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Genetic relationship between lodging and lodging components in barley (*Hordeum vulgare*) based on unconditional and conditional quantitative trait locus analyses

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ABSTRACT. Lodging (LD) is a major constraint limiting the yield and forage quality of barley. Detailed analyses of LD component (LDC) traits were conducted using 246 F_2 plants generated from a cross between cultivars ZQ320 and 1277. Genetic relationships between LD and LDC were evaluated by unconditional and conditional quantitative trait locus (QTL) mapping with 117 simple sequence repeat markers. Ultimately, 53 unconditional QTL related to LD were identified on seven barley chromosomes. Up to 15 QTL accounted for over 10% of the phenotypic variation, and up to 20 QTL for culm strength

were detected. Six QTL with pleiotropic effects showing significant negative correlations with LD were found between markers Bmag353 and GBM1482 on chromosome 4H. These alleles and alleles of QTL for wall thickness, culm strength, plant height, and plant weight originated from ZQ320. Conditional mapping identified 96 additional QTL for LD. Conditional QTL analysis demonstrated that plant height, plant height center of gravity, and length of the sixth internode had the greatest contribution to LD, whereas culm strength and length of the fourth internode, and culm strength of the second internode were the key factors for LD-resistant. Therefore, lodging resistance in barley can be improved based on selection of alleles affecting culm strength, wall thickness, plant height, and plant weight. The conditional QTL mapping method can be used to evaluate possible genetic relationships between LD and LDC while efficiently and precisely determining counteracting QTL, which will help in understanding the genetic basis of LD in barley.

Key words: Barley; *Hordeum vulgare* L.; Genetic relationship; Lodging; Lodging components; Unconditional and conditional QTL analysis

INTRODUCTION

Barley (*Hordeum vulgare* L.) is an ancient and important cereal grain crop that is used as foodstuff, animal feed, and as raw material for wine production (Newman and Newman, 2006). The need for barley with higher yield and functional foodstuff quality has become more urgent in recent years. However, lodging (LD), which involves permanent displacement of culms from their upright position, is a major constraint for breeding high-yield barley. LD is a common problem in most cereals, and in various other crops, such as barley, wheat, oats, corn, grain sorghum, soybean, tomato, tobacco, and rice. LD reduces barley yield by 28-65%, reduces grain quality, and directly affects the forage yield and malt quality (Stanca et al., 1979; Jedel and Helm, 1991). Thus, lodging resistance has become one of the major goals in crop breeding, particularly in barley. Unfortunately, LD is a complex trait related to several crop characteristics caused by a combination of complex environmental factors such as wind, rain, and hail. In addition, other factors also induce LD, such as high nitrogen fertilization, high sowing density, and drought (Sanchez et al., 2002). Thus, LD cannot be assessed based on only phenotype. Most researchers determined the correlation between morphological traits and LD resistance by observing naturally or artificially induced LD. These researchers generally focused on components of the plant and their histologic distributions (Dunn and Briggs, 1989). Subsequent studies aimed to establish mechanical models for lodging resistance based on physical formulae for barley (Berry et al., 2006) and wheat. Previous studies conducted have identified morphologic traits that are correlated with lodging, which could be used as indirect selection parameters. However, because LD is a quantitative trait that is highly affected by environmental conditions, no single trait or group of traits has proven to be a reliable index for lodging resistance (Keller et al., 1999).

The development of molecular genetic maps and DNA markers facilitated the discovery of quantitative trait loci (QTL) for complex traits, enabling the dissection of the genetic basis

of relationships among traits (Ishimaru et al., 2001). Marker-assisted selection has potential to improve the efficiency of selection for lodging resistance in breeding programs. Thus, QTL studies on the correlation between LD and other traits have been performed in several cereal crops, such as soybean, rice (Kashiwagi et al., 2008; Zhu et al., 2008), wheat (Keller et al., 1999; Zuber et al., 1999; Börner et al., 2002; Kelbert et al., 2004; Verma et al., 2005), maize (Flint-Garcia et al., 2003), and field pea. However, little information related to barley LD has been reported to date (Sameri et al., 2009). Plant height was considered to be the main and most favorable target trait for improving lodging resistance in many previous studies. Furthermore, semi-dwarfing and dwarfing genes are known to reduce barley lodging damage, and QTL were found to increase lodging resistance by reducing plant height (Sameri et al., 2009). However, due to their low plant height, farmers discriminate against semi-dwarf and dwarf barley strains where it is used as animal feed (Kandemir, 2004). Furthermore, reducing plant height to improve lodging resistance may also reduce its photosynthetic capability (Kumar et al., 1999), which then reduces the plants' biomass. Hence, finding more QTL related to other traits, such as diameter, wall thickness, ratio of wall thickness to diameter, and culm strength of internodes among others, is necessary for further improving lodging resistance without reducing plant height.

Although complex relationships between barley LD and several lodging components (LDC) have been identified through conventional statistical genetic analyses, these results can only reveal pairwise correlations between traits under the potential interference of the effects of other traits. Therefore, clarifying the actual relationship between LD and each LDC using conventional QTL analysis (unconditional QTL method) is very difficult. In addition, determining the major component that affects LD at the QTL level remains a big challenge. Recently, a method called multivariable conditional QTL analysis was proposed for analyzing the contributions of component traits to a complex trait and for investigating the genetic relationship between two traits at the QTL level (Wen and Zhu, 2005). This method could be used to determine genetic interrelationships between traits at the level of individual QTL, and to find additional QTL that are undetectable with unconditional mapping (Li et al., 2008). This method has been successfully used to analyze QTL by Guo et al. (2005), Zhao et al. (2006), Liu et al. (2008), and Cui et al. (2011).

To date, few studies have been published relating barley QTL to LD, and no study has documented the relationship between LD and its components at the QTL level. Considering the current conflict between decreased barley yields due to serious lodging and increased demand for barley, identifications of more useful genes and QTL related to the molecular mechanisms regulating lodging are urgently needed. This understanding will provide a theoretical basis for breeding programs designed to increase lodging resistance based on selecting desirable cultivars for attaining desired yield. In the present study, both unconditional and conditional mapping methods for multivariable conditional analysis were utilized. The objectives of this study were as follows: 1) to identify all of the genetic factors that affect barley lodging, and 2) to specify the genetic relationships between lodging and its components at the QTL level.

MATERIAL AND METHODS

Plant materials and field experiments

An F_2 population of 246 individuals was established by self-pollinating an F_1 hybrid

from a cross between cultivars ZQ320 and 1277, which was used for linkage map construction and QTL analysis. ZQ320 is one of the cultivars favored by Tibetans in China. Although it has a high plant height, it also has serious lodging. By contrast, 1277 is a semi-dwarf commercial cultivar with excellent lodging resistance. The 246 seeds of the F_2 population, along with parent cultivars, were sown in a field at the China West Normal University (30° 49' N, 106° 3' E) on November 4, 2009. Each row contained 10 plants that were spaced 30 x 15 cm apart. Appropriate doses of fertilizer were administered after sowing, and pesticides were applied during appropriate stages of growth.

