Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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Asian Journal of Plant Sciences 6 (3): 518-522, 2007
ISSN 1682-3974

Molecular Detection of Quantitative Trait Loci for Leaf Chlorophyll Content at Different Growth-Stages of Rice (*Oryza sativa* L.)

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Abstract: The objectives of present study are to identify and characterize QTLs for LCC at three different growth stages of rice. In present study, the Recombinant Inbred (RI) lines derived from Asominori (*Japonica*)/IR 24 (*Indica*) were used to identify Quantitative Trait Loci (QTLs) for LCC at three growth stages (45-day-old seedling, maximum tillering and heading) in rice. Continuous variations and transgressive segregation for LCC were observed in the RI population, indicating that LCC was a quantitatively inherited trait at different growth stages of rice. Ten QTLs for LCC were identified and mapped to chromosomes 1(two QTLs), 3(two QTLs), 4, 5, 7(two QTLs), 11, 12, respectively, which accounted for 9.7-25.7% of the total phenotypic variation. Interestingly, non-common QTLs were detected at all three growth stages, clearly indicating that different QTLs control the LCC during growth intervals. In addition, the alleles at the eight QTLs (*q*LCC-1-1, *q*LCC-1-2, *q*LCC-3-1, *q*LCC-3-2, *q*LCC-4, *q*LCC-7-1, *q*LCC-7-2 and *q*LCC-12) from Asominori and the remaining alleles at *q*LCC-5 and *q*LCC-11 from IR 24 contributed to the increase of LCC. The results and the tightly linked molecular markers that flank the QTLs detected in this study may be useful for improvement of photosynthetic ability at different growth stages of rice.

Key words: Leaf Chlorophyll Content (LCC), Quantitative Trait Locus (QTL), rice (*Oryza sativa* L.)

INTRODUCTION

Leaf Chlorophyll Content (LCC) is one of importantly physiological traits and closely related to photosynthetic ability in plants. Undoubtedly, an understanding the genetic basis associated with LCC in different growth stages of rice has significant implications for rice breeding. The great advances in 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; Xu, 2002). To date, numerous Quantitative Trait Loci (QTLs) controlling some importantly agronomical and physiological traits in rice have been identified and mapped using molecular makers (Xu, 2002). Also, few QTLs affecting LCC at the flourishing tillering stage (Teng *et al*., 2004) and 5 day after heading (Ishimaru *et al*., 2001) in rice were reported. However, based on the developmental genetics, QTLs/genes are expressed selectively at different growth stages and conventional at the rate of statistical genetics analysis results have revealed that gene action was distinct at various growth stages and genetic model from the final traits could not fully reflect the real gene actions during the developmental of the trait (Zhu, 1995). To our knowledge, developmental genetic analysis of QTLs for LCC in rice has not been conducted yet at different growth stages using the same population. Undoubtedly, the identification of those QTLs determining LCC during different growth stages in rice can benefit the development of varieties remaining high photosynthetic ability during whole growth stages. The objectives of present study are to identify and characterize QTLs for LCC at three different growth stages of rice using recombinant inbred population derived from cross of Asominori (*Japonica*)/IR 24 (*Indica*).

MATERIALS AND METHODS

Plant materials: Recombinant Inbred (RI) lines, kindly provided by professor A. Yoshimura of Plant Breeding Laboratory, Agricultural Faculty of Kyushu University, Japan were developed by single seed descent from the progeny of combination of a cross of japonica cultivar Asominori from Japan with indica cultivar IR 24 developed by IRRI. In past, 165 F_6 lines were

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obtained from 227 original F_2 individual plants. From these, 71 lines were randomly selected and used for mapping. The Restriction Fragment Length Polymorphism (RFLP) map covering 1275 cm in entire rice chromosomes was constructed with 375 markers from the F_6 and F_7 generations (Tsunematsu *et al.*, 1996). In the study, we used a subset of 289 RFLP markers without overlapping for all loci from the original genetic map (Tsunematsu *et al.*, 1996) to map QTLs affecting LCC in rice, for which the average interval distance between pair of markers was 4.4 cm.

