Mapping Quantitative Trait Loci for Long Coleoptile in Chinese Wheat Landrace Wangshuibai

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ABSTRACT

Wheat (*Triticum aestivum* L.) cultivars with long coleoptiles can be sown deeply for better seedling establishment in drought environments. A population of 139 recombinant inbred lines (RILs) from a cross between a long-coleoptile Chinese landrace, Wangshuibai, and short-coleoptile U.S. wheat cultivar, Wheaton, were characterized for coleoptile length and plant height. Heritabilities for coleoptile length were high (h^2 > 0.82). Interval mapping identified six significant quantitative trait loci (QTL) on 1B, 3D, 4DS, 4DL, 5AS, and 5B for coleoptile length; four of them, on 3D, 4DS, 4DL, and 5AS, showed pleiotropic effects on plant height. One major QTL for long coleoptile was mapped on the locus *Rht-D1* (*Rht2*) for reduced height (*Rht*) on chromosome 4DS and explained up to 65% of phenotypic variation for coleoptile length. Another major QTL was located on 4DL and explained up to 33% of phenotypic variation for coleoptile length. Standard height allele *Rht-D1a* from Wangshuibai appeared to have an epistatic effect on the 4DL QTL for long coleoptile. Other QTL showed only a minor effect. Although *Rht-D1a* explained a major portion of genetic variation for long coleoptile in Wangshuibai, a combination of *Rht-D1a* in Wangshuibai with gibberellic acid (GA)–sensitive *Rht* genes for reduced wheat height from other sources should be able to select long coleoptiles, semidwarf cultivars in wheat breeding programs for which long coleoptile is a breeding objective.

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Abbreviations: AFLP, amplified fragment length polymorphism; CIM, composite interval mapping; GA, gibberellic acid; LOD, logarithm of odds; PCR, polymerase chain reaction; QTL, quantitative trait locus; RIL, recombinant inbred line; SIM, simple interval mapping; SSR, simple sequence repeat.

In low-precipitation dryland wheat-growing regions, such as the southern and central Great Plains of the United States, deep seed placement is essential for wheat (*Triticum aestivum* L.) to obtain sufficient moisture to initiate germination (Schillinger et al., 1998). Quick establishment of a high-quality plant stand with early growth of seedlings to shade soil surface is also important in reducing water losses from evaporation in these dryland regions. Poor seedling emergence and plant stand also decrease wheat's competitiveness with weeds (Huel and Hucl, 1996) and can result in reduced crop water use efficiency and, eventually, grain yield (López-Castañeda and Richards, 1994). The wheat coleoptile is a sheathlike structure that permits growth of the young stem and seedling leaves from the embryo to the soil surface. Wheat cultivars with a short coleoptile may have difficulty in emerging from deep sowing, particularly when planted deeper than 5 cm, which can result in poor seedling establishment (Rebetzke et al., 2007). Occasionally, deep-sown plants

Published in Crop Sci. 50:43–50 (2010).
doi: 10.2135/cropsci2009.02.0065

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do emerge, but they emerge much later and with poor early vigor (Rebetzke et al., 2007). Cultivars with a long coleoptile can be deeply sown into soil where the surface is dry but moist soil is deep underneath, to obtain a high emergence frequency (Rebetzke et al., 2005). A long coleoptile is also important for winter wheat that needs to be planted early in the fall as a dual-purpose crop that can be grazed by cattle in late fall and early spring of the next year as forage and harvested in the summer for grain in the Great Plains (Epplin et al., 2000).

Long coleoptile is a polygenic trait (Rebetzke et al., 1999, 2001). Twelve significant quantitative trait loci (QTL) were identified for coleoptile length in four wheat cultivars, including semidwarf cultivar Cranbrook (Rebetzke et al., 2001, 2007). Two major QTL were mapped to the *Rht-B1* locus on chromosome arm 4BS and the *Rht-D1* locus on chromosome arm 4DS and explained up to 49% of phenotypic variation for coleoptile length in four cultivars (Rebetzke et al., 2007). The other QTL showed minor effects in different genetic backgrounds compared with the *Rht-B1* and *Rht-D1* loci (Rebetzke et al., 2007; Spielmeyer et al., 2007).

