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QTLs Identification and Confirmation of Field Resistance to Leaf Blast in Temperate *japonica Rice* (*Oryza sativa* L.)

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Abstract

Field resistance is defined as the resistance that allows effective control of a parasite under natural field condition and is durable when exposed to new races of that parasite. To identify the genes for field resistance to rice blast, quantitative trait loci (QTLs) conferring the resistance for races and blast nursery screening in japonica rice cultivars were detected and mapped using SSR markers. QTL analysis was carried out in 190 RILs population from the cross between Suweon365 (moderately resistant) and Chucheong (highly susceptible). Twelve QTLs against nine blast races inoculated were detected on chromosomes 1, 2, 4, 6, 7, 11 and 12. They explained from 5.1% to 34.9% of total phenotypic variation. Eight QTLs against blast nursery screening in four regions for three years were detected on chromosomes 1, 2, 4, 11 and 12. The phenotypic variation explained by each QTL ranged from 4.3% to 37.7%. Three chromosome segment substitution lines (CSSLs) of BC_2F_6 by backcross method were developed to transfer the QTLs into the susceptible cultivar Chucheong as a recurrent parent. A CSSL4-1 containing two QTLs *qLB6.2* and *qLB7* against blast races showed to the reaction of 6 to 7 at blast nursery in two regions for two years. The CSSL4-2 and CSSL93 containing QTLs, *qLB11.2* and *qLB12.1* of the resistance against leaf blast in blast nursery screening, respectively, had enhanced the resistance for blast nursery screening across two regions and in two years.

Key words: QTL, field resistance, blast, temperate *japonica*, rice

Introduction

Rice blast disease, caused by *Magnaporthe oryzae*, is one of the most serious diseases of rice, an important staple crop for more than half the world's population. The use of disease resistance (*R*) genes is the most efficient and environmental friendly way to control such disease. Rice blast resistance is generally classified into two types; qualitative (complete or major) and quantitative (partial or minor) (Ezuka 1972; Bonman and Mackill 1988; Lee et al. 1989). Over 50 major *R* genes to rice blast have been mapped to date (Salluad et al. 2003; Hayashi 2005; Liu et al. 2005; Chen et al. 2006; Jeung et al. 2007), and about 30 *R* genes have been mapped on different rice chromosomes and tightly linked DNA markers have been developed. Eight *R* genes *Pib*, *Pita*, *Pi9*, *Pid2*, *Pi2*, *Piz-t*, *Pi36* and *Pi37* have recently been isolated (Wang et al. 1999; Bryan et al. 2000; Qu et al. 2006; Chen et al. 2006; Zhou et al. 2006; Lin et al. 2007; Liu et al. 2007), and seven genes *Pib*, *Pita*, *Pi9*, *Pi2*, *Piz-t*, *Pi36* and *Pi37* are of the nucleotide binding site-leucine-rich repeat (NBS-LRR) type, while *Pid-2* encodes a receptor-like kinase. Many rice varieties with complete resistance to *M. oryzae* have been developed in recent years, but in many cases this resistance has been lost within a few years of the initial cultivation of these lines due to the emergence of stronger virulent isolates of the rice blast fungus (Bonman et al. 1986; Yaegashi

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1994; Han et al. 2001).

The partial resistance or quantitative trait locus (QTL) is stable against different pathogenic races of the rice blast fungus; hence, the use of partial resistance is one of the most promising measures for blast control. Recent studies demonstrated that genetic linkage maps constructed with various DNA markers are very useful for the analysis and detection not only of qualitative trait locus but also of quantitative trait loci (QTLs) (Bao et al. 2000; Price et al. 2000; Zenbayashi et al. 2002). Wang et al. (1994) identified ten QTLs affecting partial resistance to blast in tropical *japonica* rice Moroberekan. Four QTLs on chromosome 4, 9 and 12 from upland rice Owarihatamochi were identified and a QTL, *pi21* on chromosome 4 showing large effect on field resistance was cloned by a map-based strategy (Fukuoka and Okuno 2001; Fukuoka et al. 2007). The resistance genes and QTLs related to neck blast were reported by a few researchers (Fujii et al. 2000; Sirithunya et al. 2002; Zhuang et al. 2002; Ramalingam et al. 2003). This study was conducted to identify QTLs and confirm the effect of QTLs to rice blast using an RIL population from the cross between two *japonica* cultivars Suweon365 and Chucheong.

