

Analysis of quantitative trait loci underlying the traits related to chlorophyll content of the flag leaf in rice

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Abstract A population of 117 doubled haploid (DH) lines derived from the cross of Zhaiyeqing 8 (*indica*) × Jingxi 17 (*japonica*) was employed to map quantitative trait loci (QTL) underlying four physiological traits related to chlorophyll contents of the flag leaf. There were significantly positive correlations among chlorophyll a, chlorophyll b and chlorophyll a + b content. Chlorophyll a/b ratio was significantly negatively correlated with chlorophyll b content. These four traits were normally distributed with transgressive segregation, suggesting that they were controlled by multiple minor genes. A total of 11 QTLs were detected for the four traits and they lay on six chromosomes. Each of them explained 9.2%–19.6% of the phenotypic variations, respectively. Of these, two QTLs controlling chlorophyll a content were mapped on chromosomes 2 and 5; four QTLs underlying chlorophyll b content were mapped on chromosomes 2, 3, 5 and 9; three QTLs underlying chlorophyll a + b amount were mapped on chromosomes 3, 5 and 9; two QTLs underlying chlorophyll a/b ratio were mapped on chromosomes 6 and 11. The intrinsic relationship among the four traits and the practical implication in rice breeding are discussed.

Keywords rice, chlorophyll, traits, quantitative trait loci (QTL), correlation, breeding

1 Introduction

Modern rice breeding has undergone two stages: dwarfing breeding and heterosis utilization. Increase of rice yield percentage per unit area is attributed to the improvement of population utilization rate of light energy (Tu et al., 1995). Rice photosynthesis depends on the light capture

capability and the efficiency of light energy transformation. To further increase the rice yield potential, we have made efforts focusing on improving the coefficient of light energy transformation. Translating the key gene of the C₄ path plant to rice is one of the effective ways of improving photosynthetic efficiency and enhancing rice yield (Matsuoka et al., 1998; Ku et al., 1999; Dunwell, 2000).

Chlorophyll is a major pigment for absorbing, translating and transforming light energy. To a certain extent, chlorophyll content of the leaf is positively related to the photosynthetic velocity (Mae, 1997).

Previous researches show that both the chlorophyll content and the value of chlorophyll a/b ratio of cultivated rice are higher than in wild rice, and so is photosynthesis in the saturated light magnitude (Kourill et al., 1999). Various kinds of cultivated rice with different genotypes demonstrate significant differences both in maximal photosynthesis efficiency and in chlorophyll content (Li et al., 1984). The rice F₁ bred from different ecological types has a pronounced improvement in overall photosynthetic capability (Tu et al., 1988). Chlorophylls are divided into chlorophyll a and chlorophyll b according to their structure and characteristics of light energy transformation. Chlorophyll a is in favor of absorption of short wave light, while chlorophyll b is in favor of absorption of long wave light. The value of chlorophyll a/b is related to both photosynthetic organ development and photosynthetic activity (Tu, 1997). Thus, the content of both chlorophyll a and chlorophyll b and the value of chlorophyll a/b are important factors determining photosynthetic capacity. Clarification of their genetic patterns is of significance for improving rice photosynthetic capacity.

The combination of molecular marker techniques and quantitative genetics provides an effective method to dissect quantitative genetic law. There have been a few reports on the mapping of the quantitative trait loci (QTL) underlying the traits related to chlorophyll (Yang et al., 2003; Wang et al., 2003; Hu et al., 2006). However, those studies only investigated single traits of chlorophyll,

while systematic analyses of the traits related to chlorophyll are lacking. In the present research, a population of 117 doubled haploid (DH) lines derived from the cross of Zhaiyeqing 8 (*indica*) × Jingxi 17 (*japonica*) was employed to map QTL underlying four physiological traits related to chlorophyll content of the flag leaf. The results provide a theoretical basis for rice breeding through high, effective utilization of light energy.

2 Materials and methods

2.1 Plant materials and field planting

A population of 117 DH lines, kindly provided by Professor Qifa Zhang from the Huazhong Agricultural University, Wuhan, was used in this study. The DH lines were derived from the cross of Zhaiyeqing 8 (*indica*) × Jingxi 17 (*japonica*). The field trial was conducted according to a randomized complete block design with two replications at the Jiufeng Experimental Farm, Wuhan. The germinated seeds were sown in a seedling bed on April 29, 2004, and the seedlings were transferred to a paddy field 22 days later, with a single plant per hill. Each plot included two rows, and each row consisted of 10 plants, with a space of 16.7 cm between plants and 20 cm between rows. This planting density is lower than that in normal agricultural production, in order to reduce the competition between plants in the field. The fertilization and cultivation regimes were consistent with optimum rice production in the region.

