Euphytica (2016) 208:337–351
DOI 10.1007/s10681-015-1603-0

QTL mapping of flag leaf traits in common wheat using an integrated high-density SSR and SNP genetic linkage map

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Received: 16 January 2015 / Accepted: 15 November 2015 / Published online: 28 November 2015

Abstract Photosynthesis of carbohydrates is the primary source of grain yield in wheat. Photosynthetic organs, especially flag leaves and awns play important roles in wheat growth and development. Genetic analysis of flag leaf posture, size and shape and presence/absence of awns was conducted using a set of 269 recombinant inbred lines (RILs) derived from Yanda1817 × Beinong6. Six agronomic traits comprising flag leaf angle (FLAN), flag leaf width (FLW), flag leaf length (FLL), the ratio of length/width of flag leaf (FLR), flag leaf area (FLA) and presence/absence of awns were evaluated in Shijiazhuang (2011, 2012 and 2013) and Beijing (2012). Using the available high-density single nucleotide polymorphism and simple sequence repeats (SSR) genetic linkage map, a total of 61 putative quantitative trait loci (QTL) for FLAN, FLW, FLL, FLR and FLA were detected on 16 of the 21 wheat chromosomes excluding 1D, 4B, 5D, 6A and 7A, with single QTL in different environments explaining 2.49–42.41 % of the phenotypic variation. Among the identified QTL, 17 were for FLAN, 11 for FLW, seven for FLL, 13 for FLR and 13 for FLA. Twenty-five (41 %) QTL were detected in at least two environments, while four QTL for FLW were detected in all environments. Thirty QTL were associated with higher number of flag leaf traits originated from Yanda1817 alleles, whereas the remaining 31 QTL were derived from Beinong6. In addition, pleiotropic effects were detected for QTL on chromosomes 2D, 3B, 4A, 4D, 5A, 5B, 6B, 6D and 7D that could serve as target regions for fine mapping and marker-assisted selection in wheat

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Electronic supplementary material The online version of this article (doi:10.1007/s10681-015-1603-0) contains supplementary material, which is available to authorized users.

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breeding programs. Genetic analysis revealed that the presence/absence of awns in the RIL population is controlled by the awn-inhibitor gene *B1* linked to SSR marker *Xgwm291* on the long arm of chromosome 5A. Our results also suggest that physiological traits FLL, FLW and FLA were significantly and positively correlated to spike length (SL), grain weight per spike and grain number per spike. FLR was significantly and positively related to SL but negatively related to grain width and grain thickness (GT). In addition, the awn trait was strongly and positively correlated to thousand grain weight, grain length and GT.

Keywords Wheat · SSR · SNP · QTL mapping · Awn · Flag leaf

Introduction

Wheat is the third highest produced cereal crop after maize and rice and is the leading source of plant-based protein in human food. Leaf size, shape, posture as well as ear and awn define the photosynthetic capability of wheat plants, and also impacts significantly on important agronomic traits such as yield and stress responses (Sourdille et al. 2002; Pérez-Pérez et al. 2010).

In cereals, the uppermost three leaves, and especially the flag leaf, have been identified as the primary sources of the photo-assimilate accumulated in the grain (Foyer 1987; Hirota et al. 1990; Li et al. 1998). Xu and Zhao (1995) and Sharma et al. (2003) reported that the flag leaf contributed about 50 % to the total photosynthetic activity and about 41–43 % to carbohydrates needed for grain filling. Therefore, the morphology of flag leaf, in particular its length, width, area and angle, is one of the most important components in determining grain yield potential in cereal crops (Sakamoto et al. 2006; Xue et al. 2008). Ideal leaf size and shape should improve photosynthesis and enhance grain yield. Rajaram et al. (2002) found erect-leaf types were slightly higher yielding than their droopy counterparts in random populations, but the dynamic flag leaf which is only initially erect and then becomes droopy would be advantageous for the overall efficiency. Obtaining optimal leaf morphology has become an important aim in plant breeding programs.

Flag leaf morphology is a complex quantitative trait, which is controlled by a number of genes or QTL which is quantitatively inherited and significantly influenced by the environment (Kobayashi et al. 2003; Fan et al. 2015). Flag leaf morphology elements include FLAN, FLW, FLL, FLR and FLA (Coleman et al. 2001; Yue et al. 2006; Xue et al. 2008; Wang et al. 2011; Chen et al. 2012). With the availability of molecular markers and genetic maps, QTL analysis have been used to identify genomic regions associated with flag leaf morphology in rice and barely (Mei et al. 2005; Yue et al. 2006; Xue et al. 2008; Farooq et al. 2010). In rice, two major QTL, *qFL1* and *qFLW4*, associated with flag leaf length and width, respectively, have been fine mapped (Wang et al. 2011; Chen et al. 2012). Seven narrow-leaf mutants have been identified and genetically mapped in rice (http://www.gramene.org/), but only *Nal1* and *Nal7* have been cloned (Fujino et al. 2008; Qi et al. 2008).

To date, little information is available on the genetic mechanisms of flag leaf traits in wheat. Six chromosome regions were found to be associated with FLL and FLW using an integrated SSR and expressed sequence tag (EST) genetic linkage map (Jia et al. 2013). One of the major QTL for FLW, *QFlw.nau-5A.1*, was fine mapped in a 0.2 centiMorgan (cM) interval in the 5AL12-0.35-0.57 deletion bin (Xue et al. 2013). Genes/QTL controlling leaf erectness or droopiness were reported on chromosome 2AS (Börner et al. 2002).

The objectives of our study were to locate QTL controlling flag leaf and awn morphology, and to reveal the relationships among flag leaf, awn and yield traits using a population of RILs derived from two Chinese winter wheat varieties and an integrated high-density SSR and SNP genetic linkage map.

Materials and methods

Plant materials and field trials

The QTL mapping population comprised of 269 RILs (F_9 to F_{11}) derived from Yanda1817 × Beinong6 by single seed descent. Yanda1817 was one of the 'cornerstone parental' breeding lines for the northern China Winter Wheat Breeding Program between 1950 and 1960 (Zhuang 2003). Beinong6 is a semi-dwarf high-yielding 1B/1R derivative released in the 1990s

by the Beijing University of Agriculture. RILs of Yanda1817 × Beinong6 were selected for QTL mapping because this RIL population was known to be segregating widely for FLAN, FLW, FLL, FLA, FLR and presence/absence of awns. Compared to Yanda1817, Beinong6 showed a larger FLA and FLW but smaller FLL and FLR. Furthermore, the flag leaf of Yanda1817 was droopy while Beinong6 was erect.

