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DYNAMIC QTL ANALYSIS OF CHLOROPHYLL CONTENT DURING GRAIN FILLING STAGE IN WINTER WHEAT (*TRITICUM AESTIVUM* L.)

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ABSTRACT

A recombinant inbred line (RIL) population consisting of 306 lines was constructed from a cross between two winter wheat (*Triticum aestivum* L.) cultivars, Hanxuan 10 and Lumai 14. The dynamic quantitative trait loci (QTLs) for chlorophyll content of flag leaves were detected using the RIL population by a combination of unconditional and conditional mapping method. Chlorophyll content was measured with a SPAD meter at seven stages of grain filling under two water conditions (well-watered and drought-stress). Thirty-four simple-sequence repeat markers used in this study were derived from the candidate QTLs for chlorophyll content previously detected in a doubled haploid population of the same cross as the RIL. In the entire grain-filling period, five additive QTLs and one pair of epistatic QTLs were detected by unconditional mapping, while seven additive QTLs and one pair of epistatic QTLs were detected by conditional mapping. These QTLs for chlorophyll content were distributed on six chromosomes: 3B, 3D, 4B, 5A, 6A and 7A. The phenotypic variation explained by individual QTLs ranged from 2.12% to 12.80%. A major QTL (QChl-5A.1) was detected at multiple stages during grain filling, unaffected by genetic background and environmental conditions and might have great prospects for use in marker-assisted selection (MAS) breeding. The combination of unconditional and conditional QTL approach allowed for better understanding of both dynamic and static genetic information on the expression of QTLs for chlorophyll content of flag leaves during grain filling of wheat, providing reference data for marker-assisted selection breeding of high-yielding wheat cultivars.

Key words: chlorophyll content; marker-assisted selection; quantitative trait loci; grain filling stage; conditional and unconditional mapping; wheat.

INTRODUCTION

Chlorophyll is the main pigment involved in photosynthesis and it plays a critical role in light absorption and utilization (Zhang et al., 2009a), thereby directly affecting the rate and yield of photosynthesis in crops (Guo and Li, 1996; Thomas and Howarth, 2000). Studies have shown that cereal grain yield and biomass production can be improved by delaying leaf senescence and maintaining higher leaf chlorophyll content during grain filling, such as in sorghum (Xu et al., 2000), rice (You et al., 2007; Fu et al., 2011), maize (Wang et Zhang, 2012) and wheat (Spano et al., 2003). Additionally, cereal crops with higher leaf chlorophyll content exhibited improved resistance to biotic and abiotic stresses, such as drought (Yang et al., 2007), high temperature (Vijayalakshmi et al., 2010), and plant diseases (Joshi et al., 2007). Therefore, increasing the leaf chlorophyll content in crops may be an effective strategy to increase grain yield.

However, chlorophyll synthesis and degradation is a complex and dynamic process during grain filling period of wheat (Suzuki et al., 1997). Genes associate with chlorophyll synthesis and degradation express selectively (Wang et al., 2010), and most of them only express at a certain development stage (Atchley and Zhu, 1997). Thus, it is complex but very important to research on the dynamics genetic mechanism underlying chlorophyll content of flag leaves during grain filling of wheat for high yield breeding program.

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In recent years, molecular marker technologies have provided an opportunity for studying quantitative traits (e.g., leaf chlorophyll content) in crops (Guo et al., 2008). Unconditional QTL mapping method has been frequently used to detect QTLs for chlorophyll content of flag leaves at one certain growth stage (e.g., seeding or heading stage) of wheat (Yang et al., 2007; Guo et al., 2008; Zhang et al., 2009b; 2010; Czyczylo-Mysza et al., 2011; Li et al., 2014). However, unconditional QTL analysis is based on the phenotypic values of a single time point; the results only reflect the cumulative genetic effect from the initiation of growth to the measure time (Atchley and Zhu, 1997), without considering the effects due to distinct gene expression at different developmental stages (Wang et al., 2010)

