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REGULAR ARTICLE

Quantitative trait loci for leaf chlorophyll content at two developmental stages of rice (*Oryza sativa* L.)

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ABSTRACT

Knowledge of the genetics of leaf chlorophyll content (LCC) at tillering and heading stages should help develop rice varieties with high photosynthetic ability. In this study, 182 backcross-recombinant inbred lines (BIL), derived from Koshihikari (*japonica*)/Kasalath (*indica*)//Koshihikari, were used to identify quantitative trait loci (QTL) for LCC at the tillering and heading stages of rice. Continuous variation and transgressive segregation for LCC were observed in the BIL population, indicating that LCC was a quantitatively inherited trait. Seven QTL for LCC were identified and mapped to chromosomes 1 (two QTL), 2, 3, 4, 6, and 8, which individually accounted for 5.1 to 14.8% of the total phenotypic variation. Three QTL (*q*LCC-1-1, *q*LCC-1-2 and *q*LCC-4) were common between the tillering and heading stages. The alleles at four QTL (*q*LCC-1-1, *q*LCC-1-2, *q*LCC-2, and *q*LCC-8) from Koshihikari and the alleles at the other three QTL (*q*LCC-3, *q*LCC-4 and *q*LCC-6) from Kasalath increased LCC. The tightly linked molecular markers flanking the QTL detected in this study should be useful in improving photosynthetic ability in rice.

Key Words: *leaf chlorophyll content (LCC); quantitative trait locus (QTL); rice.*

INTRODUCTION

Leaf chlorophyll content (LCC) is one of the important physiological traits closely related to photosynthetic ability in rice (Teng et al., 2004). Undoubtedly, understanding the genetic mechanisms underlying the LCC across different developmental stages of rice has significant implications for improving photosynthetic ability in rice. The advances made in preparing

high-density marker linkage maps in rice have provided a powerful tool for elucidating the genetic basis of quantitatively inherited traits (Harushima et al., 1998; Yano and Sasaki, 1997). Until now, numerous quantitative trait loci (QTL) associated with some important agronomic and physiological traits in rice have been identified and mapped using molecular makers (Yano and Sasaki, 1997). Also, some QTL affecting LCC at the flourishing tillering stage (Teng et al. 2004) and 5d after heading (Ishimaru et al., 2001) in rice have been reported. However, developmental genetic studies have indicated that QTL/genes were expressed selectively at different developmental stages and genetic model from the final traits could not fully reflect the real gene actions during the development of the trait (Zhu, 1995). Tillering and heading are two important developmental stages of rice; the former determines biomass and the latter affects grain yield in rice (Oritani, 1984). To our knowledge, QTL analysis for LCC in rice has not been conducted at different developmental stages using the same population. Undoubtedly, the identification of novel QTL determining LCC throughout the growth period in rice should be helpful in developing varieties with high photosynthetic ability. The main objective of the present study was to identify and characterize QTL controlling LCC at two developmental stages of rice using backcross-recombinant inbred lines (BILs) from a backcross of Koshihikari (*japoncia*)/Kasalath (*indica*)//Koshihikari.

MATERIALS AND METHODS

PLANT MATERIAL

A set of 182 BILs developed by the single-seed descent method from a backcross of Koshihikari (*japoncia*)/Kasalath (*indica*)//Koshihikari (kindly provided by National Institute of Agrobiological Resources, Japan) was used. A restriction fragment length polymorphism (RFLP) map covering 1115.2 cM was constructed with 162 RFLP markers from these BC1F7 lines (Y. Takeuchi and M. Yano, unpublished data, http://www.rgrc.dna.affrc.go.jp/jp/data/KK-BIL182-20030506.xls).

CULTIVATION AND MEASURMENT

The seeds of 182 BILs and their parents, 'Koshihikari' (a leading *japonica* variety from Japan) and 'Kasalath'(an *indica* variety from India), after surface-sterilization for 7 min in 1% sodium hypochlorite solution, were germinated for 48 hr at 30°C. The germinated seeds were sown in a nursery seeding bed (Takii Seed Co. Japan) filled with commercial soil (Miyazaki Yamamune Ltd., Japan) on 10 April 2004. After 30 d, all seedlings were transplanted to a field at the Experimental Station of Miyazaki University, Japan, during summer–autumn, 2004. There was a single seedling per hill spaced at 10×15 cm. The experiment consisted of three-row plots, with five plants per row for each BIL, with two replications. The LCC was measured using the SPAD-502 Chlorophyll Meter (Minolta Co., Ltd, Japan). The SPAD-502 provides a simple, quick, portable and non-destructive method for estimating leaf chlorophyll content (Peng et al, 1993; Turner and Jund; 1991, Dwyer et al., 1991; Watanable et al., 1980). In this experiment, SPAD values of 30 uppermost fully expanded leaves at the maximum tillering stage and 20 healthy flag leaves and the 2nd leaf at heading stage were measured according to the methods described by Peng at al. (1993). Mean values for each line were used for QTL analyses.

