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Mapping Quantitative Trait Loci Associated with Leaf Senescence During Maturation of Rice (*Oryza sativa* L.)

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Abstract: In this study, the Recombinant Inbred (RI) population derived from Asominori (*japonica*)/ IR24 (*indica*) were employed to identify Quantitative Trait Loci (QTLs) for six leaf-senescence associated traits during maturation in rice, viz., the flag Leaf Chlorophyll-Content (LCC) at heading (FCH) and maturity (FCM), the second-leaf LCC at heading (SCH) and maturity (SCM), the reduction-speed of flag-leaf LCC (RFC) and the second-leaf LCC (RSC). Resultantly, all continuous variations and transgressive segregations in the RI population were observed, indicating that the six traits were quantitatively inherited. A total of eighteen QTLs for the six traits were mapped to chromosome 2 (four QTLs), 3 (four QTLs), 4 (two QTLs), 6 (two QTLs), 8 (two QTLs), 9 (two QTLs), 11, 12, which could explained 11.0 to 27.8% of the phenotypic variation. Interestingly, most LCC QTLs varied with leaf order and growth stage in rice. Among them, four QTLs for RFC and three ones for RSC, conferring the speed of flag- and the second leaf senescence, respectively, did not share the common genomic region each other and the all former alleles from *japonica* Asominori delayed leaf senescence and two of the latter alleles also from *indica* IR24, whereas the remaining one from Asominori, showing that genetic basis of leaf senescence was different between flag- and the second leaf during maturation of rice. The results and the tightly linked molecular markers that flank the QTLs detected in this study may be beneficial for selection of rice varieties with late leaf senescence.

Key words: Leaf senescence, chlorophyll content, quantitative trait locus (QTL), rice (*Oryza stativa* L.)

INTRODUCTION

During the maturation of rice, leaf senescence, the ultimately essential developmental phase due to chlorophyll degradation, is widely considered to be one of the most apparent features of leaf senescence, despite a series of other biochemical and physiological changes (Nooden, 1988; Matile, 1992). Moreover, leaf senescence resulting in the progressive declining in photosynthetic capability might induce the quality deterioration of grain and subsequently the yield loss of rice, especially under drought stressed condition (Gan and Amasino, 1995; Xu *et al*., 2000).

Leaf senescence can be induced by a lot of environmental and developmental factors, but timing of leaf senescence is determined by genetic background rather than a passive degenerative process (Buchanan-Wollaston, 1997; Wingler *et al*., 1998). To date, several studies (Ishimaru *et al*., 2001; Jiang *et al*., 2004; Abdelkhalik *et al*., 2005) had mapped few Quantitative Trait Loci (QTLs) for leaf senescence during maturation, nevertheless, they involved in QTLs either only for flag-leaf senescence or the second leaf in rice. As known, the photosynthesis of the top two leaves (flag- and the second leaf) occupied no less than 80% of the totally photosynthetic productivity of rice, which is regarded as one of the most important contributors to grain yield (Gladun and Karpov, 1993). In addition, 60%-90% of total carbon founds in rice synthesis after heading (Mae, 1997) and at least 80% of grain yield came from photosynthesis in leaves after heading (Zhai *et al*., 2002). Hence, it is very valuable to explicit the genetic basis of flag- and the second leaf senescence after heading for increasing photosynthetic activity and yield in rice. However, it is not clear yet whether there exist different genetic-basis of senescence between flag- and the second leaf during the maturation of rice. To find novel QTLs/genes for leaf senescence and understand the genetic basis of flag- and the second leaf senescence in rice during maturation, in this study, the Recombinant

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Inbred (RI) lines derived from Asominori (*japonica*) / IR24 (*indica*) were used to identify QTLs for six leaf-senescence associated traits during maturation, viz., the flag Leaf Chlorophyll-Content (LCC) at heading (FCH) and maturity (FCM), the second LCC at heading (SCH) and maturity (SCM), the reduction-speed of flag-leaf LCC (RFC) and the second-leaf LCC (RSC), which is expected to benefit the development of varieties remaining high photosynthetic rate during maturation and subsequently is helpful for increasing yield in rice.

