Leaf thickness of barley: genetic dissection, candidate genes identification and its relationship with yield-related traits

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Abstract

Leaf thickness (LT) is an important characteristic affecting leaf functions which have been intensively studied. However, as LT has a small dimension in many plant species and technically difficult to measure, previous studies on this characteristic are often based on indirectly estimations. In the first study of detecting QTL controlling LT by directly measuring the characteristic in barley, large and stable loci were detected from both field and glasshouse trials conducted in different cropping seasons. Four loci (locating on chromosome arms 2H, 3H, 5H and 6H, respectively) were consistently detected for flag leaf thickness (FLT) in each of these trials. The one on 6H had the largest effect, with a maximum LOD 9.8 explaining up to 20.9% of phenotypic variance. FLT does not only show strong interactions with flag leaf width and flag leaf area but has also strong correlations with fertile tiller number, spike row types, kernel number per spike, and heading date. Though with reduced efficiency, these loci were also detectable from assessing second last leaf of fully grown plants or even from assessing the third leaves of seedlings. Taken advantage of the high-quality genome assemblies for both parents of the mapping population used in this study, three candidate genes underlying the 6H QTL were identified based on orthologous analysis. These results do not only provide direct evidence showing the importance of LT in improving kernel yield of cereal crops but also form the bases for cloning and functional analysis of genes regulating LT in barley.

Introduction

Leaves are the most important organ in plant photosynthesis (Van Camp, 2005; White et al. 2016) and their characteristics also affect plant adaptations to different environments (Wright et al. 2004; Donovan et al. 2011). Plant with thicker leaves trends to contain higher chlorophyll, nitrogen, and photosynthetic content per unit leaf area (Yin et al. 1999a; Murchie et al. 2002; Li et al. 2009). Strong relationships exist between leaf thickness (LT) with photosynthesis ability (Smith et al. 1998; Taiz and Zeiger 2006; Li et al. 2009; Tsukaya 2013), relative water content (Afzal et al. 2017) and yield potential of crop cultivars (Sexton et al. 1997; White and Montes-R 2005; Peng et al. 2008; Liu et al. 2014). Plants adapted to arid environments tend to have thicker leaves (Wright et al. 2004; Poorter et al. 2009). Not surprisingly, LT has been intensively studied in different species (e.g., Diaz et al. 2004; Vile et al. 2005; Li et al. 2009; Tsukaya 2013; Coneva et al. 2017; Coneva and Chitwood 2018).

Due to its relatively small dimension, LT can be difficult to measure directly in some plant species. To overcome the difficulty, several surrogates have been used to estimate LT. These include specific leaf area (SLA, the ratio of leaf area to leaf dry mass), leaf dry matter content (LDMC, the ratio of leaf dry mass to saturated fresh mass = 1- leaf water content) and leaf mass per area (LMA, the ratio of leaf dry mass to leaf area) (Witkowski and Lamont 1991; Roderick et al. 1999; Poorter et al. 2009; Muir et al. 2014;). With the use of these surrogates, QTL have been detected in various species including cereals. In barley, the numbers of QTL for SLA detected among different studies varied. In analysing QTL related to yield potential in spring barley, Yin et al. (1999a) detected loci for SLA on chromosomes 2H, 3H and 4H based on the evaluation of a population consisting of 94 recombinant lines (RILs). In a study on QTL affecting

growth-related traits in wild barley (*Hordeum spontaneum*), Elberse et al. (2004) detected loci for SLA on chromosomes 3H and 4H based on assessments of an F3 population. In a recent publication studying traits related to seedling vigour in barley, Capo-chichi et al. (2021) detected as many as 26 loci for SLA based on an analysis of a RIL population and these loci were distributed on each of the seven chromosomes.

Studies on LT based on direct measurements have been reported on several plant species in recent years. They included the studies on the natural variation of LT and its correlation with yield traits in rice (Liu et al. 2014), on the genetic architecture and molecular networks underlying LT in desert-adapted tomato (Coneva et al. 2017), on the influence of LT on canopy reflectance and physiological traits in cotton (Pauli et al. 2017) and the genetic and developmental basis for increased LT in *Arabidopsis* (Coneva and Chitwood 2018). As expected, available data showed that results from direct measurements do not always agree with those from indirect estimations (Coneva et al. 2017). However, genetic studies based on direct measurements of LT have not been reported in any cereal crop species yet. We thus made such an attempt and measured LT directly for QTL detection in a barley population consisting of 201 RILs. Following the successful detections of large-effect loci across different trials, we analysed candidate genes underlying a locus with the largest effect and assessed possible interactions between LT and other traits of agronomic importance. Results obtained from the study are reported in this publication.

Materials And Methods

Plant materials

Results reported here were based on a population of recombinant lines. The population consisting of 201 F8 RILs was developed in an earlier study from a cross between Morex and AWCS276 (Zhou et al. 2021) using the single-seed descendent method based on the fast generation technique (Zheng et al. 2013). Morex is a six-row malting variety and AWCS276 is two-row wild barley.