Yanda1817, Beinong6 and the 269 RILs were grown in Beijing (E116.10°, N40.08°; 2012) and Shijiazhuang (E114.36°, N37.38°; 2011, 2012 and 2013) for phenotypic evaluations, viz., 2011 Shijiazhuang, 2012 Beijing, 2012 Shijiazhuang and 2013 Shijiazhuang. Beijing (Northern Winter Wheat Zone) and Shijiazhuang (Yellow and Huai River Valleys Facultative Wheat Zone) represent two different wheat growing agro-climatic regions in China. A randomized complete block design with three replications was used in each of the four environments, and 30 seeds were evenly planted in each row of a two-row plot with 2 m long rows spaced 0.25 m apart. Field management was the same as commonly practiced in wheat production.

Testing of flag leaf and awn traits

From the center of each of the rows, ten representative plants were selected from each plot to measure the FLL (in centimeters), FLW (in centimeters) and FLAN (angle between flag leaf and the internode below the spike, in degrees) at the main grain filling stage by using an electronic goniometer. Two derived traits, the flag leaf area (FLA = FLL × FLW × 0.7) and the ratio of length/width of the flag leaf (FLR = FLL/FLW) were calculated. The presence or absence of awns of each line was also observed. Trait values of each year-location combination (defined as one environment) were used for QTL analysis.

Yield and yield components were examined for all plants, including TGW, GL, GW, GT, SL, GWS and GNS (Wu et al. 2015a, b).

Statistical analysis

Data obtained from field evaluations in four environments were subjected to combined analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS software (SAS Institute Inc., 2000). The broad sense heritability ($h^2 = V_G/V_P$, V_G is the genetic variance, V_P is the phenotypic variance) of each trait was estimated from the variance components from the ANOVA. The correlation coefficients (r) between pairs of all six traits and yield traits of this population (Wu et al. 2015a, b) were calculated using SPSS. 20. (SPSS Inc., Chicago,IL, USA).

QTL analysis

The high-density genetic linkage map and marker data of the Yanda1817 × Beinong6 mapping population were used during QTL analysis. The map included 1062 loci on all 21 chromosomes, comprising 109 SSR, 19 EST-SSR and 2431 SNP markers. The map covered 3213.2 cM with an average marker distance of 1.26 cM (Wu et al. 2015a). The Kosambi mapping function was used to convert the recombination frequencies into cM map distance (Kosambi 1943) and the genetic linkage maps were constructed using the software Map Draw V2.1 (Liu and Meng 2003).

The inclusive composite interval mapping (ICIM) function of IciMapping 4.0 (http://www.isbreeding.net/) which is based on stepwise regression of simultaneous consideration of all marker information was performed for QTL analysis. 'Deletion' command was used to accommodate missing phenotypes. The walking speed chosen for all QTL was 1.0 cM and the *P* value inclusion threshold was 0.001. A QTL was claimed to be significant at an LOD [logarithm (base 10) of odds] value of 2.5.

Results

Phenotypic variation and correlation analysis

Phenotypes of the RILs and two parental lines were evaluated for flag leaf traits, and the means, standard deviation, ranges, skewness, kurtosis and broad sense heritability of measured traits were summarized in Table 1. For FLAN, Yanda1817 was always more than 90° while Beinong6 was always less than 90° after heading (Table 1; Fig. 1). Beinong6 consistently showed higher values than Yanda1817 for FLW in all tested environments (Table 1; Fig. 1). The FLL of Yanda1817 was larger than Beinong6 in all environments except for 2012 at Beijing (Table 1). For FLA, Beinong6 showed higher values than Yanda1817 except for 2012 at Shijiazhuang (Table 1).

Table 1 Phenotypic performance and distribution of flag leaf traits in Yanda1817 × Beinong6 RILs at four environments

Trait	Environment	Yanda1817	Beinong6	RILs				
				Mean ± SD	Range	Skewness	Kurtosis	h^2 (%)
FLAN	2011_Shijiazhuang	138.87	55.48	101.57 ± 31.75	33.05–155.71	−0.53	−0.90	70.30
(degree)	2012_Beijing	135.74	50.65	94.07 ± 21.28	42.60–137.1	−0.45	−0.46	
	2012_Shijiazhuang	164.32	44.71	103.83 ± 49.72	16.30–164.32	−0.51	−1.52	
	2013_Shijiazhuang	138.75	48.44	103.14 ± 44.00	12.36–160.77	−0.82	−0.78	
FLW	2011_Shijiazhuang	1.19	1.70	1.44 ± 0.11	1.12–1.77	0.10	0.30	91.51
(cm)	2012_Beijing	1.40	1.98	1.72 ± 0.17	1.30–2.22	0.17	−0.10	
	2012_Shijiazhuang	1.05	1.50	1.24 ± 0.16	0.90–1.81	0.54	0.24	
	2013_Shijiazhuang	1.10	1.74	1.43 ± 0.14	1.03–1.91	0.40	0.66	
FLL	2011_Shijiazhuang	21.85	18.05	21.13 ± 2.22	16.19–26.92	0.11	−0.22	84.71
(cm)	2012_Beijing	20.16	23.04	23.80 ± 2.20	18.35–28.63	0.17	−0.10	
	2012_Shijiazhuang	23.49	14.85	20.15 ± 2.77	13.35–28.91	0.21	−0.14	
	2013_Shijiazhuang	20.06	19.06	20.94 ± 2.08	15.01–27.61	0.22	0.10	
FLR	2011_Shijiazhuang	18.57	10.72	14.77 ± 1.75	10.72–19.84	0.34	−0.16	88.12
	2012_Beijing	15.26	11.65	13.94 ± 1.35	10.48–17.83	0.29	−0.11	
	2012_Shijiazhuang	21.74	10.11	16.47 ± 2.09	10.07–21.80	0.04	0.06	
	2013_Shijiazhuang	17.87	10.80	14.80 ± 1.91	10.66–20.61	0.21	−0.44	
FLA	2011_Shijiazhuang	18.44	21.84	21.32 ± 3.21	13.18–31.02	0.33	0.15	86.88
(cm^2)	2012_Beijing	19.47	31.27	28.71 ± 4.17	17.70–41.56	0.18	−0.03	
	2012_Shijiazhuang	17.28	15.44	17.61 ± 4.20	9.51–36.12	0.76	0.91	
	2013_Shijiazhuang	15.69	23.69	20.89 ± 2.92	13.01–29.59	0.30	0.42	

FLAN flag leaf angle, *FLW* flag leaf width, *FLL* flag leaf length, *FLR* the ratio of length/width of flag leaf, *FLA* flag leaf area, h^2 broad sense heritability

