper extends Rebetzke et al. (2001) to report mapping studies aimed at identifying QTL for coleoptile growth characteristics in four wheat populations evaluated at three soil temperatures.

Materials and methods

Populations

Four populations containing between 161 and 190 doubled-haploid (DH) lines were derived from crosses between Cranbrook and Halberd (hereafter C/H), Sunco and Tasman (S/T), CD87 and Katepwa (C/K), and Kukri and Janz (K/J). Coancestries among parents varied from a low 0.062 for C/H, to 0.154 and 0.361 for C/K and S/T, respectively. Across populations, the S/T, C/K and K/J populations were genetically related through Condor and WW15 as parents in the pedigrees of Sunco, Tasman, CD87 and Janz. Development of the C/H, S/T, C/K and K/J populations were described in detail in Kammholz et al. (2001). Cranbrook, Sunco, CD87 and Janz all contain the *Rht-B1b* gibberellin-insensitive dwarfing gene; Tasman and Kukri contain *Rht-D1b*; whereas Halberd and Katepwa are standard ('tall') height wheat containing no major dwarfing genes.

Coleoptile growth

Good quality seeds free of any visible damage and weighing between 40 and 45 mg were sourced from mainstems of glasshouse-grown plants for each line of the C/H population, and irrigated, field-grown seed for the other three populations. Seed of all DH and parental lines, and long- and short-coleoptile controls (cvv. Halberd and Janz, and double-dwarf Yecora in the S/T population) were sown in a row–column experimental design in wooden, seedling trays (dimensions $600(L) \times 300(W) \times 120(D)$mm) containing a fertile, compost-based potting mix, and at *ca.* 2 cm below the soil surface. Two replicates were sown of each line. Trays were moistened and then placed into darkened

growth cabinets set at constant temperatures of 11, 15 and 19°C. The three temperatures were chosen to represent the range of soil temperatures commonly encountered in the Australian wheatbelt (Rebetzke et al. 1999). Seedlings were removed from the dark at 200°Cd (assuming a base temperature of 0°C) and coleoptile lengths determined with a ruler as the distance from the scutellum to the tip of the coleoptile. Seed weight and coleoptile length were uncorrelated except in the C/H population where there was a small yet significant correlation ($r^2 = 0.01$, $P < 0.05$, $n = 2{,}109$ observations). For the 11 and 15°C temperatures, degree of spiralling was assessed for all lines using an arbitrary score ranging from 0 (no spiralling along a 2 cm section of the coleoptile) to 2 (2 or more spirals along the 2 cm section of coleoptile) in 0.5 increments midway along the coleoptile.

Cross-sectional area was determined on all lines at all temperatures in the Cranbrook/Halberd population. A previous study (Rebetzke et al. 2004) showed a strong genetic correlation between basal and upper coleoptile diameter ($r_g = 0.72$, $P < 0.01$) indicating the coleoptile sheath was uniform in size along its length. Therefore, coleoptile diameter was determined midway along the coleoptile from the width at the widest axis, and then width perpendicular to this axis. Cross-sectional area was then determined from the mean of these two diameters. A digital micrometer was used for all coleoptile diameter measurements. Seed weight showed a small albeit significant association with cross-sectional area ($r^2 = 0.03$, $P < 0.05$, $n = 2{,}109$ observations). However, the slope of the resulting regression was small at 0.01 $mm^2\,mg^{-1}$ seed weight, and so seed weight was ignored from subsequent analysis of cross-sectional area.

Statistical and genetic analyses

All coleoptile growth data were analysed after first checking for normality and error variance homogeneity across temperatures. Residuals plotted against fitted values revealed a random distribution for all traits (data not shown). Variance components, their standard errors and best linear unbiased estimators were obtained following analysis by the method of restricted maximum likelihood using the SAS procedure MIXED (Littell et al. 1996). Doubled-haploid lines and environments were considered random for the initial analyses of all data but then data were reanalysed with DH and checks (parents and other lines) as fixed effects. Statistical significance of variance components was ascertained from log likelihood ratio tests for full and reduced models. Genotypic variances (σ^2_G) for all traits were equated with the expectation of the covariance among full-sib families as: σ^2_G = Cov (among F_1-derived DH lines) = $1.0\sigma^2_A + 0\sigma^2_D + 0D_1 + 1.0\sigma^2_{AA}$, where, σ^2_A, σ^2_D, and σ^2_{AA}, refers to additive, dominance, and additive × additive epistatic genetic variances, respectively, and D_1 the covariance between additive and homozygous dominance effects for alleles at a locus (Cockerham 1983). Because alleles at a locus are equally frequent in an unselected DH population developed from inbred parents, D_1 is zero. Thus, with zero dominance, the covariance among full-sibs includes $1\sigma^2_A$ and $1\sigma^2_{AA}$ so that narrow-sense heritability (h^2) = $[1\sigma^2_A + 1\sigma^2_{AA}] / \sigma^2_P$, where σ^2_P is the phenotypic variance on a line-mean basis.