Wangshuibai is a tall Chinese wheat landrace that showed a long coleoptile in a previous study (Bai et al., 2004). However, inheritance of the long coleoptile trait in Wangshuibai remains unknown. Objectives of this study were to identify genomic regions that contribute to increased coleoptile length in the mapping population developed between Wangshuibai and short-coleoptile U.S. wheat cultivar Wheaton and elaborate their relationship with plant height gene(s) that may be present in the parents.

MATERIALS AND METHODS

Plant Materials and Trait Measurement

A population of 139 F_6 recombinant inbred lines (RILs) was developed by single-seed descent from a cross between Wangshuibai and Wheaton. Coleoptile length was measured following the blotter-paper germination protocol of Hakizimana et al. (2000), with some modifications. In brief, a germination paper towel (no. 76 germination paper, Anchor Paper Co., St. Paul, MN) was laid on top of a piece of white wax paper (Anchor Paper Co., St. Paul, MN). Fifteen large uniform seeds per line were arranged in a straight line at 1-cm apart and about 7 cm from the bottom of the germination towel. The towel was folded up together with the wax paper at about 5 cm from the bottom, rolled loosely from left to right without disturbing the seed position in the paper, and secured with a rubber band in the middle of the paper roll. Wrapped towels were arranged vertically in a metal rack. The rack was set in a deep container half full of distilled water. When germination towels were thoroughly wet, the rack was moved out of the water container to drain off excessive water. The rack with seeds in germination towers was covered with a black plastic bag and placed in a cold room at 4°C for 2 d to interrupt dormancy. The rack with samples was incubated in a growth chamber without light for 7 d at 15°C with 100% relative humidity followed by 6 d at 20°C. The experiment was repeated once and arranged in a randomized complete block design, with three replicates in each experiment. Coleoptiles were gently straightened and measured with a ruler. Materials from each replicate were measured within 4 h to minimize measurement error among RILs.

Plant heights of the RILs were measured in two experiments conducted in a greenhouse at Kansas State University in 2003 and 2004. In each greenhouse experiment, six plants per RIL were transplanted into a 13 cm × 13 cm Tora pot (Hummert Int., St. Louis, MO) filled with Metro Mix 360 soil mix (Hummert Int., St. Louis, MO) after vernalization at 4°C in a growth chamber for 6 wk. Plants were grown on a greenhouse bench at 22°C day, 15°C night, with a supplemented daylight of 16 h. Each experiment was arranged in a randomized complete block design with two replicates. Plant height in centimeters was measured from the soil surface to the top of spikes of main tillers.

Molecular Marker Analysis

Genomic DNA of each RIL was isolated from leaf tissue by using the CTAB method (Saghai Maroof et al., 1984). For analysis of amplified fragment length polymorphism (AFLP), DNA restriction digestion (with *Pst*I and *Mse*I enzymes), adaptor ligation, and polymerase chain reaction (PCR) amplification were performed as described in Bai et al. (2003). Preamplification was conducted with a PstI primer (5′- ACTGCGTACATGCAG) and an MseI primer (5′-GATGAGTCCTGAGTAA). DNA from the parents, a bulk of the 10 RILs with the longest coleoptile, and a bulk of the 10 RILs with the shortest coleoptile were screened with 110 primer combinations between 15 IRD700/800-dye-labeled PstI primers and 29 MseI primers. The polymorphic primers were used to screen the population. The IRdye-labeled AFLPs were analyzed in a Li-COR 4200 DNA analyzer (Li-Cor, Inc., Lincoln, NE). The AFLP images were stored in an attached computer and scored with Saga^{GT} software (Li-Cor, Inc. Lincoln, NE) and visually rechecked for scoring errors.

About 1300 simple sequence repeats (SSRs) from different sources were screened for polymorphism between the parental lines, including WMC (Somers et al., 2004), GWM (Röder et al., 1998), BARC (Song et al., 2005), CFA and CFD (Guyomarc'h et al., 2002; Sourdille et al., 2003), and GDM (Pestsova et al., 2000). A total of 248 polymorphic SSR markers were obtained for genotyping of the RIL population. Polymerase chain reaction amplifications of the SSR markers were performed as described previously (Yu et al., 2006). The SSR fragments were analyzed in a Li-Cor 4200 DNA analyzer under the same conditions as described for AFLP. Simple sequence repeat data were scored by visual inspection and rechecked twice for scoring errors.