Different from unconditional QTL analysis, conditional QTL analysis provides genetic information from stage t-1 to stage t (Zhu, 1995). In order to elucidate the dynamic and static genetic information on QTLs during chlorophyll development, it is necessary to perform a combined analysis by conditional and unconditional mapping. This combined approach will not only provide insights into the final results of QTL expression, but also clarify the exact time of QTL expression and the number of QTLs expressed (Liu et al., 2010). To the best of the authors' knowledge, no studies have combined conditional and unconditional QTL analysis to study the dynamics of chlorophyll development during grain filling of wheat.

In a previous study, a doubled haploid (DH) population containing 150 lines was constructed from a cross of two winter wheat (*Triticum aestivum* L.) cultivars (Hanxuan 10 and Lumai 14) by our laboratory (Wu et al., 2010). Further, unconditional QTL analysis was performed on the DH population to detect QTLs that control chlorophyll content of flag leaves during grain filling of wheat (Liang, 2013). Nineteen QTLs for chlorophyll content were identified in the DH population and considered as candidate QTL intervals. But the expression characteristics of these candidate QTLs during grain filling of wheat remained to be determined. Based on the previous work, a recombinant inbred line (RIL) population containing 306 lines was constructed in the present study using the same parents as the DH population. Conditional and unconditional QTLs for chlorophyll content of flag leaves were identified throughout the grain-filling period of wheat. The information obtained in this study provides new insights into understanding the genetic mechanism underlying the development of chlorophyll content in crops and also benefits crop genetic manipulations through molecular marker-assisted selection (MAS).

MATERIAL AND METHODS

Plant materials

A RIL population comprised of 306 lines was constructed from a cross between Hanxuan 10 and Lumai 14 at the Institute of Crop Science, CAAS, Beijing, China (Jing et al., 1999). The female parent, Hanxuan 10, is a drought resistant cultivar selected from more than 20,000 wheat germplasms and bred in 1966 by the Shanxi Academy of Agricultural Sciences, Fenyang, China. The male parent, Lumai 14, is an irrigated-land high-yielding variety bred by the Yantai Academy of Agricultural Sciences, Shandong, China.

The experimental plots were located at the agricultural experiment station of Shanxi Agricultural University, Taigu, China (37°25' N, 112°25' S). The experiment field was divided into two parts for different water treatments, including well-watered (WW) and drought-stress (DS) water regimes. The field design of each part consisted of randomised complete blocks with three replications. Each plot consisted of two rows, 2 m long with 0.25 m spacing between rows. Forty seeds were sown in each row on the 27th September 2012.

Prior to sowing, all of the plots were irrigated. After sowing, plants under DS only relied on natural rainfall throughout the growth period (total rainfall 206 mm); plants under WW were irrigated before the overwintering stage and at the jointing and flowering stages (600 mm water each time).

Phenotypic evaluation

At the flowering stage, five plants which flowered at the same day and had normal development were randomly selected and tagged in the middle of each row from each line and the parents. Chlorophyll contents of flag leaves of the tagged plants were measured using a SPAD-502 (Konica-Minolta, Japan) chlorophyll meter. Chlorophyll contents were measured once every five days until maturity (seven times in total). The measurement was performed at 7:00 to 10:00 with three replications for each plant. The average of chlorophyll contents from five plants was used as phenotypic value for each line.

The seven measure times were referred to as stage 1 to stage 7. For conditional QTL analysis, period 1 represents the time interval from the initiation of plant growth to stage 1; period 2 represents the time interval from stage 1 to stage 2; and so on.

Molecular markers and linkage map construction

A total of 34 candidate simple sequence repeat (SSR) markers were used in the study. These SSR markers were selected from ten linkage groups of wheat (on chromosomes 2A, 2D, 3B, 3D, 4B, 5A, 6A, 7A, and 7D, respectively) associated with chlorophyll content of flag leaves. All of these marker intervals were previously detected in the DH population from the same parents of the RIL population by our laboratory (Liang, 2013; Yang et al., 2007).

