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ORIGINAL PAPER

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QTL-based analysis of leaf senescence in an *indica/japonica* hybrid in rice (*Oryza sativa* L.)

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Abstract In order to identify quantitative trait loci (QTLs) for leaf senescence and related traits in rice (*Oryza sativa* L.), we developed two backcross populations, *indica*/*japonica*// *japonica* and *indica*/*japonica*// *indica*, using IR36 as the *indica* parent and Nekken-2 as the *japonica* parent. The QTLs were mapped using a set of simple sequence-repeat markers (SSRs) in the BC_1F_1 population. Senescence was characterized in these plants by measuring the leaf chlorophyll content 25 days after flowering (DAF), the reduction in chlorophyll content (the difference between the chlorophyll content at flowering and at 25 DAF), and the number of late-discoloring leaves per panicle at 25 DAF in five plants from each BC_1F_2 line. These plants were moved into a temperature-controlled growth cabinet at the time of flowering and allowed to mature under identical conditions. Eleven QTLs were detected in the two populations. The major of QTLs for senescence were found on the short arm of chromosome 6 and on the long arm of chromosome 9. Of these, one QTL on chromosome 6 and two on chromosome 9 were verified by confirming the effects of the genotypes on the phenotypes of the BC_1F_3 lines. The *japonica* parent was found to contribute to late senescence at all but one QTL. Based on a comparison of the effects of heterozygotes and homozygotes on the phenotypic values of each QTL genotype, we concluded that the differential senescence observed in the *indica-japonica* hybrid was not due to over-dominance; rather, it was the result of partial-dominance genes that were donated from either of the parents.

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Introduction

Leaf senescence is an essential developmental phase that becomes apparent through visible changes in the color of the leaves of grain plants, which turn from green to yellow as the crop ripens before harvest (Buchanan-Wollaston et al. 2003). Chlorophyll degradation is the major biochemical marker for the identifying senescence in plants as it is highly correlated with leaf aging. This process is also evaluated by the visual scoring of senescence in rice leaves (Toojinda et al. 2003; Jiang et al. 2004) and by measuring the chlorophyll content using a chlorophyll meter (Peng et al. 1995; Toojinda et al. 2003).

Late leaf senescence plays an important role in increased photosynthetic activity in plants. Mae (1997) reported that 60–90% of the total carbon found in rice panicles at the time of harvest is produced by photosynthesis after heading, while at least 80% of the nitrogen is absorbed before heading and subsequently translocated from the vegetative organs. Nitrogen recycling is considered to be a key factor in determining the rate of senescence and, therefore, the productivity of rice plants (Yamaya et al. 2002).

Differences in leaf senescence have been identified between the rice subspecies *indica* and *japonica*, with the former showing early senescence and the latter, late senescence (Yoshida 1981). However, despite the importance of leaf senescence in rice, genetic analyses have been hindered by the difficulty of measuring this trait. A few studies have recently reported on quantitative trait loci (QTLs) for leaf senescence in rice. Cha et al. (2002) mapped a QTL for leaf senescence on the long arm of chromosome 9 using a stay-green mutant. In an extensive study of submergence tolerance in rice, Toojinda et al. (2003) identified a set of major QTLs for plant survival, plant height, simulated shoot elongation, visually measured tolerance and leaf senescence on the short arm of chromosome 9. Jiang et al. (2004) conducted an extensive analysis of QTLs for delayed leaf

senescence using a double haploid population from an *indica-japonica* cross and detected a total of 46 QTLs in 25 chromosomal regions. As the contribution of each of the QTLs was relatively small in their analysis, the authors concluded that a large part of the senescence could be attributed to environmental or digenic interactions. Ishimaru et al. (2001) mapped QTLs for 23 physiological and agronomic characters, including 13 newly measured traits, such as ribulose bisphosphate carboxylase oxygenase (Rubisco) activity, chlorophyll content, reduction in chlorophyll content, and ratooning ability.

The purpose of the present study was to confirm major QTLs for leaf senescence and to compare the effects of heterozygotes and homozygotes at each locus in an *indica-japonica* rice hybrid. Two linkage maps based on the BC_1F_1 for *indica*/*japonica*//*japonica* and *indica*//*japonica*// *indica* were constructed using simple sequence repeat (SSR) markers. Senescence-related traits were measured in the BC_1F_2 lines of the two populations in a growth cabinet in order to maintain uniform temperature conditions at grain-filling stages for the early-flowering and late-flowering plants. To confirm the effects of the QTLs for chlorophyll content, we tested the contrasting genotypes at each QTL for their phenotypic effects in the BC_1F_3 lines under field conditions.