QTL mapping

Analysis of the C/H, S/T, and C/K populations in producing the genetic maps has been detailed in Chalmers et al. (2001), and more recently in Lehmensiek et al. (2005), with each map containing between 400 and 800 microsatellite, AFLP, morphological and DArT markers. Development of the K/J genetic map was undertaken elsewhere (Gulay Mann, unpublished data). QTL analysis was performed on spatially-adjusted line means estimated from mixed linear models in SAS (Littell et al. 1996). QTL detection was undertaken using mixed linear composite interval mapping in QTLNetwork 2.0 (Yang et al. 2005). Composite interval analysis was undertaken using forward–backward stepwise, multiple linear regression with a probability into and out of the model of 0.05 and window size set at 10 cM. Significant thresholds for QTL detection were calculated for each dataset using 1,000 permutations (Churchill and Doerge 1994) and a genome-wide error rate of 0.10 (suggestive) and 0.05 (significant). The final genetic model incorporated significant main additive and additive × additive epistatic genetic effects, and their interaction with environment. Additive and epistatic effects were fixed and environment effects random in the mixed model. Locations of genetic effects of individual QTL were identified from maps drawn using MapChart 2.1 (Voorips 2002), and 95% confidence intervals for each QTL location were obtained through jack-knifing.

Results

Temperature effects on coleoptile growth were large for all coleoptile growth parameters but the extent of change varied with population. Increases in soil temperature were commonly associated with significant

Table 1 Influence of soil temperature on different coleoptile characteristics measured on random F_1-derived, doubled-haploid (DH) progenies from the Cranbrook/Halberd, Sunco/Tasman, CD87/Katepwa and Kukri/Janz mapping populations

Temperature	Cranbrook/Halberd			Sunco/Tasman		CD87/Katepwa		Kukri/Janz	
	Coleoptile length (mm)	Coleoptile cross-sectional area (mm^2)	Coleoptile spiralling[a]	Coleoptile length (mm)	Coleoptile spiralling	Coleoptile length (mm)	Coleoptile spiralling	Coleoptile length (mm)	Coleoptile spiralling
11°C	119	1.65	0.62	101	0.52	86	1.08	84	0.56
15°C	113	1.73	1.65	84	0.28	89	0.54	69	–[b]
19°C	103	1.64	0.99	79	–[b]	65	–[b]	60	–[b]
l.s.d.[c]	2	0.08	0.10	4	0.20	1	0.36	6	

[a] Coleoptile spiralling was scored from 0 (no spiralling along a 2 cm section of the coleoptile) to 2 (2 or more spirals along the 2 cm section of coleoptile)