Data Analysis

Variance analyses on coleoptile length and plant height were performed by using the GLM procedure of SAS software (SAS Institute Inc, Cary, NC). Broad-sense heritabilities and their 90% confidence intervals were estimated on the basis of line means according to Shen et al. (2003) and Knapp et al. (1985), respectively. The equation used to calculate heritability was: $H^2 = \delta_g^2/(\delta_g^2 + \delta_{g\times e}^2 / r + \delta_e^2 / n)$ based on entry means,

where δ^2_g was genetic variance, $\delta^2_{g\times e}$ was RILs × environment interactions, δ^2_e was error, r was the number of experiments, and n was the number of replicates in each experiment.

Linkage maps of AFLP and SSR were constructed with the Kosambi mapping function (Kosambi, 1944) by using JoinMap 3.0 (van Ooijen and Voorips, 2001). A minimum logarithm of odds (LOD) threshold of 3 was used to determine linkage groups. The QTL analyses were conducted separately for coleoptile length and plant height. Both simple interval mapping (SIM) and composite interval mapping (CIM) were performed on the line means of individual experiments and on the line means over two experiments by using WinQTLCart 2.5 (Wang et al., 2006). Five markers and a 10-cM window size were used as a background control in CIM analysis. Permutation tests were performed to estimate appropriate thresholds to claim significant QTL for both SIM and CIM (Churchill and Doerge, 1994). On the basis of 1000 permutations, a LOD threshold of 2.0 was identified for a significant QTL in both SIM and CIM. A multiple regression model was used to estimate the total phenotypic variation explained by all the QTL together detected for the trait. This model included one single marker with the largest determination coefficient (R^2) value for each QTL detected through interval mapping. Regression analyses were performed with the SAS REG procedure. Analysis of variance (PROC GLM) and Duncan's critical range test were used for analyses of the genotypic effects of QTL-linked markers on a trait. Chromosome regions corresponding to specific QTL were determined with the 1-LOD support interval method (Lander and Botstein, 1989).

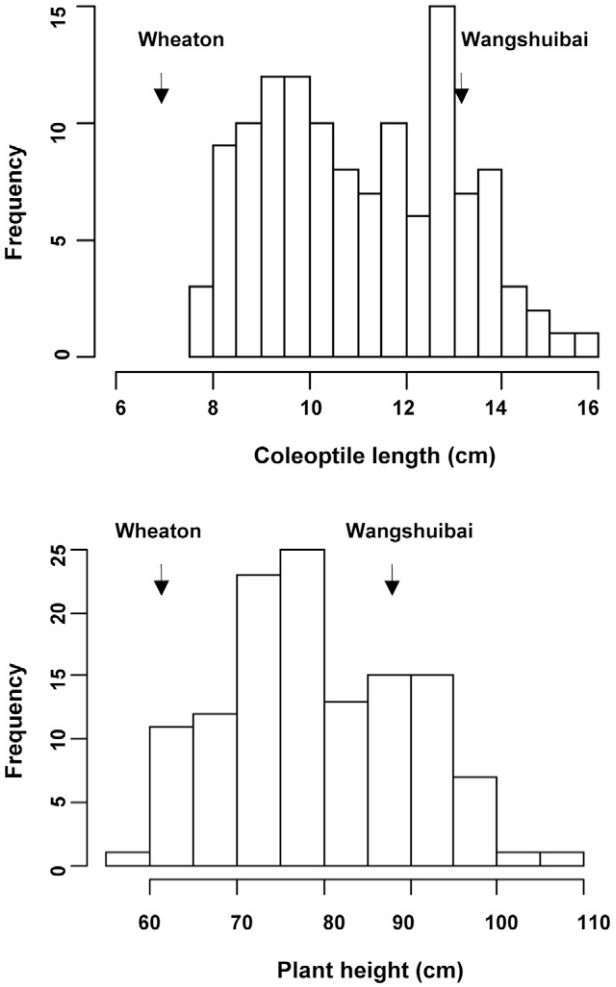

Figure 1. Frequency distribution of 139 recombinant inbred lines for mean values of coleoptile length and plant height. Arrows indicate values of the parental lines.