A genetic linkage map of the RIL population was constructed using Mapmake/Exp 3.0 (Vijayalakshmi et al., 2010). The total length of the ten linkage groups was 291.8 cM, and the average distance between adjacent markers was 8.58 cM.

Data analysis and QTL detection

The phenotypic values of chlorophyll content were analyzed using SPSS19.0 (IBM SPSS, Somers, NY, USA). Significant differences (P<0.05) between two parents at different developmental stages were determined by the *t*-test. Unconditional QTL was detected using QTLNetwork 2.0 (http://ibi.zju.edu.cn/software/qtlnetwork/ based on the mixed linear model. For conditional QTL, the net expression of t-1 to t period was calculated using QGAStation V1.0 (http://ibi.zju.edu.cn/software/qga/) then analysed using QTLNetwork 2.0. The threshold to declaring QTL was set to P value of 0.005 by permutation method. A marker interval with the P value less than 0.005 indicates the presence of a trait-associated QTL (Liang et al., 2010). The QTLs were named according to the rule of 'QTL + trait + chromosome + gene number'.

RESULTS

Phenotypic variations in chlorophyll content of wheat flag leaves

For RILs and their parents, chlorophyll content of flag leaves gradually decreased from flowering to maturity with the most rapid decline from stage 5 to stage 6 under the WW regime and from stage 4 to stage 5 under the DS regime (Table 1). At the same growth stage, chlorophyll content was higher under the WW than under the DS. The finding indicated that drought stress accelerated chlorophyll degradation in flag leaves.

Chlorophyll content of the male parent Lumai 14 was significantly higher than that of the female parent Hanxuan 10 (P<0.05) from S1 to S4 under WW regime and from S1 to S3 under DS regime. But it showed an opposite trend from S5 to S7 under WW regime and from S4 to S6 under DS regime (P<0.05).

Chlorophyll content of the RIL population was mostly between data of the two parents at different stages of development, showing a continuous distribution. Bidirectional transgressive segregation was also observed in chlorophyll content of the RIL population at stages 3 and 6 under the DS regime and stages 4, 5, and 7 under the WW regime (Table 1).

Table 1. Chlorophyll content of flag leaves during grain filling of wheat under two water regimes

Stage	Evn.	Parents		*t* value	Recombinant inbred line population					
		Hanxuan 10	Lumai 14		Mean	SD	Range	Skew	Kurt.	CV (%)
S1	WW	56.97	61.51	4.39*	57.26	2.44	49.42-64.34	-0.12	-0.08	4.27
	DS	56.37	59.67	5.99*	56.39	2.33	50.16-62.66	-0.05	-0.26	4.13
S2	WW	55.38	59.93	5.75*	56.24	2.53	48.82-64.12	0.02	-0.12	4.42
	DS	54.18	56.87	3.23ns	55.44	2.56	45.97-61.16	-0.22	0.16	4.62
S3	WW	53.88	59.34	9.09*	55.10	3.22	35.90-61.84	-0.78	2.11	5.85
	DS	51.97	52.06	0.12ns	52.71	3.27	39.58-59.92	-0.52	0.68	6.21
S4	WW	52.74	55.79	5.73*	50.97	6.44	1.48-60.55	-1.19	2.71	12.64
	DS	49.34	42.46	20.05**	46.88	8.44	3.58-57.92	-1.61	2.97	18.01
S5	WW	42.98	41.43	0.35ns	34.61	9.30	0.00-55.82	-0.65	-0.03	35.53
	DS	25.95	10.12	7.56*	18.94	12.30	0.00-49.32	0.35	-0.65	64.95
S6	WW	15.79	12.34	1.72ns	12.96	2.12	0.00-42.55	0.85	1.81	43.58
	DS	3.25	1.74	8.13*	3.97	2.87	0.00-20.38	0.70	1.20	76.82
S7	WW	3.71	2.11	4.82*	2.04	0.12	0.00-6.22	1.20	1.75	95.95
	DS	0.00	0.00	—	—	—	—	—	—	—