[b] Coleoptile spiralling not measured at this soil temperature

[c] Average least significant difference for comparing means at each temperature

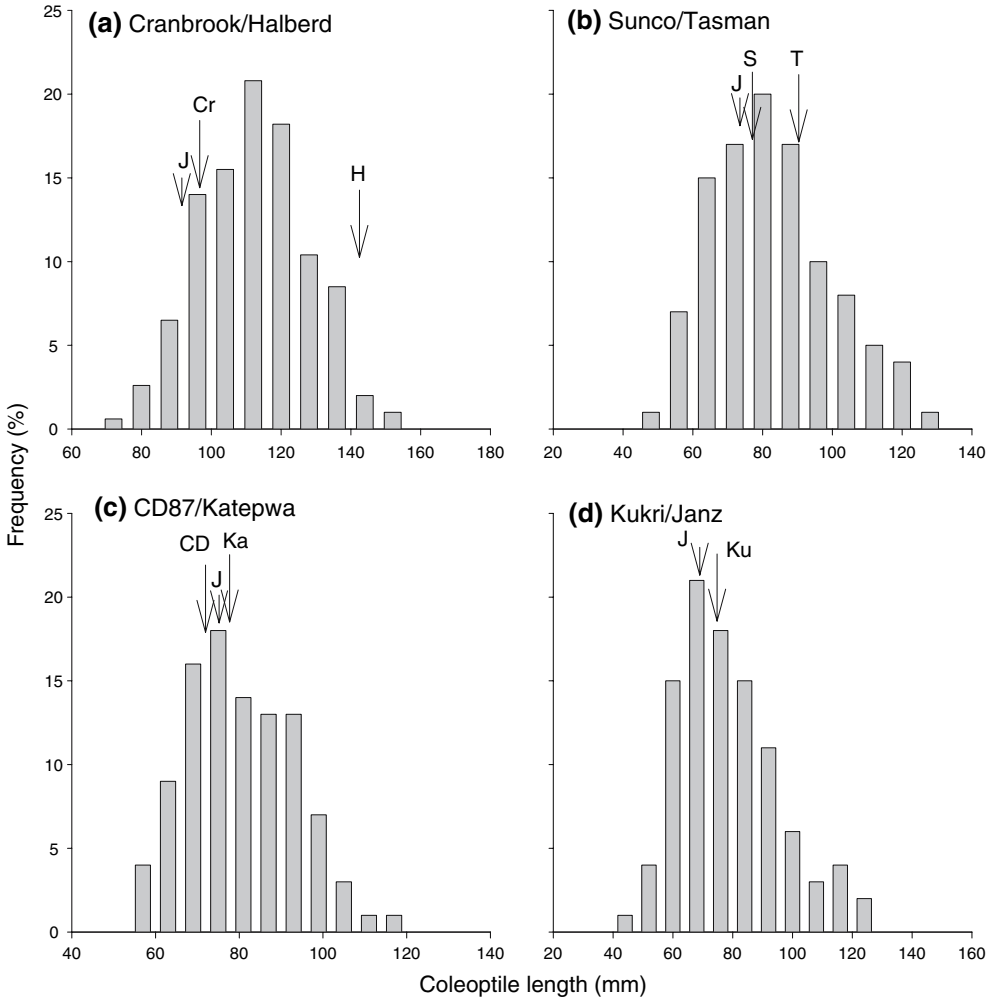

Fig. 1 Frequency distributions for coleoptile length measured on random doubled-haploid lines evaluated at 11, 15 and 19°C for **a** Cranbrook/Halberd, **b** Sunco/Tasman, **c** CD87/Katepwa, and **d** Kukri/Janz mapping populations. Mean coleoptile length for parental and control varieties Janz (J) are indicated

reductions in coleoptile length (Table 1). This change was approximately linear for the C/H, S/T and K/J populations while for the C/K population coleoptile length was significantly smaller for the 19°C temperature. There was no systematic change in coleoptile cross-sectional area (CCSA) with temperature. However, the

environmental correlation for coleoptile length and CCSA in the C/H population was moderately strong and negative ($r_e = -0.57 \pm 0.18$, $P < 0.01$) indicating environmental factors associated with greater coleoptile length contributed to reductions in CCSA. The degree of coleoptile spiralling varied significantly in some populations (Table 1) but generally showed no systematic change with temperature.

The assembled populations varied considerably in both mean and range for coleoptile length, CCSA and degree of coleoptile spiralling (Table 2). The large range among progeny sometimes mirrored large differences in parental means (e.g. coleoptile length in Cranbrook and Halberd and coleoptile spiralling in Sunco and Tasman), while in other cases parents were very similar for coleoptile growth traits. Despite the similarity in parental means for some traits, progeny were widely varying for coleoptile length, CCSA, and in most cases, coleoptile spiralling (Table 2). The large range among progeny beyond that of either parent indicated transgressive segregation (Figs. 1–3). Distributions of progeny means were continuous and *ca*. normal for most traits (Figs. 1, 2). There was some evidence for skewedness toward longer coleoptiles in the S/T, C/K and K/J populations (Fig. 1). However, together these data suggest that more than one gene is likely affecting genic expression of the different coleoptile growth traits. There was strong evidence of an association between coleoptile length and presence of the *Rht-B1b* and *Rht-D1b* dwarfing genes (e.g. Sunco/Tasman population in Fig. 3). Coleoptile length was linearly reduced ($r = 0.83$, $P < 0.01$) with increasing frequency of *Rht-B1b* and *Rht-D1b* alleles. Double-dwarfs containing both *Rht-B1b* and *Rht-D1b* alleles produced the shortest mean coleoptile length consistent with the shorter coleoptile of the double-dwarf check variety Yecora (Fig. 3). However, large and repeatable genotypic variation was still identified within each of the dwarfing gene classes. For example, mean coleoptile length ranged between 56 and 99 mm within the single dwarf (*Rht-B1b* or *Rht-D1b*) genotypic group (Fig. 3).

Progeny was observed in all populations with coleoptile lengths, CCSA and spiralling scores exceeding the tested semi-dwarf controls (parents and commercial variety Janz). Janz was common to all four studies and remained reasonably consistent between populations for coleoptile length (Fig. 1). The semi-dwarf controls were among the shortest coleoptile genotypes in each study with numerous progenies identified producing coleoptiles between 30 and 50 mm greater in length (Table 2, Fig. 1).

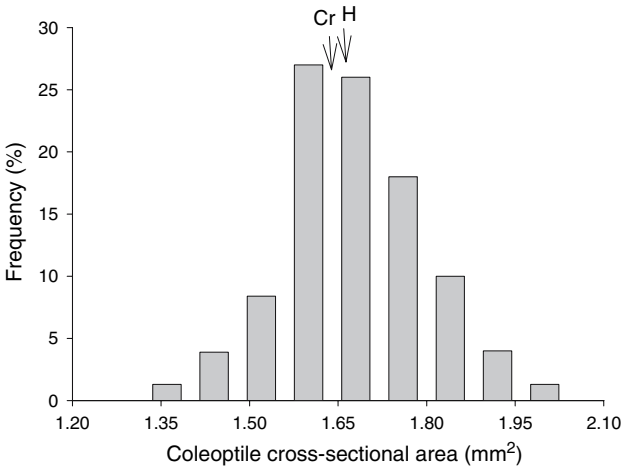

Fig. 2 Frequency distributions for coleoptile cross-sectional area measured on random Cranbrook/Halberd DH lines evaluated at 11, 15 and 19°C. Line and parental means are indicated