RESULTS

Coleoptile Length and Plant Height in Parents and Recombinant Inbred Lines

Significant contrasts in plant height and coleoptile length were observed between the two parents. On average, Wangshuibai had a coleoptile length of 13.2 cm and plant height of 88 cm, whereas Wheaton had 6.7 cm of coleoptile and a 62-cm plant height under greenhouse conditions. Frequency distributions of the RILs for mean coleoptile length and plant height exhibited continuous variation (Fig. 1). Significant transgressive segregation was observed toward long coleoptile and both tall and short plant status. Mean coleoptile lengths of RILs ranged from 7.7 to 15.9 cm, and mean plant heights of RILs ranged from 58 to 106 cm over the two experiments. The grand mean and CV were 11.1 cm and 17.8% for coleoptile length, and 79.3 cm and 13.0% for plant height in two experiments, respectively.

The RILs demonstrated significant variations in coleoptile length and plant height with high heritabilities (h^2) (Table 1). High correlations were observed between the two experiments for both coleoptile length (r = 0.80, $P < 0.01$) and plant height (r = 0.70, $P < 0.01$) in the RIL population (Table 2). Results suggest both traits are highly heritable. However, significant variations were observed between experiments (Table 1), and the interaction between experiment and genotype was also significant for both traits, suggesting the environment also affected the two traits. The correlation coefficient between coleoptile length and plant height

Table 1. Analysis of variance for coleoptile length, plant height, and the broad-sense heritabilities of the two traits estimated for 2003 and 2004 experiments.

Trait	Variable	df	Mean square	*F* value	*P* value	Heritability 90% CI†
Coleoptile length						0.89 (0.86–0.91)
	Experiments	1	198.3	147.8	<0.0001	
	RILs‡	137	21.5	16.1	<0.0001	
	RILs × experiments	137	2.4	1.8	<0.0001	
	Error	550	1.3			
Plant height						0.82 (0.77–0.86)
	Experiments	1	92334.2	1748.4	<0.0001	
	RILs	137	633.2	12.0	<0.0001	
	RILs × experiments	137	117.0	2.2	<0.0001	
	Error	550	52.8			

†CI, confidence interval.

‡RIL, recombinant inbred line.

Table 2. Correlations among coleoptile length and plant height from 2003 and 2004 experiments.

Trait†	2003 clp	2004 clp	2003 hgt
2004 clp	0.80***		
2003 hgt	0.50***	0.53***	
2004 hgt	0.59***	0.66***	0.70***

***Significant at $P < 0.0001$.

†clp, coleoptile length; hgt, plant height.

was significant ($r > 0.50$, $P < 0.01$) but lower than those between experiments for each trait, suggesting that some, but not all, QTL might be common for both traits in the population.

QTL for Coleoptile Length

Coleoptile length was evaluated in two independent experiments. Interval mapping of coleoptile length from a single experiment or from combined data over the two experiments detected six QTL on chromosomes 1B, 5AS, 5B, 3D, 4DS, and 4DL (Fig. 2). All six were significant in both experiments in SIM. The two QTL on 4DS and 4DL had a larger effect on coleoptile length than the others and explained up to 65 and 33%, respectively, of phenotypic variation of coleoptile length (Table 3). The remaining four QTL had relatively minor effects on coleoptile length and explained 7 to 21% of phenotypic variation in SIM. Wangshuibai carried QTL alleles for long coleoptile on 5AS, 4DS, and 4DL and for short coleoptile on 1B, 3D, and 5B. The 3D and 5B QTL were not significant in CIM. All these QTL together explained up to 72% of phenotypic variation of coleoptile length in the Wangshuibai/Wheaton population. The difference between the joint effects and summarized effects of all QTL is likely due to epistatic interactions among these QTL.

QTL for Plant Height

Interval mapping of plant height with data from a single experiment or combined means from two experiments detected four QTL on 5AS, 3D, 4DS, and 4DL (Fig. 2). Similar to coleoptile length, the QTL on 4DS and 4DL showed larger effects on plant height in both experiments and explained up to 59 and 40% of phenotypic variation, respectively (Table 3). The minor QTL on 5AS and 3D explained 8 to 19% of the phenotypic variation of plant height (Table 3) and were detected only by SIM. These QTL jointly explained up to 57% of phenotypic variation of plant height. In Wangshuibai, the QTL on 5AS, 4DS, and 4DL appeared to be responsible for increased plant height, whereas the QTL on 3D was for reduced plant height.