Phenotypic evaluation of agronomic traits

To evaluate the agronomic performance related to lodging, 32 traits (LD and 31 LDCs) were measured at the waxy stage of barley. The 31 LDCs examined were divided into four groups. The first group consisted of nine traits associated with plant height (PH): length of the first (LFN), second (LSN), third (LTN), fourth (LFON), fifth (LFIN), and sixth (LSIN) internodes, length of the panicle (LP), plant height center of gravity (PHCG), and PH. PHCG was measured from the soil surface to the tip of the tallest panicle (awns excluded) at the mature stage. PH was calculated as the total of the six internodes and LP (awns excluded). This method was different from those used in some previous studies because barley bends at the waxy stage and suffered from lodging, which leads to unequal PH and PHCG. The second group consisted of 18 traits associated with culm diameter, wall thickness, and the ratio of wall thickness to diameter of six internodes: diameter of first (DFN), second (DSN), third (DTN), fourth (DFON), fifth (DFIN), and sixth (DSIN) internodes; wall thickness of the first (WTFN), second (WTSN), third (WTTN), fourth (WTFON), fifth (WTFIN), and sixth (WTSIN) internodes. These traits were measured using a dial caliper (Kori Seiki Mfg. Co. Ltd., Japan) according to a previously described method (Inoue et al., 2004). The third group consisted of six traits associated with the culm strength of internodes one to six: CSFN, CSSN, CSTN, CSFON, CSFIN, and CSSIN. These traits were measured using a plant culm strength meter (YYD-IA, China). The fourth group included four traits: panicle weight (PLW), plant weight (PAW), length of flag leaf (LFL), and width of flag leaf (WFL).

LD was assessed visually using an arbitrary score. Three lodging levels were chosen according to the lodging status of the parents and the F_2 population: plants leaning 5° to 45° were scored as A, plants lodged at 45° to 85° were scored as B, and plants lodged flat were scored as C (Keller et al., 1999; Verma et al., 2005; Eduardo, 2006).

Molecular marker analysis and linkage map construction

Total genomic DNA was extracted from the young leaves of each F_2 plant using a modification of the cetyltrimethylammonium bromide method (Paterson et al., 1993). Up to 580 pairs of simple sequence repeat (SSR) primers with averages ranging from 1H to 7H were used to identify polymorphic SSR primers between ZQ320 and 1277. DNA from the parents and the 246 progeny were then screened for polymorphisms using polymerase chain reaction (PCR). The PCR amplification procedure for SSR markers was performed as described in Sameri et al. (2009). The amplified products were electrophoresed on 8 to 10% non-denaturing polyacrylamide gels. Finally, the gels were visualized through improved silver staining (Gao et al., 2009).

A total of 580 SSR primer pairs chosen from GrainGenes 2.0 (http://wheat.pw.usda.gov/GG2/index.shtml), which covers the entire barley genome, were screened for polymorphisms between parents. Of all primer pairs, 117 exhibited polymorphisms and were used to construct linkage maps. Markers were linearly aligned in each linkage group using a logarithm of odds (LOD) score of 3.0 via Joinmap 4.0. The recombination frequency was converted to genetic distance (centimorgans, cM) using Kosambi's (1943) mapping function.

Data analysis and QTL mapping

Basic statistical analysis and distribution histograms were implemented using the SPSS 15.0 software (SPSS, Chicago, IL, USA). Broad sense heritability (H^2) was estimated for all measured traits using the following formula: $H^2 = V_G/V_P$, where V_G is the genotypic variance, V_P ($V_P = V_G + V_E$) is the phenotypic variance of the F_2 measured traits, and V_E is the environmental variance estimated from the mean of the trait variances of the parents using the formula $V_E = (V_{P1} + V_{P2}) / 2$.

Conditional genetic analysis was conducted based on the phenotypic values of LD conditioned by each of the other 31 LDCs, which were obtained using the method described by Zhu (1995). Conditional phenotypic values y(LD|LDC) indicate LD values without the influence of LDC. The observed phenotypic values of the 32 traits obtained were used for unconditional QTL analysis and the calculated conditional phenotypic values [y(LD|LDC)] were used for conditional QTL mapping analysis. Composite interval mapping was used for unconditional and conditional QTL analyses in WinQTLcart 2.5. The walking speed for all QTL was 1.0 cM and LODs were determined by 1000 rounds of permutations.

RESULTS

Phenotypic variation in traits and correlations with lodging

Significant differences between ZQ320 and 1277 were detected for all measured traits, except for LTN, DFIN, and WTFIN (Table 1). The PH and PHCG of ZQ320 were higher than those of 1277, which is consistent with its relatively longer internodes. The 1277 cultivar had a higher wall thickness to diameter ratio than ZQ320, which resulted from the relatively smaller internode diameter but high internode wall thickness of 1277. The culm strength of ZQ320 was lower than that of 1277. Phenotypic variations between populations showed tremendous transgressive segregation for all traits. The frequency distribution of the traits was approximately continuous and followed a normal distribution (Figure 1). This result indicated that the traits were typical quantitative traits controlled by a few minor genes, and that the data were suitable for QTL analysis. All measured traits were highly heritable except for LFON and LFIN, with an average heritability of 65.70, ranging from 20.42% for LFIN to 95.81% for WTSN (Table 1).

Pairwise correlation analysis of the 32 traits showed that most of the traits were correlated with each other (**Supplementary Table**). For example, a significant positive correlation was observed between PH and PHC, especially between PH/PHCG and LFIN ($r = 0.607/0.622$, $P < 0.001$) and between PH/PHCG and LSIN ($r = 0.705/0.687$, $P < 0.001$). PLW was significantly correlated with the diameters and wall thicknesses of the six internodes. All

diameters were significantly correlated with the culm strength of the six internodes. Both positive and negative correlations were observed between LD and LDC. LD was positively correlated with plant height components (the first group traits), which was particularly significant among PH, LTN, LFON, and LFIN. The negative influence on LD was mostly contributed by internode diameter, wall thickness, ratio of wall thickness to diameter, and culm strength. Among these traits, WTSN, WTTN, WTFON, WTFIN, WTSIN, CSSN, CSFIN, and CSSIN were negatively correlated with LD, which indicated that these traits could be used for selecting indices of lodging resistance in barley.

Table 1. Summary statistics for lodging component traits related to lodging resistance in the 246 ZQ320/1277 F_2 populations.