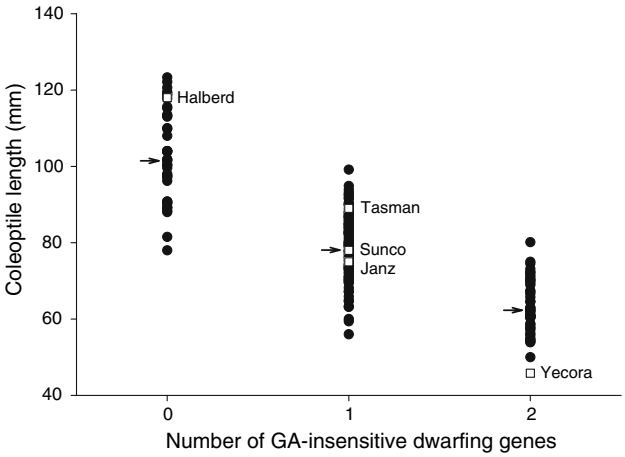

Fig. 3 Influence of increasing frequency of GA-insensitive dwarfing genes (*Rht-B1b* and *Rht-D1b*) on coleoptile length for lines within the Sunco/Tasman DH population evaluated at 11, 15 and 19°C. *Points* indicate the mean coleoptile length for each line (*filled circle*) when averaged across all temperatures. The equation for this relationship was: Coleoptile length = 101–20 × ($r^2 = 0.68$, $P < 0.01$). *Arrows* indicate the mean coleoptile length for each dwarfing gene class. Mean coleoptile lengths for control varieties (*square*) are indicated

Large among DH differences translated into significant ($P < 0.05$) genotypic and subsequent additive genetic variances for most coleoptile growth traits (Table 3). Among all populations, there was a strong, positive correlation between midparent and DH progeny mean for coleoptile length ($r = 0.97$, $P < 0.05$) and coleoptile spiralling (0.93, $P < 0.05$). Narrow-sense heritabilities were large for coleoptile length and CCSA indicating a generally high degree of genetic determination. Heritability for coleoptile spiralling was generally small or not different from zero suggesting

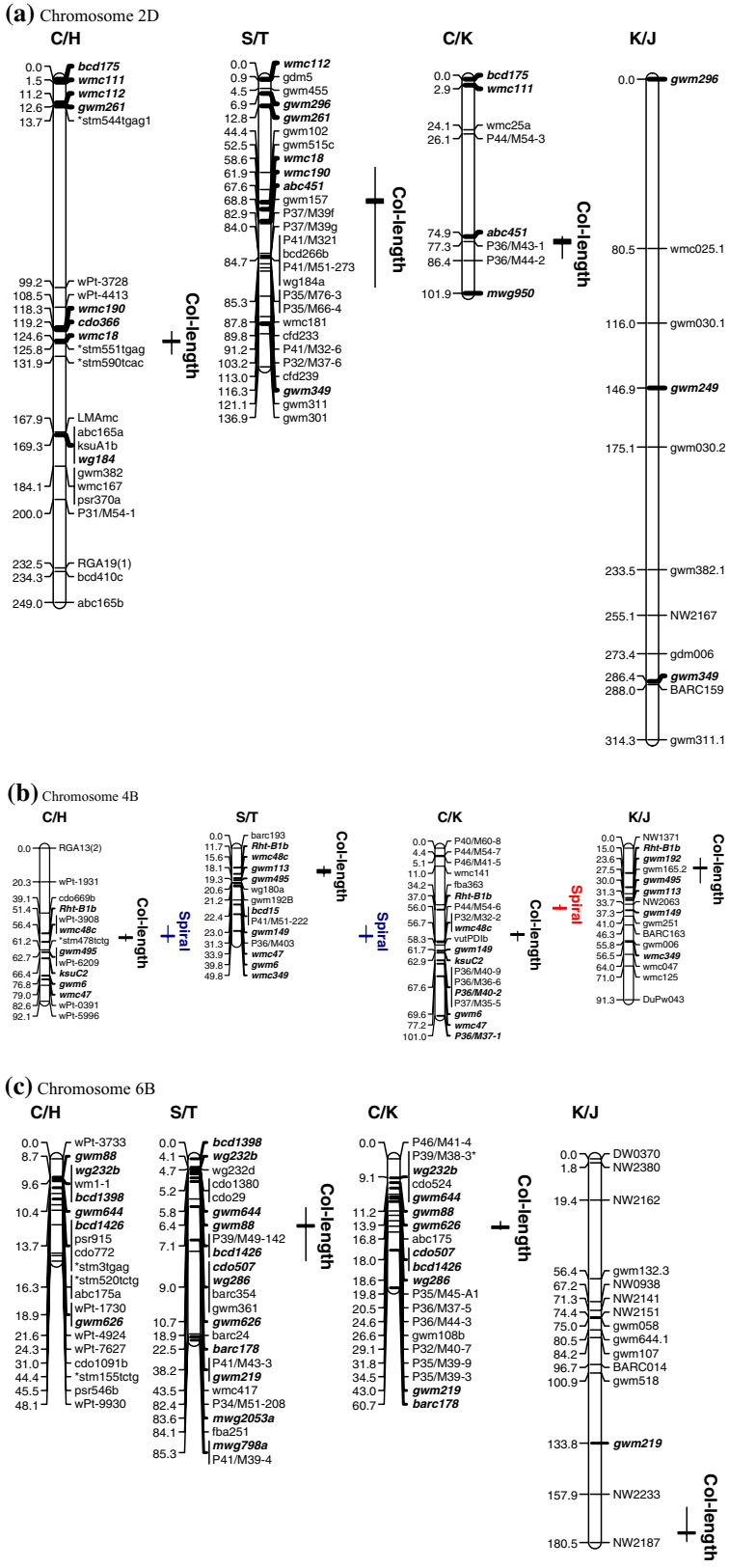

Fig. 4 Chromosomal locations (and 95% confidence intervals) for mean coleoptile length ('Col-length') and coleoptile spiralling ('Spiral') for chromosomes **a** 2D, **b** 4B, and **c** 6B in the Cranbrook/Halberd (*C/H*), Sunco/Tasman (*S/T*), CD87/Katepwa (*C/K*), and Kukri/Janz (*K/J*) mapping populations. Molecular markers and genetic distance (cM) are indicated. Markers in common across populations are indicated (*italicized* and **in bold**)