DETECTION OF QTL

Composite interval mapping (CIM) analysis was applied to trait average and marker data to identify precisely the QTL locations in accordance with Zeng (1994). The CIM analysis was performed by QTL Cartographer computer software version 2.0 (Wang et al., 2003) using forward regression with a walk speed of 2 cM and a window size of 10 cM. A locus with a LOD threshold value higher than 2.5 was declared a putative QTL. In addition, the additive effect and percentage of variation explained by an individual QTL were estimated. The QTL were named according to the suggestions of McCouch et al. (1997).

RESULTS

FREQUENCY DISTRIBUTION OF SPAD VALUES REPRESENTING LCC IN SEGREGATING POPULATION

Figure 1 represents mean LCC SPAD values of both the parents (Koshihikari, Kasalath) and the frequency distributions of BILs at the two developmental stages. Continuous phenotypic variation was observed and most BILs were distributed between LCC SPAD values of the parents.

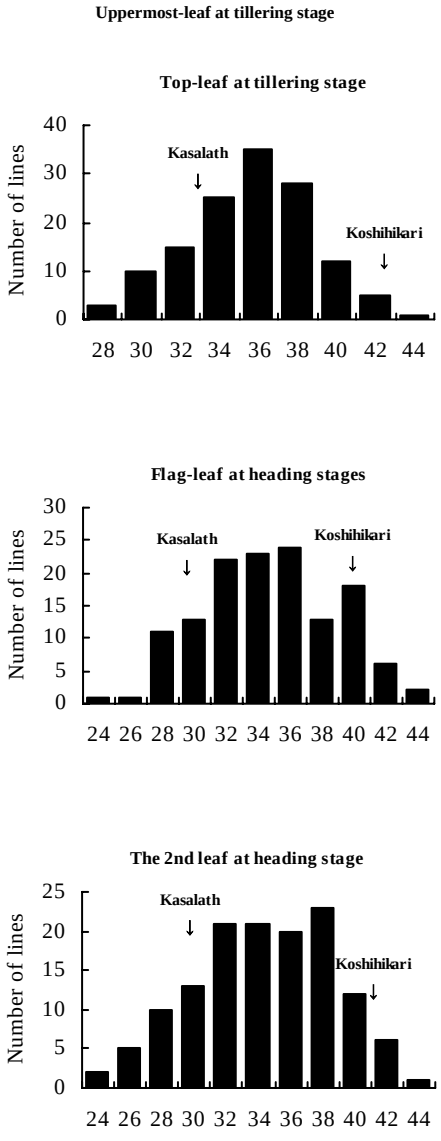

Figure 1. Frequency distributions for SPAD values of LCC across two developmental stages in 182 backcross inbred lines derived from progeny of Koshihikari/Kasalath//Koshihikari.

LCC at uppermost-leaf (at the maximum tillering stage) and both flag-leaf and the 2nd leaf (at heading stage) in the BIL population had an approximate normal distribution in the BIL population (data not shown). These results indicated that LCC was a quantitative trait at the two developmental stages. In addition, the correlation coefficients (data not shown) for LCC values were significant at 1% possibility level: 0.88 between flag-leaf and the 2nd leaf at heading, 0.52 between uppermost-leaf at the maximum tillering stage and flag-leaf at heading, and 0.59 between uppermost leaf at maximum tillering stage and the 2nd leaf at heading, which showed the high consistency of the LCC values for BILs at various growth stages.

MAPPING QTL FOR LCC

Seven QTL controlling LCC were identified and mapped to chromosomes 1, 2, 3, 4, 6, and 8 (Table 1 and Figure 2), and tentatively designated as *q*LCC-1-1, *q*LCC-1-2, *q*LCC-2, *q*LCC-3, *q*LCC-4, *q*LCC-6, and *q*LCC-8, respectively. Interestingly, three QTL (*q*LCC-1-1, *q*LCC-1-2 and *q*LCC-4) were common for the two developmental stages. The *q*LCC-2 was identified at both uppermost-leaf at tillering and flag-leaf at heading, but was not detected for the 2nd leaf chlorophyll content. On the other hand, the *q*LCC-6 was detected for both the flag-leaf and the 2nd leaf chlorophyll content at heading, but was not detected at the tillering stage. Individually, *q*LCC-1-1, located near R10920S, and *q*LCC-1-2, located near C813, both on the chromosome 1, explained 5.1~8.1% and 5.4~7.8 % of total phenotypic variation, respectively. The *q*LCC-2 was detected in the interval between R1826 and C424 markers on chromosome 2; it accounted for 6.4 % of the total phenotypic variation. The *q*LCC-4, located near C1016 on chromosome 4, explained 7.6~14.8% of total phenotypic variation. The *q*LCC-6, located near R2123 on chromosome 6, explained 8.1 % of the total phenotypic variation. The *q*LCC-3, located near R3226, and *q*LCC-8, located near R1963, were detected only at the tillering stage; they explained 14.4% and 8.8% of total phenotypic variation, respectively. Furthermore, the alleles at four QTL (*q*LCC-1-1, *q*LCC-1-2, *q*LCC-2, and *q*LCC-8) from Koshihikari and the alleles at the other three QTL (*q*LCC-3, *q*LCC-4, and *q*LCC-6) from Kasalath contributed toward increasing LCC.