Traits	ZQ320 (P_1)	1277 (P_2)	P_1-P_2	F_2 population			
	(Means ± SD)	(Means ± SD)		(Means ± SD)	Range	CV (%)	H^2 (%)
PHCG (cm)	98.25 ± 9.19	80.19 ± 6.46	18.06**	97.77 ± 11.35	65.70-122.80	11.61	51.04
PH (cm)	106.41 ± 6.46	80.19 ± 6.66	26.22**	100.27 ± 11.45	65.20-131.00	11.42	67.19
PLW (g)	26.90 ± 2.55	19.83 ± 1.91	7.07**	23.35 ± 6.93	7.10-48.00	29.69	89.43
LP (cm)	11.15 ± 0.74	10.02 ± 0.66	1.13**	10.34 ± 1.56	4.80-14.50	15.06	79.83
PAW (g)	7.65 ± 0.90	5.76 ± 0.56	1.89**	6.48 ± 2.25	4.20-9.50	34.68	88.76
LFL (cm)	20.73 ± 1.76	14.95 ± 1.91	5.78**	22.94 ± 4.48	10.40-33.50	19.53	83.24
WFL (cm)	2.42 ± 0.11	1.62 ± 0.22	0.80**	2.22 ± 0.38	1.10-3.20	17.32	80.18
LFN (cm)	2.93 ± 0.88	1.38 ± 0.75	1.56**	5.99 ± 1.95	1.05-5.50	32.59	82.56
LSN (cm)	10.45 ± 0.98	6.40 ± 0.65	4.05**	9.47 ± 2.14	3.60-13.40	22.55	84.79
LTN (cm)	12.16 ± 1.36	9.37 ± 0.82	2.79	12.15 ± 1.83	5.90-16.70	15.09	60.47
LFON (cm)	12.89 ± 2.58	11.72 ± 1.80	1.17**	13.53 ± 1.99	5.32-20.70	14.70	35.43
LFIN (cm)	35.38 ± 3.93	17.02 ± 1.23	2.57**	17.98 ± 2.27	11.00-23.40	12.62	20.42
LSIN (cm)	35.38 ± 3.93	27.08 ± 3.49	8.30**	32.75 ± 6.45	12.60-44.80	19.70	66.83
DFN (mm)	6.56 ± 0.56	5.32 ± 0.85	1.25**	6.43 ± 1.06	4.13-9.08	16.41	53.80
DSN (mm)	7.31 ± 0.45	5.98 ± 0.65	1.33**	6.80 ± 1.01	4.12-9.59	14.89	69.85
DTN (mm)	7.14 ± 0.69	6.41 ± 0.52	0.73**	6.80 ± 0.90	4.51-9.18	13.23	54.31
DFON (mm)	7.12 ± 0.51	6.09 ± 0.52	1.03**	6.56 ± 0.90	3.74-8.88	13.69	66.44
DFIN (mm)	6.94 ± 0.60	5.68 ± 0.31	1.26	6.13 ± 0.76	4.08-8.03	12.47	61.07
DSIN (mm)	5.03 ± 0.38	4.81 ± 0.57	0.23**	4.38 ± 0.67	2.46-6.45	15.26	47.65
WTFN (mm)	0.76 ± 0.068	1.35 ± 0.16	-0.59**	1.08 ± 0.26	0.50-1.98	24.50	79.09
WTSN (mm)	0.74 ± 0.072	1.09 ± 0.02	-0.36**	0.97 ± 0.26	0.41-1.81	26.30	95.81
WTTN (mm)	0.60 ± 0.072	0.87 ± 0.10	-0.27**	0.78 ± 0.19	0.39-1.41	25.41	79.90
WTFON (mm)	0.52 ± 0.068	0.78 ± 0.11	-0.26**	0.67 ± 0.18	0.34-1.18	27.26	74.03
WTFIN (mm)	0.49 ± 0.074	0.53 ± 0.06	-0.04	0.54 ± 0.13	0.30-0.96	24.07	71.18
WTSIN (mm)	0.35 ± 0.049	0.42 ± 0.04	-0.07**	0.39 ± 0.09	0.24-0.77	23.63	77.61
CSFN (N)	13.26 ± 3.28	29.90 ± 1.80	-16.64**	24.48 ± 7.35	9.60-42.00	30.03	82.75
CSSN (N)	10.34 ± 2.54	24.20 ± 3.56	-13.86**	16.46 ± 5.71	6.20-35.90	34.69	70.51
CSTN (N)	8.39 ± 1.99	17.19 ± 2.30	-8.80**	11.56 ± 3.65	4.50-26.80	31.62	65.45
CSFON (N)	6.81 ± 1.76	10.81 ± 1.52	-4.01**	9.03 ± 2.91	2.30-18.70	32.19	62.65
CSFIN (N)	5.34 ± 1.57	9.02 ± 1.38	-3.68**	6.44 ± 2.03	2.00-12.30	31.59	47.09
CSSIN (N)	3.55 ± 1.88	6.86 ± 1.12	-3.31*	4.73 ± 1.63	1.30-9.30	34.55	61.43

PHCG = plant height center of gravity; PH = plant height; PLW = plant weight; LP = length of panicle; PAW = panicle weight; LFL = length of flag leaf; WFL = width of flag leaf; LFN = length of first internode; LSN = length of second internode; LTN = length of third internode; LFON = length of fourth internode; LFIN = length of fifth internode; LSIN = length of sixth internode; DFN = diameter of first internode; DSN = diameter of second internode; DTN = diameter of third internode; DFON = diameter of fourth internode; DFIN = diameter of fifth internode; DSIN = diameter of sixth internode; WTFN = wall thickness of first internode; WTSN = wall thickness of second internode; WTTN = wall thickness of third internode; WTFON = wall thickness of fourth internode; WTFIN = wall thickness of fifth internode; WTSIN = wall thickness of sixth internode; CSFN = culm strength of first internode; CSSN = culm strength of second internode; CSTN = culm strength of third internode; CSFON = culm strength of fourth internode; CSFIN = culm strength of five internode; CSSIN = culm strength of sixth internode. *P < 0.05 and **P <0.01 or 0.001. Significance levels based on *t*-tests between the parents. H^2 (%) are the heritability estimates of the traits.