it to be more sensitive to environment than either coleoptile length or CCSA to differentially affect genotype response. Among coleoptile growth traits measured on the C/H population, genotypic increases in coleoptile length were associated with increases in coleoptile spiralling ($r_g = 0.57 \pm 0.14$, $P < 0.05$) and

Table 2 Ranges and means for different coleoptile characteristics measured on control varieties, parents and F_1-derived, doubled-haploid (DH) progenies from the Cranbrook/Halberd, Sunco/Tasman, CD87/Katepwa and Kukri/Janz mapping populations evaluated at three soil temperatures (11, 15, and 19°C)

Entry	Cranbrook/Halberd			Sunco/Tasman		CD87/Katepwa		Kukri/Janz	
	Coleoptile length (mm)	Coleoptile cross-sectional area (mm^2)	Coleoptile spiralling[a]	Coleoptile length (mm)	Coleoptile spiralling	Coleoptile length (mm)	Coleoptile spiralling	Coleoptile length (mm)	Coleoptile spiralling
DH lines									
Minimum	73	1.33	0.0	53	0.0	58	0.0	49	0.0
Maximum	149	2.00	1.1	125	2.0	111	2.0	143	2.0
l.s.d.[b]	14	0.21	0.3	21	0.4	16	0.6	24	0.6
DH mean	112	1.69	0.3	81	0.5	82	0.7	71	0.6
Controls									
Parent 1[c]	96	1.63	0.3	78	0.7	72	0.8	75	0.8
Parent 2[c]	140	1.64	0.3	89	1.0	78	1.0	71	0.5
Halberd	140	1.64	0.3	118	0.3	113	0.8	104	0.3
Janz	94	1.60	0.5	75	0.4	74	0.7	71	0.5
l.s.d.[d]	10	0.18	0.2	12	0.6	11	1.4	16	0.9

[a] Coleoptile spiralling was scored from 0 (no spiralling along a 2 cm section of the coleoptile) to 2 (2 or more spirals along the 2 cm section of coleoptile)

[b] Average least significant difference ($P = 0.05$) for comparing among DH lines

[c] Parent 1 is the first parent in the cross and parent 2 is the second parent

[d] Average least significant difference for comparing control and parental varieties with DH line means

reductions in CCSA (-0.84 ± 0.08, $P < 0.01$). Genotypic differences in coleoptile length were also correlated with coleoptile spiralling in the S/T population ($r_g = 0.53 \pm 0.12$, $P < 0.05$) but this correlation could not be estimated in the C/K and K/J populations owing to a lack of genetic variance for coleoptile spiralling in these two populations.

Comprehensive molecular maps of the four DH populations enabled the identification of QTL for all coleoptile growth characteristics (Tables 3, 4). The largest genetic effect on coleoptile length was associated with QTL on chromosomes 4BS, 4DS or both. There was sufficient marker resolution to indicate the location of these QTL in close proximity to the dwarfing genes *Rht-B1b* (4BS) and/or *Rht-D1b* (4DS) (Fig. 4). Both QTL were associated with significant reductions in coleoptile length with these estimates being approximately the same across populations (e.g. *Rht-B1b* in C/H and C/K). In populations where both *Rht-B1b* and *Rht-D1b* were varying (e.g. S/T and K/J), genetic reductions in coleoptile length associated with *Rht-D1b* were significantly ($P < 0.05$) greater than for *Rht-B1b* (Table 4). These dwarfing-gene QTL showed little or no interaction with temperature.

Up to six other significant QTL were identified for coleoptile length in each of the four populations (Table 4, Fig. 4). These QTL were of small to moderate genetic effect and were identified on all group chromosomes. Most were significant across all temperatures (no QTL × environment interaction) while some (e.g. 2BS, 2DS, 3BS, 4AS, 5DS and 6BL) were mapped to the same approximate location in two or more populations (e.g. Fig. 4). For example, the chromosome 2DS coleoptile length QTL mapped close to the centromere (near molecular markers *wmc18* or *abc451*) in populations C/H, S/T and C/K (Fig. 4a). Individually these QTL produced small genetic effects of between 1.7 (2DS in S/T) and −4.9 (5AL in C/H) mm (Table 4). However, when combined, these QTL can together produce superior genotypes with predictably up to 50 mm greater coleoptile length (e.g. C/H, Table 4). There was little evidence of epistatic interactions among QTL although small, significant epistatic effects were detected for coleoptile length in the C/H and C/K populations.