2.2 Chlorophyll content measurement

On the seventh day after heading, four plants of each DH line were sampled from the middle of each plot for measurement of the chlorophyll content of the main stem flag leaf scored. The middle regions of the flag leaves were chosen, clipped into fragments and mixed together. The solution that was used to extract chlorophyll from the leaves was confected according to the alcohol: acetone: water ratio of 5 : 4.5 : 1. The photo-density was detected at 645 nm and 663 nm wavelengths on a spectrophotometer. The concentrations of chlorophyll a and chlorophyll b were calculated according to the following formula:

$$OD_{663} = 82.04 \cdot C_{\text{chlorophyll a}} + 9.27 \cdot C_{\text{chlorophyll b}}$$

$$OD_{645} = 16.75 \cdot C_{\text{chlorophyll a}} + 45.6 \cdot C_{\text{chlorophyll b}}$$

The chlorophyll content was measured three times for each plot, and the mean of the trait value was used in the data analysis. The means of the two replications were used for QTL analysis.

2.3 Molecular markers and linkage map construction

The molecular marker data included those for restriction length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP), isoenzyme, and so on. The linkage map consisted of 234 markers and covered a total of 1990.14 cM, with an average interval of about 8.5 cM between adjacent marker loci.

2.4 Data analyses

Basic statistics and Pearson phenotypic correlations between the traits were calculated with SPSS 11.0 for Windows. Single-locus QTLs were analyzed by composite interval mapping (Zeng 1993; 1994), using the computer program of the Mapmaker/QTL. The threshold for LOD score was 2.0. A significance level of 0.05 was used to declare QTL. Genetic parameters associated with significant QTLs were estimated at the positions of respective maximum LOD peaks in individual putative QTLs. The proportion of the phenotypic variance explained by each QTL was also calculated. QTL nomenclature followed that of McChouch et al. (1997).

3 Results

3.1 Performance of the traits

The phenotypic values of chlorophyll a, chlorophyll b, chlorophyll a+b and chlorophyll a/b are presented in Table 1. For the contents of chlorophyll a, chlorophyll b and chlorophyll a+b, the differences between their parents were significant. But the values of chlorophyll a/b were approximately equivalent. Within the population, all the traits showed a continuous variation. The variation coefficient of chlorophyll b was maximal, and that of chlorophyll a/b was minimal. An obvious transgressive segregation for both traits was observed in both directions. The mean value for each trait was close to the mid-parent value (Fig. 1), suggesting that the traits were controlled by multiple genes and the data for the traits were suitable for QTL mapping.