Note: WW, well watered; DS, drought stress; Evn, environment; "*" and "**", Significance at 0.05 and 0.01 probability levels, respectively; ns, not significant; S1, the first measure time; S2, the second measure time; and so on; "—", means no date.

Unconditional QTL analysis

Under the WW regime, three additive QTLs and one pair of epistatic QTLs for chlorophyll content were detected by unconditional QTL mapping. These unconditional QTLs were mapped on chromosomes 3B, 5A, and 7A (Tables 2 and 3, Figure 1), with a phenotypic contribution of 4.35–8.34%. The favourable allele of QChl-5A.2 was derived from the female parent Hanxuan 10, whereas the favourable alleles of QChl-3B.1 and QChl-5A.1 were derived from the male parent Lumai 14. These results showed that both the parents provided favourable alleles for QTLs under the WW regime. In addition, QChl-5A.1 was closely linked with marker interval xgwm1 56-xgwm154 on chromosome 5A and stably expressed at stages 2 and 3, with a total phenotypic contribution of 14.15%. However, none of QChl-3B.1, QChl-5A.2, or QChl-5A.3–QChl-7A.1 was detected at multiple stages during grain filling.

Under the DS regime, four additive QTLs for chlorophyll content were detected at different stages by unconditional QTL mapping method, whose favourable positive alleles were all derived from the male parent Lumai 14 (Table 2, Figure 1).

Table 2. Additive effect of quantitative trait loci (QTLs) for chlorophyll content of flag leaves during grain filling of wheat detected by unconditional mapping

Stage	Evn.	QTL	Flanking marker	Site (cM)	A	R^2 (%)
S1	WW	QChl-3B.1	xgwm566-xgwm285	2.0	-0.46	7.99
	DS	QChl-3B.1	xgwm566-xgwm285	1.0	-0.32	7.49
		QChl-5A.1	xgwm156-xgwm154	0.0	-0.51	9.11
S2	WW	QChl-5A.1	xgwm156-xgwm154	0.0	-0.87	6.69
	DS	QChl-5A.1	xgwm156-xgwm154	0.0	-1.17	10.41
S3	WW	QChl-5A.1	xgwm156-xgwm154	0.0	-1.25	7.46
	DS	QChl-4B.1	xgwm368-xgwm495	5.6	-1.09	6.87
		QChl-5A.1	xgwm156-xgwm154	0.0	-1.01	6.40
S4	DS	QChl-6A.1	xgwm570-xpsp3071	0.0	-1.89	12.80
S6	WW	QChl-5A.2	xgwm595-xwmc410	0.0	0.88	8.34

Note: WW, well watered; DS, drought stress; Evn, environment; QTL, quantitative trait locus; A, additive effect; R^2 (%), rate of contribution explained by the additive QTL; S1, the first measure time; S2, the second measure time; and so on.