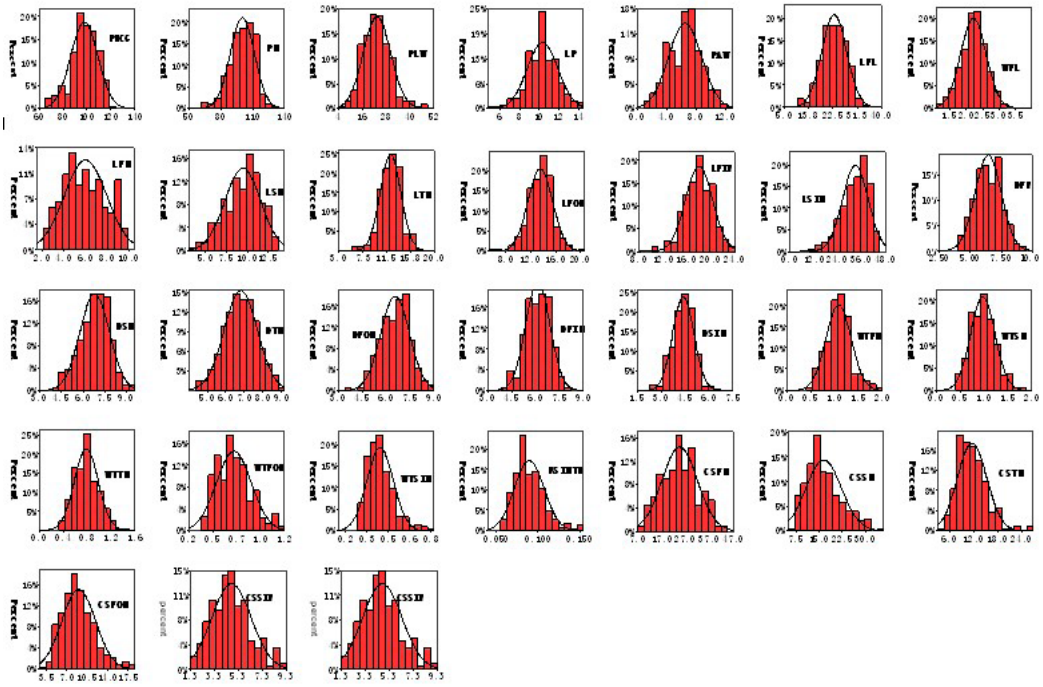

Figure 1. Frequency distribution of 31 traits related to LD. For abbreviations, see Table 1.

Construction of the genetic linkage map

The constructed genetic map included 117 SSR markers of 580 (20.17%) on barley chromosomes, which spanned a total of 909.63 cM (ranging from 100.59 to 183.12 cM) with an average interval of 7.77 cM. The map distance for the genome-wide QTL scan had an interval length of less than 10 cM (Doerge, 2002). Hence, the maps generated herein were suitable for genome-wide QTL scanning. The chromosomal locations and the orders of the markers in our maps were generally in agreement with reports published in GrainGenes 2.0 (http://wheat.pw.usda.gov/ggpages/map_shortlist.html).

Unconditional QTL mapping

Up to 53 QTL were detected based on selected thresholds (Figure 2; Table 2). Together, these QTL accounted (*qWTSN*) for up to 48.47% (*qCSFN*) of the phenotypic variation in the individual traits, with an average of 14.70%. Among these QTL, 15 accounted for more than 10% of the phenotypic variation. The major QTL (*qCSFN1*), flanking the region GBM1143-GBM1272, is an allele from ZQ320 with a positive additive effect of 4.87 cm, which accounted for 17.26% of the phenotypic variance.

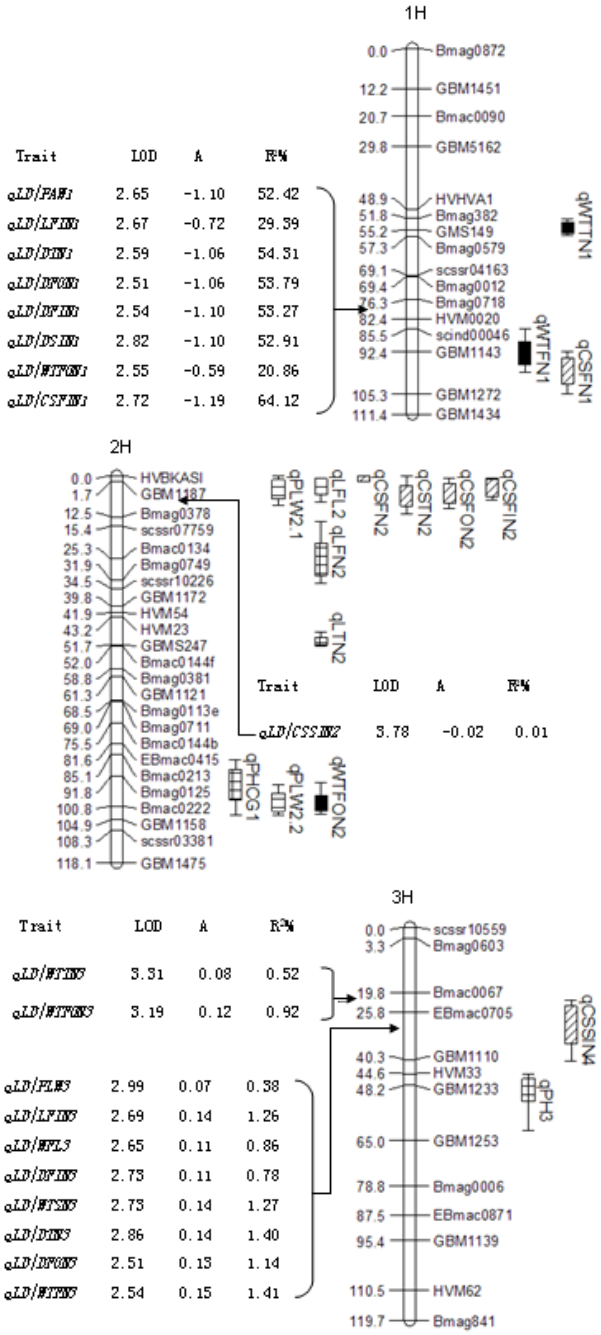

Figure 2. Linkage map showing conditional QTL and unconditional QTL detected; positions are to the left of chromosomes and markers are to the right. The symbols with *q* followed abbreviated trait name are the unconditional QTL, arrows indicate the position of the peak LODs for conditional QTL.

Continued on next page

Figure 2. Continued.