Materials and methods

Plant materials and test conditions

An IR36 variety showing early senescence was crossed with a *japonica* line, Nekken-2 (NK2) with late senescence and a 'wide compatibility gene' that prevents hybrid sterility in the resultant lines (Yanagihara et al. 1995). In conventional *indica-japonica* hybrids, the partial sterility of the panicles might impair the translocation of carbohydrates and nitrogen from the leaves to the panicles, thus resulting in late senescence. Two sets of BC_1F_1 populations, IR36/NK2//IR36 and IR36/NK2//NK2, were mapped using SSR markers. Five plants from each of the BC_1F_2 lines were initially grown outdoors in separate pots (one plant per pot) at the College of Bioresource Sciences, Nihon University, Japan. These plants were moved into a temperature-controlled growth cabinet immediately after flowering in order to evaluate leaf senescence. Because of the limited space in the growth cabinets, the IR36/NK2//IR36 population was evaluated for senescence in 2001, and the IR36/NK2//NK2 population was tested in 2003; 105 and 135 lines, respectively, were available for QTL analysis. In addition, the effects of the genotypes of three QTLs on phenotypic values were measured under field conditions in 2004 using 100–105 lines per individual QTL.

In order to avoid the effects of unequal fertilization, which might bias the results, an equal amount of fertilizer, namely 52.5 kg N, 52.5 kg P_2O_5 and 52.5 kg K_2O/ha, was applied at the time of transplanting the plants into the pots. As the two populations were grown in different years (as described above), both were transplanted at a fixed time—June 8, 2001 and 2003—in order to avoid any significant bias from the difference in day length. The daily averages of humidity, temperature, and solar radiation from June to October in 2001 and 2003 were measured using the meteorological observation system (Bisara, Tokyo, Japan). To ensure a uniform temperature at the grain-filling stage, we moved all of the plants into a controlled environmental chamber at the emergence of the third panicle and left them there for 25 days under a 28/21°C day/night cycle. The humidity inside the cabinet varied between 75% and 80%.

Measurements of traits

All of the traits evaluated in this experiment were measured for five plants per line in both populations. Plant height at the vegetative stage and the number of tillers were measured at the maximum-tiller-stage, which was approximately 3 weeks before the earliest flowering. The chlorophyll content of the second leaf from the top was measured using a chlorophyll meter (SPAD-502, Minolta Camera Company, Japan). The meter readings are presented using the soil-plant analysis development (SPAD) values defined by the manufacturer, which indicate the relative chlorophyll contents. At 25 days after flowering (DAF), the plants were moved out of the growth cabinet. Chlorophyll content, the number of panicles, and the number of late-discoloring leaves were measured. If three-quarters of the leaf area remained green, the leaf was classified as a late-discoloring leaf. The difference between the chlorophyll content at flowering and at 25 DAF was calculated as an indicator of the reduction in chlorophyll content; the greater the rate of reduction, the earlier the senescence, and vice versa. The number of late-discoloring leaves per panicle at 25 DAF was used as one of the indicators of senescence.

Confirmation of the effect of QTL in the backcross lines

The effects of the genotypes of the QTLs on chlorophyll content 25 DAF and on the reduction in the chlorophyll content was tested in the BC_1F_3 lines. Five seeds from each BC_1F_2 line were used to generate BC_1F_3 hill plots for the two-backcross populations, which were grown under field conditions in 2004. The chlorophyll content for the second leaf was measured at flowering and at 25 DAF for two plants from each BC_1F_3 hill plot.

SSR linkage map construction

DNA was extracted from the leaf tissues of 143 BC_1F_1 plants of each of the two-backcross populations using

the CTAB method (Murray and Thompson 1980). Two hundred and twenty SSR markers covering the 12 chromosomes were selected according to the description by Temnykh et al. (2000) and McCouch et al. (2002). Amplification was performed using the GenAmp PCR System 9700 (Applied Biosystems, Foster City, Calif.). The amplified products were separated by electrophoresis through 4% polyacrylamide denaturing gels with Sequi-Gene GT (BioRad, Hercules, Calif.) and were silver-stained according to the method developed by Bassam et al. (1991).

Statistical analysis

The genetic maps of the polymorphic SSR markers were constructed using the computer program, MAPL 97 (Ukai et al. 1991). Interval mapping was performed. The significant threshold at 1% was calculated on the basis of the 1,000 permutations of the data. The logarithmic odds (LOD) score of 2.5 was found to be the significant threshold for most of the detected multiple QTLs. A LOD score of 2.7 was found to be suitable for CCFJ-9. The percentage of phenotypic variation associated with an individual QTL was calculated by simple interval mapping, while the multiple contributions were calculated by multiple regression analysis. All of the procedures were carried out using QGENE VER. 3.07 (Nelson 1997). The QTLs were designated following the system reported by McCouch et al. (1997) with minor modifications. The first three letters were selected from the character name and the fourth letter was either "I" for the *indica* backcross IR36/NK2//IR36 or "J" for the *japonica* backcross IR36/NK2//NK2; for example, qLDPJ-6 represents the QTL for the number of late-discoloring leaves per panicle (LDP) on chromosome 6 in the IR36/NK2//NK2 backcross population.

Results

Meteorological data in 2001 and 2003

The monthly averages of humidity, temperature and solar radiation from June to October in 2001 and 2003 are shown in Table 1. The *t*-test was performed to evaluate the differences in the daily average of all three parameters. The data shows that there were highly significant differences ($P \leq 0.01$) in these three weather parameters of July 2001 and 2003.

Phenotypic variation of the traits

The mean and range of each trait for the parent plants and both hybrid populations are shown in Table 2. The difference in values for the parental lines in both years was tested using the *t*-test, which revealed that the only trait showing a significant variation between years (5% level) was the number of late-discoloring leaves per plant at 25 DAF .