Field experiment and measure of LCC values: The germinated seeds of 68 RI lines along with its parents, Asominori (P_1) and IR 24 (P_2) were sown on 25, May, 2006 (Shanghai, China). After 25 days all seedlings were transplanted to Experiment Farm of Shanghai Normal University (Shanghai, China) with single seedling per hill spaced at 10 by 15 cm, respectively. Each plot included one line with six plants per line. The other managements followed the local conventional methods. LCC were measured using *Chlorophyll meter* (SPAD-502, Minolta Co. Ltd. Japan), which can provide a simple, quick portable and non-destructive method to measure LCC (Dwyer *et al.*, 1991; Peng *et al.*, 1993; Turner and Jund; 1991; Watanabe *et al.*, 1980). In this experiment, four uppermost fully expanded leaves at 45-day-old seedling stage and maximum tillering stage and flag leaves at heading stage were selected from each plot to measure their SPAD values representing LCC according to the methods described by Peng *at al.* (1993). Average SPAD values for each line were used for QTL analysis.

Detection of QTLs: Composite Interval Mapping (CIM) analysis was applied to trait average and marker data to more precisely identify the QTL locations (Zeng, 1994). The CIM analysis were performed by QTL Cartographer computer program software (Wang *et al.*, 2003) version 2.0 using forward regression with the walk speed of 2 cm and the window size of 10 cm. A locus with a LOD threshold value of more than 2.3 was to be declared a putative QTL. In addition, the additive effect and percentage of variation explained by an individual QTL were also measured. The QTLs were named according to the suggestions of McCouch *et al.* (1997).

RESULTS

Frequency distribution of SPAD values representing LCC in RI population: Figure 1 shows the average SPAD values of the both parents (Asominori, IR24) and the frequency distributions of RI lines at the different three

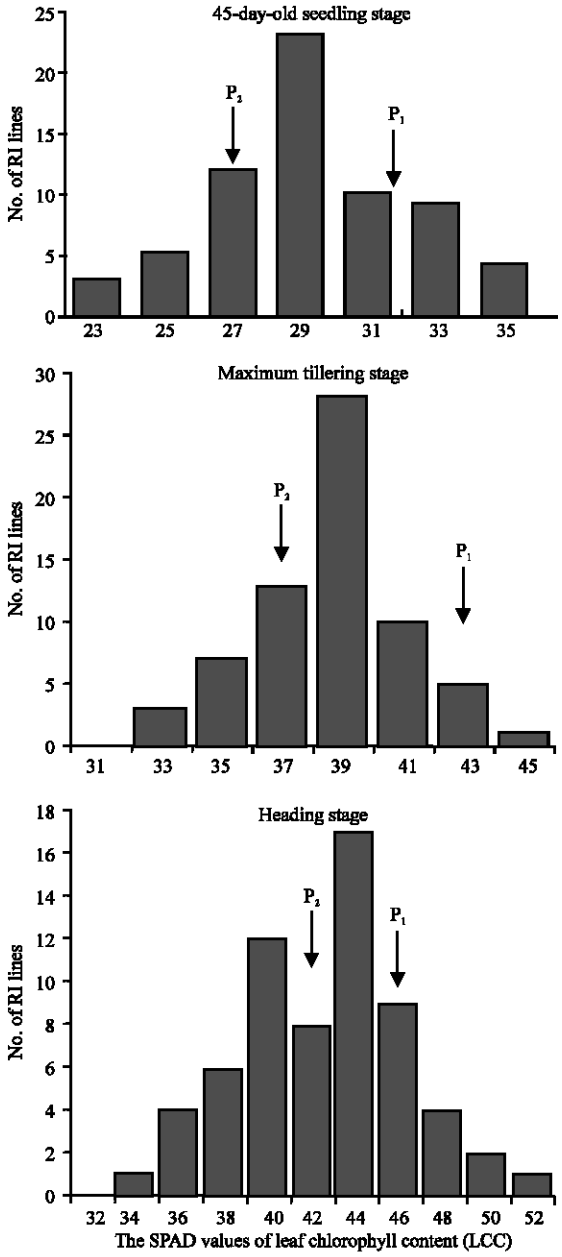

Fig. 1: Frequency distributions for SPAD values of LCC in RI population derived from cross between Asominori (P_1) and IR24 (P_2)