Phenotypic evaluation

Data on flag leaf (FL) and second last leaf (2LL) were collected from two field trials and two glasshouse trials. In making sure that all lines could reach flowering stage, these trials were all conducted using vernalized seedlings. For vernalization, seeds were germinated in Petri dishes on two layers of filter paper saturated with water and placed in a 4°C cold room with constant lighting for five weeks.

The field trials were conducted at CSIRO Gatton Research Station (27°33'S, 152°16'E), one in 2019 and the other in 2020 (designated as FD19 and FD20, respectively). Each of the field trials contained two replicates, each replicate with ten spaced planted (20 cm apart) seedlings in a single row with 25 cm row spacing. The two glasshouse trials were conducted at Queensland Bioscience Precinct (QBP), one in 2019 and the other in 2020 (designated as GH19 and GH20, respectively). Each of the glasshouse trials consisted of three replicates. Three plants, each in a separate 2.0 L pot with stem sterilized University of California mix C (UC mix) (50% sand and 50% peat v/v), were used in each of the replicates. A random

block design was used for all the trials. Measurements of flag leaf thickness (FLT), flag leaf length (FLL), flag leaf width (FLW), flag leaf area (FLA), flag leaf length to width ratio (FLWR) the second last leaf thickness (2LLT) were taken from the main tiller of each plant after anthesis.

Due to LT is sensitive to leaf water status, a standardized protocol described by Garnier et al. (2001) was applied on samples for rehydration. Briefly, leaf samples were collected at least 2–3 h after sunrise and 3–4 h before sunset and were immediately wrapped in moist paper bags and conserved in a cold box until return to the lab. Then, the bags were placed into water and stored in a dark and cold room (4°C) for at least 6 h before measurement. LT were measured by an electronic thickness gauge (SIDA, model SD-201) as the thickness in the middle section of the leaf on both sides as near the main midrib as possible. The leaf midrib was avoided and average of two readings were used to represent the thickness of the leaf; leaf length (LL) was measured as the distance from the bottom to the tip of the leaf; leaf width (LW) was measured as the width of the widest section of the leaf. Leaf area (LA) and leaf length to width ratio (LWR) are derivative characters, and their algorithms are LA = LW × LL × 0.75 (Spagnoletti Zeuli and Qualset 1990) and LWR = LL/LW (Zhang et al. 2015).

Plant height (PH) were obtained from the five tallest tillers in each row and the average of the five measurements was used for analysis. Heading date (HD) was recorded on the day on which approximately 50% of spikes emerged from main tillers. Thirty seeds per line were scanned with Epson Expression 10000 XL, and kernel length (KL) and kernel width (KW) were calculated using WinSEEDLE (Regent Instruments Canada Inc). Spike row type (SRT) was determined by 2 or 6 row types. Kernel number per spike (KNPS) was calculated from the main tiller of each plant. Fertile tiller number (FTN) was scored by counting tiller numbers for five plants in the middle section of each row from the field trials and each of the three plants from the glasshouse trials. Data for thousand-kernel weight (TKW) was obtained from the previous study (Zhou et al. 2021).

To investigate if any similar loci can be detected from young seedlings, two trials were conducted in 2021 (designated as GH21a and GH21b) at QBP glasshouse with two replicates in each trial and each replicate contained seven seedlings. Seeds with similar size were soaked in 70% ethanol for 30 s to sterilize and then washed two or three times with distilled water. Sterilized seeds were germinated in petri dishes on two layers of filter paper saturated with water under room temperature for 1–2 days. Seedlings with coleoptiles about 0.5 cm were planted into square punnets of a 56-well tray (Rite Grow Kwik Pots, Garden City Plastics, Australia) containing stem sterilized UC mix. Measurements of the 3rd leaf thickness (3LT), length (3LL), width (3LW), area (3LA) and length to width ratio (3LWR) were taken from each of the seven seedlings when the collars of the 4th leaves become visible on about 50% of the plants as described before. A random block design was used for both seedling trials.

QTL analysis

A high-density genetic map of this population based on genotyping by sequencing (GBS) data was constructed according to the previous study (Zhou et al., 2021). The total length of the linkage map is about 1022.4 cM with an average distance of 0.7 cM. MapQTL 6.0 (Van Ooijen and Kyazma 2009) was

used for QTL analysis. For each trial, a test of 1,000 permutations was performed to identify the LOD threshold corresponding to a genome-wide false discovery rate of 5% ($P < 0.05$). A linkage map showing the QTL positions was drawn using MapChart (Voorrips 2002).

Identification of candidate genes underlying QTL for LT

Markers flanking QTL were used to delineate the physical intervals. Tag sequences in GBS dataset were used to blast against the Morex Assembly of RefSeq v2.0 (Mascher et al. 2017) to get physical positions. Gene lists in the candidate regions were downloaded from the website ftp://ftp.ensemblgenomes.org/pub/release-44/plants/gff3/ hordeum_vulgare. At the same time, gene sequences related to leaf size, leaf development, organ development and cell elongation were collected from rice and used to blast against Morex genes in the putative QTL intervals. Genes detected in Morex were then used to blast against the genome assembly of AWCS276 (Liu et al. 2020) to detect sequence differences between the two parents and only those genes differing in sequences were treated as candidates.