MATERIALS AND METHODS

Plant materials: The Recombinant Inbred (RI) lines in this study, 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 IR24 developed by IRRI. In past, one hundred sixty-five F_6 lines were 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. 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 QTL associated with leaf senescence during maturing stage in rice, for which the average interval distance between pair of markers was 4.4 cM.

Field experiment and measurements: The germinated seeds of 71 RI lines along with its parents, Asominori (As) and IR24 were sown on 25, May, 2007 (Shanghai, China). After 25 days all seedlings were transplanted to rice paddy field in Experiment Farm of Shanghai Normal University (Shanghai, China) with single seeding per hill spaced at 10 by 15 cm. Each plot included one line with three plants per line. The other managements followed the local conventional methods. Leaf chlorophyll content (LCC), representing the degree of leaf senescence, in rice was measured using Chlorophyll meter (SPAD-502, Minolta Co. Ltd. Japan), which can provide a simple, quick portable and non-destructive method for estimating the relative LCC (Dwyer *et al*., 1991; Peng *et al*., 1993; Turner and Jund, 1991; Watanabe *et al*., 1980). In this experiment, each six flag-leaves and respective the second leaves for one plant were selected to evaluate FCH, FCM, SCH, SCM, by mean of reading SPAD data of the flag- and the second leaf of rice plants for each RI line on the day of heading and 35 days after heading (maturity) with three replicates, respectively. To determine the speed of leaf senescence during maturation in rice, the reduction-speed of flag-leaf LCC (RFC) and the second-leaf LCC (RSC) were calculated by the formula: RFC=(FCH-FCM)/35 and RSC=(SCH-SCM)/35, respectively. All average values for each line were used for QTL analysis.

Detection of QTLs: Composite Interval Mapping (CIM) analysis was applied to identify the QTLs locations more precisely (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 2cM and the window size of 10cM. A locus with a LOD 2.5 was to be declared a putative QTL. In addition, the addition 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

Trait variation and correlation: All average SPAD values of parents [Asominori(As), IR24] and the frequency distribution of RI lines at both heading and maturity (35 days after heading) and the reduction-speed of LCC during maturation were shown in Fig. 1a-f. Continuous phenotypic variations and transgressive segregations in RI population were observed. These results indicated that no major genes were involved in six leaf-senescence associated traits. In addition, the *japonica* parent, Asominori (As) always had larger values for all six traits than the *indica* parent, IR24, suggesting that Asominori both retained relatively high chlorophyll level especially at heading and exhibited more rapidly leaf senescence during maturation regardless of flag- and the second-leaf in rice (Fig. 1).

Correlation coefficients among the all six traits were listed in Table 1. Significantly positive correlations ($r>0.84$, $p<0.01$) between FCH and SCH, between FCM and SCM and between RFC and RSC, respectively, showed the high consistency of both LCC and leaf-senescence speed between flag- and the second leaf. In addition, it can be understandable that there existed strongly negative correlations ($r>0.78$, $p<0.01$) between FCM and its leaf-senescence speed regardless of flag- and the second leaf. More interestingly, it was found that LCC at heading were not related with both LCC at maturity and leaf senescence speed during maturation, which showed that rice plants with high LCC at heading can not lighten leaf senescence speed during maturation.