growth stages. Continuous phenotypic variations were observed, indicating that LCC was quantitative trait at three growth stages in the RI population. In addition, the correlation coefficients (data not shown) were 0.36 (significant at 5% level) between 45-old-day seedling stage and maximum tillering stage, 0.57 (significant at 1% level) between heading and maximum tillering stage, respectively, however, there did not exist relationship

between heading stage and seedling stage, which indicated that expressed QTLs/genes controlling the LCC might vary with growth stage.

Mapping QTLs for LCC: A total of ten QTLs for LCC were identified and mapped to chromosomes 1 (two QTLs), 3 (two QTLs), 4, 5, 7 (two QTLs), 11, 12 (Table 1 and Fig. 2) and tentatively named as *q*LCC-1-1, *q*LCC-1-2, *q*LCC-3-1, *q*LCC-3-2, *q*LCC-4, *q*LCC-5, *q*LCC-7-1, *q*LCC-7-2, *q*LCC-11 and *q*LCC-12, respectively. At 45-old-day seedling stage, two QTLs (*q*LCC-1-1 and *q*LCC-1-2) on the same chromosome 1 and another QTL (*q*LCC-11) on chromosome 11 were detected. Among them, *q*LCC-1-1 with the largest effects (LOD = 5.6) was located between C 904 and Y 5714 L, which accounted for 25.6% of total variation. At maximum tillering stage, four