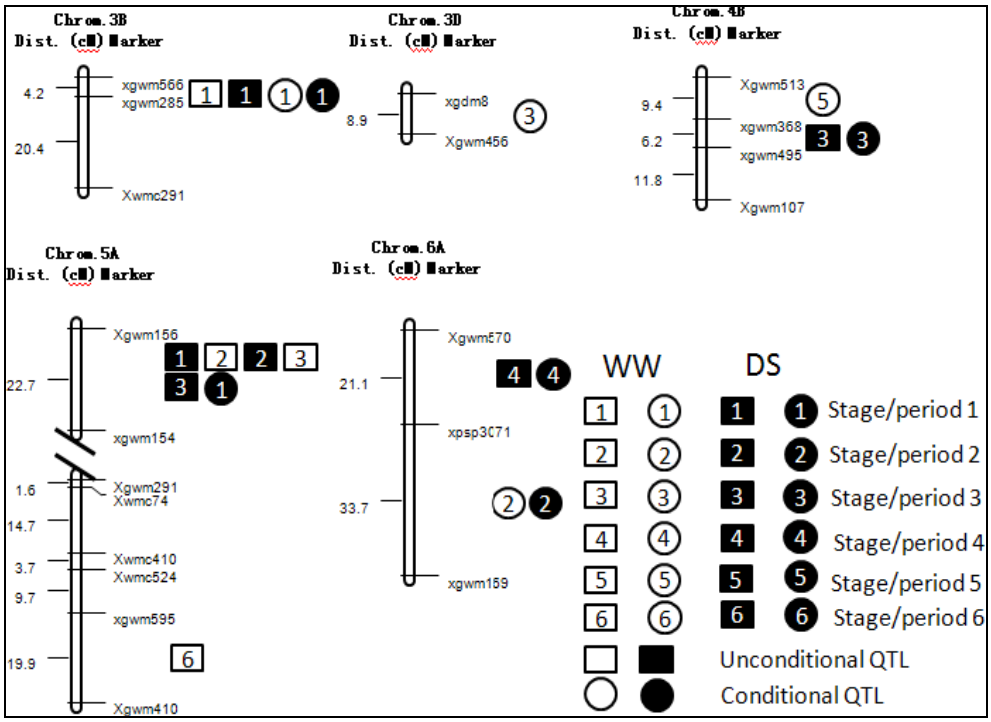

Figure 1. The distribution of quantitative trait loci (QTLs) for chlorophyll content of flag leaves in genetic linkage groups constructed on a recombinant inbred line population of wheat from the Hanxuan 10 × Lumai 14 cross

Table 3. Epistatic effect of quantitative trait loci (QTLs) for chlorophyll content of flag leaves during grain filling of wheat detected by unconditional mapping

	Treatment	QTL	Flanking marker	Site (cM)	QTL	Flanking marker	Site (cM)*	AA	R^2 (%)
S2	WW	QChl-5A.3	xgwm410-xwwm410	25.8	QChl-7A.1	xwmc9-xgwm260	17.2	0.51	4.35
P1	WW	QChl-5A.2	xgwm595-xwmc410	3.0	QChl-7A.1	xwmc9-xgwm260	10.1	0.47	3.21

Note: WW, well watered; DS, drought stress; QTL, quantitative trait locus; AA, epistatic effect; R^2 (%), rate of contribution explained by the epistatic QTL; S2, the second measure time; P1, the time interval from the initiation of plant growth to the first measure.

Table 4. Additive effect of quantitative trait loci (QTLs) for chlorophyll content of flag leaves during grain filling of wheat detected by conditional mapping

Period	Evn.	QTL	Flanking marker	Site (cM)	A	R^2 (%)
P1	WW	QChl-3B.1	xgwm566-xgwm285	2.0	-0.46	7.99
	DS	QChl-3B.1	xgwm566-xgwm285	0.0	-0.32	7.49
		QChl-5A.1	xgwm156-xgwm154	0.0	-0.51	9.11
P2	WW	QChl-6A.2	xpsp3071-xgwm169	10.2	-0.28	4.78
	DS	QChl-6A.2	xpsp3071-xgwm169	10.2	-0.34	5.19
P3	WW	QChl-3D.1	xgdm8-xgwm456	16.0	0.16	2.12
	DS	QChl-4B.1	xgwm368-xgwm495	5.6	-0.53	5.56
P4	DS	QChl-6A.1	xgwm570-xpsp3071	0.0	-0.73	3.73
P5	WW	QChl-4B.2	xgwm513-xgwm368	5.8	0.52	6.61

Note: WW, well watered; DS, drought stress; Evn, environment; QTL, quantitative trait loci; A, additive effect; R^2 (%),rate of contribution explained by the additive QTL; P1, the time interval from the initiation of plant growth to the first measure; P2, the time interval from the first measure to the second measure; and so on.