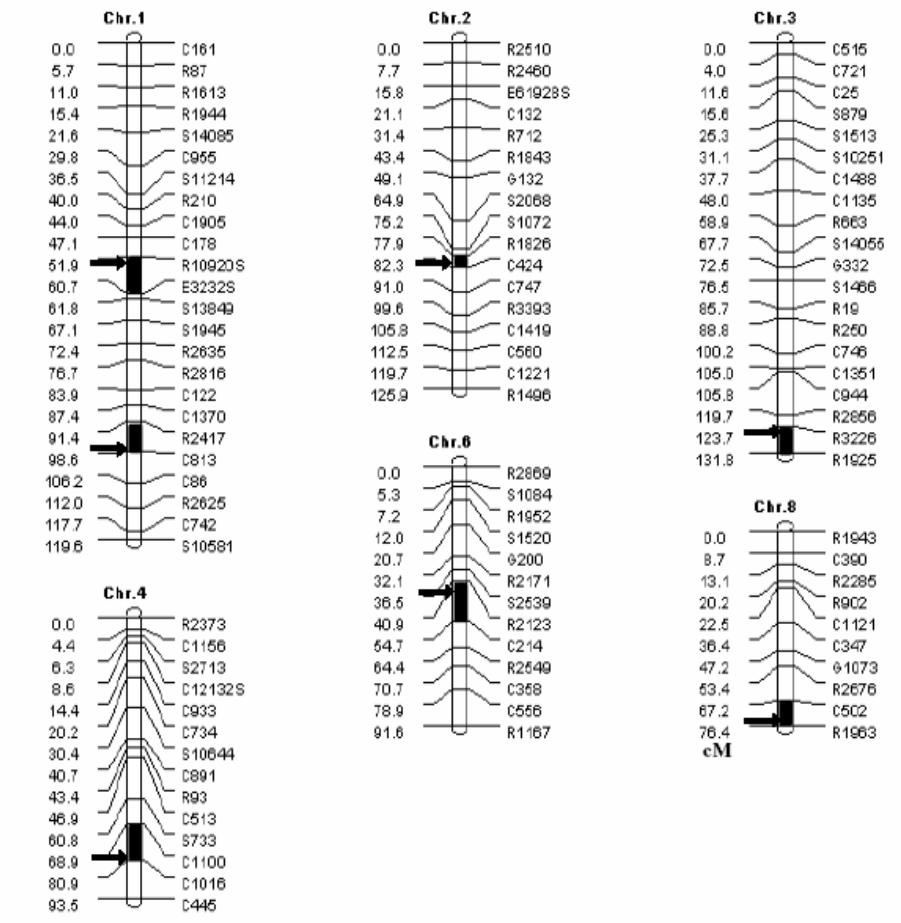

Figure 2. Locations of QTL associated with leaf chlorophyll content (LCC) in 182 backcross inbred lines derived from progeny of Koshihikari/Kasalath//Koshihikari. Arrowheads indicate the location of peak LOD for QTL detected.

Table 1. QTL for leaf chlorophyll content (LCC) in rice based on CIM methods (Wang et al., 2003).

Name of QTL	Chromosome number	Distance (cM)	Marker interval[a]	Peak LOD value	Additive effects[b]	Variation (%)[c]
			Uppermost-leaf at tillering stage			
*q*LCC-1-1	1	51.9	R10920S-E3232	3.1	0.86	8.1
*q*LCC-1-2	1	97.4	R2417-C813	2.6	0.77	7.8
*q*LCC-2	2	82.0	R1826-C424	2.5	0.61	6.4
*q*LCC-3	3	121.7	R2856-R3226	5.5	–1.42	14.4
*q*LCC-4	4	76.9	C1100-C1016	2.5	–0.72	7.6
*q*LCC-8	8	73.2	C502-R1963	2.9	0.95	8.8
					Total	52.2
			The flag-leaf at heading stage			
*q*LCC-1-1	1	51.9	R10920S-E3232	2.5	1.05	5.1
*q*LCC-1-2	1	97.4	R2417-C813	2.6	1.27	5.4
*q*LCC-2	2	82.0	R1826-C424	2.8	1.01	6.4
*q*LCC-4	4	76.9	C1100-C1016	7.3	–2.28	14.8
*q*LCC-6	6	40.5	R2123-C214	3.2	–1.61	8.1
					Total	40.1
			The 2nd leaf at heading stage			
*q*LCC-1-1	1	51.9	R10920S-E3232	2.5	1.14	5.8
*q*LCC-1-2	1	97.4	R2417-C813	3.9	1.35	7.1
*q*LCC-4	4	76.9	C1100-C1016	5.1	–1.69	10.3
*q*LCC-6	6	40.9	R2123-C214	3.3	–1.38	8.1
					Total	31.4