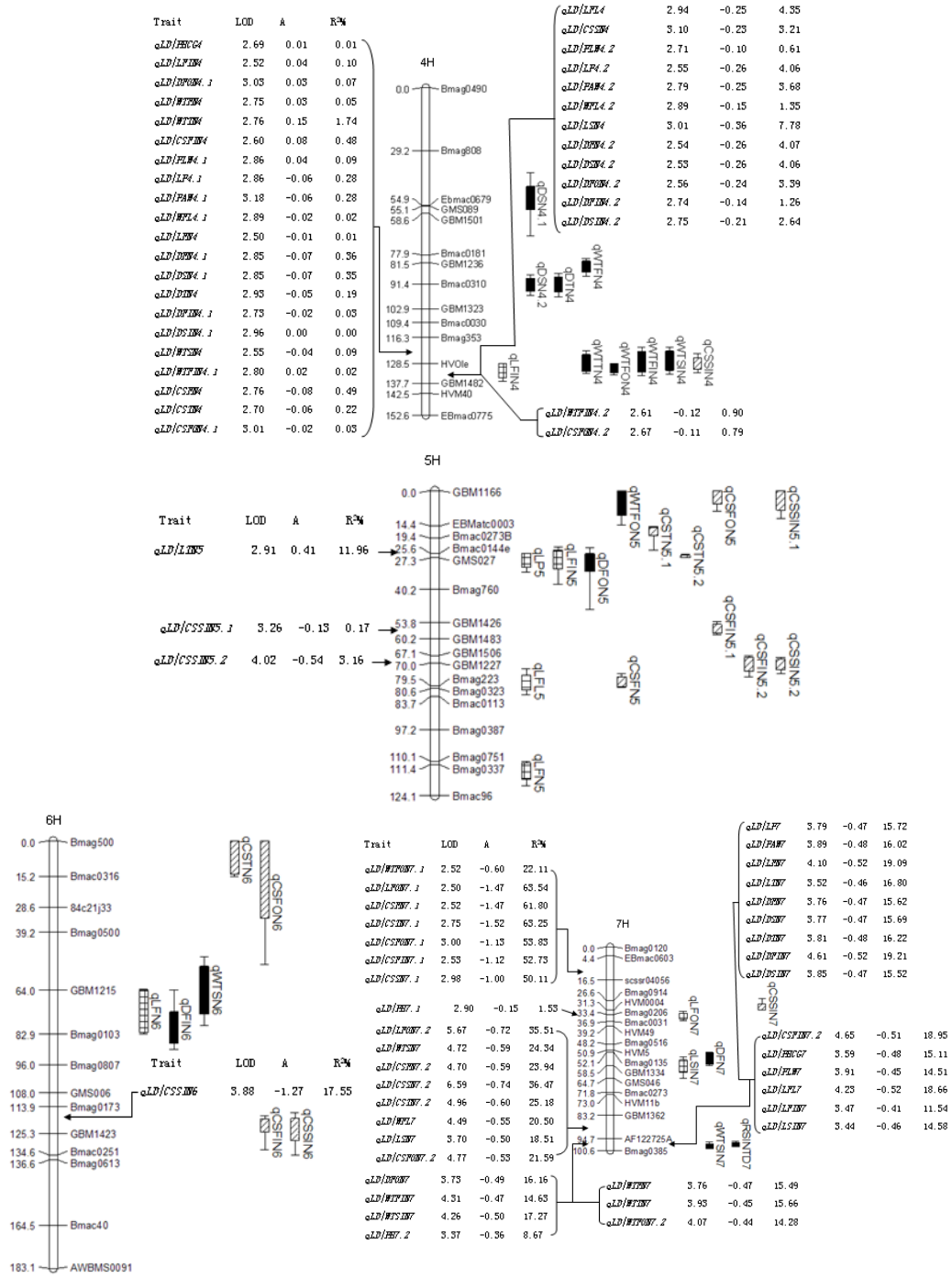

Table 2. Unconditional QTL with all traits.

Trait	QTL	Flank markers	Chr	Position	LOD	A	R^2 (%)
PHCG	*qPHCG2*	*Bmag0125-Bmac0222*	2	93.81	3.42	2.29	1.86
PH	*qPH3*	*HVM33-GBM1233*	3	46.61	2.51	-0.92	0.29
PLW	*qPLW2.1*	*GBM1187-Bmag0378*	2	3.81	2.74	-0.37	0.13
	qPLW2.2	*Bmag0125-Bmac0222*	2	100.81	3.04	2.40	5.61
LP	*qLP5*	*Bmac0144e-GMS027*	5	26.61	4.44	1.00	18.75
LFL	*qLFL2*	*GBM1187-Bmag0378*	2	1.81	2.60	0.01	0.00
	qLFL5	*Bmag223-Bmag0323*	5	79.51	2.51	1.90	8.64
LFN	*qLFN2*	*Bmac0134-Bmag0749*	2	28.31	2.71	0.70	5.53
	qLFN5	*Bmag0337-Bmac96*	5	113.41	2.52	0.90	8.90
	qLFN6	*GBM1215-Bmag0103*	6	74.01	4.62	0.81	8.77
LTN	*qLTN2*	*HVM23-GBMS247*	2	51.71	3.47	0.79	8.30
LFON	*qLFON7*	*Bmag0206-Bmac0031*	7	34.41	3.17	0.67	5.30
LFIN	*qLFIN4*	*HVOle-GBM1482*	4	132.51	2.69	-0.68	3.92
	qLFIN5	*Bmac0144e-GMS027*	5	25.61	2.70	0.46	1.97
LSIN	*qLSIN7*	*Bmag0135-GBM1334*	7	58.11	2.55	-2.82	8.50
DFN	*qDFN7*	*Bmag0135-GBM1334*	7	55.11	2.78	-0.59	11.63
	qDSN4.1	*Ebmac0679-GMS089*	4	55.01	2.54	-0.38	7.18
	qDSN4.2	*GBM1236-Bmac0310*	4	91.41	2.92	0.34	6.58
DTN	*qDTN4*	*GBM1236-Bmac0310*	4	90.51	2.58	0.27	5.18
DFON	*qDFON5*	*Bmac0144e-GMS027*	5	26.61	3.33	0.48	13.25
DFIN	*qDFIN6*	*Bmag0103-Bmag0807*	6	82.91	2.65	-0.04	0.13
WTFN	*qWTFN1*	*scind00046-GBM1143*	1	92.41	2.87	0.00	0.02
	qWTFN4	*GBM1236-Bmac0310*	4	83.51	2.93	0.00	0.01
WTSN	*qWTSN6*	*GBM1215-Bmag0103*	6	64.01	3.12	0.00	0.01
WTTN	*qWTTN1*	*Bmag382-GMS149*	1	54.81	2.63	-0.07	4.74
	qWTTN4	*HVOle-GBM1482*	4	129.51	3.68	0.08	8.26
WTFON	*qWTFON2*	*Bmag0125-Bmac0222*	2	100.81	2.82	0.06	4.67
	qWTFON4	*HVOle-GBM1482*	4	130.51	4.04	0.07	7.20
	qWTFON5	*GBM1166-EBMatc0003*	5	3.01	2.74	0.07	8.29
WTFIN	*qWTFIN4*	*Bmag353-HVOle*	4	126.31	2.51	0.06	9.97
WTSIN	*qWTSIN4*	*Bmag353-HVOle*	4	125.31	3.23	0.05	12.73
	qWTSIN7	*AF122725A-Bmag0385*	7	98.71	2.68	-0.02	1.24
CSFN	*qCSFN1*	*GBM1143-GBM1272*	1	98.41	2.70	4.87	17.26
	qCSFN2	*HVBKASI-GBM1187*	2	1.01	3.34	-4.60	15.90
	qCSFN5	*GBM1227-Bmag223*	5	78.01	4.16	4.34	15.32
CSTN	*qCSTN2*	*GBM1187-Bmag0378*	2	6.81	2.50	-0.82	2.20
	qCSTN5.1	*EBMatc0003-Bmac0273B*	5	16.41	2.73	2.02	12.73
	qCSTN5.2	*Bmac0144e-GMS027*	5	26.61	3.41	2.07	14.16
	qCSTN6	*Bmag500-Bmac0316*	6	2.01	2.97	-1.54	8.73
CSFON	*qCSFON2*	*GBM1187-Bmag0378*	2	5.81	3.04	-0.53	1.45
	qCSFON5	*GBM1166-EBMatc0003*	5	0.01	5.68	1.77	15.77
	qCSFON6	*Bmac0316-84c21j33*	6	21.31	3.05	-1.24	8.22
CSFIN	*qCSFIN2*	*GBM1187-Bmag0378*	2	3.81	3.78	-0.02	0.01
	qCSFIN5.1	*GBM1426-GBM1483*	5	56.91	3.26	-0.13	0.17
	qCSFIN5.2	*GBM1506-GBM1227*	5	69.11	4.02	-0.54	3.16
	qCSFIN6	*Bmag0173-GBM1423*	6	121.91	3.88	-1.27	17.55
CSSIN	*qCSSIN4*	*EBmac0705-GBM1110*	3	28.81	2.76	-0.71	8.73
	qCSSIN4	*HVOle-GBM1482*	4	128.51	3.73	0.37	2.66
	qCSSIN5.1	*GBM1166-EBMatc0003*	5	0.01	3.54	0.75	10.21
	qCSSIN5.2	*GBM1227-Bmag223*	5	70.01	3.19	-0.46	3.66
	qCSSIN6	*Bmag0173-GBM1423*	6	122.91	2.66	-0.74	9.46
	qCSSIN7	*Bmag0914-HVM0004*	7	29.61	2.56	-0.05	0.04

