RESEARCH ARTICLE

Genetic dissection of chlorophyll content at different growth stages in common wheat

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Abstract

Quantitative trait loci (QTLs) for chlorophyll content were studied using a doubled haploid (DH) population with 168 progeny lines, derived from a cross between two elite Chinese wheat cultivars Huapei 3 × Yumai 57. Chlorophyll content was evaluated at the maximum tillering stage (MS), the heading stage (HS), and the grain filling stage (GS), at three different environments in 2005 and 2006 cropping seasons. QTL analyses were performed using a mixed linear model approach. A total of 17 additive QTLs and nine pairs of epistatic QTLs were detected. Ten of 17 additive QTLs for chlorophyll content were persistently expressed at more than two growth stages, which suggest developmentally regulated loci controlling genetics for chlorophyll content in different growth stages in wheat. One novel major QTL for chlorophyll content was closely linked with the PCR marker *Xwmc215* and was persistently expressed at three growth stages.

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Introduction

Chlorophyll a and b contained in leaves of higher plants are the main pigments of photosynthesis in the chloroplasts, and have important functions in the absorption and exploitation of the light energy, thereby influence photosynthetic efficiency (Pan and Dong 1995). Some studies have demonstrated that chlorophyll content is positively correlated with photosynthetic rate (Araus *et al*. 1997; Thomas *et al*. 2005). Increasing the chlorophyll content in crops may be an effective way to increase biomass production and grain yield (Wang *et al*. 2008). Therefore, understanding the genetic mechanism of chlorophyll content would be very important for yield improvement in wheat.

Genes controlling chlorophyll content constitute a multilocus system. Although QTLs influencing leaf chlorophyll content have been well identified in rice (Ishimaru *et al*. 2001; Wang *et al*. 2003; Fang *et al*. 2004; Yang *et al*. 2006; Shen *et al*. 2007), to our knowledge, only two reports of mapping the QTLs for chlorophyll content in wheat have been published (Cao *et al*. 2004; Yang *et al*. 2007). Cao *et al*. (2004) detected seven QTLs for chlorophyll content on chromosomes 2B, 4A, 5B, 6A, 7A, and 7D under nitrogen (N) sufficient environment, while nine QTLs were identified for chlorophyll content on chromosomes 2D, 3A, 4B, 5B, and 6A when wheat seedlings are grown under N deficient environment. Yang *et al*. (2007) reported that four additive QTLs controlling chlorophyll content under conditions of both rainfed and well watered mapped on chromosomes 1A, 5A, and 7A at GS.

In the course of development, QTLs/genes are expressed selectively at different growth stages. Atchley and Zhu (1997) demonstrated that the genetic mechanisms of controlling a complex quantitative traits changed distinctly in on-

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togeny. Conditional QTL mapping may be a valid way to reveal dynamic gene expression for the development of quantitative traits (Zhu 1998). To our knowledge, developmental stage-specific analysis of QTLs for chlorophyll content in wheat has not been conducted yet.

We investigated chlorophyll content for 168 DH lines at three different growth stages (MS, HS, and GS). The primary goal of the study described here was to detect QTLs with additive effects, epistatic effects, and QTL × environment (QE) interactions for chlorophyll content, and to identify markers that can be used in molecular marker-assisted selection (MAS) in wheat breeding programmes.

Materials and methods

Plant materials

A population of 168 DH lines was produced from a cross between two Chinese elite wheat cultivars (Huapei 3 × Yumai 57) and used for the construction of a genetic linkage map. Huapei 3 is an elite variety with desirable agronomic characteristics for early maturity and high resistance to several diseases (Hai and Kang 2007), whereas Yumai 57 is a variety widely cultivated for its yield stability under different ecological conditions (Guo *et al*. 2004). Huapei 3 and Yumai 57 were registered by Henan in 2006 (Hai and Kang 2007) and by state (China) in 2003 (Guo *et al*. 2004), respectively. Yumai 57 has higher chlorophyll content than Huapei 3.

Field trials and measurement of chlorophyll content

The field trials were conducted in Taian, Shandong province, in 2005 and 2006, and in Suzhou, Anhui province, in 2006, providing data for three environments. In the autumn of 2005, all DH lines and parents were grown in a plot with three rows in 2-m length and 25 cm between rows. In the autumn of 2006, the lines were grown in a plot with four 2-m rows spaced 25 cm apart. Crop management was carried out following the local practice.