Few QTL were identified for CCSA in the C/H population and coleoptile spiralling in the different populations. Three independent QTL were identified for CCSA in the C/H population (Table 5); the parent Cranbrook contributing alleles for reduced CCSA on chromosomes 2A, 3A and 5B. Across populations, QTL for coleoptile spiralling were mapped to either *Rht-B1* or *Rht-D1* loci. Presence of the *Rht-B1b* or *Rht-D1b* alleles was associated with reduced spiralling along the coleoptile. Additional QTL for coleoptile spiralling were identified on chromosomes 1A, 3D and 7A (Table 5). These QTL were generally of small effect. There was no evidence for epistatic interactions for either CCSA or coleoptile spiralling.

Table 3 Genetic (σ^2_G) and genotype × environment interaction (σ^2_{GE}) (±standard errors), and narrow-sense heritability estimates on a genotype-mean basis (±standard errors) for a range of coleoptile characteristics measured on random F_1-derived, doubled-haploid progeny from Cranbrook/Halberd, Sunco/Tasman, CD87/Katepwa and Kukri/Janz mapping populations evaluated at three soil temperatures (11, 15, and 19°C)

Parameter	Cranbrook/Halberd			Sunco/Tasman		CD87/Katepwa		Kukri/Janz	
	Coleoptile length	Coleoptile cross-sectional area	Coleoptile spiralling	Coleoptile length	Coleoptile spiralling	Coleoptile length	Coleoptile spiralling	Coleoptile length	Coleoptile spiralling
σ^2_G	182 ± 30**	0.0106 ± 0.0017*	0.073 ± 0.028*	207 ± 29**	0.030 ± 0.015*	135 ± 23**	0.000 ± 0.000	194 ± 30**	0.000 ± 0.000
σ^2_{GE}	10 ± 1**	0.0001 ± 0.0007	0.024 ± 0.045	206 ± 14**	0.270 ± 0.022	108 ± 14**	0.390 ± 0.070*	130 ± 34**	0.271 ± 0.058*
h^2	0.90 ± 0.14	0.77 ± 0.16	0.41 ± 0.09	0.80 ± 0.21	0.31 ± 0.09	0.79 ± 0.20	–	0.82 ± 0.17	–

*, ** variance component is statistically different from zero at $P = 0.05$ and $P = 0.01$, respectively

– not estimated as genetic variance estimate was zero

Discussion

Large phenotypic variation for coleoptile growth characteristics enabled the mapping of genomic locations associated with coleoptile length, cross-sectional area and degree of coleoptile spiralling. All but degree of spiralling were highly heritable and under strong genetic control. Further, the range and distribution of progeny indicated polygenic variation in each population. Polygenic control has been reported previously for coleoptile length assessed across different wheat populations (e.g. Fick and Qualset 1976; Singhal et al. 1985; Rebetzke et al. 1999). Using a diallel cross of twelve wheat parents, coleoptile length was largely under additive genetic control whereas genotypic variation for CCSA reflected both additive and non-additive gene action (Rebetzke et al. 2004). Both traits were of moderate-to-high heritability when assessed across soil temperatures ranging between 11 and 23°C.

Large phenotypic variation and high narrow-sense heritabilities allowed good resolution of QTL associated with coleoptile length. A number of the coleoptile QTL reported herein were repeatable across temperatures while some were repeatable across populations. The *Rht-B1b* and/or *Rht-D1b* dwarfing genes varying in all four populations 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 in all four populations confirms previous studies (e.g. Fick and Qualset 1976; Whan 1976; Allan 1989; Rebetzke et al. 2001; Ellis et al. 2004) reporting pleiotropic effects of these dwarfing genes on reductions in seedling growth. Further, there was good evidence that reduction in coleoptile length was greater for *Rht-D1b* than for *Rht-B1b*. The shorter coleoptile associated with *Rht-D1b* is consistent with deep-sowing studies by Allan (1989) where *Rht-D1b* near-isogenic lines emerged slower and produced fewer seedlings when compared with *Rht-B1b* near-isogenic counterparts.

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 coleoptile growth QTL. This robustness increases confidence in those QTL identified independent of *Rht-B1b* and *Rht-D1b*. Further, many of these QTL mapped to the same approximate chromosomal locations across different populations providing some validation and increasing their value for use in marker-aided selection. In the case of the C/H population, accumulation of favourable coleoptile QTL could increase coleoptile length approximately 50 mm. Furthermore, replacement of the *Rht-B1b* dwarfing gene

Table 4 Estimated genetic [additive (*a*) and additive × environment (*ae*)] effects, percent additive genetic variance and chromosomal location (and corresponding 95% confidence intervals) of QTL for coleoptile length measured on random doubled-haploid progenies from the Cranbrook/Halberd, Sunco/Tasman, CD87/Katepwa and Kukri/Janz mapping populations evaluated at three soil temperatures (11, 15 and 19°C). *Ep* indicates statistically significant epistatic interactions