Interaction between QTL with a Major Effect on Long Coleoptile

Two markers linked to QTL that associated with both coleoptile length and plant height traits on chromosomes 4DS and 4DL were chosen for use in evaluating their possible interaction by comparing phenotypic data of the two traits between two allelic groups of RILs at the two marker loci (Table 4). The Wangshuibai allele *Rht-D1a* demonstrated a large positive effect on both traits. Mean coleoptile length and plant height of the RIL group with *Rht-D1a* were 3-cm longer and 15-cm taller than those of the RIL group with *Rht-D1b* allele from Wheaton. The effect of 4DL QTL was slightly smaller than that of 4DS on both traits. However, plants having both Wangshuibai alleles at two QTL on 4D did not show a significant increase in coleoptile length and plant height compared with plants having only *Rht-D1b*.

DISCUSSION

A blotter-paper germination protocol (Dilday et al., 1990) has been successfully used for phenotyping coleoptile length in wheat and rice (*Oryza sativa* L.) (Dilday et al., 1990, Hakizimana et al., 2000; Pereira et al., 2002; Bai et al., 2004). This protocol geminates seeds under dark, moist conditions that allow maximum coleoptile growth. In this study, the protocol clearly differentiated two parents with a great contrast, and high heritability was obtained for long coleoptile. In addition, the major coleoptile growth QTL detected in this study, *Rht-D1*, was also detected in previous studies that used a deep-soil protocol to evaluate coleoptile length (Rebetzke et al., 2001; Spielmeyer et al., 2007). Therefore, the protocol for coleoptile length evaluation used in this study is suitable for mapping coleoptile growth QTL.

Wheat coleoptile length is a quantitative trait that may be controlled by both major and minor genes (Rebetzke et al., 2007). Inheritance of wheat coleoptile length has been characterized in only a few sources. Several mapping works, mainly from CSIRO Plant Industry, Canberra, Australia, identified QTL on chromosomes 6A (Spielmeyer et al., 2007), 4B (Rebetzke et al., 2001), 2B, 2D, 4A, 5D, and 6B (Rebetzke et al., 2007) of Australian wheat cultivars by using a deep-soil germination method. Strong negative correlations were established between the presence of dwarfing genes *Rht-B1* or *Rht-D1* and long coleoptile (Rebetzke et al., 2001, 2007). The QTL with large effects on coleoptile growth were directly mapped to the *Rht-B1* on 4BS or *Rht-D1* on 4DS dwarfing gene loci in some cultivars (Whan, 1976; Rebetzke et al., 1999, 2001, 2007). Using gene markers for *Rht-D1b* (Ellis et al., 2002), we confirmed that long coleoptile parent Wangshuibai carries *Rht-B1a* and *Rht-D1a* for standard height and short coleoptile parent Wheaton has *Rht-D1b* for reduced height. The RIL population of Wangshuibai–Wheaton was phenotyped for both coleoptile length and plant height. Allelic variation of two 4D chromosome regions explained most of the phenotypic variation for both coleoptile length and plant height. The two QTL were consistent over two experiments. The *Rht-D1* locus on 4DS showed a major effect on both coleoptile length and plant height in the Wangshuibai–Wheaton population. This QTL

identified on 4DS appeared to have a larger effect than *Rht-B1b* (Rebetzke et al., 2001), which supports results from a previous study (Rebetzke et al., 2007).

Another QTL associated with SSR marker *Xgwm194* on the long arm of 4D also significantly affected both traits. This QTL was located at about 20 cM from the *Rht-D1* on