The nomination of QTL is comprised of four parts. The first part "q" stands for QTL. The second part is the abbreviation of traits. The third part, the number stands for chromosome. The fourth part is the serial number of QTL on the same chromosome. A = additive effect of the QTL, positive values indicate that the alleles for increasing trait values are contributed by ZQ320; negative values indicate that the alleles for increasing trait values are contributed by another parent 1277. R^2 (%) = percentage of phenotypic variance explained by the QTL. For abbreviations, see Table 1.

Six QTL that affected culm diameter were detected on chromosomes 4H (*qDSN4.1*, *qDSN4.2*, and *qDTN4*), 5H (*qDFON5*), 6H (*qDFIN6*), and 7H (*qDFN7*). These QTL collectively accounted for 43.96% of the total phenotypic variation (Figure 2). The ZQ320 alleles at *qDFON5*, *qDSN4.2*, and *qDTN4*, as well as the 1277 alleles at *qDFIN6*, *qDFN7*, and *qDSN4.1*

increased culm diameter. Eleven QTL that affect wall thickness were mapped to all seven of the barley chromosomes except for 6H. These QTL collectively accounted for 57.14% of the total phenotypic variation, with *qWTSIN4* accounting for 12.73% of the phenotypic variation. An increase in wall thickness was associated with the ZQ320 alleles at nine loci (*qWTFIN4*, *qWTFN1*, *qWTFN4*, *qWTFON2*, *qWTFON4*, *qWTFON5*, *qWTSIN4*, *qWTSN*, and *qWTTN4*) and with the 1277 alleles at the remaining two QTL (*qWTTN1* and *qWTSIN7*).

Up to 20 QTL for culm strength were detected on the seven chromosomes. Among these QTL, three affecting CSFN, which accounted for 48.47% of the total phenotypic variation, were detected on 1H (*qCSFN1*, 17.26%), 2H (*qCSFN2*, 15.90%), and 5H (*qCSFN5*, 15.32%), and had major effects of 4.87, 4.60, and 4.34 cm, respectively. Four QTL that affected CSTN were mapped to 2H, 5H, and 6H. They collectively accounted for 37.82% of the total phenotypic variation. The ZQ320 alleles at *qCSTN5.1* and *qCSTN5.2* increased CSTN by 2.02 and 2.07 cm, respectively, with R^2 values of 12.73 and 14.16%, respectively. Three QTL (*qCSFON2*, *qCSFON5*, and *qCSFON6*) that affected CSFON accounted for 25.44% of the total phenotypic variation, with *qCSFON5* contributing the most at 1.77 cm with an R^2 of 15.77% between GBM1166 and EBMatc0003. Four QTL that affected CSFIN were detected on chromosomes 2H, 5H, and 6H with the highest R^2 (17.55%). These QTL, which increased CSFIN, were the 1277 alleles at *qCSFIN2*, *qCSFIN5.1*, *qCSFIN5.2*, and *qCSFIN6*. Six QTL that affected CSSIN were detected on 3H (*qCSSIN3*), 4H (*qCSSIN4*), 5H (*qCSSIN5.1*, *qCSSIN5.2*), 6H (*qCSSIN6*), and 7H (*qCSSIN7*). They collectively accounted for 34.76% of the total phenotypic variation.

QTL affecting plant height-related traits and other traits

Three QTL that affected LFN were mapped onto chromosomes 2H, 5H, and 6H. These QTL accounted for 23.20% of the total phenotypic variation. An increase in LFN was associated with the ZQ320 alleles at *qLFN2*, *qLFN5*, and *qLFN6*. Two QTL that affected LFIN were identified and mapped onto 4H and 5H. The 1277 allele at *qLFIN4* and the ZQ320 allele at *qLFIN5* were associated with the increase in LFIN. Only one QTL was mapped for each of LTN, LFON, LSIN, PHCG, and PH, which were on 2H, 7H, 7H, 2H, and 3H, respectively. These QTL accounted for 24.26% of the total phenotypic variation. The ZQ320 alleles at *qLTN2*, *qLFON7*, and *qPHCG2* were associated with increased LTN, LFON, and PHCG, whereas the 1277 alleles at *qLSIN7* and *qPH3* increased LSIN and PH, respectively. Two PLW QTL were mapped onto 2H, which accounted for 5.74% of the total phenotypic variation. The ZQ320 allele at *qPLW2.2*, which had an effect of 2.40 cm (R^2 = 5.61%), and the 1277 allele at *qPLW2.1*, which had an effect of 0.37 cm, were associated with an increase in PLW. Two QTL that affected LFL were mapped onto 2H and 5H, and accounted for 8.65% of the total phenotypic variation. Both QTL came from the ZQ320 alleles at *qLFL2* and *qLFL5*, and the latter had a positive additive effect of 1.90 cm accounting for 8.64% of the phenotypic variance. This effect was detected between markers Bmag223 and Bmag0323. One QTL from the ZQ320 allele, *qLP5*, increased LP, which accounted for 18.75% of the phenotypic variation with an effect of 1.0 cm; it was localized between markers Bmac0144e and GMS027.

Conditional QTL mapping related to LD

When LD was conditioned on the other 31 traits (LDC), 96 QTL that affected LD were detected across all seven chromosomes. These QTL accounted for 10.20 to 136.28% of the to-

tal phenotypic variation (Figure 2). On chromosome 1H, eight QTL (*qLD|PAW1*, *qLD|LFIN1*, *qLD|DTN1*, *qLD|DFON1*, *qLD|DFIN1*, *qLD|DSIN1*, *qLD|WTFON1*, and *qLD|CSFIN1*) were localized in one region between markers Bmag0718 and HVM0020. These QTL accounted for 20.86 to 64.12% of the phenotypic variation. However, only one QTL (*qLD|CSSIN2*) was detected on chromosome 2H between markers GBM1187 and Bmag0378 when the effect of CSSIN on LD was excluded. Up to 10 QTL for LD were mapped in two regions (Bmac0067-EBmac0705 and EBmac0705-G9GBM1110) on 3H, which accounted for less of the phenotypic variation. Up to 35 QTL for LD were mapped onto 4H, which were localized in the regions Bmag353-HVOle and HVOle-GBM1482. Three QTL that affected LD were detected and distributed among three regions on 3H, which controlled for the influence of LFIN on LD. *qLD|LTN5* was localized between markers Bmac0144e and GMS027, which accounted for 11.96% of the phenotypic variation. However, only one QTL, *qLD|CSSIN6*, was detected and mapped onto 6H, which accounted for 17.55% of the phenotypic variation when the effect of CSSIN on LD was excluded. Most of the conditional QTL for LD (up to 38) mapped on 7H, which highly accounted for the phenotypic variation. These QTL were localized in four regions, namely EBmac0603-scssr04056, Bmag0206-Bmac0031, GBM1362-AF122725A, and AF122725A-Bmag0385.