For leaf chlorophyll content analyses, flag leaves were taken from five plants per plot at the grain filling stage (20 days after flowering). Chlorophyll a and b contents were estimated, adapting the procedure described by Porra *et al.* (1989). Samples of approximately 0.2 g of leaf tissue (taken from the middle of the leaves) were homogenized in 80% acetone at 4°C, the homogenates centrifuged, and fluorescence measured at 662 nm and 645 nm with a spectrophotometer UV-4802 (Shanghai, China). Leaf chlorophyll content was obtained according to the formula given by Zhang (1990).

Data analysis and QTL identification

Analysis of variance and correlation were performed using the statistical software SPSS version 13.0 (SPSS, Chicago, USA). With a previously constructed linkage map (Zhang *et al*. 2008), QTLs for chlorophyll content were detected using the QTLNetwork version 2.0 (Yang and Zhu 2005) with the composite interval mapping of the mixed linear model (Wang *et al*. 1999). Composite interval analysis was undertaken using forward–backward stepwise, multiple linear regression with a probability into and out of the model of 0.05 and window size set at 10 cM. QTL was declared if the phenotype was associated with a marker locus at $P < 0.005$. The final genetic model incorporated significant additive effects and epistatic effects, and their interactions with the environment.

Results

Phenotype distribution

Mean values of chlorophyll a and b contents for the parents Huapei 3 and Yumai 57, as well as the 168 DH lines under different growth stages in the years 2005 and 2006 are shown in figure 1. Yumai 57 had larger values than Huapei 3 for chlorophyll a and b contents. The distribution of chlorophyll a and b contents were continuous in the DH lines, showing their quantitative nature (figure 1). Meanwhile, a transgressive separation was found from the DH lines. It indicated that favourable alleles governing target traits had been widely separated in the DH lines. Therefore, the distributive character of phenotypic data was suitable for QTL analysis.

Strong and positive correlations were observed between chlorophyll a and b contents at three growth stages in wheat (table 1). Chlorophyll a content at GS had the highest positive correlations with chlorophyll b content at GS ($r = 0.936$; $P < 0.01$).

QTL analysis

A total of 17 additive QTLs were detected for chlorophyll content on chromosomes 1B, 2D, 4A, 5A, 5D, 6B, 7A, and 7B at three different stages (table 2; figure 2). Among them, eight additive QTLs showed additive × environment (AE) effects. Nine pairs of QTLs with epistatic effects and/or epistasis × environment (AAE) effects were detected for chlorophyll content (table 3). Two pairs of epistatic QTLs were identified on the basis of interaction effects with the environment.

Eight additive QTLs were detected for chlorophyll a content on chromosomes 1B, 4A, 5D, 6B, and 7A at three different stages (table 2). The variance explained by the QTLs ranged from 0.84% to 13.96%. Five of eight alleles with negative effects came from Yumai 57, which could account for 85.96% of total additive QTLs. The results were consistent with Yumai 57 having much larger chlorophyll a content. The *qHChla5D* had significant additive effects at HS, which could explain 13.96% of the phenotypic variation. Three major QTLs (*qMChla5D, qHChla5D*, and *qGChla5D*) were closely linked with the PCR marker *Xwmc215* on chromosome 5D, and consistently presented at three different stages. Two QTLs (*qHChla4A* and *qGChla4A*) were detected at both HS and GS and were mapped very near the loci of *Xwmc718* on chromosome 4A. A total of five pairs of epistatic QTLs were detected for chlorophyll a content in all stages (table 3).