A major QTL (QChl-5A.1) closely linked with marker interval xgwm156-xgwm154 on chromosome 5A was expressed at stage 2, which explained 10.41% of the phenotypic

variation. Additionally, QChl-5A.1 was detected at multiple stages 1 to 3, with a total phenotypic contribution of 25.92%.

Conditional QTL analysis

Under the WW regime, a total of four additive QTLs and one pair of epistatic QTL for chlorophyll content were detected by conditional QTL analysis. None of these conditional QTLs was expressed in multiple periods (Tables 3 and 4). One additive QTL for chlorophyll content was detected during each of the following four periods: period 1 (QChl-3B.1), period 2 (QChl-6A.2), period 3 (QChl-3D.1), and period 5 (QChl-4B.2). These four additive QTLs explained 2.12-7.99% of the phenotypic variation. Except for QChl-3B.1, the remaining three additive QTLs (QChl-6A.2, QChl-3D.1, and QChl-4B.2) were not detected by the unconditional QTL analysis.

Under the DS regime, totally five additive QTLs for chlorophyll content were detected in different periods, which explained 3.73–9.11% of the phenotypic variation (Table 3). None of these conditional QTLs was expressed in multiple periods, and QChl-6A.2 was not detected by the unconditional QTL analysis.

DISCUSSION

Temporal specificity of QTL expression

Using the combined approach, a total of seven unconditional QTLs and six conditional QTLs for chlorophyll content of flag leaves were detected in the RIL population during different stages/periods of grain filling (Tables 2 and 4; Figure 1). Most of the QTLs expressed at one certain stage/period, only one QTL (QChl-5A.1) worked at multiple stages, none of the genes controlling chlorophyll content expressed throughout the entire period of chlorophyll development (Tables 2 and 4). This data showed that the expression of QTLs for chlorophyll content of wheat flag leaves during grain filling exhibited temporal specificity. Similar phenomena were reported in dynamic QTL analysis for other quantitative traits such as stay-green property in potato (Hurtado et al., 2012), tiller number in rice (Liu et al., 2012), and plant height in triticale (Wuerschum et al., 2014).

In the RIL population, the expression characteristics of QTLs for chlorophyll content during different stages or periods of grain filling could be classified into the four categories:

(I) Unconditional and conditional QTLs were highly consistent. For example, the QTL (QChl-6A.1) closely linked with marker interval xgwm570-xpsp3071 on chromosome 6A was detected by both unconditional (stage 4) and conditional QTL analysis (period 4) (Tables 2 and 4). QChl-6A.1 was not detected at any other stages or periods, indicating that this QTL for chlorophyll content was exclusively expressed during period 4.

(II) Conditional QTLs were identified while unconditional QTLs were not. For example, the conditional QTL (QChl-3D.1) was detected in period 3, but the corresponding unconditional QTL was not detected. QChl-3D.1 only expressed from the fifth to tenth day after flowering, for 2.12% of phenotypic variation (Tables 2 and 4). This observation implied that a gene with small net genetic effect would not be detected by unconditional QTL mapping method for the total cumulative effect. This phenomenon also reflects that conditional QTL analysis enables the detection of more minor QTLs than unconditional QTL analysis. Similar results have been reported in a study of grain starch accumulation in wheat by conditional and unconditional QTL mapping (Tian et al., 2011).

(III) Unconditional QTLs were detected while conditional QTLs were not. For example, the QTL (QChl-5A.2) was detected by unconditional QTL mapping method at stage 5, but was not identified by conditional QTL mapping strategy in corresponding period (Tables 2 and 4). One possible explanation was that the additive effect of QChl-5A.2 was positive, whereas other unconditional QTLs showed negatively additive effects. All of these QTLs with opposite genetic effects were detected at previous development periods. Thus, the variation of net gene effects might be diminished to the point of being undetectable.