Fig. 1 The performance of FLAN, FLW and FLL between Yanda1817 and Beinong6 in 2013 at Shijiazhuang

The frequency distributions of the investigated traits revealed continuous variations and transgressive segregation in the RIL population (Table 1). The skewness and kurtosis for all traits except FLAN in 2012 at Shijiazhuang, indicated a normal distribution of phenotypic data for FLAN, FLW, FLL, FLR and FLA, suggesting these traits were controlled by multiple loci. The broad-sense heritability for FLAN, FLW, FLL, FLR and FLA were 70.30, 91.51, 84.71, 88.12 and 86.88 %, respectively, indicating that flag leaf traits are stable and mainly under genetic control (Table 1). Among the

Table 2 Correlation coefficients between flag leaf traits and yield-related traits in the Yanda1817 × Beinong6 RIL population

Traits	FLAN	FLW	FLL	FLR	FLA	TGW	GL	GW	GT	SL	GWS	GNS
FLW	−0.056											
FLL	0.033	0.328**										
FLR	0.059	0.521**	0.617**									
FLA	−0.012	0.799**	0.811**	0.059								
TGW	−0.005	0.049	−0.035	−0.081	0.021							
GL	0.104	0.053	0.163**	0.099	0.152*	0.555**						
GW	0.016	0.094	−0.035	−0.124*	0.046	0.791**	0.203**					
GT	−0.102	0.092	−0.111	−0.184**	−0.007	0.766**	0.209**	0.598**				
SL	0.108	0.216**	0.406**	0.171**	0.386**	−0.072	0.153*	−0.059				
GWS	−0.068	0.255**	0.206**	−0.029	0.288**	0.616**	0.242**	0.573**	0.465**	0.110		
GNS	−0.044	0.316**	0.254**	−0.027	0.337**	−0.264**	−0.259**	−0.092	−0.223**	0.197**	0.435**	
AWN	0.063	0.031	0.033	0.009	0.036	0.261**	0.282**	−0.019	0.285**	−0.070	0.115	−0.096

FLAN flag leaf angle, *FLW* flag leaf width, *FLL* flag leaf length, *FLR* the ratio of length/width of flag leaf, *FLA* flag leaf area

* and ** indicate significance levels at P = 0.05 and 0.01 (2-tailed), respectively

five flag leaf traits, FLW had the highest heritability, followed by FLR and FLA.

Yanda1817 was awnless while Beinong6 was awned. The RILs segregated as 118 were awnless and 151 awned, fitting a 1:1 single Mendelian loci ratio ($\chi^2 = 2.02$, $P > 0.05$). The result suggested that the presence/absence of awns in the RIL population was controlled by a single-locus.

Correlation coefficients (r) among the flag leaf traits, awnedness and yield-related traits in different environments were calculated. No significant correlation was detected between FLAN and all other traits (Table 2). FLW was significantly and positively correlated with FLL ($r = 0.328$, $P < 0.01$), FLR ($r = 0.521$, $P < 0.01$) and especially FLA ($r = 0.799$, $P < 0.01$). Strong correlations were also identified between FLL and FLR ($r = 0.617$, $P < 0.01$), FLA ($r = 0.811$, $P < 0.01$) and GL ($r = 0.163$, $P < 0.01$) (Table 2). In addition, FLL, FLW and FLA were significantly and positively correlated to SL, GWS and GNS, while FLR was positively correlated to SL but negatively correlated to GW and GT. Furthermore, the awn trait was significantly positively correlated to TGW, GL and GT (Table 2).

QTL analysis of the flag leaf traits

A total of 61 QTL for the examined flag leaf traits were detected on all 21 chromosomes except 1D, 4B, 5D, 6A and 7A chromosomes with individual QTL contributing between 2.49 and 42.41 % to the phenotypic variance in different environments (Table S2; Fig. 2). Twenty-five QTL (41 % of the mapped QTL) were identified in more than one environment and the highest number of QTL was detected on chromosome 5A. Among the 61 QTL, 30 (49 %) were associated with increased FLAN, FLW, FLL, FLR and FLA through the Yanda1817 alleles, mainly distributed on chromosomes 1B, 2D, 3A, 3B, 4A, 5A and 6D (Table S2). Co-localized QTL for different flag leaf traits were found on chromosomes 1B, 2B, 2D, 3A, 3B, 4A, 4D, 5A, 5B, 6B, 6D and 7D.

Seventeen QTL for FLAN were mapped on chromosomes 1A, 1B, 2A, 3A, 3B, 4A, 5A, 5B, 6B and 6D with phenotypic variations ranging from 2.49 to 42.41 % (Table S2; Fig. 2). However, 13 of these were detected in only one environment. *QFlan.cau-1A*, *QFlan.cau-1B.2* and *QFlan.cau-6B.2* were detected in three environments while *QFlan.cau-5A.2* was identified in two environments, suggesting that these were stable QTL. Positive values of additive effects for 10 QTL indicated that the Yanda1817 alleles contributed to the increase of the FLAN (Table S2).

Eleven QTL for FLW were detected on chromosomes 1B, 2B, 2D, 3A, 4D, 5A, 6B and 7D with individual QTL explaining 2.77–17.98 % of the phenotypic variance (Table S2; Fig. 2). Four QTL, *QFlw.cau-3A*, *QFlw.cau-5A.1*, *QFlw.cau-5A.3* and *QFlw.cau-7D* were detected in all four environments and *QFlw.cau-5A.1* had the largest effect (Table 3).

QFlw.cau-2D was identified in two environments while *QFlw.cau-4D*, *QFlw.cau-5A.2* and *QFlw.cau-6B* were detected in three environments. Only three QTL (*QFlw.cau-1B.1*, *QFlw.cau-1B.2* and *QFlw.cau-2B*) were identified in only one environment. *QFlw.cau-1B.1* and *QFlw.cau-2D* both contributed to broader FLW through the Yanda1817 alleles while the other QTL were derived from Beinong6 alleles (Table S2).

QTL for FLL were found at seven chromosome regions with phenotypic variations ranging from 3.48 to 23.86 % (Table S2, Fig. 2). *QFll.cau-2D* was detected in two environments with the strongest association with FLL and explained up to 23.86 % of the phenotypic variance. *QFll.cau-5B* was detected in three environments, indicating it was a stable QTL. *QFll.cau-3B* and *QFll.cau-4A* were identified in two environments and the remaining three QTL were found in only one environment. The chromosome regions that contributed to FLL were associated with chromosomes 1B, 2B and 5B associated with the Beinong6 allele, and chromosomes 2D, 3B and 4A for the Yanda1817 allele, respectively (Table S2).