Controlling for the effect of CSFIN on LD, the conditional mapping identified four QTL distributed on 1H, 4H, and 7H, which accounted for the highest proportion of the total phenotypic variation (Table 3). Five QTL for LD were detected when the influence of DFIN and DFON were excluded, which accounted for 74.57 and 74.54% of the total phenotypic variation, respectively. Excluding the effect of LFON on LD, two QTL were mapped on 7H and accounted for 63.57% (*qLD|LFON7.1*) and 35.51% (*qLD|LFON7.2*) of the total phenotypic variation. Three QTL were detected when the influence of CSSN, CSTN, and CSFN on LD were excluded, and accounted for 89.78, 88.65, and 86.23% of the total phenotypic variation, respectively. In addition, four QTL (*qLD|CSFON*, *qLD|PAW*, *qLD|DTN*, and *qLD|DSIN*) were detected when LD was conditioned on CSFON, PAW, DTN, DSIN, WTFON, LFIN, WFL, CSSIN, and PLW, which accounted for more than 70.0% of the total phenotypic variation. When the influence of WTSN, DSN, LP, DFN, WTTN, WTFN and WTFIN on LD were excluded, three QTL were detected, which accounted for relatively less of the phenotypic variation. Two QTL were detected when the influence of LTN, LSN, LFL, LFN, PHCG, and PH on LD were excluded.

Table 3. Conditional QTL detected on LD as conditioned trait.

Trait[a]	TN	TR^2 (%)	Trait[a]	TN	TR^2 (%)
qLD|CSFIN	4	136.28	qLD|LFL	2	23.02
qLD|LFON	2	99.05	qLD|WFL	4	22.73
qLD|CSSN	3	89.78	qLD|CSSIN	4	20.88
qLD|CSTN	3	88.65	qLD|DSN	3	20.10
qLD|CSFN	3	86.23	qLD|LP	3	20.06
qLD|CSFON	4	76.24	qLD|DFN	3	20.05
qLD|DFIN	5	74.57	qLD|LFN	2	19.09
qLD|DFON	5	74.54	qLD|WTTN	3	17.91
qLD|PAW	4	72.40	qLD|WTSIN	1	17.27
qLD|DTN	4	72.11	qLD|WTFN	3	16.94
qLD|DSIN	4	71.07	qLD|PLW	4	15.58
qLD|WTFON	4	58.16	qLD|WTFIN	3	15.55
qLD|LFIN	4	42.30	qLD|PHCG	2	15.12
qLD|LTN	2	28.77	qLD|LSIN	1	14.58
qLD|LSN	2	26.30	qLD|PH	2	10.20
qLD|WTSN	3	25.69			

[a]Conditional QTL (qLD|X) indicate the QTL for LD without the influence of X. TN = total number of conditional QTL on each condition; TR^2 (%) = percentage of total phenotypic variance explained by each conditional QTL. For abbreviations, see Table 1.

DISCUSSION

Morphological traits as indirect selection parameters for LD resistance

LD in cereals is defined as a permanent displacement of culms from their upright position, and several morphological traits have been shown to influence LD (Berry et al., 2006). Most of the studies conducted so far have aimed to identify the morphological traits that are correlated with LD for use as indirect selection parameters. In the present study, the physiological traits were measured during the waxy period because barley is most vulnerable to LD after grain filling, which elevates the center of gravity of the plants to their highest points. We analyzed LD and various related morphological traits (31 LDC traits), particularly plant height components and culm characteristics during the waxy stage, to identify potential genetic targets that could be used for improving LD resistance. The information we gathered in this analysis is the first comprehensive study on barley LD. PH and PH components (LTN, LFON, and LFIN) were significantly correlated with LD, which suggests that an increase in PH and the length of the three internodes contributed to greater LD damage. This result is in agreement with previous studies on barley (Stanca et al., 1979) and wheat. Moreover, some researchers have investigated correlations between stem characteristics, including culm diameter, wall thickness, and culm strength and LD (Sameri et al., 2009). Studies on wheat and soybean showed that the degree of LD was negatively correlated with stem diameter (Zuber et al., 1999; Tripathi et al., 2003). Jellum (1962) reported that two LD-resistant cultivars had larger stem diameters and wider culm walls compared to three LD-susceptible cultivars in a study on five oat cultivars with different LD susceptibilities. However, Kelbert et al. (2004) did not consider stem diameter to be a significant factor in LD resistance, which was corroborated in the present study; although the diameters of all six internodes were negatively correlated with LD, the effect was not significant. Nevertheless, wall thickness, the ratio of wall thickness to diameter, and culm strength were all negatively correlated with LD, which suggests that these traits are important for LD resistance. These results correspond with those obtained by Tripathi et al. (2003) in their field studies on wheat. Furthermore, most of these highly heritable traits are relatively less influenced by environmental factors. Thus, selecting LD-resistant barley genotypes can be accomplished through indirect selection based on wall thickness, the ratio of wall thickness to diameter, and culm strength, without having to decrease plant height. Moreover, stem weight is generally found to be positively correlated with stem diameter (Tripathi et al., 2003); stem weight increases the PLW of cereals. In the present study, stem diameter and wall thickness were highly correlated with PLW, which suggests that thicker culms and thicker walls increase plant weight, which is negatively correlated with LD. Therefore, thicker stems and walls in barley are most likely responsible for greater LD resistance and higher plant yield.