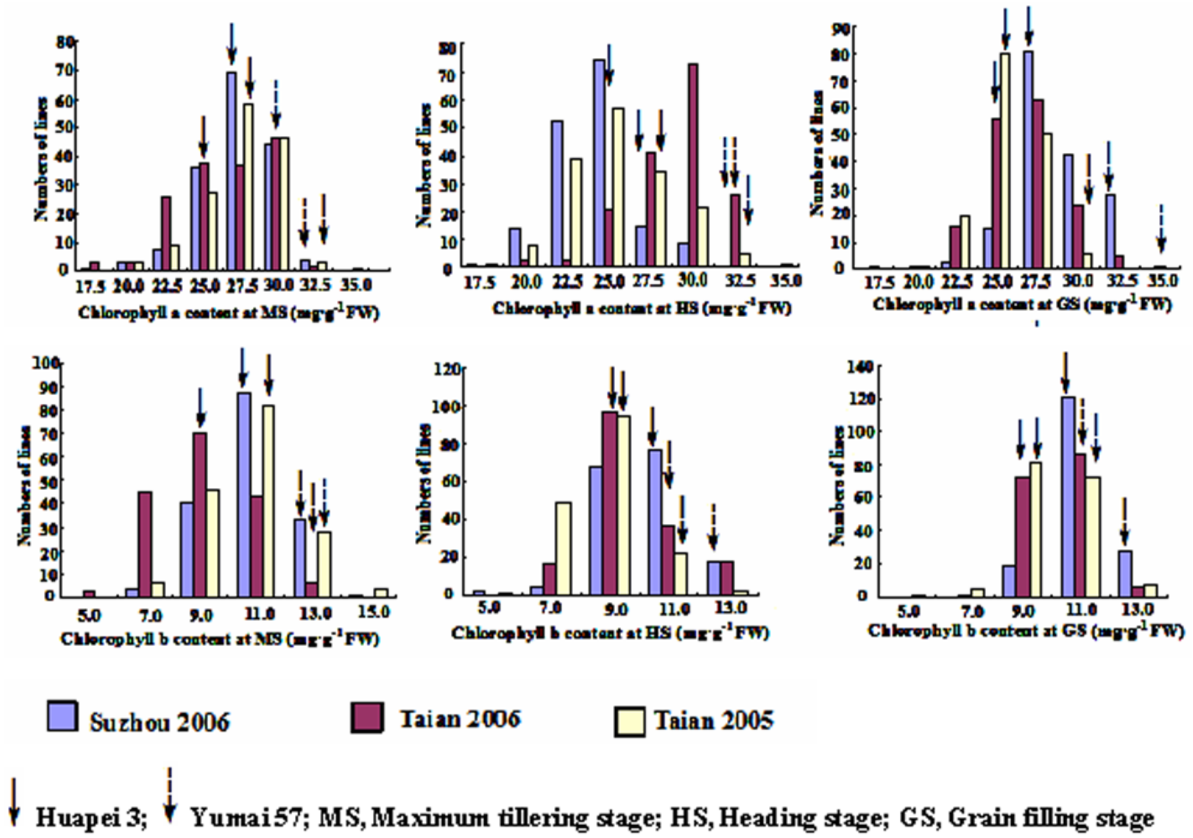

Figure 1. Phenotypic distribution of DH progeny from the cross Huapei 3/Yumai 57 for chlorophyll a and b contents at three different stages.

Table 1. Coefficients of pair-wise correlations of chlorophyll a and b contents for the DH progeny evaluated at different growth stages.

	Chl a			Chl b	
Trait	MS	HS	GS	MS	HS
Chl a at HS	0.424**	–	–	–	–
Chl a at GS	0.322**	0.264**	–	–	–
Chl b at MS	0.783**	0.375**	0.312**	–	–
Chl b at HS	0.383**	0.849**	0.302**	0.453**	0.383**
Chl b at GS	0.359**	0.301**	0.936**	0.409**	0.222**

*Significance at 0.05 level of probability and **significance at 0.01 level of probability, respectively Chl a, chlorophyll a; Chl b, chlorophyll b; MS, maximum tillering stage; HS, heading stage; GS, grain filling stage.

Further, two pairs of epistatic QTLs (*qHChla2A*/*qHChla2B* and *qGChla2A*/*qGChla2B*) were identified at both HS and GS. QTL × environment (QE) interaction was another important factor, which could account for 43.57% of the phenotypic variation.

Nine additive QTLs were identified for chlorophyll b content at different developmental stages on chromosomes 2D, 4A, 5A, 5D, and 7B (table 2). Yumai 57 contributed the most alleles for increasing chlorophyll b content, which could explain 98.12% of the total additive QTLs. The *qGChlb5D* had the highest contribution to chlorophyll b content at GS, accounting for 23.29% of the phenotypic variation. Of these nine QTLs, three major QTLs (*qMChlb5D, qHChlb5D*, and *qGChlb5D*) were closely linked with the loci of *Xwmc215* on chromosome 5D and consistently expressed at three different stages. Two QTLs (*qHChlb4A* and

Table 2. Estimated additive (A) and additive × environment (AE) interactions of QTLs for chlorophyll content at different growth stages.