Statistical analysis

The average values of five plants from the field trials and three plants from glasshouse trials for each line were employed in the succeeding analysis during 2019 to 2020, while the average value of seven plants from the seedling trials for each line was employed in 2021. The best linear unbiased prediction (BLUP) of target traits and the broad-sense heritability (H^2) were calculated using SAS V8.0 (SAS Institute, Cary, NC, USA; https://www.sas.com). H^2 for each trait was estimated as $H^2 = \sigma^2_g / (\sigma^2_g + \sigma^2_{ge}/n + \sigma^2_e/nr)$, where σ^2_g is the genetic variance, σ^2_{ge} is the G × E variance, σ^2_e is the error, n is the number of environments, and r is the number of replicates. SPSS18.0 software (SPSS, Chicago, IL, USA) was used to perform Student's *t* test ($P < 0.05$) and correlation analysis of phenotype values in different trials.

Results

Phenotypic data analysis and correlations

LT values of Morex measured from each of the three different leaves (flag and 2nd last leaves from fully grown plants and 3rd leaf from young seedlings) were significantly higher than those of AWCS276 in each of the trials conducted (Table 1). Transgressive segregation with normal distribution for each set of these values was detected in each of the trials (Fig. 1, Fig. S1 and S2). The broad-sense heritability ranged from 0.68 to 0.86 for the three characteristics (Table 1). Significant and positive correlations were detected between FLT and 2LLT among different trials as well as 3LT (Table S1).

Table 1
Phenotypic variation and heritability of leaf thickness for the parents and population assessed in different environments

Trait	Trial	Parents		Population					
		Morex	AWCS276	Min	Max	Mean	SD	CV(%)	H^2
FLT(μm)	FD19	254.2	145.2	108.8	272.5	195.0	26.2	13.4	0.86
	GH19	270.9	130.5	108.5	295.0	191.3	28.2	14.8	
	FD20	236.8	136.9	120.6	266.8	199.0	26.5	13.3	
	GH20	257.3	129.9	120.6	265.4	196.4	26.2	13.9	
2LLT(μm)	FD19	289.9	143.8	110.0	309.7	207.3	37.1	17.9	0.74
	GH19	279.2	125.3	118.9	306.5	206.7	36.1	17.5	
	FD20	284.9	124.4	118.2	328.0	190.4	30.9	16.2	
	GH20	295.7	143.6	95.0	324.5	203.3	37.8	18.6	
3LT(μm)	GH21a	233.3	196.1	150.2	288.6	221.2	23.8	10.7	0.68
	GH21b	239.4	185.6	150.7	266.7	215.8	22.2	10.3	

FLT flag leaf thickness, *2LLT* the second last leaf thickness, *3LT* 3rd leaf thickness, *SD* standard deviation, *CV* coefficient of variation, H^2 the broad-sense heritability

Similar correlations between LT and other traits were found between the measurements of FL and 2LL. LT obtained from these two leaves showed very strong correlation with FTN, SRT, and KNPS as well as FLW. They also showed strong correlation with HD, FLA and FLWR. However, LT measured from these leaves were not correlated with FLL, KW, TKW, and PH (Table 2).

Table 2
Coefficients of pairwise Pearson correlations between leaf thickness and other traits

Traits	FLT	2LLT	3LT
3LT	0.25**	0.24**	1.00
3LL	0.16*	0.17*	0.56***
3LW	0.22**	0.20**	0.68***
3LA	0.20**	0.21**	0.65***
3LWR	0.05	0.07	0.22**
FLL	0.12	0.12	0.03
FLW	0.34***	0.30***	0.28***
FLA	0.27**	0.24**	0.20**
FLWR	-0.22**	-0.23**	-0.26**
TKW	-0.05	-0.04	0.08
KL	-0.16*	-0.11	-0.08
KW	0.07	0.10	0.17*
SRT	0.29***	0.28***	0.03
KNPS	0.29***	0.30***	0.01
FTN	-0.34***	-0.36***	-0.11
PH	0.12	0.12	0.04
HD	0.20**	0.23**	0.21**

FLT flag leaf thickness, *2LLT* the second last leaf thickness, *3LT* 3rd leaf thickness, *3LL* 3rd leaf length, *3LW* 3rd leaf width, *3LA* 3rd leaf area, *3LWR* 3rd leaf length and width ratio, *FLL* flag leaf length, *FLW* flag leaf width, *FLA* flag leaf area, *FLWR* flag leaf length and width ratio, *TKW* thousand kernel weight, *KL* kernel length, *KW* kernel width, *SRT* spike row type, *KNPS* kernel number per spike, *FTN* fertile tiller number, *PH* plant height, *HD* heading date

Correlations between LT and other traits are very different between the results obtained from 3LT and the other two leaves. The only similarity among measurements from the three different leaves is that LT was strongly correlated with HD. Apart from that, 3LT correlates strongly only with other leaf characteristics including those taken from ether seedlings (3LL, 3LW, and 3LA) or fully grown plants (FLW, FLA and FLWR). Different from FLT and 2LLT, 3LT does not correlated with any of the yield-related traits including FTN, SRY and KNPS (Table 2).