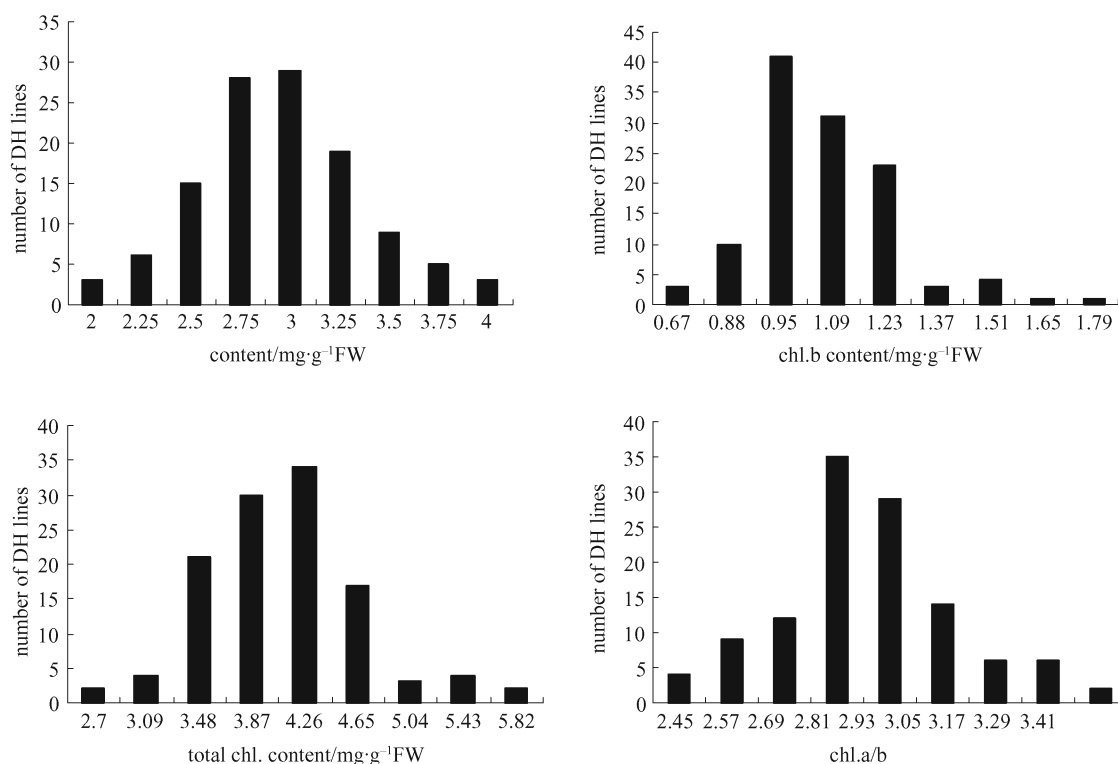
3.2 Trait correlation analysis

The phenotypic relationships show a significant positive correlation among chlorophyll a, chlorophyll b, and chlorophyll a+b. Both chlorophyll b and chlorophyll a+b were significantly negatively correlated with chlorophyll a/b, while chlorophyll a was not correlated significantly with chlorophyll a/b (Table 2).

Table 1 Phenotypic analysis of chlorophyll a, chlorophyll b, chlorophyll a+b and chlorophyll a/b

materials	parameters	chl. a/mg·g ⁻¹ FW	chl. b/mg·g ⁻¹ FW	chl. (a+b)/mg·g ⁻¹ FW	chl. a/b
Zhaiyeqing 8	mean	3.550	1.240	4.790	2.863
Jingxi 17	mean	2.098	0.720	2.821	2.879
	<i>t</i> value	3.218*	3.485*	3.288*	0.303
DH population	mean	3.050	1.063	4.105	2.891
	min	2.127	0.689	2.855	2.411
	max	4.154	1.808	5.916	3.434
	S D	0.413	0.181	0.584	0.203
	C.V.(%)	13.541	17.027	14.227	7.022

* indicate 5% significance level. chl. refers to chlorophyll. S D: standard deviation; C.V. : coefficient of variation.

**Fig. 1** Frequency distribution of chlorophyll a, chlorophyll b, chlorophyll a+b and chlorophyll a/b

3.3 QTL mapping

The chromosomal regions and estimated genetic effects of QTLs affecting the traits related to the chlorophyll content are presented in Table 3 and Figure 2. A total of 11 chromosome regions showed significant QTLs for chlorophyll a, chlorophyll b, chlorophyll a+b and chlorophyll

Table 2 Correlation analysis of chlorophyll a, chlorophyll b, chlorophyll a+b, and chlorophyll a/b

	chl. a	chl. b	chl. (a+b)
chl. b	0.903**		
chl. (a+b)	0.946**	0.881**	
chl. a/b	-0.188	-0.577**	-0.236*

* indicates 5% significance level, ** indicates 1% significance level. chl. refers to chlorophyll.

a/b. These regions were mapped on six out of 12 rice chromosomes.

Two QTLs underlying chlorophyll a content were detected, respectively explaining 9.2% and 12.6% of the total phenotypic variations. These QTLs were mapped in the interval C132-G1327 on chromosome 2 and in the interval RG13-RG573 on chromosome 5. Jingxi 17 (*japonica*) alleles at two of the QTLs, *qChla-2* and *qChla-5*, were in the direction of increasing chlorophyll a content.

Four QTLs underlying chlorophyll b were detected. These QTLs were mapped on chromosomes 2, 3, 5 and 9, respectively. Each QTL alone explained 10.0%–19.0% of phenotypic variations. The additive effects of half of these QTLs were negative, suggesting that the alleles came from Zhaiyeqing 8 (*indica*); while the other half had