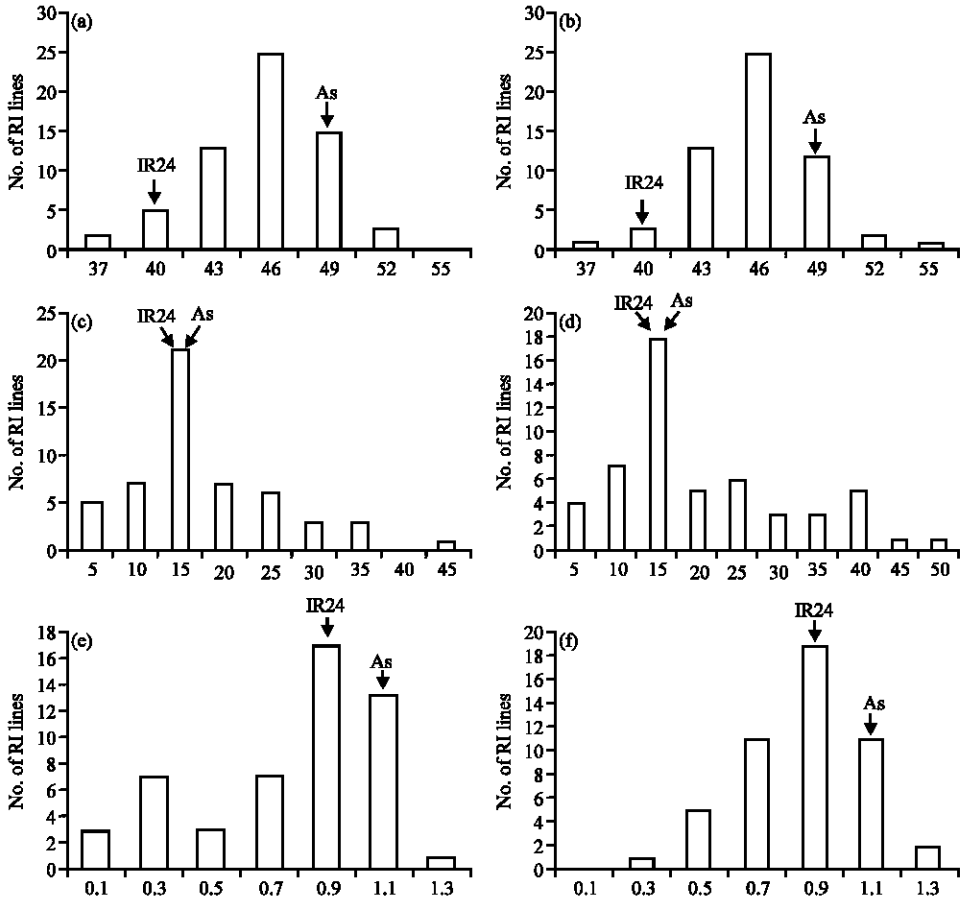

Fig. 1: Frequency distributions for SPAD values of LCC during maturation in RI population derived from Asominori (As)/IR24. (a, b) The abbreviation of FCH and SCH indicate the LCC of flag- and the second leaf at heading stage, respectively; (c, d) FCM and SCM indicate the LCC of flag- and the second leaf at maturity (35 days after heading), respectively; (e, f) RFC and RSC indicate the reduction-speed of flag-leaf and the second-leaf LCC, respectively. Arrowheads indicate the average values of Asominori (As) and IR24

Table 1: Correlation coefficients of six leaf-senescence associated traits in RI population

Trait[1]	FCH	SCH	FCM	SCM	RFC
SCH	0.86**				
FCM	0.01	0.14			
SCM	0.11	0.29	0.84**		
RFC	0.25	0.09	-0.96**	-0.78**	
RSC	0.17	0.04	-0.83**	-0.95**	0.85**

*Represent significant at p = 0.01 level. [1]Trait abbreviations are shown in Fig. 1

QTLs for six traits associated with leaf senescence: Two QTLs for flag-leaf chlorophyll content at heading (FCH) and three QTLs for the second-leaf chlorophyll content at heading (SCH) (Table 2, Fig. 2) were detected on chromosome 2(qSCH-2), 3(qSCH-3) 9 (qFCH-9 and qSCH-9) and 12 (qFCH-12), which accounted for 11.0 to 22.9% of the total phenotype variation, respectively. Among them, qFCH-12 with the largest effect (LOD = 4.44) to FCH and qSCH-2 with the largest effect (LOD = 4.84) to

Table 2: QTLs for LCC at heading and maturity in rice in RI population from Asominori/IR24

Name of QTL	Chromosome	Marker interval[1]	LOD	Add[2]	Var (%)[3]
Heading					
Flag leaf					
qFCH-9	9	XNpb103-C397	3.44	1.24	14.7
qFCH-12	12	XNpb193- XNpb24-2	4.44	-2.03	20.2
The second leaf					
qSCH-2	2	C560-C370	4.84	1.51	22.9
qSCH-3	3	C595-C1468	2.67	1.07	11.7
qSCH-9	9	C506-G1445	2.55	-1.03	11.0
Maturity					
Flag leaf					
qFCM-2	2	XNpb227-C132	2.95	4.37	12.9
qFCM-3	3	C1468-C1351	4.12	5.57	19.0
qFCM-4	4	XNpb49- XNpb271	3.39	4.88	16.0
qFCM-8	8	C621C-R727	4.16	5.70	19.2
The second leaf					
qSCM-3-2	3	C595-C1468	4.17	3.75	19.2
qSCM-6	6	C1677B- XNpb386	3.04	3.82	17.7