QTL for leaf thickness

Permutation tests found that a LOD score of 2.9 was the threshold for the trials conducted in this study. Based on this threshold, a total of five QTL controlling FLT were detected across the first four trials. They were located on chromosomes 2H, 3H, 5H.1, 5H.2 and 6H, respectively (Table 3). Four of these five QTL were consistently detected in each of the four trials as well as with the use of the BLUP values. Among them, the most significant QTL (designated as *Qflt.caf-6H*) was identified on chromosome 6H. This locus had a LOD value of 9.8 and explained up to 20.9% of phenotypic variance (Table 3; Fig. 2). Phenotypic variances explained by the other three QTL ranged from 7.3 to 11.5% with the LOD scores between 3.2 and 5.4 (Fig. 3; Table 3).

Table 3
QTL for FLT and 2LLT identified in the population of Morex/AWCS276

Traits	Trials	Chromosomes	Interval	LeftMarker	RightMarker	LOD	PVE (%)
FLT	FD19	2H	80.5–97.1	GBS_MST11178	GBS_MST1324	4.2	9.2
		3H	49.2–59.8	GBS_MST1659	GBS_MST2019	4.3	9.5
		6H	31.6–80.9	GBS_MST4489	GBS_MST3979	9.8	20.9
	GH19	2H	81.5–97.1	GBS_MST1202	GBS_MST1324	3.9	8.4
		3H	49.2–59.8	GBS_MST1659	GBS_MST2019	4.2	9.1
		5H.1	39.9–48.9	GBS_MST3835	GBS_MST3752	3.7	8.1
		6H	31.6–80.9	GBS_MST4489	GBS_MST3979	7.4	16.7
	FD20	2H	81.5–97.1	GBS_MST1202	GBS_MST1324	5.1	10.9
		3H	49.2–59.8	GBS_MST1659	GBS_MST2019	4.1	8.9
		5H.1	39.9–48.9	GBS_MST3835	GBS_MST3752	3.4	7.6
		5H.2	118.0-127.2	GBS_MST3270	GBS_MST3245	3.2	7.0
		6H	31.6–80.9	GBS_MST4489	GBS_MST3979	9.0	19.2
	GH20	2H	81.5–97.1	GBS_MST1202	GBS_MST1324	3.8	8.4
		3H	49.2–59.8	GBS_MST1659	GBS_MST2019	5.4	11.5
		5H.1	39.9–48.9	GBS_MST3835	GBS_MST3752	3.2	7.0
		5H.2	118.0-127.2	GBS_MST3270	GBS_MST3245	3.3	7.3
		6H	31.6–80.9	GBS_MST4489	GBS_MST3979	8.3	17.9
	BLUP	2H	81.5–97.1	GBS_MST1202	GBS_MST1324	4.6	10.1
		3H	49.2–59.8	GBS_MST1659	GBS_MST2019	4.2	9.1
		5H.1	39.9–48.9	GBS_MST3835	GBS_MST3752	3.3	7.3
		6H	31.6–80.9	GBS_MST4489	GBS_MST3979	9.5	20.6
2LLT	FD19	2H	81.5–97.1	GBS_MST1202	GBS_MST1324	3.6	8.0
		5H.1	39.9–48.9	GBS_MST3835	GBS_MST3752	3.7	8.2

FLT flag leaf thickness, *2LLT* the second last leaf thickness, *GH* glasshouse trial, *FD* field trial, *BLUP* best linear unbiased prediction

Traits	Trials	Chromosomes	Interval	LeftMarker	RightMarker	LOD	PVE (%)
		5H.2	118.0-127.2	GBS_MST3270	GBS_MST3245	4.2	9.2
		6H	31.6–80.9	GBS_MST4489	GBS_MST3979	6.2	11.4
	GH19	2H	81.5–97.1	GBS_MST1202	GBS_MST1324	3.8	8.5
		5H.2	118.0-127.2	GBS_MST3270	GBS_MST3245	4.4	9.6
		6H	31.6–80.9	GBS_MST4489	GBS_MST3979	5.3	11.7
	FD20	6H	31.6–80.9	GBS_MST4489	GBS_MST3979	6.7	15.0
	GH20	6H	42.4–53.6	GBS_MST4467	GBS_MST4142	7.0	15.5
	BLUP	2H	81.5–97.1	GBS_MST1202	GBS_MST1324	4.8	10.3
		3H	48.9–54.7	GBS_MST1635	GBS_MST1986	3.6	7.8
		6H	31.6–80.9	GBS_MST4489	GBS_MST3979	10.0	20.8

FLT flag leaf thickness, *2LLT* the second last leaf thickness, *GH* glasshouse trial, *FD* field trial, *BLUP* best linear unbiased prediction

For 2LLT, five QTL were identified sharing similar positions of FLT on chromosomes 2H, 3H, 5H.1, 5H.2 and 6H in different trials (Fig. 3). However, only the QTL on chromosome 6H was constantly detected in all the four trials. LOD values of the locus on 6H varied from 5.3 to 10.0, explaining between 11.4 to 20.8% of phenotypic variance. Of the remaining QTL, the two located on chromosomes 3H and 5H.1 were only detected in one trial, and the ones on chromosomes 2H and 5H.2 in only two of the trials. Surprisingly, the locus on 3H was not detected from any of the trials but it was picked up with the use of BLUP values (Table 3).