QTL analysis: corroborations and new findings

Genomic studies of QTL analyses for LD of barley have lagged behind those of wheat and rice. Hayes et al. (1993) identified six QTL in 150 lines that accounted for 72% of the variation in barley. Tinker et al. (1996) found six QTL in 146 barley lines using composite interval mapping. In both studies, each parent contributed positive and negative alleles for LD. Backes et al. (1995) obtained only three QTL for LD in 250 barley double-haploid lines, which accounted for 26% of the genotypic variance. Some studies have found that QTL for

LD resistance were linked to or located in the same regions as QTL for plant height (Keller et al., 1999; Börner et al., 2002). In the present study, 53 QTL for 32 morphological traits were identified. One QTL (*qPH3*) was mapped on 3H, which agrees with results of three separate studies (Yu et al., 2010). Researchers have increasingly focused on finding new QTL for culm characteristics without decreasing plant height to provide more information regarding LD resistance. Kashiwagi et al. (2008) revealed a QTL, *sdm8*, which improves LD resistance by increasing rice diameter. Sameri et al. (2009) mapped an unexpected QTL (*qCUL*) on 7HL for reducing the length of the third and fourth culm internodes, which were associated with reducing LD in two independent experiments. Our results revealed a large number of new QTL for culm characteristics that could contribute to LD resistance. One QTL (*qLFON7*) that affects the length of the fourth internode and accounts for 5.30% of the phenotypic variation was detected on 7H. The correlation analysis suggested that culm strength (CSSN, CSFIN, and CSSIN) is negatively correlated with LD. This finding indicates that increasing culm strength enhances LD resistance. Up to 20 QTL were detected for the culm strength of six internodes, which are distributed among the seven chromosomes. Culm strength results from chemical and biochemical components. Generally, lignin or cellulose determines physical strength, as lower lignin and cellulose contents produce brittle culms (Kashiwagi et al., 2008). Thus, detecting QTL related to culm components is necessary.

Multiple alleles for QTL for wall thickness, culm strength, plant weight, and plant weight were found to have originated from cultivar ZQ320, which suggests that selecting LD-resistant barley genotypes can be accomplished without decreasing plant height and weight. Furthermore, QTL for culm strength, diameter, wall thickness, and the ratio of wall thickness to diameter were adjacent to one another or overlapped by different degrees on all seven chromosomes. In particular, six QTL were found to demonstrate pleiotropic effects on five traits (WTTN, WTFON, WTFIN, WTSIN, and CSSIN) between Bmag353 and GBM1482 on 4H, which accounted for 89.51% of the total phenotypic variation. Moreover, these traits were negatively correlated with LD, and their alleles all came from ZQ320, which indicates that the flanking markers Bmag353 and GBM1482, along with the middle marker HVOle, of these QTL will serve as a useful tool for marker-assisted LD resistance selection in barley.

Relationship between LD and LDC at the QTL level

Zhu (1995) introduced a new methodology for conditional genetic analysis, which was later used to study developmental quantitative genetics in mice (Atchley and Zhu, 1997), rice (Shi et al., 2001), and cotton (Zhu, 1995; Ye et al., 2003). More recently, a method for multivariable conditional analysis was proposed for analyzing the contributions of individual component traits to a complex trait (Wen and Zhu, 2005). By combining the conditional genetic analysis method with QTL mapping (unconditional QTL analysis), the method was extended to map conditional QTL for the molecular dissection of complex traits (Guo et al., 2005; Zhao et al., 2006; Liu et al., 2008; Cui et al., 2011). Therefore, conditional QTL mapping analysis is an efficient tool for determining relationships between complex traits and their components. The present study is the first to combine unconditional and conditional QTL methods for analyzing genetic relationships between LD and LDCs of barley. Four results are possible when performing a conditional QTL analysis of LD conditioned on PH (LD|PH): 1) a QTL detected using the unconditional method could be identified with a similar or equal effect, which would indicate that the QTL for LD is independent of PH; 2) a QTL detected using the

unconditional method could be identified with either a greatly reduced or a greatly enhanced effect, which would suggest that the QTL for LD is partially associated with PH; 3) a QTL detected using the unconditional method cannot be identified, which would indicate that the QTL for LD is entirely contributed by PH; 4) an additional QTL could be detected using the conditional mapping method, but not detected using the unconditional method, which would imply that the expression of the QTL for LD is completely suppressed by PH and the effects are only identifiable by eliminating the effects of PH. This result would suggest that additional QTL have an opposite additive effect on LD and PH (Cui et al., 2011). LD is a complex trait affected by many interacting morphological traits (**Supplementary Table**). Thus, identifying the genetic factors that affect LD is difficult, and analyzing the genetic relationships between LD and its components at the QTL level is particularly challenging. In our study, 53 QTL related to LD were detected, but no QTL for LD was detected using unconditional QTL analysis. Nevertheless, 96 QTL for LD were detected when the influence of each LDC on LD was excluded using conditional QTL analysis. The fact that detecting QTL depended on the ratio between the variance caused by the effect of the QTL, the total variance of the traits, and the size of the mapping population may account for this phenomenon (Zhao et al., 2006). Another potential reason is that the occurrence of LD is a very complex phenomenon, and therefore the genetic dissection of LD is considerably difficult (Cao and Zhu, 2007). Previous studies using molecular markers have indicated some putative QTL that control LD and its components. However, in these studies, the phenotypic values of the final trait were used for QTL mapping. We mapped QTL for LDC and investigated the net effects of LDC on LD using conditional analysis. The same results were found when other components were used for comparison. Therefore, our findings demonstrated that 31 LDCs suppressed QTL expression of LD to different extents under the unconditional analysis. The present study demonstrated that conditional QTL mapping could identify a greater number of QTL, as well as evaluate the genetic relationships among different significantly correlated traits at the molecular level. Such information could be used in marker-assisted selection for simultaneously improving objective traits (Li et al., 2008). Excluding the influences of CSFIN, CSSN, CSTN, CSFN, CSFON, DFIN, and DFON on LD resulted in the identification of a large number of new QTL that accounted for a high proportion of the phenotypic variation. This result is consistent with the significant correlation analysis, which indicates that these traits are considerably important for LD and that they substantially contribute to LD resistance. In addition, the fewest number of additional QTL that accounted for the lowest phenotypic variation for LD were revealed when the influence of PH on LD was excluded, followed by the exclusion of PHCG and LSIN, which indicates they had greatest contribution to LD at the QTL level. CSFIN had the strongest influence on LD, followed by CSTN and CSSN. The results described above demonstrate that LD is affected by many complex traits, depending on whether the QTL could be identified using conditional analysis. However, CSFIN, CSTN, and CSSN could be considered as the most important traits for LD resistance, which is in agreement with results of the correlation analysis. Moreover, conditional QTL for LD were clustered on chromosomes 1H (between Bmag0718 and HVM0020), 3H, 4H, and 7H. This clustering demonstrates that these chromosomal regions have potential QTL that contribute to LD resistance. For example, on 4H, conditional QTL were clustered in two regions (Bmag353-HVOle and HVOle-GBM1482) where conditional QTL related to LD were distributed. Interestingly, these two regions are also the areas where the six unconditional QTL with pleiotropic effects are located,

which indicates that the alleles of these QTL suppress LD, thereby improving LD resistance. Moreover, these results confirm that the flanking markers of these QTL are important regions for marker-assisted selection of LD resistance in barley.

In conclusion, wall thickness and culm strength are potential targets for breeding to improve LD resistance. Multiple alleles of QTL that contribute to LD resistance, plant height, and plant weight originated from the ZQ320 cultivar. These QTL are required for selecting for LD resistance without decreasing plant biomass. In addition, this study demonstrated that the combination of conditional and unconditional mapping methods could precisely determine the factors that affect LD and evaluate possible genetic relationships between LD and LDCs at the QTL level.

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Supplementary material

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