(IV) Unconditional QTLs were identified at multiple stages, while conditional QTLs were detected in only one single period. The conditional QTL (QChl-5A.1) was only expressed in period 1, explaining 9.11% of the phenotypic variation, whereas the corresponding unconditional QTL was detected at stages 1, 2, and 3. The reason might be that QChl-5A.1 expressed before flowering with a strong net genetic effect. As a result, it could be detected by unconditional mapping approach from the total cumulative effect at multiple stages.

Taken together, the above results demonstrated the efficiency of dynamic QTL analysis combining conditional and unconditional QTLs. In other words, the combined approach allows for better understanding of both dynamic and static genetic information on the expression of QTLs for chlorophyll content of flag leaves during grain filling of wheat.

Regardless of the choice of the mapping approach, six and five additive QTLs were detected under the WW regime and the DS regime, respectively (Tables 2 and 4; Figure 1). Three QTLs (QChl-5A.2, QChl-3D.1 and QChl-4B.2) were exclusively expressed under WW regime, and two QTLs (QChl-4B.1 and QChl-6A.1) were only detected under DS regime. Although three QTLs (QChl-3B.1, QChl-5A.1 and QChl-6A.2) were consistently expressed under different water regimes, the contributions for phenotypic were different. It was not difficult to find that there were some differences in QTL mapping data between the WW and DS regimes. Thus, the results imply that there were different QTLs expression patterns under different water regimes. A similar phenomenon has been reported in wheat grown under normal and abiotic stress conditions. (Yang et al., 2007; Guo et al., 2008; Li et al., 2012; Czyczyło-Mysza et al., 2013).

Stability of QTL expression

The choice of molecular markers influences the efficiency of QTL mapping (Wang et al., 2010), whereas the size of mapping population influences the accuracy and precision of QTL mapping (Austin and Lee, 1996; Jia et al., 2013). Previously, a genetic linkage map was constructed for the DH population containing 150 lines derived from the same parents as used in the present study (Liang, 2013; Yang et al., 2007). The total length of the genetic linkage map was 3904 cM, including 132 amplified fragment length polymorphism markers and 263 SSR markers. Unconditional QTL analysis was performed using the DH population and 19 QTLs controlling chlorophyll content were detected.

In the present study, 34 SSR markers derived from the candidate marker intervals controlling chlorophyll content previously detected in the DH population were used (Liang, 2013). Furthermore, the size of the mapping population was increased in order to reduce the possibility of false positive results of QTLs. An increased the number and size of mapping population would allow for mutual verification and improvement in the accuracy of QTL mapping. Totally eight additive QTLs for chlorophyll content were detected in the RIL population (QChl-3B.1, QChl-3D.1, QChl-4B.1, QChl-4B.2, QChl-5A.1, QChl-5A.2, QChl-6A.1, and QChl-6A.2). All of these QTLs were also detected in the DH population previously (Liang, 2013; Yang et al., 2007). This result indicated that the expression of these eight QTLs were stable and unaffected by genetic background. Additionally, the present study also demonstrated that QChl-5A.1 is expressed stably without being affected by environmental conditions, with a relatively high contribution to phenotypic variation. Thus, QChl-5A.1 may have great prospects for use in MAS breeding.

CONCLUSIONS

Based on the obtained results it can be concluded that combining conditional and unconditional QTL mapping method could reveal more important information for understanding the molecular mechanism governing quantitative traits in plants. The most important merit of the present study is to

uncover the dynamic characterization on the expression of QTLs for chlorophyll content of flag leaves during grain filling of wheat under two water conditions. Eight QTLs having a potential for MAS were identified using the two populations.

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