Thirteen QTL affecting FLR were detected on chromosomes 2A, 3A, 3B, 3D, 4A, 4D, 5A, 5B, 6D, 7B and 7D with contributions to phenotypic variations of individual QTL ranging from 2.70 to 14.79 % (Table S2; Fig. 2). *QFlr.cau-5A.1* was the most stable QTL and was detected in four environments, explaining the highest phenotypic variation (Table 3). *QFlr.cau-4A* and *QFlr.cau-5B* were identified in three environments while *QFlr.cau-3A.2* and *QFlr.cau-6D* were detected in two environments. The remaining eight QTL were mapped in only one environment. The increasing effects of FLR originated from Beinong6 alleles for *QFlr.cau-3D* and *QFlr.cau-5B* and Yanda1817 alleles of all the other QTL (Table S2).

Thirteen chromosome regions were found to be associated with FLA explaining 3.33–26.13 % of the phenotypic variance (Table S2, Fig. 2). *QFla.cau-2D* and *QFla.cau-5A.1* were detected in three environments while *QFla.cau-5A.3* and *QFla.cau-5B.2* were identified in two environments. Among them, *QFla.cau-2D* had the strongest effects on FLA with a LOD score of 9.42, explaining 26.13 % of the phenotypic variation at Shijiazhuang in 2012. The other nine QTL were only detected in one environment. The Yanda1817 allele of *QFla.cau-2D*, *QFla.cau-3B* and *QFla.cau-4A* are associated with larger leaves (Table S2).

Fig. 2 QTL for FLAN, FLW, FLL, FLR and FLA detected in the Yanda1817 × Beinong6 RIL population. Supported intervals for QTL are indicated by *vertical bars*, the length of the *bar* shows a one LOD confidence interval. LOD max is indicated by a *triangle*. E1: 2011 Shijiazhuang; E2: 2012 Beijing; E3: 2012 Shijiazhuang; E4: 2013 Shijiazhuang

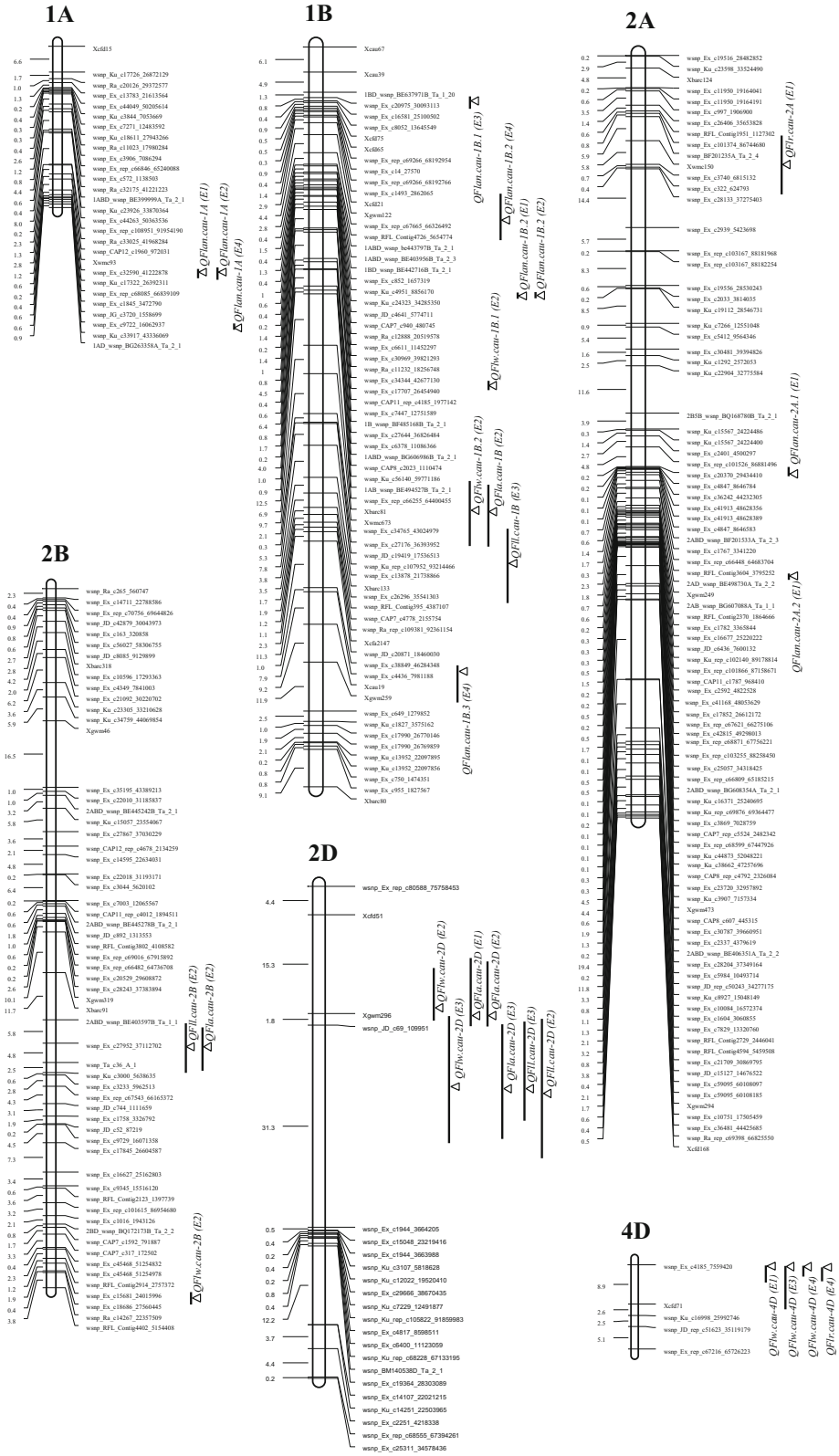
Identification of the locus controlling the presence of awns

A locus controlling the presence or absence of awns was identified on chromosome arm 5AL between SNP marker *wsnp_Ku_rep_c72362_72059764* and SSR marker *Xgwm291* with genetic distances of 3.8 and 5.4 cM, respectively. This locus could be the awn-inhibitor gene *B1* (Du et al. 2010; McIntosh et al. 2013).