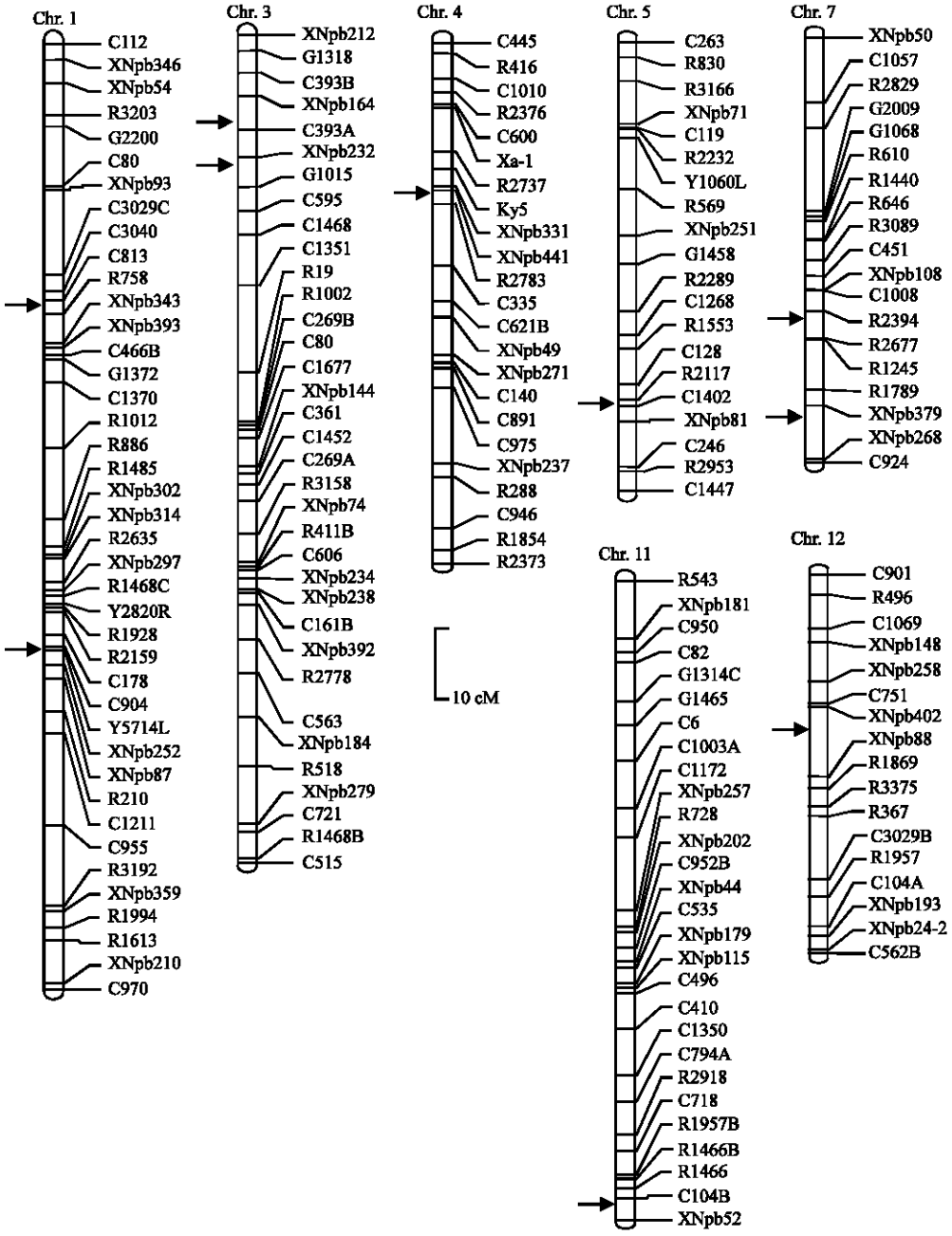

Fig. 2: Chromosomal location of QTLs controlling LCC in RI population derived from cross of Asominori/IR24. Arrowheads indicate the location of peak LOD for QTLs detected

Table 1: QTLs for Leaf Chlorophyll Content (LLC) rice in RI population derived from Asominori/IR24

Name of QTLs	Chromosome No.	Distance (cm)	Marker interval[1)]	Peak LOD value	Additive effects[2)]	Variation[3)] (%)
45-day-old seedling stage						
*q*LCC-1-1	1	106.3	*C904*-.Y5714L	5.6	1.44	25.6
*q*LCC-7-1	7	93.7	*XNpb379*-XNpb268	3.4	1.08	12.8
*q*LCC-11	11	98.4	*C104B*-XNpb52	3.3	-1.24	14.1
Maximum tillering stage						
q.LCC-3-1	3	23.0	*XNpb232*-G1015	3.2	0.87	12.2
q.LCC-5	5	76.2	*R2117*-C1402	3.4	-0.89	12.3
q.LCC-7-2	7	69.8	*R2394*-R2677	3.7	0.94	14.2
q.LCC-12	12	39.7	*XNpb402*-XNpb88	5.2	1.29	25.7
Heading stage						
q.LCC-1-2	1	45.3	*C813*-R758	2.4	1.26	9.7
q.LCC-3-2	3	16.8	*XNpb164*-C393A.	2.3	1.25	9.9
q.LCC-4	4	27.8	*XNpb441*-R2783	3.0	1.44	12.6

[1]Markers in italic .letters indicate the nearest ones linked to putative QTL. [2]Positive values of additive effects indicate Asominori alleles are in the direction of increasing LCC. Percentage of explained phenotypic variation

QTLs (*q*LCC-3-1, *q*LCC-5, *q*LCC-7-2 and *q*LCC-12) were detected on chromosome 3, 5, 7, 12, which accounted for 12.2-25.7% of the total variation, respectively. Among them, *q*LCC-12 near XNpb402 had the largest genetic effect, which accounted for 25.7% of the total variation. At heading stage, three QTLs (*q*LCC-1-2, *q*LCC-3-2 and *q*LCC-4) detected for LCC were detected on chromosome 1, 3, 4, which accounted for 9.7, 9.9 and 12.6% of the total variation, respectively. It was noted that non-common QTLs were detected at all three growth stages, clearly indicating that different QTLs control the LCC during different growth intervals. In addition, the alleles at the eight QTLs (*q*LCC-1-1, *q*LCC-1-2, *q*LCC-3-1, *q*LCC-3-2, *q*LCC-4, *q*LCC-7-1, *q*LCC-7-2 and *q*LCC-12) from Asominori and the alleles at remaining two QTLs (*q*LCC-5 and *q*LCC-11) from IR24 contributed to the increase of LCC.