To validate whether QTL detected from fully grown plants could also be found from seedlings, two additional trials were conducted. Three of the five loci detected by assessing FL and 2LL from fully grown plants were detected in these two seedling trials. However, none of them were detected from both seedling trials. Two of them, located on chromosomes 3H and 6H, were detected in one of the trials, while only the locus on chromosome 2H was detected in the other trial. When BLUP values were used, all three loci were detected. Like the results obtained from measuring fully grown plants, the locus on chromosome 6H again gave the largest effect, explaining up to 9.7% phenotypic variance with a LOD value of 4.0 (Table S2).

Candidate genes underlying the major locus on chromosome 6H

As the QTL on chromosome 6H did not only show the largest effect but was also consistently detected, candidate genes underlying this locus were identified based on an orthologous analysis. Three candidate genes were identified, including *HORVU6Hr1G057630, HORVU6Hr1G060990 and HORVU6Hr1G068370*. They were orthologous to rice genes *OSPRR1*, *OsVPE3* and *GS2*, respectively. Five SNPs in exons of *HORVU6Hr1G057630* were detected between Morex and AWCS276. Two of them were non-synonymous mutations, producing amino acid residue substitutions at positions 1219 (Threonine→Alanine) and 1994 (Serine→Proline), respectively. Four SNPs were identified in exons of *HORVU6Hr1G060990.* Two of them were non-synonymous mutations (T→G transversion at position 95 and A→G transition at position 99, respectively). The other two were synonymous mutations (G→A transition at position 122 and C→T transition at position 3933, respectively). Only one SNP (G→T) was detected between the two parental genotypes for *HORVU6Hr1G068370*, producing an amino acid substitution (Aspartic acid→Tyrosine) (Table 4).

Table 4
Candidate genes and their orthologs underlying the locus controlling leaf thickness on chromosome 6H

Barley Orthologs	Physical position (bp)	Rice gene	Identity	SNP#	Amino acids#
HORVU6Hr1G057630	chr6H: 374866561-374869556	OsPRR1	76.1	T/C(108)	T/A(215)
				A/G(1219)	S/P(434)
				C/T(1705)	
				G/T(1690)	
				T/C(1994)	
HORVU6Hr1G060990	chr6H: 407203000-407209104	*OsVPE3*	84.0	T/G(95)	I/M(32)
				A/G(99)	R/G(34)
	chr6H: 473135773-473139363			G/A(122)	
				C/T(3933)	
HORVU6Hr1G068370		*OsGRF4*	80.7	G/T(1111)	D/Y(371)
# the numbers in brackets represents the positions of differences in nucleotide or amino acid sequences between Morex and AWCS276 relative to initiation codons.					
Supplementary File					

Discussion

The importance of LT in plant adaptation and crop production has been well documented. Due to the limited dimensions of LT, previous genetic studies on this characteristic in cereals has all been based on indirect estimations. In the study reported here, we demonstrated for the first time that targeting LT

directly in the genetic studies is now feasible. By assessing the RIL population consisting of 201 lines, we did not only detect QTL for LT in barley but also showed that QTL detected for FLT are larger and more stable compared with those for other leaf characteristics including length, width, and area. Although with reduced magnitudes, QTL for LT with similar locations were also detected from measuring 2LL after anthesis as well as from measuring the 3rd leaves of developing plants. These results indicate that the thicknesses of different leaves in a plant are correlated, and it likely has a more simpler inherence than other leaf characteristics. The importance of FLT and 2LLT is shown by its strong correlation with HD, FTN, SRT, KL and KNPS. Taken advantage of the high-quality genome assemblies for both parents of the mapping population used in this study, we also identified candidate genes underlying the most significant QTL on chromosome 6H based on the orthologous analysis.

In addition to the major locus on chromosome 6H, several other loci detected from fully grown plants were also detected from measuring 3L of seedlings especially with the use of the BLUP values. However, the magnitudes of the loci detected from seedlings were all significantly smaller. Importantly, the strong correlations between LT and yield-related traits obtained from measuring leaves of fully-grown plants were not detected from measuring seedlings. One of the possible reasons for these differences could be caused by the likelihood that HD could have a larger effect on the 3L in developing seedlings compared with that on leaves of fully grown plants. However, it is difficult to understand why the magnitude and consistency of the loci detected from the two different leaves of fully-grown plants also differ so much. Our results show that, when possible, data from FL should be collected for LT.

In mapping loci for traits related to seedling vigour, Capo-chichi et al. (2021) detected multiple QTL for SLA on each of the seven chromosomes in barley. Of them, six were on chromosome 6H. It is likely that one of these six loci shares a similar location with the one on 6HL detected in this study. However, none of the six loci reported earlier comes close to the latter in regarding to either the magnitude or stability. Loci for SLA have been reported previously based on assessing either plants after anthesis (Yin et al. 1999a, b) or young seedlings (Elberse et al. 2004; Poorter et al. 2005). However, loci on chromosome 6H was not detected in any of these studies. The different results between the study reported here and those earlier ones could be due to direct vs indirect measurements as found in the study on desert-adapted tomato (Coneva et al. 2017). As only loci segregating in a population can be detected, another likely reason for the different results is due to the different materials used among these studies.