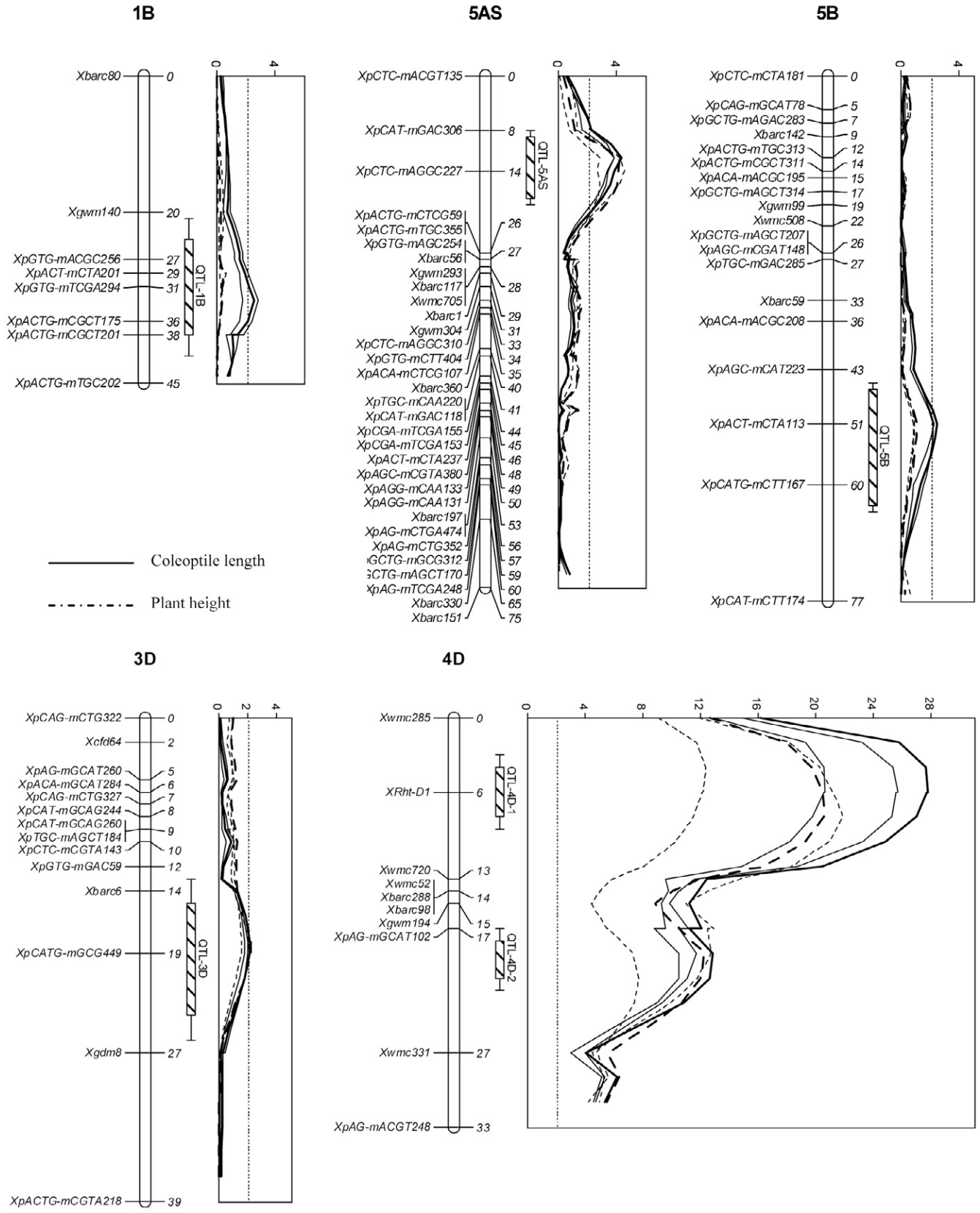

Figure 2. Log likelihood ratio (LOD) contours obtained from interval mapping of the Wangshuibai × Wheaton population that show quantitative trait loci (QTL) locations for wheat (*Triticum aestivum* L.) coleoptile length and plant height traits. Bars indicate QTL locations, and bar lengths represent 1-LOD support intervals.

Table 3. Estimates of the phenotypic effects of quantitative trait loci on coleoptile length and plant height in the RI population of Wangshuibai/Wheaton.

Method†	Trait‡	Chrom.	Marker	Source§	2003		2004		Mean	
					LOD¶	R^2	LOD	R^2	LOD	R^2
IM	Clp									
		1B	*XpGTG-mTCGA294*	WTN	1.8	0.07	2.9	0.12	2.5	0.11
		3D	*XpCATG-mGCG449*	WTN	1.8	0.07	2.0	0.07	2.1	0.08
		4DS	*XRht-D1*	WSB	20.7	0.55	25.8	0.63	27.8	0.65
		4DL	*Xgwm194*	WSB	5.2	0.29	5.4	0.27	6.2	0.33
		5AS	*XpCAT-mGAC306*	WSB	3.8	0.21	3.8	0.19	4.3	0.22
		5B	*XpACT-mCTA113*	WTN	2.2	0.11	2.3	0.12	2.5	0.13
Total						0.62		0.69		0.72
	Hgt									
		3D	*XpCATG-mGCG449*	WTN			2.1	0.08	2.3	0.08
		4DS	*XRht-D1*	WSB	12.4	0.41	22	0.59	20.6	0.56
		4DL	*Xgwm194*	WSB	5.6	0.28	5.3	0.23	6.4	0.29
		5AS	*XpCAT-mGAC306*	WSB	4.5	0.19	2.9	0.15	4.2	0.23
Total						0.42		0.56		0.57
CIM	Clp									
		1B	*XpGTG-mTCGA294*	WTN			2.7	0.10	2.3	0.08
		4DS	*XRht-D1*	WSB	21.6	0.50	28.2	0.56	30.9	0.55
		4DL	*Xgwm194*	WSB	10.4	0.29	10.7	0.29	11.6	0.30
		5AS	*XpCAT-mGAC306*	WSB					2.1	0.06
Total						0.59		0.66		0.69
	Hgt									
		4DS	*XRht-D1*	WSB	10.1	0.25	23.5	0.58	19.8	0.42
		4DL	*Xgwm194*	WSB	4.2	0.12	13.6	0.40	10.0	0.26
		5AS	*XpCAT-mGAC306*	WSB	2.6	0.09				
Total						0.38		0.56		0.54