Table 3 QTLs for chlorophyll content of flag leaf in rice

traits	loci	chromosome	marker interval	LOD score	variance/%	additive effect/mg·g ⁻¹
chlorophyll a	<i>qChla-2</i>	2	C132-G1327	2.15	9.2	-0.25
	<i>qChla-5</i>	5	RG13-RG573	2.94	12.6	-0.31
chlorophyll b	<i>qChlb-2</i>	2	G1327-CT16	3.45	14.4	-0.14
	<i>qChlb-3</i>	3	RG450-RG266	3.16	12.8	0.13
	<i>qChlb-5</i>	5	RG776B-RM164	3.36	19.0	-0.16
	<i>qChlb-9</i>	9	G103-G93F	2.13	10.0	0.11
	<i>qTotalchl-3</i>	3	RG450-RG266	2.35	9.8	0.37
total chlorophyll	<i>qTotalchl-5</i>	5	RG13-RG573	2.66	11.4	-0.41
	<i>qTotalchl-9</i>	9	CT410-CT6	2.61	10.8	0.39
	chlorophyll a/b	<i>qChlalb-6</i>	6	RG433-G342	4.07	19.6
<i>qChlalb-11</i>		11	CT553-G320	2.61	11.9	-0.14

Positive values indicate Zhaiyeqing 8 alleles increase trait values.

positive additive effects, suggesting that the alleles came from Jingxi 17 (*japonica*).

Three QTLs controlling chlorophyll a+b were detected. They were distributed on chromosome 3 (*qTotalchl-3*), chromosome 5 (*qTotalchl-5*), and chromosome 9 (*qTotalchl-9*). Each QTL explained 9.8%–11.4% of the phenotypic variations. Among these, *qTotalchl-5* showed a negative additive effect (-0.41 mg/g), and *qTotalchl-3* and *qTotalchl-9* showed a positive additive effect (0.37 mg/g and 0.39 mg/g). It is noted that there are two co-located QTLs (interval RG13-RG573 on chromosome 5 and interval RG450-RG266 on chromosome 3) showing the overlap region between *qTotalchl-5* and *qChla-5* and the overlap region between *qTotalchl-3* and *qChlb-3*.

Two QTLs (*qChlalb-6*, *qChlalb-11*) controlling chlorophyll a/b were also mapped on chromosome 6 (RG433-G342) and chromosome 11 (CT553-G320), and they accounted for 19.6% and 11.9% of the phenotypic variations, respectively. The QTL *qChlalb-6* showed a positive

additive effect (0.18 mg/g), whereas the QTL *qChlalb-11* showed a negative additive effect (-0.14 mg/g).

4 Discussion

Rice is considered as a C₃ plant, which has powerful light respiration and a low photosynthetic rate. Chlorophyll is the basic material for photosynthesis. Therefore, increasing chlorophyll content of the leaves can enhance the rice yield and quality. Recently, research on chlorophyll has drawn more and more attention. Yang et al. (2003) have detected 5 QTLs controlling the total chlorophyll content on chromosomes 1, 2 and 3 using a backcross inbred lines population. Wang et al. (2003) have mapped 6 QTLs for chlorophyll a and 6 QTLs for chlorophyll b at four different developmental stages. Of these, 5 pairs of QTLs are in overlap regions. Hu et al. (2006) have detected 6 QTLs controlling the total chlorophyll content in drought