High quality genome assemblies are available for both parental genotypes of the mapping population used in this study (Liu et al. 2020), which made it easier to identify candidate genes targeting a given region based on orthologous analysis (Zhou et al. 2021). Based on such an analysis, three candidate genes were detected for the major locus on chromosome arm 6HL. One of these genes, *HORVU6Hr1G057630*, is orthologous to *OSPRR1* in rice which is involved in tiller bud outgrowth (Strable 2020). The orthologs of this gene are involved in photoperiodic flowering response in barley and *Arabidopsis* (Matsushika et al. 2000; Pruneda-Paz et al. 2009; He et al. 2019). The second gene *HORVU6Hr1G060990* is homologous with *OsVPE3* in rice. It has been reported that suppression of this gene could decrease the leaf width and guard cell length (Lu et al. 2016). The ortholog for the third gene

HORVU6Hr1G068370 is *OsGRF4* in rice and it is a positive regulator of genes that promote cell proliferation (Hu et al. 2015; Sun et al. 2016) and activates transcription of expansin promoters in protoplasts leading to a potential function in cell expansion (Liebsch and Palatnik 2020). Orthologs of this gene have also been found to be involved in multiple development processes in various species (Liebsch and Palatnik 2020). All three genes contain non-synonymous variations in their exons between the two parental genotypes which lead to amino acid substitutions. They form the primary targets to identify the gene(s) underlying this major locus for LT.

Correlations among various traits are common in any plant species thus it is not surprising that strong correlations between LT and several other traits were detected. Covariance analysis has been widely used to estimate the effects of such interaction between traits, but the power of such statistical analysis can be limited. One of the examples is the locus conferring plant height on chromosome 3H in barley. This locus was also shown to confer crown rot resistance and the effect of the locus on crown rot was still highly significant when the effect of plant height was removed by a covariance analysis (Li et al. 2009). However, further analyses based on near isogenic lines (NILs) for various plant height genes showed that all of height genes affect CR severity significantly (Liu et al. 2010) and the attempt to exploiting the 3H locus in generating barley breeding lines suggest that the observed CR resistance at this locus was a by-product of plant height (Unpublished). NILs have been effectively used to study the effect of a given locus for different traits in various plant species (Liu et al. 2010; Yan et al. 2011; Ma et al. 2012; Habib et al. 2016; Gao et al. 2019; Chen et al. 2021). With the adoption of techniques in rapidly generating materials with high-level of homozygosity (Zheng et al. 2013; Liu et al. 2016; Yan et al., 2017; Wanga et al. 2021), generating NILs for a given locus in many plant species is not a time-consuming process anymore. The size and stability of the loci detected for LT in this study suggest that developing NILs for some of these loci can be straightforward. As only two isolines need to be compared, effects of a given LT locus in multiple genetic backgrounds can be conveniently and accurately assessed in different environments once a few sets of NILs become available.

Declarations

Authors declare that there are no conflicts of interest.

Author contributions

JJ conceived the study. CL, ZZ and HH designed the experiments. ZZ, HH, SG, HZ, WL, and UK conducted the experiments, collected, and analysed data. ZZ, CL and HH prepared the first draft of the manuscript. All authors approved the final manuscript.