[1]Markers in italic letters indicate the nearest ones linked to putative QTL. [2]Positive values indicate Asominori alleles are in the direction of increasing traits. [3]Percentage of explained phenotypic variation

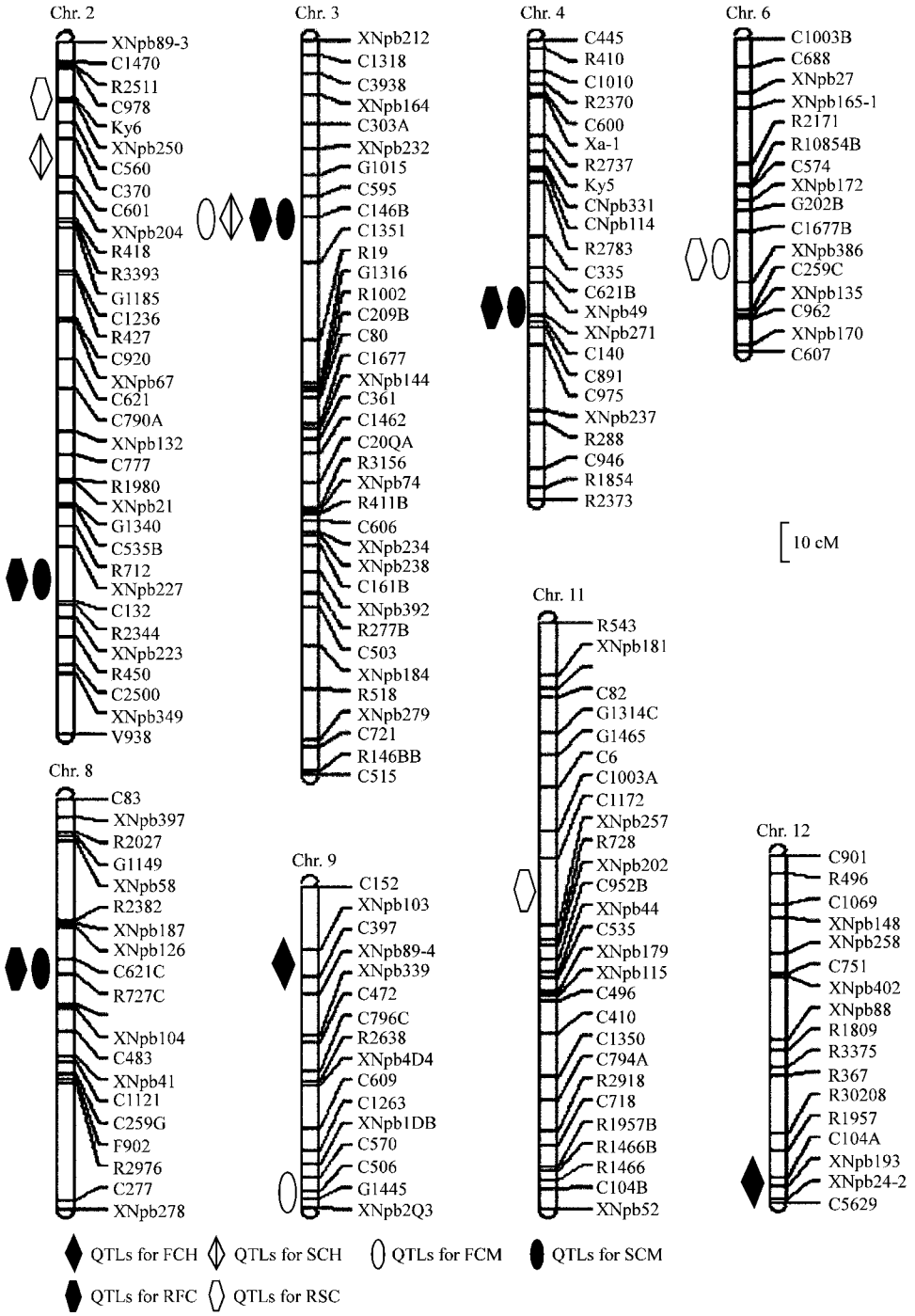

Fig. 2: Chromosomal location of QTLs for six leaf-senescence associated traits on the molecular linkage map. Traits abbreviations are shown in Fig. 1

SCH explained 20.2, 22.9% of the total phenotype variation, respectively. It was noted that no common QTLs were found between flag- and the second-leaf, clearly indicating that different QTLs controlled the LCC of flag- and the second-leaf at heading. In addition, the alleles at the three QTLs (qFCH-9, qSCH-2 and qSCH-3) from Asominori increased LCC, whereas the alleles at both qFCH-12 and qSCH-9 from IR24 increased LCC.