†IM, interval mapping; CIM, composite interval mapping.

‡Clp, coleoptile length; Hgt, plant height.

§WSB, Wangshuibai; WTN, Wheaton.

¶LOD, logarithm of odds.

4DS, and an obvious valley was observed between the two QTL. Genetic effects on both coleoptile length and plant height of the 4DL QTL were smaller than those of *Rht-B1* QTL but repeatable across the experiments. In a previous study, Rebetzke et al. (2001) reported a second QTL associated with coleoptile length on chromosome 4BL, in addition to *Rht-B1* on 4BS. The 4BL QTL showed a smaller effect (R^2 = 27%) than the *Rht-B1* locus (R^2 = 45%) for coleoptile length. Similarly, the 4DL QTL identified in this study explained up to 33% of phenotypic variation for long coleoptile, whereas the *Rht-D1* locus accounted for up to 55% of phenotypic variance. The 4DL QTL identified in this study has not been reported in previous studies and may be a homeologous locus to the one on 4BL. Nevertheless, analysis on combined effects of the two QTL on 4D indicated that addition of the 4DL QTL to the *Rht-D1* gene in wheat did not increase the effect of the *Rht-D1* gene on either coleoptile length or plant height. The *Rht-D1* gene may have an epistatic effect on the expression of 4DL QTL, and presence of the *Rht-D1* gene may repress expression of the QTL on 4DL. Therefore, pyramiding the two QTL on 4DL may not be an effective approach to increase coleoptile length.

Minor QTL for coleoptile length were identified on several wheat chromosomes (Rebetzke et al., 2001, 2007; Spielmeyer et al., 2007). Chromosome regions in 1A, 2B, 2D, 4A, 5A, 5B, 5D, 6A, and 6B have all been associated with coleoptile length (Matsui et al., 1998; Rebetzke et al., 2001, 2007). Genetic variations explained by these individual QTL were usually less than 10%, which was much smaller than variations of QTL on chromosomes 4B and 4D. Some of the reported QTL also showed pleiotropic effects on plant height, but others did not (Rebetzke et al., 2001, 2007; Spielmeyer et al., 2007). In this study, four minor QTL for wheat coleoptile length were identified on 1B, 3D, 5AS, and 5B by interval mapping and explained 7 to 22% of phenotypic variance of coleoptile length. Two (3D and 5AS) also showed a significant effect on plant height. Quantitative trait loci on chromosome 1B and 5AS were significant in CIM; hence, they are likely real QTL for coleoptile length. The QTL on 1B has not been reported before and did not affect plant height; thus, it may be useful for improving long coleoptiles with increased plant height. Nevertheless, validation of genetic effects of these QTL in other genetic backgrounds may be essential before they can be used to select for lines having long coleoptiles in breeding programs.

Expression of the genes for plant height may be affected by other genetic and environmental factors. Three loci on 5AL, *VRN-A1*, *Q*, and *Qt.ocs-5A.1* have been reported to reduce plant height (Kato et al., 1999). However, promotion of vernalization appeared to reduce this dwarfing effect (Kato et al., 1999). Plant height measured in different field conditions may be ideal for identification of all possible QTL for plant height. In this study, two parents used were spring type (Wheaton) and facultative type (Wangshuibai) and could not survive winter weather in the field in Kansas. Therefore, plant height was evaluated in the greenhouse conditions. A high correlation in plant height between the two experiments indicates that the plant height data used in this study are reliable. By providing vernalization and long photoperiods for plant growth, we detected four QTL for

plant height, and none of them has been mapped to the genomic regions that harbor known QTL or genes for photoperiod and vernalization responses. The results suggest that vernalization and long photoperiods might eliminate the potential effects of photoperiod and growth habit genes on plant height.