Linkage maps of the two-backcross populations

In IR36/NK2//IR36, 105 SSR markers were mapped on 12 chromosomes, with a total length of 1,836 cM. In IR36/NK2//NK2, 117 SSR markers were mapped with a total of length of 1,740 cM. SSR mapping for each population together with the interval distance between the SSR markers for each linkage group is shown in Table 3. A common set of SSR markers was applied to both populations for a single-locus comparison. However, the mapping distance of the markers differed in the two populations, which might have been partly due to sampling errors. In addition, a few of the markers were mapped only in one population and not in the other as their segregation deviated from 1:1 ratio. Finally, some SSR markers were used only at one locus, which magnified the LOD score for specific QTLs.

Chlorophyll content

Three QTLs (qCCFJ-4, qCCFJ-9-1 and qCCFJ-9-2) for chlorophyll content at flowering were identified on chromosomes 4 and 9 in the IR36/NK2//NK2 cross population (Fig.1). The effects of these QTLs on increasing the chlorophyll content were contributed by the NK2 allele of the *japonica* parent . The heterozyg-

Table 1 The meteorological data collected during the experiments in 2001 and 2003

Parameter	June		July		August		September		October	
	2001	2003	2001	2003	2001	2003	2001	2003	2001	2003
Humidity (%)	78.5 ns	78.7	73.6**	82.3	77.9 ns	80.9	76.2 ns	72.9	72.0 ns	68.7
Temperature (°C)	22.2 ns	22.4	27.1**	22.6	25.9 ns	25.4	22.4 ns	23.6	17.8 ns	17.0
Solar radiation (MJ/m^2)	9.0 ns	9.0	15.1**	7.5	8.5 ns	9.2	6.9 ns	8.4	5.0 ns	4.5

*Significant at 1% level; ns, not significant

Table 2 Traits related to senescence of parents and the two-backcross populations in 2001 and 2003

Trait	Year	Parents		Population			
		IR36	NK2	IR36/NK2//IR36 in 2001		IR36/NK2//NK2 in 2003	
				Range	Mean	Range	Mean
Chlorophyll content at flowering (SPAD) ns	2001	39.4	43.5	27.2–43.1	35.6		
	2003	39.8	43.8			36.9–52.8	44.3
Number of late-discoloring leaves per plant at 25 DAF*	2001	25.8	32.0	3.0–59.5	29.8		
	2003	18.3	25.0			10.6–45.6	24.3
Chlorophyll content at 25 DAF (SPAD) ns	2001	25.4	31.6	5.4–36.5	22.0		
	2003	24.0	32.2			13.4–43.9	32.2
Reduction in chlorophyll content (SPAD) ns	2001	14.0	11.9	4.3–29.2	10.9		
	2003	15.8	11.6			1.3–32.1	12.0
Number of late-discoloring leaves per panicle at 25 DAF ns	2001	2.0	2.1	0.4–4.2	2.4		
	2003	1.4	1.6			0.8–3.6	1.8
Number of panicles per plant ns	2001	12.5	15.3	2.4–24.0	12.5		
	2003	13.0	15.6			8.6–28.0	12.9

*Significant at the 5% level; ns, not significant

otes showed low levels of chlorophyll similar to the those of the *indica*//*indica* homozygotes (Table 4). The multiple phenotypic variances explained by these three QTLs was 14% (Table 4).

One QTL (qCCAI-9) for chlorophyll content at 25 DAF was detected on the long arm of chromosome 9 in IR36/NK2//IR36 (Fig.2). The effect of this QTL on increasing the chlorophyll content at 25 DAF was contributed by the IR36 allele (Table 5). Likewise, in IR36/NK2//NK2, one QTL (qCCAJ-9) for chlorophyll content at 25 DAF was detected in a region on chromosome 9 similar to that on IR36/NK2//IR36 (Fig.1). The NK2 allele contributed to the effect of this QTL on increasing the chlorophyll content (Table 5). Homozygotes of both parental alleles at these two closely located QTLs contributed to the effect of increasing the

Table 3 SSR mapping in the two-backcross populations

Chromosome number	Backcross population							
	IR36/NK2//IR36				IR36/NK2//NK2			
	SLG[a]	MSM[b]	CA[c] (cM)	SSR ID[d]	SLG	MSM	CA (cM)	SSR ID
1	SLG-1	8	184.4	26.3	SLG-1	11	191.5	19.2
2	−1	5	127.7	32.0	−1	8	146.3	21.0
	−2	10	156.3	17.4	−2	5	57.1	14.3
3	−1	3	35.1	17.6	−1	6	99.5	20.0
	−2	4	31.6	10.6				
4	−1	7	116.2	19.4	−1	10	156.4	17.4
5	−1	7	132.4	22.0	−1	9	92.8	11.6
	−2	3	28.5	14.3	−2	3	22.3	11.2
6	−1	10	232.4	25.8	−1	12	223.1	20.3
	−2	2	38.4	38.4	−2	2	44.5	44.5
7	−1	6	131.5	26.3	−1	6	97.2	19.4
8	−1	5	101.7	25.4	−1	9	160.0	20.0
	−2	2	35.6	35.6				
9	−1	12	198.2	18.0	−1	13	186.7	15.6
10	−1	5	58.1	14.5	−1	3	23.8	12.0
	−2	2	31.0	31.0	−2	2	10.2	10.2
11	−1	5	71.7	18.0	−1	8	98.5	14.0
	−2	2	23.2	23.2				
12	−1	5	66.0	16.5	−1	10	129.6	14.4
	−2	2	35.7	35.7				
Total		105	1836			117	1740	