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Figures

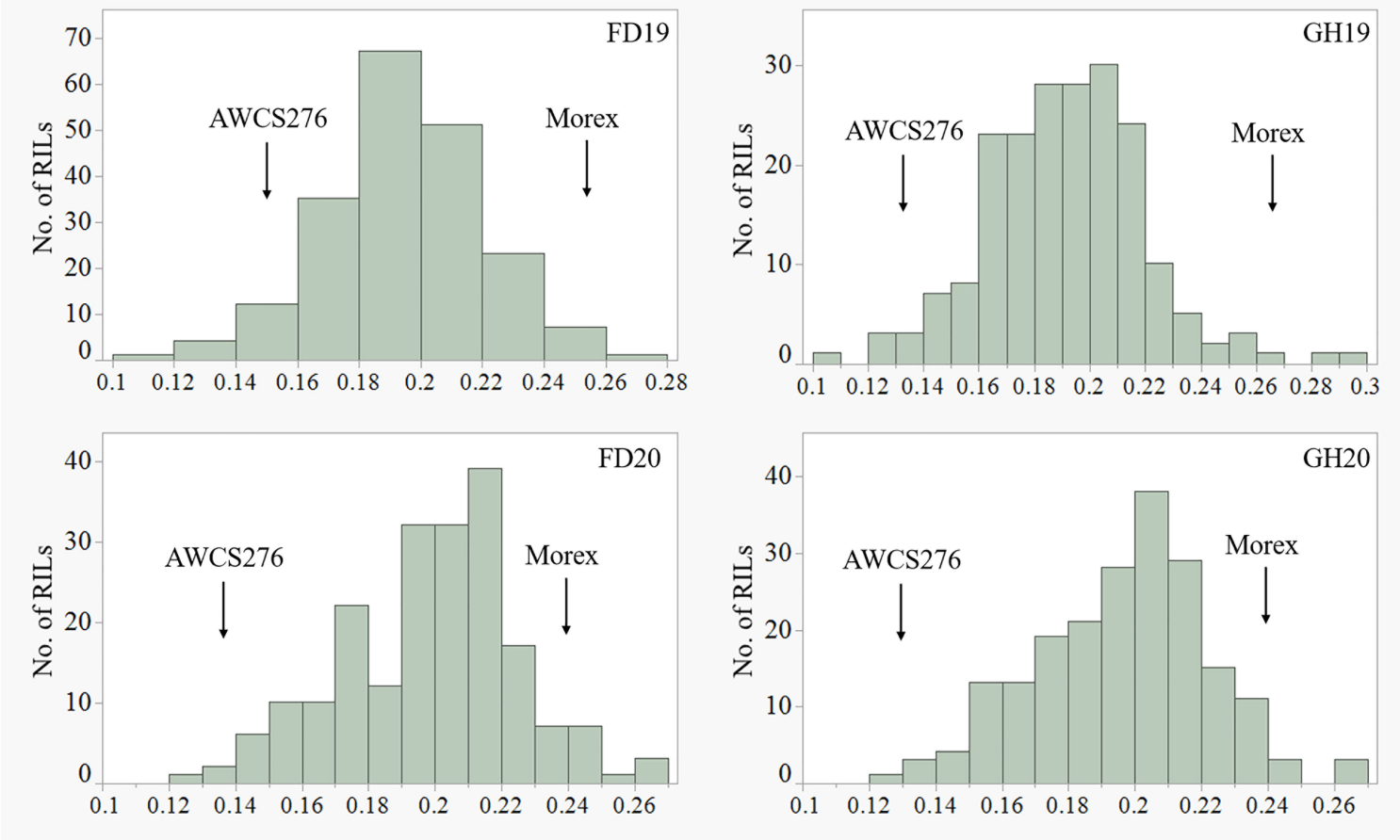

Figure 1

Frequency distributions for flag leaf thickness (FLT) obtained from the population of Morex/AWCS276 in different trials.