In addition, four QTLs for flag-leaf chlorophyll content at maturity (35 days after heading) (FCM) and two

Table 3: QTLs for the reduction-speed of LCC during maturation in RI population derived from Asominori/IR24

Name of QTL	Chromosome	Marker interval[1]	LOD	Add[2]	Var (%)[3]
Flag leaf					
qRFC-2	2	*XNpb227*-C132	3.70	-0.13	16.4
qRFC-3	3	*C1468*-C1351	4.27	-0.15	19.5
qRFC-4	4	XNpb271-*C140*	2.97	-0.12	12.7
qRFC-8	8	*C621C*-R727	5.70	-0.18	27.8
The second leaf					
qRSC-2	2	*C978*-Ky6	2.73	0.08	11.3
qRSC-6	6	C1677B- *XNpb386*	2.79	-0.10	17.3
qRSC-11	11	C1172- *XNpb357*	2.65	0.09	15.6

[1]Markers in italic letters indicate the nearest ones linked to putative QTL. [2]Positive values indicate Asominori alleles are in the direction of increasing traits. [3]Percentage of explained phenotypic variation

QTLs for the second-leaf chlorophyll content at maturity (SCM) (Table 2, Fig. 2) were identified, respectively. In the flag leaf, the four QTLs for FCM were located on chromosome 2, 3, 4, 8, respectively, which accounted for 12.9-19.2% of the total variation. Among them, the qFCM-8 with the largest effect (LOD = 4.16) was located in the interval of markers between C621C and R727, which accounted for 19.2% of the total phenotype variation. In the second-leaf, two QTLs (qSCM-3, qSCM-6) were located between C595 and C1468 on chromosome 3 and between C1677B and XNpb386 on chromosome 6, explaining 19.2 and 17.7% of the total variation, respectively. In addition, it was noted that only single common QTL (qFCM-3 and qSCM-3) for FCM and SCM was located on the same genomic region due to commonly linked to C1468, clearly indicating that a majority of QTLs for LCC are different between flag-leaf and the second-leaf at maturity. More interestingly, all Asominori alleles at six QTLs detected contributed to the increase of LCC values. Four QTLs (qRFC-2, qRFC-3, qRFC-4 and qRFC-8) for the reduction-speed of flag-leaf LCC (RFC) and three QTLs (qRSC-2, qRSC-6 and qRSC-11) for the reduction-speed of the second-leaf LCC (RSC) were detected on chromosome 2 (two QTLs), 3, 4, 6, 8, 11, respectively, with 11.3-27.8% of total phenotypic variation explained by individual QTL (Table 3, Fig. 2). Among them, the qRFC-8 with the largest effect (LOD = 5.70) to RFC, accounting for 27.8% of the total variation, was located in the interval of markers between C621C and R727 and the qRSC-6 with the largest effect to RSC, accounting for 17.3% of the total variation, was located between C1677B and XNpb386. Moreover, all four delaying flag-leaf senescence alleles come from Asominori. However, the qRSC-6 allele from Asominori and the alleles at both qRSC-3 and qRSC-11 from IR24 delayed the second-leaf senescence. More interestingly, the existence of non-common QTL between RFC and RSC showed the different genetic mechanisms of leaf senescence between flag- and the second leaf in rice.