[a]Number of sub-linkage group (SLG) per chromosome
[b]The number of mapped SSR markers (MSM)
[c]Coverage area (CA)
[d]Interval distance (ID) between two SSR markers

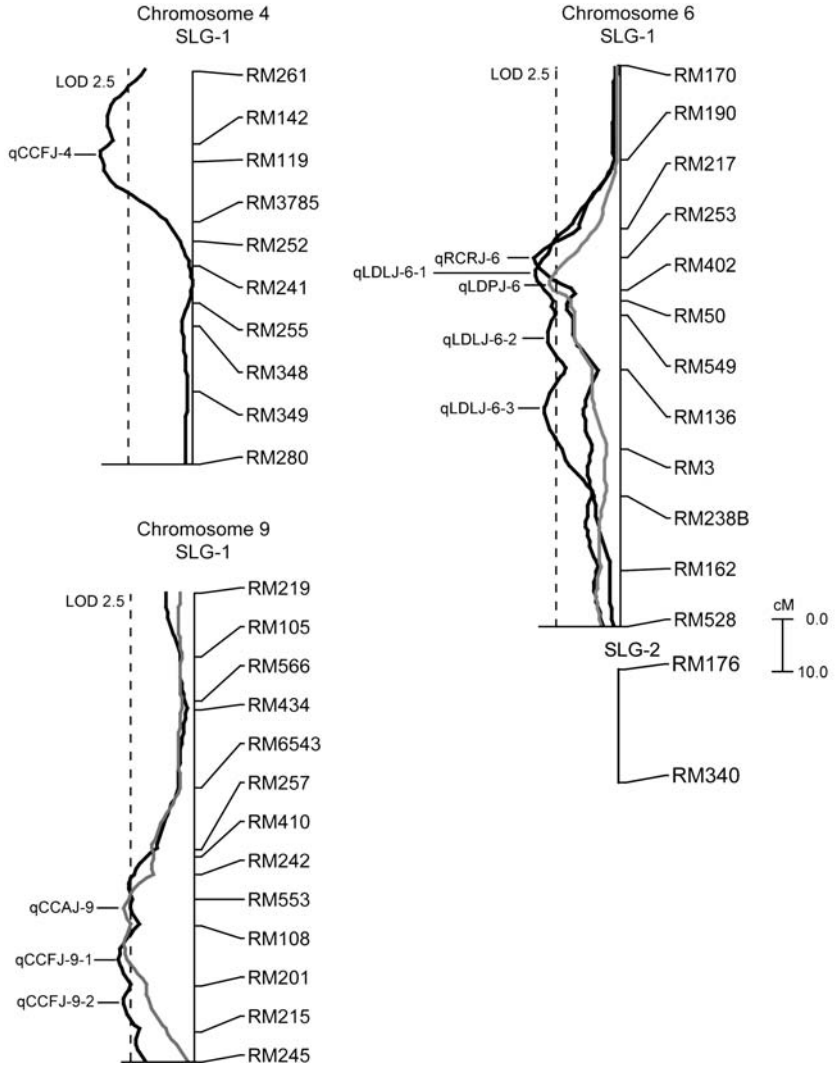

Fig. 1 QTL positions in the backcross population IR36/NK2//NK2. Only chromosomes containing QTLs are shown. The names of the QTLs are indicated to the *left* of the chromosome. A LOD *dotted line* of 2.5 is shown in the first QTL curve. *CCFJ*, chlorophyll content at flowering; *LDLJ*, number of late-discoloring leaves per plant at 25 DAF; *LDPJ*, number of late-discoloring leaves per panicle at 25 DAF; *RCRJ*, reduction in chlorophyll content; *CCAJ*, chlorophyll content at 25 DAF

chlorophyll content. The percentage of phenotypic variance explained by these QTLs was 13.7% and 8.7%, respectively (Table 5).

One QTL (qRCRJ-6) for reduced chlorophyll content was found on the short arm of chromosome 6 in the IR36/NK2//NK2 population (Fig.1). The effect of this

Table 4 QTLs for chlorophyll content at flowering detected in both populations

Traits	QTL	Chromosome	Marker interval	Variance explained (%)	IR36/NK2//IR36I/J//I		IR36/NK2//NK2I/J//J		Comparison of genotypes[b]
					LOD[a]	Genotype[b]	LOD	Genotype[b]	
Chlorophyll content at flowering	qCCFJ-4	4	RM119-RM142	11.0	ND	IJ≈II	3.65	IJ < JJ	II≈IJ < JJ
	qCCFJ-9-1	9	RM201-RM108	9.0	ND	IJ≈II	2.91	IJ < JJ	II≈IJ < JJ
	qCCFJ-9-2	9	RM215-RM201	8.4	ND	IJ≈II	2.70	IJ < JJ	II≈IJ < JJ
Multiple contribution				14.0					