[a] Markers in italic letters indicate the nearest ones linked to putative QTL.
[b] Positive values of additive effects indicate Koshihikari alleles are in the direction of increasing LCC values.
[c] Percentage of explained phenotypic variation.

DISCUSSION

In this study, we discuss the results of QTL mapping for LCC at two main developmental stages (tillering and heading) of rice using the BILs derived from backcross Koshihiakri/Kasalath //Koshihikari with 162 RFLP markers. Seven QTL (*q*LCC-1-1, *q*LCC-1-2, *q*LCC-2, *q*LCC-3, *q*LCC-4, *q*LCC-6, and *q*LCC-8) for LCC were detected and mapped to chromosomes 1 (two QTL), 2, 3, 4, 6, and 8 (Table 1 and Figure 2). In addition, alleles with increasing and decreasing effects for LCC at the two developmental stages were detected from both the parents; Koshihikari had the increasing alleles for LCC at *q*LCC-1-1, *q*LCC-1-2, *q*LCC-2, and *q*LCC-8, but decreasing alleles at *q*LCC-3, *q*LCC-4, and *q*LCC-6, whereas Kasalath had the opposite effects. These results explained the observed transgressive segregation and continuous distributions for LCC in the BIL population. The QTL number for LCC varied with the growth periods. Interestingly, *q*LCC-1-1, *q*LCC-1-2, and *q*LCC-4 were effectively detected regardless of the developmental stage and leaf order, indicating that these three QTL were stably expressed at different stages. However, *q*LCC-2 was detected only at uppermost-leaf at tillering and flag-leaf at heading, but not at the 2nd leaf. This indicated that the *q*LCC-2 expression pattern probably varied with leaf order.

To increase photosynthetic ability in rice through genetic manipulation of LCC QTL, a prerequisite is that there should neither be a tight linkage nor pleiotropic effects between

LCC QTL and those genes/QTL for decreased LCC during the later developmental stages, and that those LCC QTL be effectively expressed during the entire growth period. Teng et al. (2004) reported that three QTL for LCC at flourishing tillering stage of rice were located on chromosome 1, 3 and 8. In addition, six QTL for LCC at 5 d after heading, located on chromosomes 1, 3 (two QTL), 5, 8, and 12, and the other four QTL for decreased LCC at 5 to 25 d after heading, located on chromosomes 4, 6, 8, and 9, were detected using the BIL population from Nipponbare/Kasalath//Nipponbare (Ishimaru et al., 2001). In comparing the genomic positions of those QTL associated with LCC (Ishimaru et al., 2001; Teng et al., 2004) with the seven QTL detected in our study, the qLCC-1-1 is tightly linked to/allelic to the QTL reported by Ishimaru et al. (2001) because they are located near the common C178 marker; the *q*LCC-1-2 is closely linked to/allelic to the QTL reported by Teng et al. (2004) because they are located near the common C813 makers; and the *q*LCC-3 on chromosome 3 is closely linked to/allelic to the QTL reported by Ishimaru et al. (2001) because they are located near the common R3226 marker. In addition, because *q*LCC-4 and *q*LCC-8 are allelic/tightly linked to each QTL for decreased LCC, which was detected 5 to 25 d after rice heading (Ishimaru et al., 2001), it could be deduced that function of *q*LCC-4 and *q*LCC-8 might be easily lost at the late developmental stages or might be involved in leaf senescence during milk-ripe stage. The remaining two QTL (*q*LCC-2 and *q*LCC-6) are reported for the first time in this paper.

High photosynthetic ability will be the target of rice breeding in the 21st century. Knowledge of genetic mechanisms of photosynthesis will benefit rice breeders. Five QTL (*q*LCC-1-2, *q*LCC-1-2, *q*LCC-2, *q*LCC-3, and *q*LCC-6), possibly having stable expression for increasing LCC, detected in our study, should accelerate breeding of new varieties with higher LCC, which can produce higher yield in rice.

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