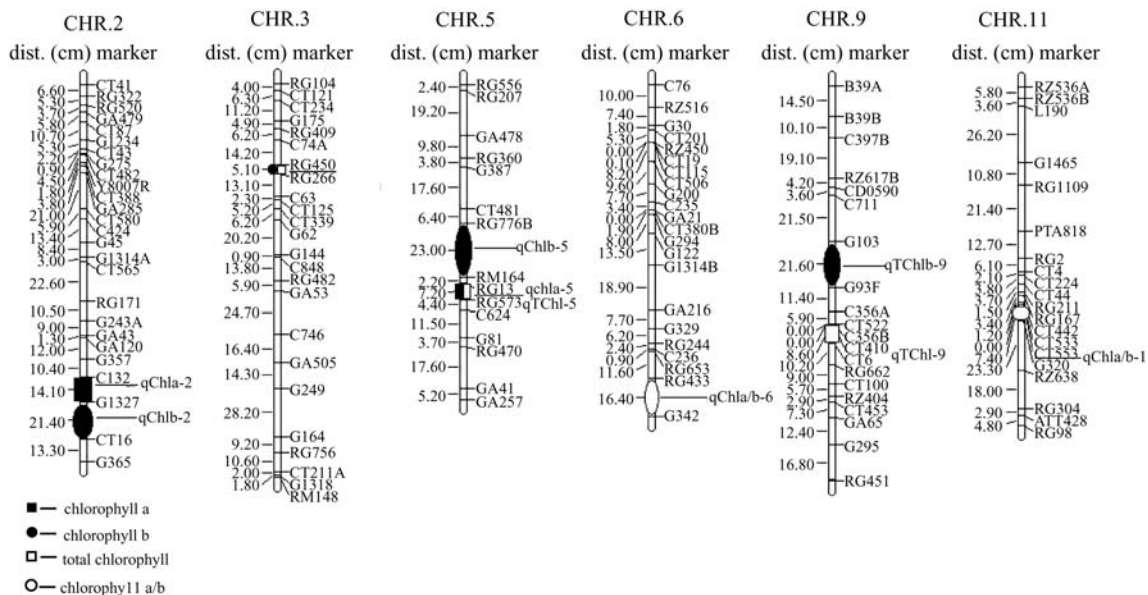


Fig. 2 Molecular linkage map showing locations of QTLs underlying chlorophyll content

handling situations and 7 QTLs in the control. In our study, a population of 117 DH lines derived from the cross of Zhaiyeqing 8 (*indica*) × Jingxi 17 (*japonica*) was used to map QTL underlying four physiological traits related to chlorophyll content of the flag leaf. A total of 11 QTLs were detected for the four traits and they lay on six chromosomes, explaining 9.2%–19.6% of the phenotypic variations, respectively. Two QTLs underlying chlorophyll a were mapped on chromosomes 2 and 5; four QTLs underlying chlorophyll b were mapped on chromosomes 2, 3, 5 and 9; three QTLs underlying chlorophyll a+b were mapped on chromosomes 3, 5 and 9, and two QTLs underlying chlorophyll a/b were mapped on chromosomes 6 and 11. Compared with previous research results, there is no common QTL, suggesting a complex molecular genetic mechanism. Using different populations to dissect the genetic mechanism of chlorophyll will provide more insight and possible ways for rice breeding.

Among our DH population, chlorophyll a, chlorophyll b and chlorophyll a+b contents showed significant correlations with each other. The QTL mapping result revealed that *qChla-5* and *qTotalchl-5* are co-located at the interval RG13-RG573 on chromosome 5, with the same negative additive effect. On the other hand, *qChlb-3* and *qTotalchl-3* are co-located at the interval RG450-RG266 on chromosome 3, with the same positive additive effect. Moreover, *qChla-2* and *qChlb-2* are closely linked together, with the same negative additive effect. Each of these chromosomal regions could be assumed as one putative QTL controlling both traits. These map locations should be a single locus with pleiotropic effects on multiple traits or a group of tightly linked loci, which might be one genetic explanation for the correlations between the traits.

Chlorophyll a is a main component of the reactive center complex. The function of chlorophyll a is energy transformation. Chlorophyll b is a key component of the pigment protein complex capturing light. It functions in capturing and transporting light energy. Both chlorophyll a and chlorophyll b are the components of the photosynthetic membrane. Generally the value of the chlorophyll a/b ratio is about 3 (Lichtenthaler, 1987). In the present study, the chlorophyll a/b ratio had no significant difference between parents, and its variation coefficient in the population was minimal; the value for each line was close to 2.9. This result is in accordance with previous conclusions. The value of chlorophyll a/b was negatively significantly correlated with both chlorophyll b and chlorophyll a+b content, but not with chlorophyll a. In addition, the variation coefficient of chlorophyll b was greatest in the population, which suggests that the factor affecting the chlorophyll a/b value was neither the chlorophyll a content, nor the chlorophyll a+b content, but the chlorophyll b content.

In summary, our research reveals that, firstly, the co-location in the interval RG13-RG573 on chromosome 5 shows the same direction for both chlorophyll a and chlorophyll a+b, and the co-location in the interval RG450-RG266 on chromosome 3 shows the same direction for both chlorophyll b and chlorophyll a+b. This suggests that a genotype increasing the three trait values simultaneously could be obtained by pyramiding the positive alleles in the two loci *via* marker-assisted selection (MAS). Consequently, the capability of capturing and transporting light energy is enhanced. Secondly, chlorophyll a/b value is related to the developmental status of photosynthetic organs and photosynthetic activity (Tu, 1997). The high chlorophyll a/b value indicates a low chlorophyll b content, which could help avoid light inhibition caused by absorbing excessive light. We detected QTL *qChla/b-11* in the interval CT553-G320 on chromosome 11, which controlled the value of chlorophyll a/b. Marker CT553 is so closely adjacent to marker G320 that it is easy for MAS manipulation. Pyramiding this QTL will improve the makeup structure rationally, thereby increasing the photosynthetic efficiency of rice.

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