Discussion

Different responses to abiotic stresses

Between Beinong6, a modern wheat variety and Yanda1817, a landrace Yanda1817, a wheat landrace, derived from Pingyao Xiaobaimai of Shanxi Province, is highly tolerant to drought, winter hardiness and poor soil fertility. In contrast, Beinong6 is a semi dwarf, high-yielding modern variety adapted to the irrigated and fertilized environment. The FLL of Beinong6 showed higher values than Yanda1817 in 2012 at Beijing in contrast to the other three environments. This might be due to this environment being irrigated and being a highly fertile field. The smaller FLA of Beinong6 in 2012 at Shijiazhuang than that of Yanda1817 might be due to drought stress at flag leaf stage, resulting in a reduction of FLL. Results revealed a better adaptation of landrace Yanda1817 to water limitations during its growth and developmental stages, in agreement with previous studies (Ceccarelli 1996; Chaves et al. 2002; Moragues et al. 2006; Rodriguez et al. 2008). In response to drought stress, morphological changes in FLL, peduncle length, and loss of yield have been reported in crops (Chaves et al. 2002). Landraces represent an important source of


1A
1B
2A
2B
2D
4D
QFlan.cau-1A (E1)
QFlan.cau-1A (E2)
QFlan.cau-1A (E4)
QFlan.cau-1B.1 (E3)
QFlan.cau-1B.2 (E4)
QFlan.cau-1B.2 (E1)
QFlan.cau-1B.2 (E2)
QFlw.cau-1B.1 (E2)
QFlw.cau-1B.2 (E2)
QFla.cau-1B.1 (E2)
QFla.cau-1B (E3)
QFll.cau-1B (E3)
QFlan.cau-1B.3 (E4)
QFlw.cau-2A (E1)
QFlan.cau-2A.1 (E1)
QFlan.cau-2A.2 (E1)
QFll.cau-2B (E2)
QFla.cau-2B (E2)
QFlw.cau-2B (E2)
QFlw.cau-2D (E2)
QFla.cau-2D (E1)
QFla.cau-2D (E2)
QFla.cau-2D (E3)
QFll.cau-2D (E3)
QFll.cau-2D (E2)
QFlw.cau-4D (E1)
QFlw.cau-4D (E3)
QFlw.cau-4D (E4)
QFll.cau-4D (E4)