Trait	Stage	Chromosome	QTL	Nearest marker	Peak (cM)	F value	A^{a}	H^2 (A,%)^{b}	H^2 (AE,%)^{c}
Chl a	MS	5D	*qMChla5D*	*Xwmc215*	69.2	19.84	−1.03	10.10	4.69
–	–	6B	*qMChla6B*	*Xcfa2187*	0.0	7.65	0.51	2.51	2.73
–	HS	4A	*qHChla4A*	*Xwmc718*	1.0	9.12	−0.64	5.51	–
–	–	5D	*qHChla5D*	*Xwmc215*	73.3	32.81	−1.01	13.96	16.88
–	GS	1B	*qGChla1B*	*Xbarc008*	38.6	5.20	0.34	1.89	–
–	–	4A	*qGChla4A*	*Xwmc718*	3.0	6.64	−0.53	4.41	–
–	–	5D	*qGChla5D*	*Xwmc215*	74.3	34.69	−0.90	12.95	22.27
–	–	7A	*qGChla7A*	*Xcfa2123*	100.6	5.00	0.23	0.84	–
Chl b	MS	5D	*qMChlb5D*	*Xwmc215*	68.2	13.72	−0.41	6.94	–
–	–	7B	*qMChlb7B*	*Xbarc050*	7.3	7.49	−0.28	3.23	–
–	HS	2D	*qHChlb2D*	*Xcfd53*	2.8	30.76	−0.48	14.31	–
–	–	4A	*qHChlb4A*	*Xwmc718*	0.0	12.44	−0.29	5.21	–
–	–	5D	*qHChlb5D*	*Xwmc215*	68.2	34.41	−0.42	10.61	10.39
–	GS	2D	*qGChlb2D*	*Xcfd53*	2.8	8.14	−0.14	1.70	2.94
–	–	4A	*qGChlb4A*	*Xwmc718*	0.0	14.74	−0.26	6.15	1.32
–	–	5A	*qGChlb5A*	*Xcwem32.2*	7.0	7.05	0.12	1.37	1.55
–	–	5D	*qGChlb5D*	*Xwmc215*	73.3	42.7	−0.51	23.29	–

[a] The additive effect. A positive value indicates that the Huapei 3 allele has a positive effect on the trait, and a negative value represents the Yumai 57 allele having a positive effect.

[b] H^2(A,%), indicates the contribution explained by putative additive QTL.

[c] H^2(AE,%), indicates the contribution explained by additive QTL × environment interaction Chl a, chlorophyll a; Chl b, chlorophyll b; MS, maximum tillering stage; HS, heading stage; GS, grain filling stage.

qGChlb4A) were detected at HS and GS and were mapped very near the loci of *Xwmc718* on chromosome 4A. A total of four pairs of epistatic QTLs were detected for chlorophyll b at MS and HS (table 3), while no pair of epistatic QTLs were identified for chlorophyll b content at GS. QE interaction was another important component, which explained 16.2% of the phenotypic variation.

Discussion

In the present study, on the basis of the mixed linear model approach (Wang *et al*. 1999), we detected QTLs with additive and epistatic effects, as well as their QE interactions for chlorophyll content using DH progeny of two elite wheat cultivars which were tested at three growth stages. A total of 17 additive QTLs and nine pairs of epistatic QTLs were detected for chlorophyll content at $P \leq 0.005$. The analyses also revealed that eight additive QTLs and two pairs of epistatic QTLs had significant interactions with the environment.

Zhu (1995) described the conditional genetic analysis method through in an experiment on cotton fruiting, to compare unconditional with conditional genetic variances and additive effects. He indicated that analysis of conditional QTLs will provide a way for exploring quantitative traits in different developmental stages. This method had been successfully used for researching the developmental behaviour of root morphological traits in rice (Qu *et al*. 2008) and chlorophyll content in rice (Zuo *et al*. 2007). Qu *et al*. (2008) reported that 12 of 84 additive QTLs for root morphological traits in rice were persistently expressed in at least two stages, and no QTLs were detected at all stages. Zuo *et al*. (2007) detected no common QTLs for leaf chlorophyll content at different growth stages in rice. In the present study, 10 of 17 additive QTLs for chlorophyll content were persistently expressed at two or more growth stages. On the other hand, two of nine pairs of epistatic QTLs were identified at two different stages. These results are in agreement with the concepts that QTLs controlling developmental traits are expressed selectively at different growth/developmental stages (Atchley and Zhu 1997).