[a] ND, No QTL was detected
[b] I, IR36; J, NK2

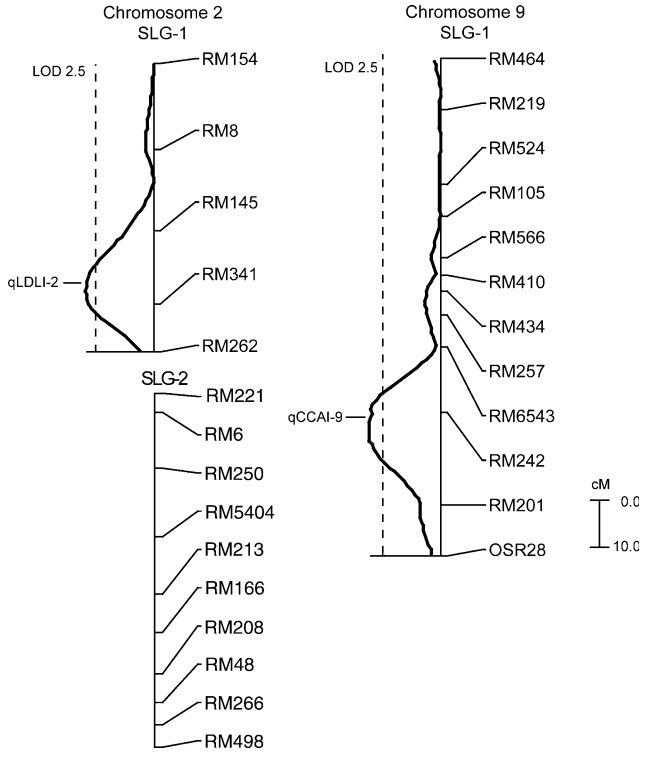

Fig. 2 QTL positions in the backcrossed population IR36/NK2//IR36. Only chromosomes containing QTLs are shown. The names of the QTLs are indicated to the *left* of the chromosome. A LOD *dotted line* of 2.5 is shown in the first QTL curve. *CCAI*, chlorophyll content at 25 DAF; *LDLI*, number of late-discoloring leaves per plant at 25 DAF

QTL on chlorophyll reduction at 25 DAF was contributed by the IR36 allele (Table 5).

Number of late-discoloring leaves per panicle and per plant at 25 DAF

In the IR36/NK2//NK2 population, one QTL (qLDPJ-6) for number of late-discoloring leaves per panicle at 25 DAF was found on the short arm of chromosome 6 (Fig.1). The effect of qLDPJ-6 was contributed by the NK2 allele (Table 5). This is consistent with the general view that the *japonica* subspecies maintains its leaf activity for a longer period during maturation than does the *indica* subspecies. Three QTLs (qLDLJ-6-1, qLDLJ-6-2 and qLDLJ-6-3) for the number of late-discoloring leaves per plant at 25 DAF were detected in the region ranging from the short to the long arm of chromosome 6 in the IR36/NK2//NK2 population (Fig.1). The effect of these QTLs was contributed by the NK2 alleles (Table 5). The multiple variances explained by these QTLs was 10%. Another QTL for the number of late-discoloring leaves per plant at 25 DAF (qLDLI-2) was found on the long arm of chromosome 2 in IR36/NK2//IR36, and 10.3% of the total variance was explained by the qLDLI-2 (Fig. 2, Table 5). The *japonica* parent contributed to the number of late-discoloring leaves (Table 5).

Table 5 QTLs detected in both populations at 25 DAF

Trait	QTL	Chromosome	Marker interval	Variance explained (%)	IR36/NK2//IR36I/J//I		IR36/NK2//NK2I/J//J		Comparison of genotypes[a]
					LOD	Genotype[a]	LOD	Genotype	
Chlorophyll content at 25 DAF	qCCAI-9	9	RM242-RM6543	13.7	3.17	IJ < II	–[c]	–	IJ < II
	qCCAJ-9	9	RM108-RM242	8.7	–[c]	–	2.70	IJ < JJ	IJ < JJ
Reduction in chlorophyll content	qRCRJ-6	6	RM253-RM217	9.5	ND[d]	IJ≈II	3.14	IJ > JJ	II≈IJ > JJ
Number of late-discoloring leaves per panicle at 25 DAF	qLDPJ-6	6	RM402-RM253	8.4	ND	IJ≈II	2.70	IJ < JJ	II≈IJ < JJ
Number of late-discoloring leaves per plant at 25 DAF	qLDLJ-6-1	6	RM402-RM253	10.0	ND	II≈II	3.26	IJ < JJ	II≈IJ < JJ
	qLDLJ-6-2	6	RM136-RM549	8.0	ND	IJ≈II	2.70	IJ < JJ	II≈IJ < JJ
	qLDLJ-6-3	6	RM3-RM136	8.5	ND	IJ≈II	2.90	IJ < JJ	II≈IJ < JJ
Multiple contribution for LDL				10.0					
	qLDLI-2[b]	2	RM341-RM145	9.5	2.97	II < IJ	ND	IJ≈JJ	II < IJ≈JJ