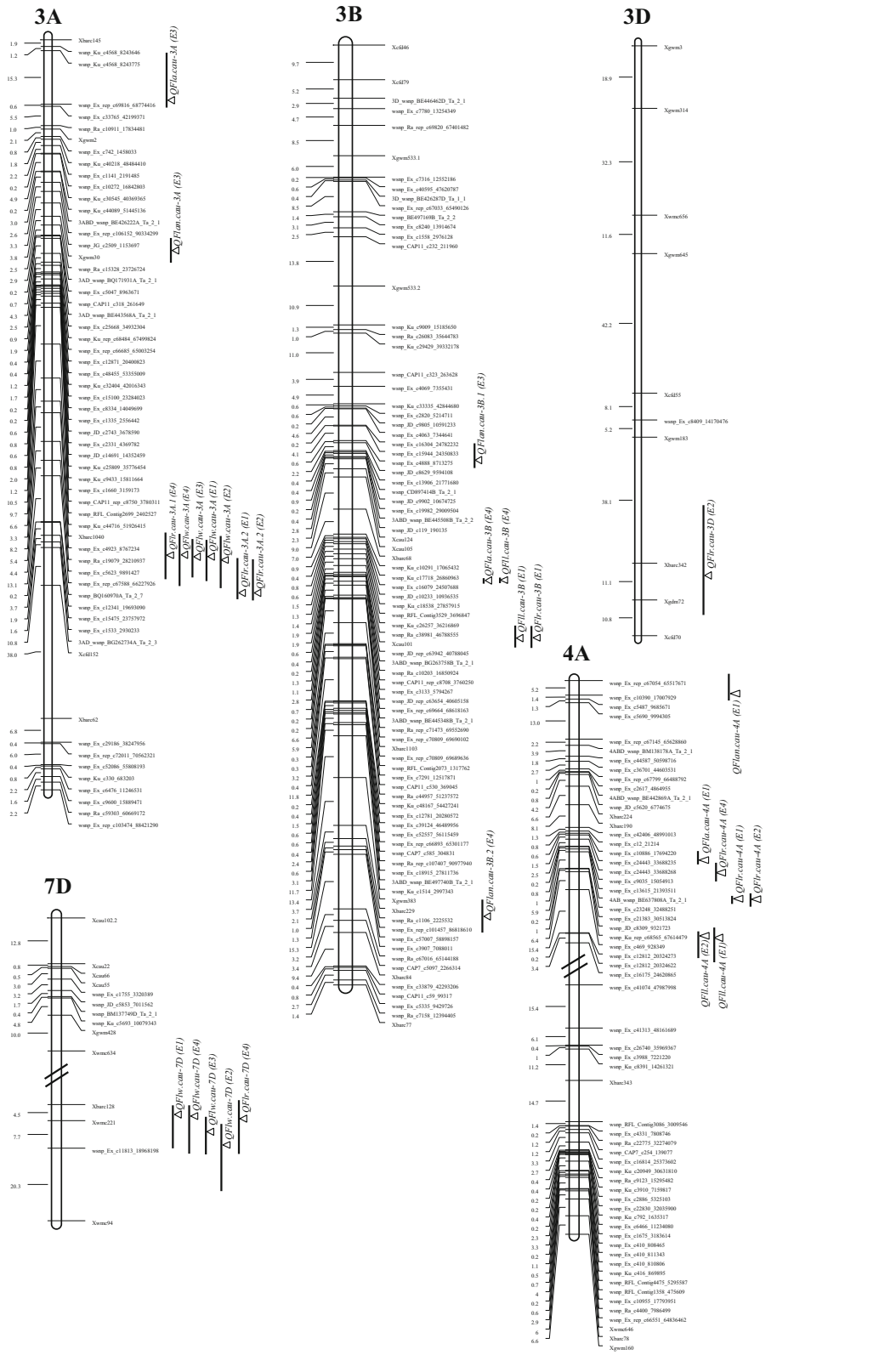


Fig. 2 continued

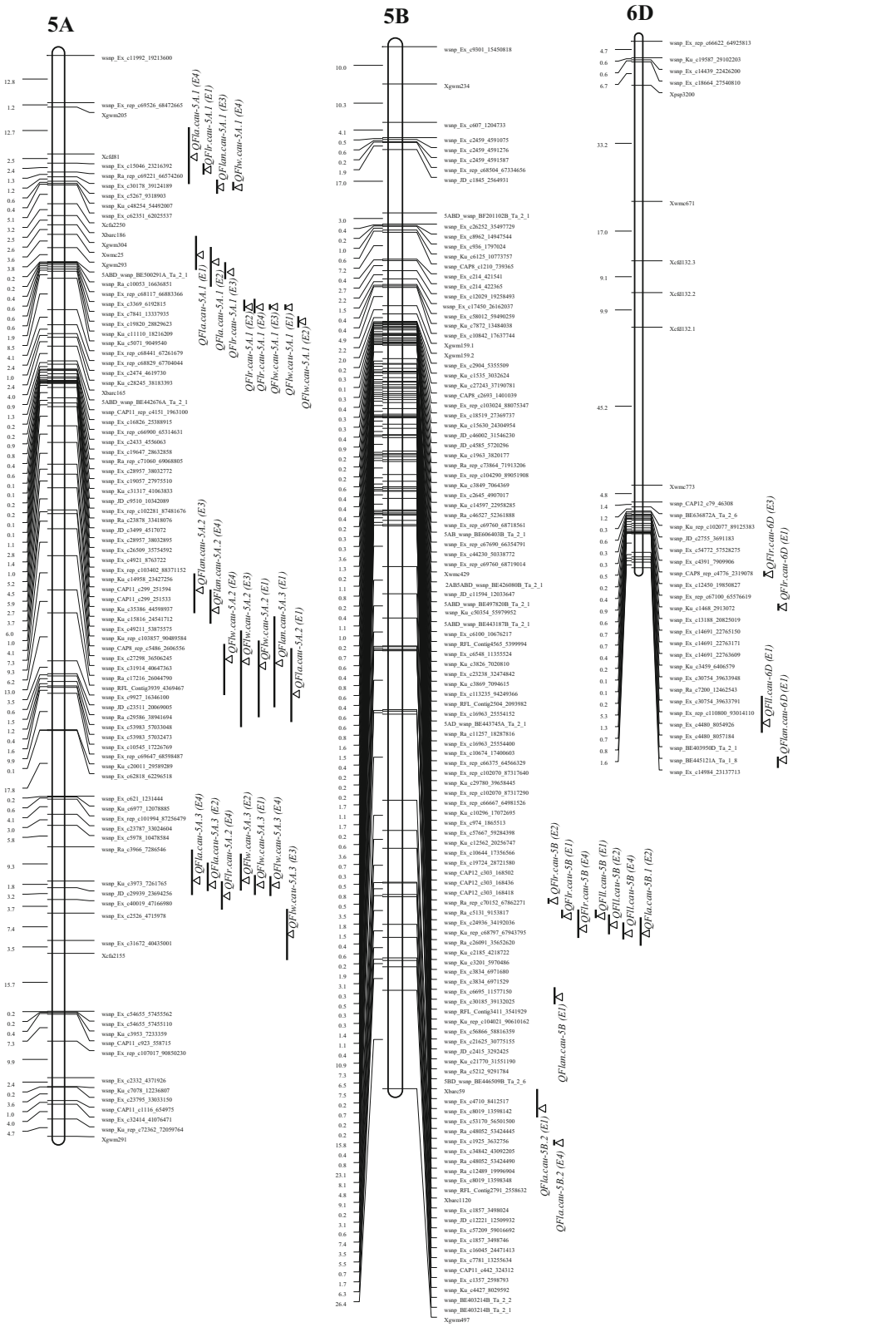


Fig. 2 continued

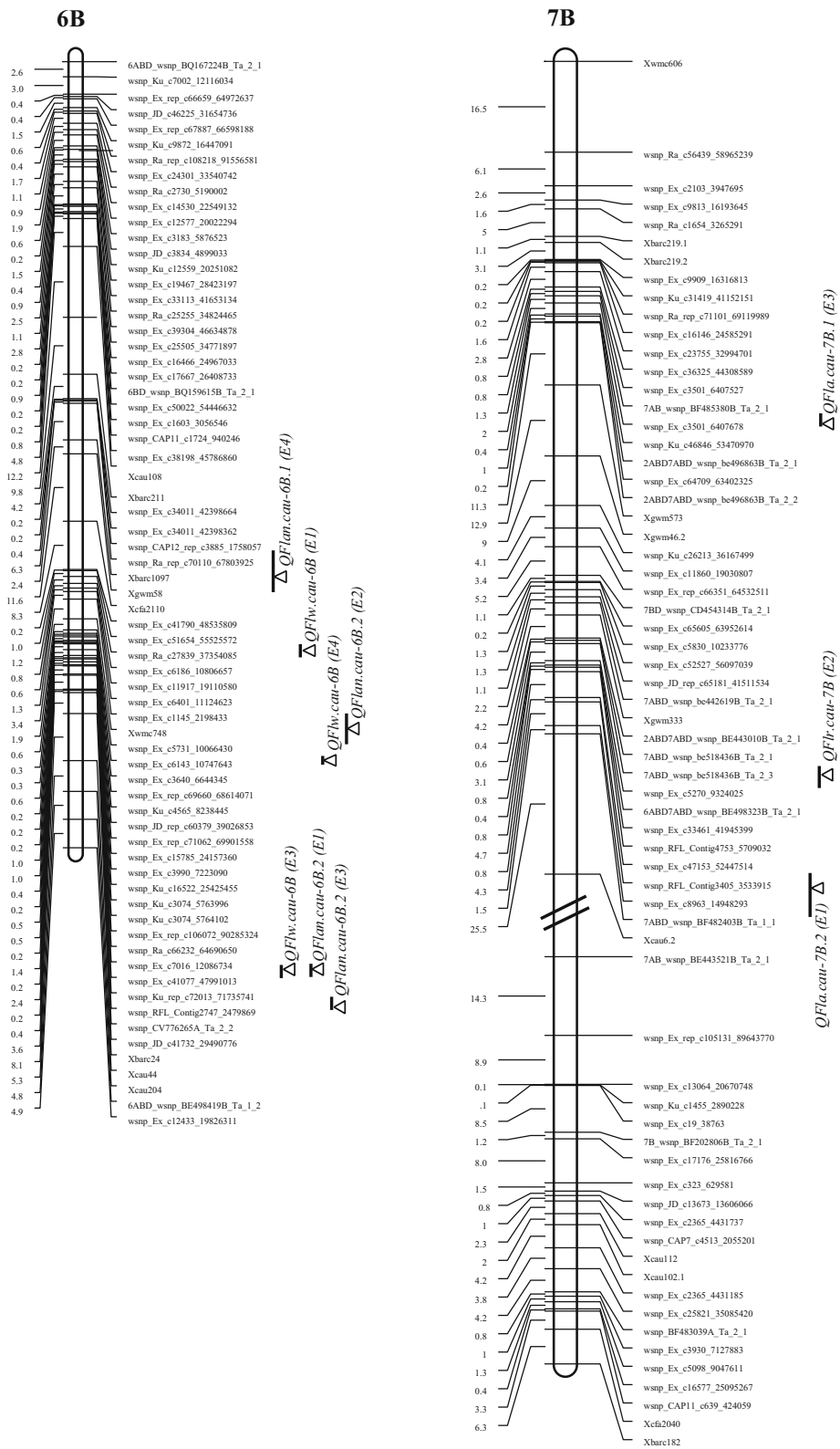


Fig. 2 continued

Table 3 QTL for FLW and FLR detected in all the environments in the Yanda1817 × Beinong6 RIL population