DISCUSSION

In the study, we report the results of QTL mapping for LCC at three growth stages (seedling, tillering and heading) of rice using the RI population derived from cross between Asominori and IR24. Consequently, ten QTLs (*q*LCC-1-1, *q*LCC-1-2, *q*LCC-3-1, *q*LCC-3-2, *q*LCC-4, *q*LCC-5, *q*LCC-7-1, *q*LCC-7-2, *q*LCC-11 and *q*LCC-12) for LCC were detected and mapped to chromosomes 1 (two QTLs), 3 (two QTLs), 4, 5, 7 (two QTLs), 11, 12 (Table 1 and Fig. 2), respectively. Furthermore, alleles with increasing and decreasing effects for LCC at both seedling and tillering stages were detected from the both parents and Asominori had increasing alleles for LCC at *q*LCC-1-1, *q*LCC-1-2, *q*LCC-3-1, *q*LCC-3-2, *q*LCC-4, *q*LCC-7-1, *q*LCC-7-2 and *q*LCC-12, but decreasing alleles at *q*LCC-5 and *q*LCC-11, while IR24 alleles had the opposite effects. These results could explain the transgressive segregations and continuous distributions for LCC in the RI population. From these results of the present study, it was shown that expressed QTLs for LCC in rice varied with different growth interval. In past, several researchers of time-related QTL mapping have reported the detection of different QTLs at different growth stages (Price and Tomos 1997; Xing *et al.* 2001; Xu *et al.*, 2004). For example, no common QTLs for root dry weight (Xu *et al.*, 2004) was detected at two growth stages. And there is no common QTLs for plant height between the two seedling stages and the maturity stage (Xing *et al.*, 2001). Price and Tomos (1997) found the two most significant QTLs for maximum root growth on chromosomes 6 and 11, which appeared to display profoundly different influences. These results, including our results in this study, are in agreement with the developmental genetics that QTLs/genes are expressed selectively at different growth stages (Zhu, 1995).

Undoubtedly, understanding all expressed QTLs increasing LCC regardless of at a certain/whole growth stage benefits the selection of high photosynthetic ability in rice. In the past, Teng *et al.* (2004) reported three QTLs for LCC at flourishing tillering stage of rice, located at chromosome 1, 3 and 8, respectively, using double haploid population from ZYQ8/JX17. In addition, six QTLs for LCC at 5d after heading, located on chromosomes 1, 3 (two QTLs), 5, 8, 12, respectively, were detected (Ishimaru *et al.*, 2001) using the BIL population from Nipponbare/Kasalath/Nipponbare. In comparing the genomic regions of those QTLs associated with LCC (Ishimaru *et al.*, 2001; Teng *et al.*, 2004) with the ten QTLs detected in present studies, *q*LCC-1-1 is tightly linked to/allelic to the QTL reported by Ishimaru *et al.* (2001) and the QTL reported by Teng *et al.* (2004), respectively, *q*LCC-4 on chromosome 4, *q*LCC-5 on chromosome 5 and *q*LCC-12 on chromosome 12 might be closely linked to/allelic to the each one QTL (Ishimaru *et al.*, 2001), respectively. However, other six QTLs (*q*LCC-1-2, *q*LCC-3-1, *q*LCC-3-2, *q*LCC-7-1, *q*LCC-7-2 and *q*LCC-11) in this study were the first reported. In a word, high

photosynthetic ability always is the target of rice breeding. Any knowledge of genetic mechanism of *q*LCC will be benefit to rice breeders. Thus, the results and the tightly linked molecular markers that flank the QTLs detected in this study may be useful for improvement of photosynthetic ability in rice at different growth stages.

ACKNOWLEDGMENTS

We are greatly indebted to Professor A. Yoshimura (plant breeding laboratory, Agricultural Faculty of Kyushu University, Japan) for kindly providing the RI lines and molecular data. This research was partly supported by Shanghai Municipal Education Commission of China (No. 05DZ25 and 06ZZ21), Shanghai Municipal Science and Technology Commission of China (No. 06PJ14074) and the 948 Program from Agricultural Department of China (No. 2006-G1).