So far, the QTL for coleoptile length with the largest genetic effects were gibberellic acid (GA)–insensitive plant-height-reducing genes *Rht-B1* and *Rht-D1* on homeologous chromosomes 4BS and 4DS. The shorter plant status controlled by semidwarf genes *Rht-B1b* and *Rht-D1b* arises through their influence on GA-insensitivity of leaf and stem tissues (Keyes et al., 1989; Hoogendoorn et al., 1990). These height-reducing genes encode proteins involved in GA signal transduction (Peng et al., 1999). By conferring insensitivity to GA, these genes can significantly reduce plant height and also coleoptile length and seedling leaf area (Whan 1976; Rebetzke et al., 2001). However, in GA-sensitive wheats, plant height and coleoptile length appear to be largely under independent genetic control (Rebetzke et al., 1999). When the standard plant height (*rht*) gene is combined with GA-sensitive *Rht8/9* semidwarf genes, wheat varieties produced about 25 to 40% longer coleoptiles than semidwarf varieties containing GA-insensitive *Rht-B1b* and *Rht-D1b* (Rebetzke et al., 1999; Botwright et al. (2001). In this study, three long-coleoptile QTL were mapped in Wangshuibai. Obviously, the major QTL for long coleoptile length on 4DS had the pleiotropic effect on plant height. Plants with long coleoptile controlled by this locus also have standard plant height. To use this QTL as a long coleoptile donor, cultivars that carry either *Rht-B1b* or *Rht-D1b* for reduced height should not be used as another parent in crosses. Gibberellic acid–sensitive *Rht* genes such as *Rht4*, *Rht5*, *Rht8*, *Rht9, Rht12*, *or Rht13* may facilitate plant height reduction (Rebetzke et al., 2007). Because height and coleoptile length are under independent genetic control in GA-sensitive wheat (Rebetzke et al., 2007), it is possible to select shorter-height, longer-coleoptile wheat under GA-sensitive *Rht* genes (Rebetzke et al., 1999).

Table 4. Individual and combined phenotypic effects of marker loci linked to quantitative trait loci on 4DS and 4DL for wheat (*Triticum aestivum* L.) coleoptile length and plant height.

Locus	Genotype	Coleoptile length		Plant height		Two years combined data	
		2003	2004	2003	2004	Clp†	Hgt‡
		cm					
XRht-D1 (4DS)	A§ (L)	11.8a¶	12.9a	74.2a	97.3a	12.5a	85.7a
	B (S)	8.9b	9.8b	61.7b	80.6b	9.4b	71.1b
	dif	2.9	3.1	12.5	16.7	3.1	14.6
Xgwm194 (4DL)	A (L)	11.6a	12.7a	73.1a	96.4a	12.2a	84.7a
	B (S)	9.1b	10.1b	63.4b	81.7b	9.7b	72.5b
	dif	2.5	2.6	9.7	14.7	2.5	12.2
XRht-D1/Xgwm194	A/A (L/L)	11.9a	13.1a	73.7a	97.8a	12.6a	85.7a
	A/B (L/S)	11.1a	12.6a	75.6a	92.2a	11.9a	83.9a
	B/A (S/L)	9.1b	9.6b	61.9b	82.5b	9.4b	72.2b
	B/B (S/S)	8.8b	9.8b	61.8b	80.2b	9.4b	70.9b
	dif	3.1	3.5	14.8	17.6	3.2	14.8

†Clp, coleoptile length.

‡Hgt, plant height.

§A = 'Wangshuibai', B = 'Wheaton', and dif = phenotypic difference between genotype means. A and B are long (L) and short (S) alleles of the linked markers.

¶Different letter indicates significant difference at $P < 0.05$ in Duncan's critical range test.

Acknowledgments

We gratefully acknowledge the contributions of Dr. Wen-Chun Zhou and Frederic Kolb, University of Illinois, for RIL population. Research is partly funded by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, Coordinated Agricultural Project grant number 2006-55606-16629.

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