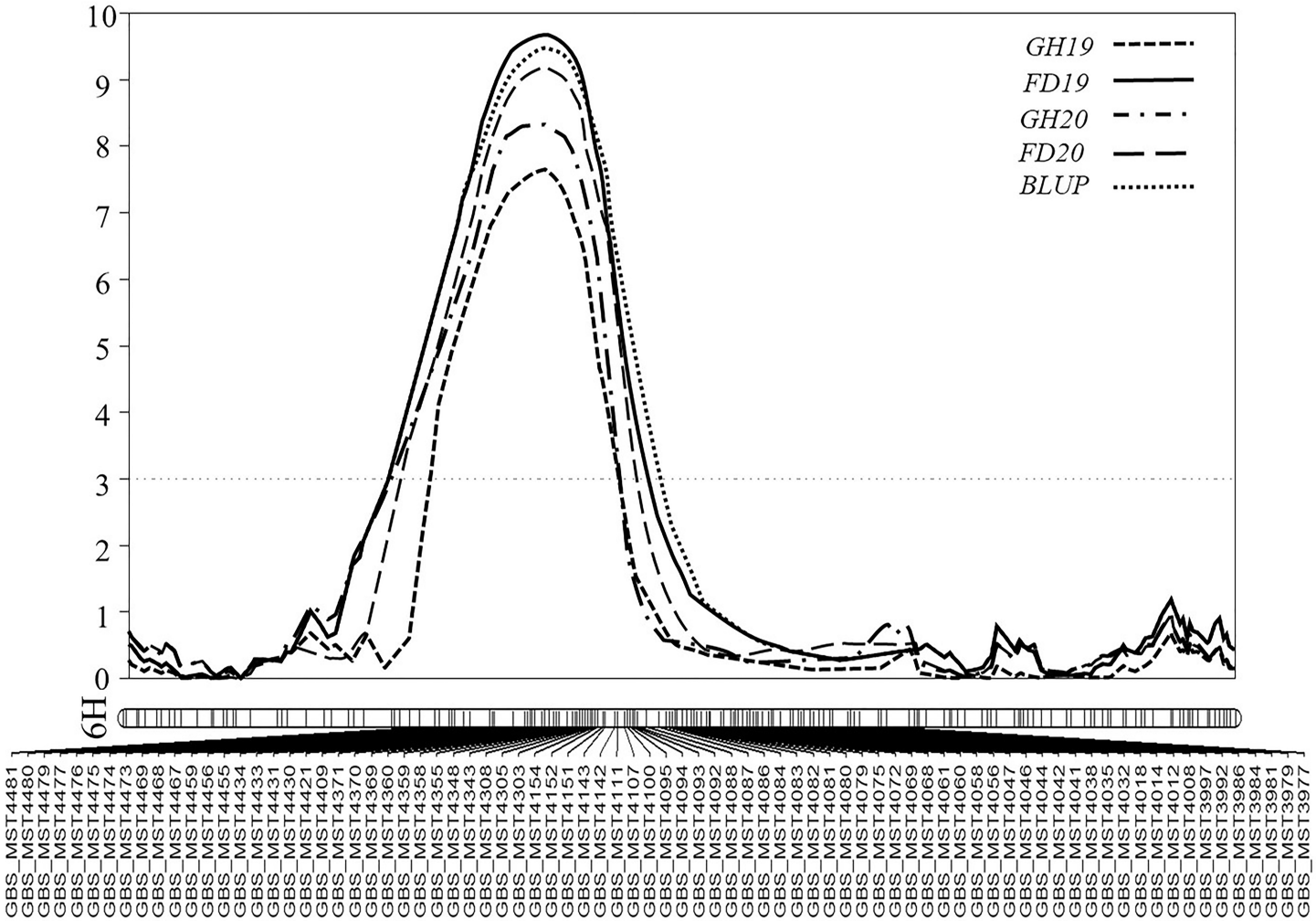

Figure 2

QTL conferring flag leaf thickness detected on chromosome 6H with interval mapping from the population of Morex/AWCS276. The LOD values from each centimorgan of the chromosome were plotted against the chromosome, and the vertical dotted line indicates the average significant threshold (LOD=2.9) derived from permutation test.

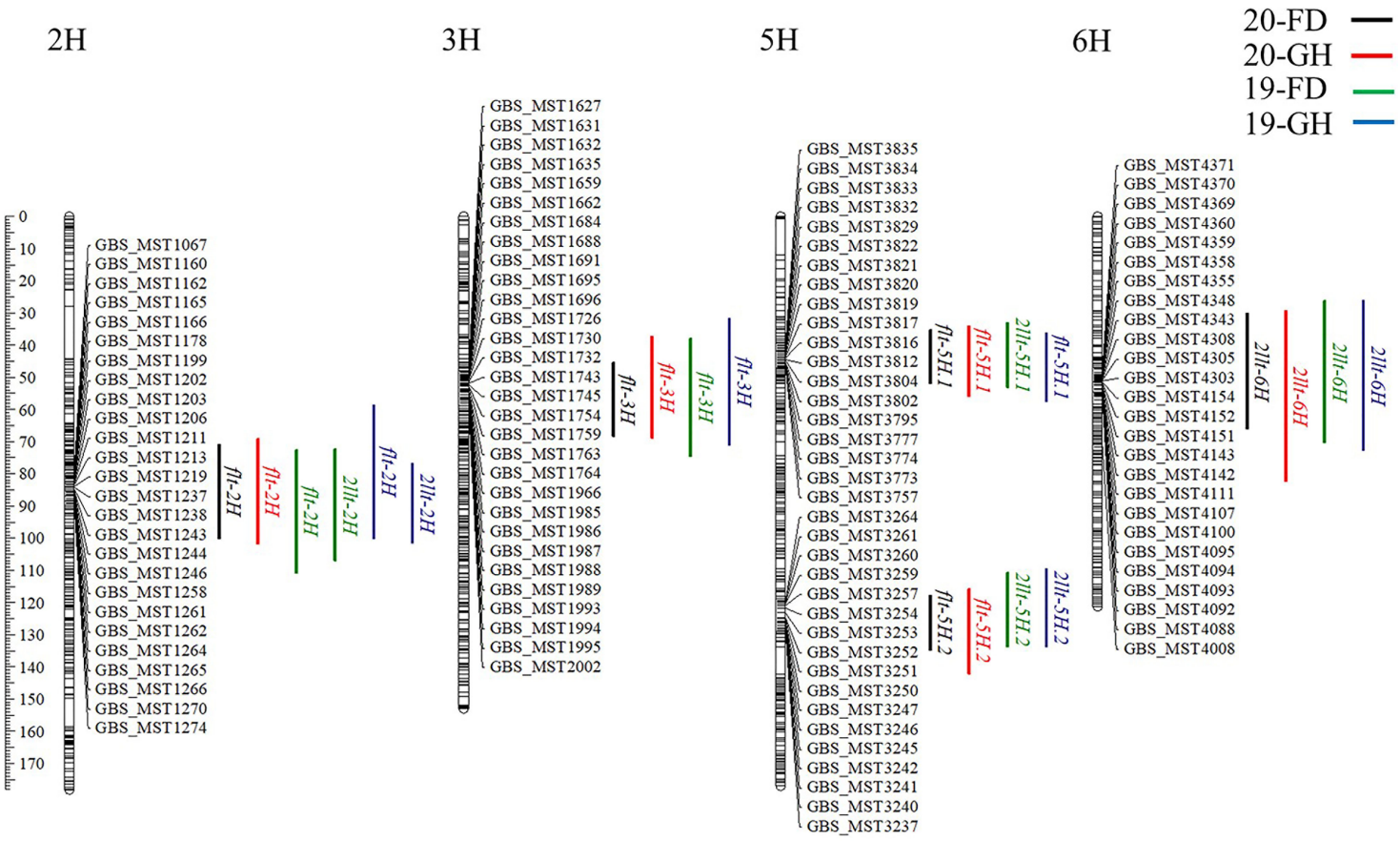

Figure 3

Locations of QTL for flag leaf thickness and second last leaf thickness in the population of Morex/AWCS276. QTL regions are marked with bars in different colours: QTL detected in FD20 (black), in GH20 (red), in FD19 (green) and in GH19 (blue).

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