REFERENCES

Dwyer, L.M., M. Tollenaar and L. Houwing, 1991. A nondestructive method to monitor leaf greenness in corn. Can. J. Plant Sci., 71: 505-509.

Harushima, Y., M. Yano, A. Shomura, M. Sato, T. Shimano, Y. Kuboki, T. Yamamoto, S.Y. Lin, B.A. Antonio, A. Parco, H. Kajiya, N. Huang, K. Yamamoto, Y. Nagamura, N. Kurata, G.S. Khush and T. Sasaki, 1998. A High-density rice genetic linkage map with 2275 markers using a single F2 population. Genetics, 148: 479-494.

Ishimaru, K., M. Yano, N. Aoki, K. Ono, T. Hirose, Y. Lin, L. Monna, T. Sasaki and R. Ohsugi, 2001. Toward the mapping of physiological and agronomic characters on a rice function map: QTL analysis and comparison between QTLs and expressed sequence tags. Theor. Applied Genet., 102: 793-800.

McCouch, S.R., Y.G. Cho, M. Yano, E. Paul and M. Blinstrub, 1997. Report on QTL nomenclature. Rice Genet. Newslett., 14: 11-13.

Peng, S., F.V. Garcia, R.C. Laza and K.G. Cassman, 1993. Adjust for specific leaf weight improves chlorophyll meter's estimates of rice leaf nitrogen concentration. Agron. J., 85: 987-990.

Price, A.H. and A.D. Tomos, 1997. Genetic dissection of root growth in rice (*Oryza sativa* L.). II. Mapping quantitative trait loci using molecular markers. Theor. Applied Genet., 95: 143-152.

Teng, S., Q. Qian, D. Zeng, Y. Kunihiro, K. Fujimoto, D. Huang and L. Zhu, 2004. QTL analysis of leaf photosynthetic rate and related physiological traits in rice (*Oryza sativa* L.). Euphytica, 135: 1-7.

Turner, F.T. and M.F. Jund, 1991. Chlorophyll meter to predict nitrogen topdress requirement for semidwarf rice. Agron. J., 83: 926-928.

Tsunematsu, H., A. Yoshimura, Y. Harushima, Y. Nagamura, N. Kurata, M. Yano and N. Iwata, 1996. RFLP framework map using recombinant inbred lines in rice. Breed. Sci., 46: 279-284.

Watanabe, S., Y. Hatanaka and K. Inada, 1980. Development of a digital chlorophyll meter: I Structure and performance. Jpn. J. Crop. Sci., (special issue) 49: 89-90.

Wang, S., J. Basten and Z. Zeng, 2003. Windows QTL Cartographer 2.0. Department of Statistics, North Carolina State University, Raleigh, NC. (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm).

Xing, Y.Z., Y.F. Tan, C.G. Xu, J.P. Hua and X.L. Sun, 2001. Mapping and isolation of quantitative trait loci controlling plant height and heading date in rice. Acta. Bot. Sin., 43: 840-845.

Xu, Y.B.C., 2002. Global view of QTL: Rice as a mode1. In: Quantitative Genetics Genomics and Plant Breeding. Kang, M.S. (Ed.), Oxford University Press, pp: 109-134.

Xu, C.G., X.Q. Li, Y. Xue, Y.W. Huang and J. Gao, 2004. Y.Z. Xing, Comparison of quantitative trait loci controlling seedling characteristics at two seedling stages using rice recombinant inbred lines. Theor Applied Genet., 109: 640-647.

Yano, M. and T. Sasaki, 1997. Genetic and molecular dissection of quantitative traits in rice. Plant Mol. Biol., 35: 145-153.

Zeng, B.Z., 1994. Precision mapping of quantitative trait loci. Genetics, 136: 1457-1468.

Zhu, J., 1995. Analysis of conditional genetic effect and variance components in developmental genetics. Genetics, 141: 1633-1639.