[a] I, IR36; J, NK2
[b] Except for the variance qLDLI-2 obtained in the other population
[c] The corresponding QTL is in a similar position
[d] ND, No QTL was detected

The trait of number of late-discoloring leaves at 25 DAF per panicle was introduced to overcome the difference in the number of late-discoloring leaves per plant in the parental lines that was observed between 2001 and 2003. To determine the effect of the number of panicles on the QTLs for the number of late-discoloring leaves both per panicle and per plant, we searched for QTLs for the number of panicles itself. No QTL was detected in either of the two backcross populations. This result suggests that the number of late-discoloring leaves per panicle and the number of panicles are genetically independent traits.

Confirmation of the effects of three QTLs for chlorophyll content at 25 DAF and the reduction in chlorophyll content

Although the two backcross populations were tested in different years (2001 and 2003), no QTL was detected at which the heterozygotes (*Aa*) showed a higher value than the homozygotes (*AA* and *aa*) simultaneously in both populations. This suggests that the two populations showed consistent phenotypic values following a linear order of genetic effect: $AA > Aa > aa$ or $AA < Aa < aa$. Only at qCCAJ-9 and qCCAI-9, which were located near to one another on the long arm of chromosome 9, did the homozygotes show a higher level of chlorophyll content at 25 DAF than the heterozygotes in both populations. As a result, the effect of the genotypes at these QTLs on the actual phenotypic values were further examined in the BC_1F_3lines.

An initial comparison of the BC_1F_2 lines revealed that the NK2 homozygotes and IR36 homozygotes showed higher chlorophyll contents at 25 DAF than the heterozygote genotypes at qCCAJ-9 and qCCAI-9 (Fig.3, upper two histograms). The genotypic effects were then tested in the BC_1F_3 lines (Fig.3, lower two histograms). The NK2 homozygotes and IR36 homozygotes showed significantly higher phenotypic values than the heterozygotes ($P \leq 0.05$), suggesting that these two QTLs are different despite their close proximity.

A similar analysis was performed to verify the QTL for the reduction in chlorophyll content, which was located on the short arm of chromosome 6 (Fig.4, upper two histograms). The IR36 homozygotes and NK2 homozygotes were selected from the BC_1F_2 of the IR36/NK2//IR36 and IR36/NK2//NK2 populations, respectively, and the effects of their genotypes on the phenotypic values were tested in the BC_1F_3 lines. The results confirmed that the IR36 homozygotes showed a significantly greater reduction in chlorophyll content than the NK2 homozygotes ($P \leq 0.001$, Fig.4, lower histogram).

Discussion

One of the most divergent traits in cultivated rice is senescence, with early and late senescence being exhibited by the *indica* and *japonica* subspecies, respectively (Yoshida 1981). Senescence is thought to affect the photosynthetic activity of leaves during maturation and to contribute to yield performance (Yoshida 1981). However, measuring this trait has proved to be difficult, particularly in segregating populations where differences in the flowering time of individual plants expose the plants to different conditions during maturation. In the present study, plants derived from segregating populations were transplanted singly into pots and transferred to a temperature-controlled growth cabinet during the period from flowering to maturity. The 'wide compatibility gene' (Yanagihara et al. 1995) carried by NK2 (*japonica*) overcomes the hybrid sterility that usually occurs in *indica*/*japonica* crosses and which affects both the grain-filling process and senescence.

We have analyzed here QTLs for senescence-related traits in two-backcross populations, *indica*/*japonica*// *japonica* and *indica*/ *japonica*// *indica*, by comparing the effects of homozygotes and heterozygotes at each locus. Leaves were sampled for DNA extraction and mapping in the BC_1F_1 generation, and the targeted traits were measured in the BC_1F_2 lines. This method was adopted in order to partially solve the problems associated with using an F_2 population for QTL analysis, in which a portion of plants are mutilated by cutting off leaves for DNA extraction and the homozygote sample size is often inadequate compared with that of the heterozygotes for the precise measurement of allelic effects. In addition, data sets obtained on a single-plant basis in an F_2 population might be subject to wider environmental biases. For these reasons, double haploid or recombinant-inbred lines are often used in QTL analyses. One drawback to the design in our study was the limited size of the growth cabinets. Due to space restrictions, the backcross populations were tested separately in two different years. Furthermore, only five plants could be measured per BC_1F_2 line. The genotypes that were determined to be heterozygous in the BC_1F_1 might therefore contain three genotypes in the BC_1F_2 lines with the predicted frequencies of $1/4\ AA + 1/2\ Aa + 1/4\ aa$. Despite these problems, noteworthy QTLs were detected by comparing two marker genotypes (*AA* and *Aa*, or *Aa* and *aa*) in each of the backcross populations, although each *Aa* group contained *aa* or *AA* in a minor ratio in the BC_1F_2 line. No QTL was identified at which the heterozygotes (*Aa*) showed a higher value than the homozygotes (*AA* and *aa*) simultaneously in the two-paired populations. This result indicates that the two populations provided consistent phenotypic values in the measured traits following a linear order of genetic effect; $AA > Aa > aa$ or $AA < Aa < aa$. These findings also suggest that over-dominance was not present.