QTL	Environment	Position	Left marker	Right marker	LOD[a]	PVE (%)[b]	Add[c]
QFlw.cau-3A	2013_Shijiazhuang	113	*wsnp_Ex_c4923_8767234*	*wsnp_Ra_c19079_28210937*	8.02	9.37	−0.04
	2012_Shijiazhuang	114	*wsnp_Ex_c4923_8767234*	*wsnp_Ra_c19079_28210937*	7.04	8.57	−0.05
	2011_Shijiazhuang	114	*wsnp_Ex_c4923_8767234*	*wsnp_Ra_c19079_28210937*	6.83	6.91	−0.03
	2012_Beijing	115	*wsnp_Ra_c19079_28210937*	*wsnp_Ex_c5623_9891427*	3.48	3.87	−0.03
QFlw.cau-5A.1	2013_Shijiazhuang	33	*wsnp_Ex_c30178_39124189*	*wsnp_Ex_c5267_9318903*	11.8	12.85	−0.05
	2012_Shijiazhuang	57	*wsnp_Ex_c3369_6192815*	*wsnp_Ex_c7841_13337935*	9.61	11.44	−0.05
	2011_Shijiazhuang	57	*wsnp_Ex_c3369_6192815*	*wsnp_Ex_c7841_13337935*	16.77	17.98	−0.05
	2012_Beijing	58	*wsnp_Ex_c19820_28829623*	*wsnp_Ku_c11110_18216209*	12.03	14.26	−0.06
QFlw.cau-5A.3	2012_Beijing	223	*wsnp_Ra_c3966_7286546*	*wsnp_Ku_c3973_7261765*	5.19	6.1	−0.04
	2011_Shijiazhuang	224	*wsnp_Ku_c3973_7261765*	*wsnp_JD_c29939_23694256*	4.01	3.87	−0.02
	2013_Shijiazhuang	224	*wsnp_Ku_c3973_7261765*	*wsnp_JD_c29939_23694256*	6.97	7.37	−0.04
	2012_Shijiazhuang	238	*wsnp_Ex_c2526_4715978*	*wsnp_Ex_c31672_40435001*	3.64	4.56	−0.03
QFlw.cau-7D	2011_Shijiazhuang	2	*Xbarc128*	*Xwmc221*	7.37	7.72	−0.03
	2013_Shijiazhuang	3	*Xbarc128*	*Xwmc221*	7.25	8.03	−0.04
	2012_Shijiazhuang	6	*Xwmc221*	*wsnp_Ex_c11813_18968198*	6.84	8.56	−0.05
	2012_Beijing	10	*Xwmc221*	*wsnp_Ex_c11813_18968198*	5.74	7.18	−0.04
QFlr.cau-5A.1	2011_Shijiazhuang	31	*wsnp_Ex_c15046_23216392*	*wsnp_Ra_rep_c69221_66574260*	7.21	7.88	0.48
	2012_Shijiazhuang	55	*Xgwm293*	*5ABD_wsnp_BE500291A_Ta_2_1*	9.28	14.79	0.79
	2012_Beijing	57	*wsnp_Ex_c3369_6192815*	*wsnp_Ex_c7841_13337935*	5.61	6.65	0.35
	2013_Shijiazhuang	57	*wsnp_Ex_c3369_6192815*	*wsnp_Ex_c7841_13337935*	6.73	7.98	0.54

[a] Maximum-likelihood LOD score for the QTL calculated by IciMapping 4.0

[b] PVE (%) = phenotypic variance estimated from marker regression against phenotype

[c] ± Additive effect. Positive values indicate a positive effect of Yanda1817 alleles, whereas negative values indicate the contribution of the Beinong6 allele

germplasm in unfavourable environments, as they are often grown in stressful environments with limited agronomic input (Ceccarelli and Grando 2000). High yielding genotypes often have inferior performances under poor growing conditions (Ceccarelli 1996) but modern wheat varieties often show a clear superiority with respect to landraces in favourable environments (Rodriguez et al. 2008).

QTL for flag leaf morphology

The improvement of flag leaf posture, size and shape has been an important objective in many cereal breeding programs. However, only a few studies reported on the genetic control of flag leaf traits in wheat. Due to the diversity of available mapping populations, molecular markers and genetic coverage of linkage maps used for QTL mapping, we used the integrated high-density SSR genetic linkage map (Somers et al. 2004) as a reference to anchor SSR markers and to compare our QTL mapping data with published reports.

We detected 11 QTL for FLW of which seven were observed in three or four environments, indicating that FLW is a stable trait which is in complete agreement with its high heritability of 91.51 %. Three of the four most stable QTL for FLW, *QFlw.cau-5A.1*, *QFlw.cau-5A.2* and *QFlw.cau-5A.3* were detected on chromosome 5A. A major QTL for FLW, *Qflw.nau-5A*, was reported on chromosome arm 5AS linked to Fusarium head blight QTL *Fhb5* using a RIL population developed from Nanda2419 × Wangshuibai (Ma et al. 2008; Xue et al. 2011; Jia et al. 2013). Using a secondary F_2 population, the *Qflw.nau-5A* QTL was fine mapped between SSR markers *Xgwm293* and *Xgwm304* (Xue et al. 2013). In our mapping population, a stable QTL, *QFlw.cau-5A.1*, explaining the largest portion of the phenotypic variance was detected at the similar location as *Qflw.nau-5A*. Another major QTL, *QFlw.cau-5A.3* linked to *Xcfa2155* was also reported by Jia et al. (2013) at a similar genetic region. *QFlw.cau-2D* was closely linked to SSR markers *Xcfd51* and *Xgwm296* that may correspond to the QTL for FLW reported by Coleman et al. (2001). The remaining QTL for FLW were newly identified.

Out of the seven QTL detected for FLL, only one was reported in previous studies and the remaining six may be new loci. *QFll.cau-2B* is closely situated to a QTL for FLL on chromosome 2B near the centromeric region reported by Coleman et al. (2001). QTL for FLL on chromosome 4A was also described by Jia et al. (2013). However, the *QFll.cau-4A* QTL detected in two environments during our study is not located in the same genetic region as previously reported.