To our knowledge, there are only two previous reports of mapping the QTLs for chlorophyll content in wheat (Cao *et al*. 2004; Yang *et al*. 2007). Further, the previous QTLs mapping studies on chlorophyll content of wheat were only based upon one growth stage. The present study revealed some QTLs/genes selectively express at different growth stages in wheat. Several additive QTLs for chlorophyll content detected in this study seemed to have similar or

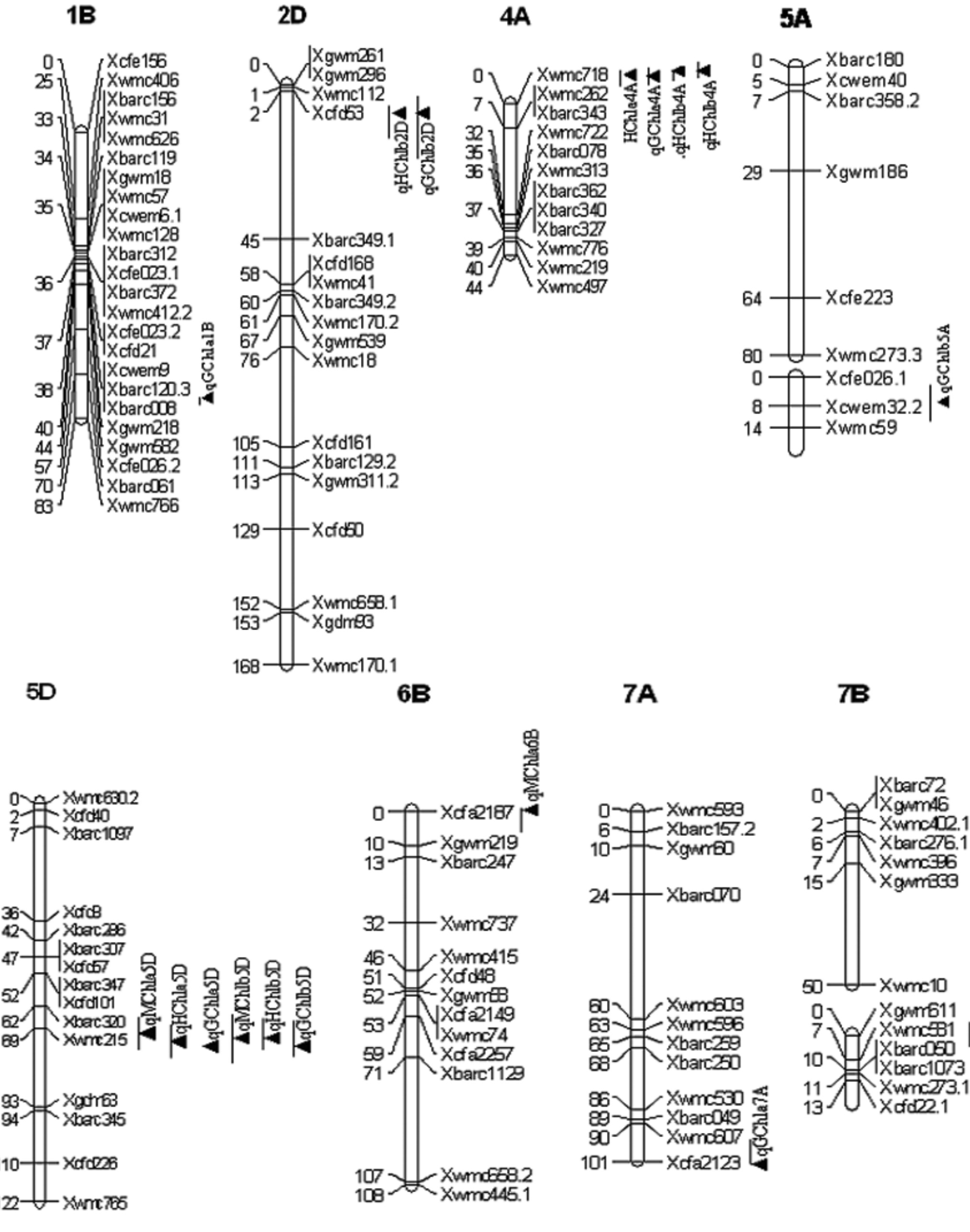

Figure 2. A genetic linkage map of wheat showing additive QTLs for chlorophyll a and b contents at three different stages.