The homozygotes showed a higher chlorophyll content than the heterozygotes at qCCAJ-9 followed by qCCAI-9, which were located close to one another on the long arm of chromosome 9 in the two populations. The effect of the genotypes at these QTLs on the actual

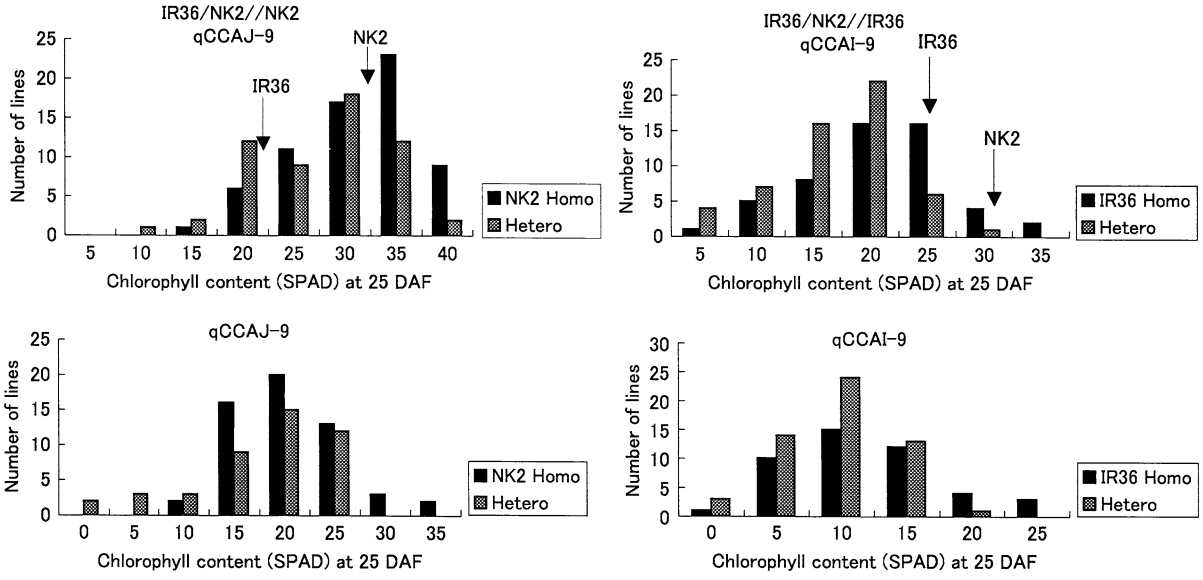

Fig. 3 Frequency distribution of the effects of qCCAJ-9 and qCCAI-9 on chromosome 9 with RM242 in both populations (*upper histogram*). The homozygous genotype (*dark bars*) is of higher value than the heterozygous genotype (*hatched bars*). The *lower histogram* shows the phenotypic effects of both QTLs in the BC_1F_3 lines. The homozygous genotypes are of higher value than the heterozygous genotypes for both IR36 and NK2