Chromosome	Nearest marker	QTL position (cM)	*a* genetic effect[a] (mm)	Additive genetic variance (%)	*ae* at 11°C (mm)	*ae* at 15°C (mm)	*ae* at 19°C (mm)
Cranbrook/Halberd							
1AS	*ksuG9c*	54.6 (28.1–81.5)	−2.9**	3.6	0.0	0.0	0.0
2BS	*wPt-0615*	59.5 (47.9–75.8)	−2.5*	2.7	0.0	0.0	0.0
2DS	*Stm55ltgag*	124.2 (119.2–129.8)	4.1**	5.9	0.0	0.0	0.0
3BS	*wPt-8855*	13.3 (12.8–19.3)	−2.0*	2.8	−0.7	0.1	0.7
4BS	*Rht-B1b*	54.4 (51.4–55.9)	−9.5**	35.4	1.8*	0.6	−2.3*
5AL	*psr426*	129.0 (121.8–130.5)	−4.9**	8.6	0.0	0.0	0.0
5DS	*psr326b*	69.0 (34.6–83.0)	3.6**	4.1	0.0	0.0	0.0
*Ep1: 4BS*7BS*	*Rht-B1b, wmc76*	*53.9, 66.3*	−1.7**	2.1	−0.2	0.1	0.1
*Ep2: 4BS*7BS*	*Rht-B1b, wPt-6463*	*53.9, 80.1*	−1.2¶	1.6	−0.0	0.0	0.0
*Ep3: 2DL*5BS*	*stm55ltgag, RGA74.15*	*124.7, 132.4*	−2.6**	2.7	0.0	0.0	0.0
Sunco/Tasman							
2BS	*wmc474*	46.4 (41.3–51.1)	−3.1**	2.8	1.3	−0.5	−0.4
2DS	*gwm515c*	58.4 (52.4–98.7)	1.7¶	1.2	2.5	0.1	−2.8*
4AS	*gwm165*	0.0 (0.0–9.0)	2.8**	2.3	0.0	0.0	0.0
4BS	*Rht-B1b*	13.7 (11.7 –15.6)	−8.4**	20.6	−0.3	−0.1	0.9
4DS	*Rht-D1b*	1.8 (0.0 –5.8)	10.0**	29.0	0.2	0.4	−0.2
6BL	*barc178*	31.0 (23.0–48.0)	2.7**	2.2	−0.1	0.6	0.1
CD87/Katepwa							
2DS	*P36/M43-1*	77.3 (74.9–85.3)	−2.8**	3.8			
3BS	*abg75c*	122.8 (107.8–122.8)	2.7**	3.6	−0.1	0.1	−0.3
4BS	*Rht-B1b*	52.0 (50.0–56.0)	−9.8**	28.2	0.0	0.0	0.0
5DS	*abg3a*	83.1 (73.1–105.9)	2.1*	2.8	0.4	0.0	−0.6
6BL	*P35/M39-9*	32.2 (29.5–36.2)	3.0**	4.1	1.4	0.7	−1.4*
7AL	*KsuH9c*	127.4 (124.9–132.8)	−3.1**	4.4	0.4	0.0	−0.4
*Ep1: 1AL*5DS*	*bcd310, P41/M51-2*	*81.1, 150.8*	0.1	0.5	0.3	0.8	−0.8*
Kukri/Janz							
4AS	*gwm637*	41.8 (36.2–50.8)	−0.1¶	0.3	3.8**	−3.7**	0.0
4BS	*Rht-B1b*	12.0 (6.0–21.0)	6.9**	22.5	−0.9	0.7	0.0
4DS	*Rht-D1b*	1.8 (0.0–5.8)	−8.5**	27.3	0.0	0.0	−0.3
6BL	*gwm219*	176.0 (164–180)	4.1*	6.7	0.3	0.0	0.0

a additive effect estimated as one-half the difference in homozygotes carrying either parental allele

[a] a positive additive effect indicates the first parent is contributing a positive allele and the second parent a negative allele

¶, *,** indicates marker effect is statistically different from zero at $P = 0.10$, 0.05 and 0.01

for a gibberellin-responsive dwarfing gene such as *Rht8* (Rebetzke et al. 1999; Ellis et al. 2004) could increase coleoptile length an additional 20 mm whilst retaining semi-dwarf stature. Other studies (e.g. Rebetzke et al 1999, 2007; Ellis et al. 2004) showed coleoptile length and height to be independent in dwarf wheat containing alternative, gibberellin-sensitive dwarfing genes such as *Rht4*, *Rht5*, *Rht8*, *Rht12* or *Rht13*.

There are few studies reporting QTL for early seedling growth in wheat. Rebetzke et al. (2001) reported three QTL for coleoptile length in the Cranbrook/Halberd population assessed separately at four temperatures. One QTL was identified on chromosome 5AL and the other two identified either side of the *Rht-B1* locus (approximately 25 cM apart) on chromosome 4B. In the current study, incorporation of all environment data together in a one-step QTL analysis has resolved the two 4B QTL into a single QTL mapping directly to the *Rht-B1* locus (Fig. 4). Furthermore, by locating directly to *Rht-B1*, the genetic effect of the *Rht-B1b* allele on coleoptile length is correctly estimated. The negative effects of dwarfing genes *Rht-B1b* and *Rht-D1b* on coleoptile length were repeatable across populations reaffirming the difficulty of obtaining long coleoptiles in *Rht-B1b* or *Rht-D1b* semi-dwarf progeny.