the same chromosomal locations in different mapping populations, as compared with previous reports. The *qChla7A* was located at a similar position as *qCHO-7A* (Cao *et al.* 2004) and *QChlc.cgb-7A* (Yang *et al.* 2007). The *qChlb4A* was very close to the *qCHO-4A* at the wheat seedling stage in the Opata 85 × W7984 cross (Cao *et al.* 2004). The *qChlb5A* mapped to a location similar to *QChlc.cgb-5A-2* (Yang *et al.* 2007). The QTLs closely linked with *Xwmc215* on chromosome 5D were detected for chlorophyll a and b contents at all stages and made high contributions (ranging from 6.94% to 23.29%) to the traits, which was in accordance with the significant positive correlations observed between chlorophyll a and b contents at three growth stages. Therefore, the *Xwmc215* can be used for improvements in chlorophyll contents by MAS in wheat breeding programmes. However, the major QTLs for chlorophyll a and b contents on chromosome 5D could not be detected by Cao *et al.* (2004) and Yang *et al.* (2007). The lack of a common element between the QTLs identified in different populations could be due to a combination of various factors, such as the type and size of

Table 3. Estimated epistasis (AA) and epistasis × environment (AAE) interactions of QTLs for chlorophyll content at different growth stages in 2005 and 2006.

Trait	Stage	QTL	Nearest marker	Peak (cM)	QTL	Nearest marker	Peak (cM)	AA[a]	H^2 (AA,%)[b]	H^2 (AA,%)[c]
Chl a	MS	*qMChla3A*	*Xwmc264*	94.2	*qMChla4B*	*Xwmc48*	18.4	–	–	3.90
–	HS	*qHChla2A*	*Xbarc296*	69.4	*qHChla2B*	*Xwmc477*	77.6	0.43	2.47	–
–	GS	*qGChla2Aa*	*Xbarc296*	69.0	*qGChla2B*	*Xwmc477*	77.6	0.57	3.97	–
–	–	*qGChla2Ab*	*Xbarc264*	75.1	*qGChla3B*	*Xcfe009*	51.8	–	–	1.62
–	–	*qGChla2Ac*	*Xgwm515*	104.9	*qGChla3B*	*Xcfe009*	51.8	-0.34	1.86	–
Chl b	MS	*qChlb3A*	*Xgwm155*	118.3	*qChlb4B*	*Xwmc48*	18.4	-0.26	2.73	–
–	HS	*qChlb2A*	*Xbarc015*	70.1	*qChlb3D*	*Xgdm72*	10.0	0.19	2.17	–
–	–	*qChlb2Db*	*Xcfd50*	125.1	*qChlb4D*	*Xgwm194*	67.0	0.26	4.18	–
–	–	*qChlb6A*	*Xwmc553*	57.3	*qChlb6B*	*Xcfa2187*	1.0	0.32	6.20	–

[a] Epistatic effects; a positive value means that the parent-type effect is greater than the recombinant-type effect, and the negative value means that the parent-type effect is less than the recombinant-type effect.

[b] Contribution explained by epistatic QTL.

[c] Contribution explained by epistatic QTL × environment interactions; Chl a, chlorophyll a; Chl b, chlorophyll b; MS, maximum tillering stage; HS, heading stage; GS, grain filling stage.

mapping populations, different experimental environments, and the methodology employed for QTL detection. Similarly, many agronomic traits were located in a similar location on chromosome 5D in the 'International Triticeae Mapping Initiative' mapping population of Opata 85 × W7984 (Börner *et al*. 2002). Therefore, further studies on the possibility of a tight linkage or pleiotropism on the chromosome 5D region will be necessary, so as to elucidate the genetic nature of the grain yield and physiological traits, and to use them in wheat improvement programmes.

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