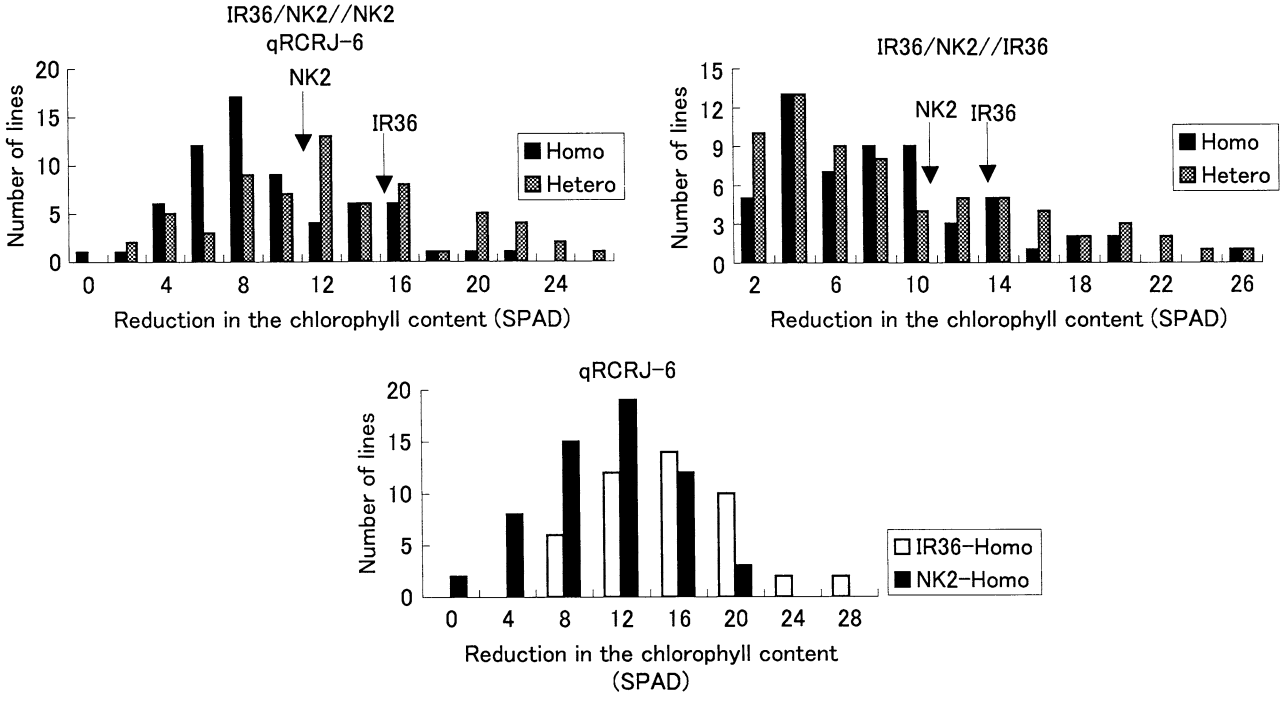

Fig. 4 Frequency distribution of the effects of qRCRJ-6 on chromosome 6 with RM253 in the IR36/NK2//NK2 population (*right-hand histogram*). The heterozygous genotype (*hatched bars*) is of higher value than the homozygous genotype (*dark bars*). The histogram on the *left-hand side* shows the frequency distribution for the IR36/NK2//IR36 population, in which no QTL was detected at this locus. The *lower histogram* shows the phenotypic effects of qRCRJ-6 in the BC_1F_3 lines. A comparison of the effects of the homozygous genotype on the phenotypes for both populations show that IR36-homozygous is superior to NK2-homozygous

phenotypic values was therefore tested in the BC_1F_3 lines, and the results confirmed the higher values contributed by the homozygotes, although the comparison might have been biased to some extent by the genotypes determined in the BC_1F_1. However, in view of the linear genetic effect discussed above, it is possible that these two loci for increased chlorophyll content at 25 DAF are independent of one another.

Some recent reports have investigated QTLs for senescence in rice (Ishimaru et al. 2001; Cha et al. 2002; Toojinda et al. 2003; Jiang et al. 2004). In particular, Toojinda et al. (2003) identified a set of major QTLs, including one for leaf senescence on the short arm of chromosome 9. QTLs for senescence have also been reported on the long arm of this chromosome by the other authors.

In the present study, three QTLs for chlorophyll content at flowering were discovered in the IR36/NK2//NK2 population: one was located on the long arm of chromosome 4, and the others were located in two regions of the long arm of chromosome 9. One QTL for chlorophyll content at 25 DAF was detected on the long arm of chromosome 9 in the IR36/NK2//NK2 population, and a second QTL for the same trait was found at a similar location in the IR36/NK2//IR36 population. One QTL for the reduction in chlorophyll content and another for the number of late-discoloring leaves per panicle were detected on the short arm of chromosome 6, on which three additional QTLs for the number of late-discoloring leaves were located at intervals; two on the short arm and one on the long arm.

A QTL for chlorophyll content at flowering that was detected in the RM142 region of chromosome 4 might correspond to one of the QTLs for the retention of greenness and green area of the flag leaf reported by Jiang et al. (2004). No corresponding QTLs were detected by Ishimaru et al. (2001).

The QTL for the number of late-discoloring leaves per panicle that we identified on the short arm of chromosome 6 along with one of the three QTLs for the number of late-discoloring leaves that was also detected on this arm might correspond to the QTL for the reduction in chlorophyll content identified in a similar region and verified using the BC_1F_3 lines (Fig.4). These QTLs could also be equivalent to one of the two QTLs for retention of the green area of the flag leaf and the second leaf identified by Jiang et al. (2004) in a region above RM136 on chromosome 6. Furthermore, one of the QTLs for the number of late-discoloring leaves identified in our study might be related to one of the QTLs for retention of the green area of the flag leaf and the second leaf that was observed by Jiang et al. (2004) near RM3 on the short arm of chromosome 6. No QTLs reported by Ishimaru et al. (2001) appear to correspond to these QTLs on chromosome 6.

The QTL for chlorophyll content at flowering (qCCFJ-9-1) and the two QTLs for chlorophyll content at 25 DAF (qCCAJ-9 and qCCAI-9) identified in our study were located near RM242 on the long arm of chromosome 9. These QTLs appear to correspond to a QTL for the retention of greenness of the flag leaf that was reported by Jiang et al. (2004) near RM257 on chromosome 9. A QTL for decreased chlorophyll content was also detected on the long arm of this chromosome by Ishimaru et al. (2001) and Cha et al. (2002).

In summary, we identified significant QTLs on the short arm of chromosome 6 and the long arm of chromosome 9 that were individually verified in the BC_1F_3 lines (Figs.3, 4). The NK2 allele of the *japonica* parent contributed to late senescence at all of the QTLs for senescence-related traits with the exception of one QTL on chromosome 9 in terms of increasing the chlorophyll content and the number of late-discoloring leaves per panicle. The IR36 allele of the *indica* parent was found to contribute to late senescence only at qCCAI-9 on chromosome 9. Further research should be undertaken to determine whether these QTLs for late senescence have a significant effect on the yield of rice crops.

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