FLA was significantly and positively correlated with FLW ($r = 0.799$) and FLL ($r = 0.811$). As expected, five and three coincided loci were detected between FLA and FLL, and FLA and FLW, respectively. For example, QTL *QFla.cau-2B*, *QFla.cau-4A* and *QFla.cau-5A.1* were found in the same genomic regions as QTL *QFll.cau-2B*, *QFll.cau-4A* and *QFlw.cau-5A.1*. Even more interesting, a genetic region on chromosome 2D is responsible for FLL, FLW and FLA with three QTL *QFla.cau-2D, QFlw.cau-2D* and *QFll.cau-2D* mapping to the same position. This finding could partially explain the phenomenon of QTL clustering in some chromosome regions.

In our study, co-located QTL were found on chromosomes 2D, 5B and 6B, especially on chromosome 5A. The QTL cluster on chromosome arm 5AS was involved in a FLAN QTL identified in one environment, a FLW QTL detected in four environments, a FLR QTL found in four environments and a FLA QTL observed in three environments. Another QTL cluster was located on chromosome arm 5AL and involved QTL controlling FLW, FLA and FLR. QTL for FLL, FLR and FLA were also found on genetic region between *wsnp_Ra_rep_c70152_67862271* and *wsnp_Ku_rep_c68797_67943795* on chromosome 5B. Furthermore, a genetic interval on chromosome 6B was responsible for FLAN and FLW simultaneously.

Only a few publications are available for FLAN or flag leaf posture. Börner et al. (2002) reported the discovery of two major QTL for FLAN on chromosome arms 2AS and 2DL, both transmitted by 'Opata 85'. In our study, two new QTL were identified on chromosome arm 2AL (*QFlan.cau-2A.1* and *QFlan.cau-2A.2*) for FLAN. *QFlan.cau-2A.1*, explaining the largest portion of the phenotypic variance 42.41 %, had the highest LOD score of all QTL detected in the study (LOD = 32.46).

Up to now, no QTL for FLR was reported. In our mapping population, thirteen QTL affecting FLR were detected with contributions to phenotypic variations of individual QTL ranging from 2.70 to 14.79 %. Among which, five QTL were detected in at least two environments. *QFlr.cau-5A.1* was detected in four

environments, explaining the highest phenotypic variation, while *QFlr.cau-5B* detected in three environments with the highest LOD score (LOD = 10.65). These stable QTL can be used in MAS to obtain optimal leaf morphology and improve yield potential.

Furthermore, some QTL were found to be associated with genomic regions of known photoperiod and vernalization genes. QTL cluster (*QFla.cau-2D*, *QFlw.cau-2D* and *QFll.cau-2D*) was located on chromosome arm 2DS and linked with SSR marker *Xgwm296*, closely linked to the photoperiod gene *Ppd-D1* (Gasperini et al. 2012). QTL *QFll.cau-5B*, *QFlr.cau-5B* and *QFla.cau-5B.1* were located on chromosome 5BL between markers *wsnp_Ra_rep_c70152_67862271* and *wsnp_Ra_c26091_35652620* and this location is close to *Vrn-B1* (Barrett et al. 2002; Somers et al. 2004; Chu et al. 2011). However, the relationship of *Ppd-D1* and *Vrn-B1* to these QTL need to be further characterized.

The influence of flag leaf and awn on yield related traits

The flag leaf and awns are thought to be the most significant contributor of photo-assimilate to the developing grain. Motzo and Giunta (2002) found that awns increase the surface area of the ear from 36 to 59 % and contribute about 40–80 % to the total spike carbon exchange rate depending on the species (Blum 1985). Li et al. (2006) reported that awns were superior to flag leaves on a cellular and physiological level throughout the grain-filling period and could possess a strong capacity to photosynthesize and promote assimilation products to grain mass. Positive and significant correlations between flag leaf characteristics and yield traits, such as GNS, GWS and SL suggested that FLW, FLL and FLA contribute to increasing yield. We have detected QTL clusters controlling flag leaf traits and yield related traits, such as TGW, GW, GL and GT, on chromosomes 1A, 2D, 4D, 5A, 5B, and 7D (Wu et al. 2015a, b). Therefore, the stable QTL for flag leaf characteristics that showed consistent relationships with yield-related traits could be more easily and effectively utilized in MAS.

Significant and positive correlations were observed between the presence of awn and yield components TGW, GW, GL and GT. The wheat awn is controlled by five major genes *B1*, *B2*, *B3*, *A*, and *Hd* and some minor genes (McIntosh et al. 2013). Three dominant inhibitors, *Hd*, *B1* and *B2*, were previously reported on chromosomes 4AS, 5AL and 6BL, respectively (Sears 1954; Snape et al. 1985; Kato et al. 1998; McIntosh et al. 2013). A yield component QTL cluster were detected near the awn-inhibitor gene *B1* that is located at the same genetic region of a TGW QTL detected in two environments, a GL QTL detected in six environments, a GT QTL detected in four environments, and a GW QTL identified in one environment (Wu et al. 2015a).

In summary, we detected 61 putative QTL for flag leaf traits including FLAN, FLW, FLL, FLR and FLA, among which 25 could be observed in at least two environments, providing useful information for genetic improvement of flag leaf morphological types in wheat through QTL pyramiding. Five QTL (*QFlw.cau-3A*, *QFlw.cau-5A.1*, *QFlw.cau-5A.3*, *QFlw.cau-7D* and *QFlr.cau-5A.1*) could be identified in all the four environments, indicating stable expression of these five QTL. Molecular makers closely linked to these five stable QTL are of great value in MAS designed to improve wheat flag leaf size and yield potential in wheat breeding programs. Furthermore, development of near isogenic lines through advanced backcrossing could be conducted in fine mapping and cloning of these major stable QTL and QTL regions with pleiotropic effects.

Acknowledgments The authors are grateful to Prof. Andrew O. Jackson of the University of California at Berkeley for editing the manuscript. This work was financially supported by the 973 project (2011CB100104), National Natural Science Foundation of China (31271710, 31301312) and Ministry of Education (MOE) of China (111-02-3).

Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest. Although co-author Chengguo Yuan is currently employed by Gaoyi Stock Seed Farm, China, all of his contributions to the current manuscript were completed while he was a graduate student at China Agricultural University (CAU). Thus, the author does not have any conflict of interest to report here. This does not alter our adherence to Euphytica policies on sharing data and materials.

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