DISCUSSION

This study reported results of QTL mapping for six leaf-senescence associated traits of the top two leaves during maturation were explored by employing the recombinant inbred lines derived from *japonica* Asominori and *indica* IR24 with 289 RFLP markers. As results, we detected a total 18 QTLs for six leaf-senescence associated traits, which were located on chromosome 2 (four QTLs), 3(four QTLs), 4 (two QTLs), 6 (two QTLs), 8 (two QTLs), 9 (two QTLs), 11, 12, respectively (Fig. 2). Among all eleven LCC QTLs detected in this study, with except of the single common QTL (qFCM-3 and qSCM-3), there was no the same genomic region for LCC, which clearly indicated that most LCC QTLs varied with both leaf order and growth stage of rice. In addition, the no common QTLs for the reduction-speed of LCC (Table 3, Fig. 2) in this study showed the different genetic basis of leaf senescence between flag- and the second leaf in rice. These results strongly supported the previous results of different QTLs for chlorophyll a and b content (Shen *et al*., 2007) and the different activities of SOD (superoxide dismuase), POD (peroxidase) and peptidase (Li *et al*., 2005) at different order leaves.

In addition, the trait correlations could be contributed by either gene linkage or pleiotropy (Xu, 1997). In this study, five common genomic regions (Fig. 2) conferring at least two leaf-senescence associated traits, tightly linked to XNpb227 (Chr. 2), C1468 (Chr. 3), XNpb271 (Chr. 4), XNpb386 (Chr. 6), C621C (Chr. 8), respectively and the existence of at least two QTLs for leaf-senescence associated traits on each chromosome except chromosome 11 and 12 (Fig. 2), were observed, which, in part, could explain significant relationships among certain traits studied. For example, the genetic basis of high correlations ($r = -0.96$, $p<0.01$) can largely be explained by the co-localization of all four QTLs for FCM with corresponding QTLs for RFC. However, both qSCH-3 and qSCM-3 overlapped the same genomic region on chromosome 3, but there seems no significant association each other. Likewise, that there existed no common /linked QTLs between FCH and SCH could not explain its high correlations ($r = 0.86$, $p<0.01$). The similar results were observed in the previous studies of Ishimaru *et al*. (2001), who reported that the QTLs for yield were located near those QTLs for LCC but without significant correlation between them.

In comparing the genomic regions of the QTLs/genes for LCC and its related traits in previous studies (Ishimaru *et al*., 2001; Jiang *et al*., 2004; Abdelkhalik *et al*., 2005; Cha *et al*., 2002) with eighteen QTLs detected in our

studies, the four QTLs (qFCM-4, qRFC-4, qFCM-8 and qRFC-8) might be allelic/tightly linked to those QTLs for decreased chlorophyll content on chromosome 4 and 8 (Ishimaru *et al*., 2001), respectively. The qSCH-9 for the second leaf LCC might simultaneously be allelic to the single recessive gene sgr(t) for a stay green mutant (Cha *et al*., 2002) and the QTL for LCC at flowering in rice (Abdelkhalik *et al*., 2005). In addition, the qSCH-2 for the second leaf LCC at heading might be also related to the QTL for the retention degree of greenness of the second-leaf (Jiang *et al*., 2004). The remaining twelve QTLs should be new QTLs for leaf-senescence associated traits in rice. Therefore, it could be concluded that number of QTL/genes for leaf-senescence associated traits varied with rice variety.

In addition, all four delaying flag-leaf senescence QTLs detected in this study come from *japonica* Asominori, not *indica* IR24, which strongly supported the results of all Yoshida (1981), Sheng *et al*. (2004) and Chen *et al*. (1995), who reported the *japonica* subspecies maintains its flag leaf activity for a longer period than the *indica* subspecies during maturation. However, among all three delaying the second-leaf senescence QTLs (Table 3) detected in this study, two QTLs (qRSC-2 and qRSC-11) came from IR24, whereas qRSC-6 from IR24. From these results, it can be concluded that the genetic basis of leaf senescence is complicated and varies with leaf order, which will bring about difficult to breed rice varieties with late senescence in all leaves.

In summary, a total of 18 QTLs for six leaf senescence traits were identified in this paper. In particular, all four Asominori alleles (qRFC-2, qRFC-3, qRFC-4 and qRFC-8) were found to contribute to delaying the flag-leaf senescence, two IR24 alleles (qRSC-2 and qRSC-11) and the Asominori allele (qRSC-6) contributed to late the second-leaf senescence in rice. In addition, further research should be undertaken to determine whether these QTLs for late leaf senescence have a significant effect on the yield of rice. Undoubtedly, the results and the tightly linked molecular markers that flank the QTLs detected in this paper may be useful for breeding of late leaf senescence in rice.

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