In an *Aegilops tauchii* Coss.-derived population containing no GA-insensitive dwarfing genes, a number of D-genome QTL were associated with genotypic variation in cell and leaf growth rates (ter Steege et al. 2005). Botwright et al. (2001) demonstrated genotypic differences in coleoptile length to be largely attributable to variation in coleoptile growth rates. Similarly, QTL for various seedling-related traits were identified

Table 5 Estimated genetic [additive (*a*) and additive × environment (*ae*)] effects and chromosomal location (and corresponding 95% confidence intervals) of QTL for coleoptile cross-section area (CCSA) and degree of coleoptile spiralling measured on random doubled-haploid progenies from the Cranbrook/Halberd, Sunco/Tasman, CD87/Katepwa and Kukri/Janz populations evaluated at three different soil temperatures

Population trait	Chromosome	Nearest marker	QTL Position (cM)	*a* genetic effect[a] (mm)	% additive genetic variance	*ae* at 11°C (mm)	*ae* at 15°C (mm)	*ae* at 19°C (mm)
Cranbrook/Halberd								
CCSA	2AS	*psr131*	20.3 (11.3–32.4)	−0.1*	6.4	0.00	−0.01	0.00
	3AL	*RGA61.2*	107.6 (87.5–120.6)	−0.1*	6.7	0.00	0.00	0.00
	5BS	*cdo749*	54.9 (38.5–63.0)	0.1¶	3.9	0.00	0.00	0.00
Coleoptile spiralling	1AS	*psr161*	61.4 (56.4–70.2)	−0.2**	6.9	0.00	0.00	0.00
	4BS	*Rht-B1b*	52.9 (46.6–56.9)	−0.2**	11.1	0.00	0.00	0.00
Sunco/Tasman								
Coleoptile spiralling	3DS	*TeloPA662*	44.1 (39.4–50.8)	−0.1*	9.3	0.0	0.0	–
	4BS	*wmc48a*	15.6 (3.0–40.0)	−0.1*	8.9	0.0	0.0	–
	4DS	*Rht-D1b*	1.8 (0.01–23.3)	0.1*	5.6	0.0	0.0	
	7AL	*wmc17*	115.6 (78.0–131.2)	−0.1*	4.2	0.1	−0.1	–
CD87/Katepwa								
Coleoptile spiralling	4BS	*fba363*	35.2 (34.2–37.0)	−0.2*	17.3	−0.01	0.01	–
Kukri/Janz								
Coleoptile spiraling	3DL	*gdm008A*	106.3 (87.1, 118.3)	0.2*	4.3	–	–	–
	4DS	*Rht-D1b*	0.01 (0, 22.3)	−0.2*	7.8	–	–	–

a Additive effect estimated as one-half the difference in homozygotes carrying either parental allele

[a] A positive additive effect indicates the first parent is contributing a positive allele and the second parent a negative allele

¶, *,** indicates marker effect is statistically different from zero at $P = 0.10, 0.05$ and 0.01

on group 2, 3, 4, 5, and 7 chromosomes in a spelt (*T. spelta* L.) × bread wheat population varying for coleoptile growth rates (St. Burgos et al. 2001). In other species, Redona and Mackill (1996) report two small QTL for coleoptile length in mapping studies conducted in rice while a single QTL for coleoptile length was identified on chromosome 5H in barley (Takahashi et al. 2001). Chromosomal substitution lines have been used to putatively identify wheat chromosomes associated with genotypic variation in coleoptile length (e.g. Allen and Vogel 1964; Matsui et al. 1998). However, the identification of many chromosomes associated with small changes in coleoptile length may reflect the large experiment-wise error rate and potentially high numbers of false positives associated with multiple range tests used in these studies.

A number of studies (e.g. Rebetzke et al. 2007; Takeda and Takahashi 1999) have demonstrated factors in addition to coleoptile length can impact on seedling establishment. For example, genotypic increases in coleoptile diameter were associated with greater shoot strength and the ability of pasture grass (Andrews et al. 1997) and sorghum (Mason et al. 1994) seedlings to push through hard and crusted soils. Genotypic variation exists for coleoptile diameter or CCSA in wheat (Marais and Botma 1987; Matsui et al. 2002) while we reported that large genotypic differences among F_1 progeny for CCSA in wheat was attributable to both additive and non-additive gene action (Rebetzke et al. 2004). The current study confirms the heritable basis for differences in CCSA in wheat, and the identification of QTL associated with progeny differences in the C/H population. The potential also exists to improve seedling emergence by the way a coleoptile moves through a soil. Coleoptile spiralling is a phenomenon we commonly observe and may assist seedlings in moving through a soil. Heritability for this trait was not high, and appears population-dependent. Despite this low heritability, QTL for coleoptile spiralling were identified mapping to *Rht-B1b* and *Rht-D1b* with both dwarfing alleles associated with reductions in coleoptile spiralling.

In conclusion, this study has identified genomic regions associated with genotypic variation in a range of coleoptile growth characteristics in bread wheat. Furthermore, a number of QTL were identified affecting coleoptile length independent of the GA-insensitive *Rht-B1b* and *Rht-D1b* dwarfing genes. Resulting coleoptile length QTL showed little interaction with temperature suggesting their robustness across environments. A number of these QTL appeared to co-locate across different populations suggesting their value in marker-aided selection. However, their relatively small genetic effect reduces their value particularly when the cost of screening coupled with their heritability should produce good genetic gain at low cost.

Acknowledgments The authors would like to thank Anke Lehmensiek (University of Southern Queensland), Lynette Rampling and Gulay Mann (CSIRO) for providing genetic maps.

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