Materials and Methods

Plant materials

Suweon365, a *japonica* rice, was crossed as a female parent with Chucheong in 1992. It was derived from the cross between Seonam and Iri353, a semi-dwarf, high-yielding, blast resistant and medium eating-quality cultivar (NICS, 1988). Chucheong or Akibare in Japanese (developed from a cross between Mandainishiki and an F_5 line from a cross of Wakaba/Kinmase) was introduced from Japan in 1969. It is late flowering, tall, highly susceptible to blast but has good eating-quality (RDA, 1975). The F_1 plants were self-pollinated to produce F_2 seeds, and then the 231 F_{11} lines were developed from the resultant F_2 plants by the single-seed descent method. One hundred ninety RILs of F_{11} were selected to construct genetic linkage map and analyzed the resistance QTLs associated with blast. To verify the effect of Suweon365 alleles in QTLs for blast resistance, two desirable RILs carrying QTL segments were successively backcrossed to the susceptible parent Chucheong with selection using linked DNA markers until BC_2F_6 generations.

DNA extraction and SSR analysis

Genomic DNA was extracted from the fresh young leaves of the 190 RILs using the methods described by Causse et al. (1994). PCR amplification using SSR markers and gel electroporesis was done using the methods described by Temnykh et al. (2000) and McCouch et al. (2002). PCR was performed with the following conditions: 5min at 95°C, followed by 60 sec at 94°C, 60 sec at 55°C, and 120 sec at 72°C for 35 cycles, and additional incubation period of 10 min at 72°C. Amplified PCR products were mixed with a half volume of formamide dye. After denatured at 95°C for 5min, 4μL of the products was separated on a 4% polyacrylamide denatured gel and detected by a silver staining methods as described by Panaud et al. (1996).

Screening for blast races and nursery test

One hundred and ninety RILs and two parents were screened against nine Korean blast isolates KJ-105, KJ-201, KJ-301, KJ-401, KI-197, KI-313, KI-409, KI-1113 and KI-1117. At 21 days after seeding (DAS), the single-race spore suspension was adjusted to a cell count of 20~30 spores per visual field under 100x magnification, and inoculated by spray method. The inoculated seedlings were kept inside the incubation chamber at 26±1 ℃ with saturated humidity for 24 hours and then transferred to the greenhouse until scoring time. The degree of incidence against each race was evaluated at 7 days after inoculation (DAI). Disease reactions for QTL analysis were scored on a scale from 0 to 9 based on diseased leaf area (DLA), lesion shape and size (Ramalingam et al. 2003).

The resistance in the blast nursery screening was tested in four regions in Korea namely; Suwon, Cheolwon, Namyang and Jinbu. The incidence scores ranged from 0 (no lesion) to 9 (necrosis of all leaves and sheaths) using the standard evaluation method of the International Rice Research Institute (IRRI).

Linkage map construction

Mapmaker/exp ver3.0 program was used to construct a genetic linkage map (Lander et al., 1987). All pairs of linked markers were identified using the "group" command with an LOD value of 3.0. The marker order was determined using the "orders" and the "compare" commands and verified using the "ripple" command. The frequency of recombination between two markers was converted to genetic distance using Kosambi map function (Kosambi 1944). Assignment of linkage groups to the respective chromosomes was based on genetic maps developed by Temnykh et al. (2000) and Gramene Annotated Nipponbare Sequence map (http://www. gramene.org). Chi-square tests were performed to examine the segregation ratios at the marker loci for deviation from the expected ratio 1:1.

QTL analysis

Composite interval mapping (CIM) was performed to identify additional QTLs and to increase the resolution of QTL locations (Basten et al. 1997). Significance thresholds for CIM were determined using 1,000 permutations for each trait. The proportion of observed phenotypic variation attributable to a particular QTL was estimated by the coefficient of determination (RSq). The total phenotypic variance was estimated by fitting a model including all putative QTLs for the respective traits.

Results

Evaluation of resistance for blast races and in the nursery

The distributions of the degree of incidence in four out of nine races screened by inoculation method in 190 RIL popula-

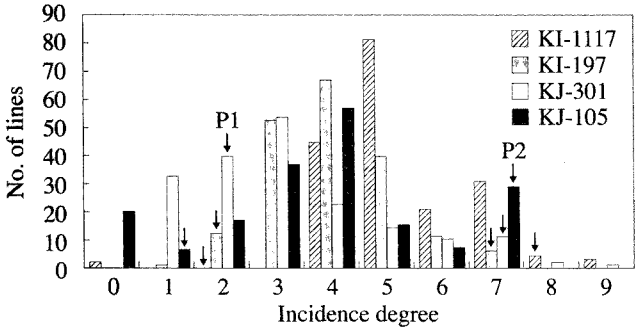
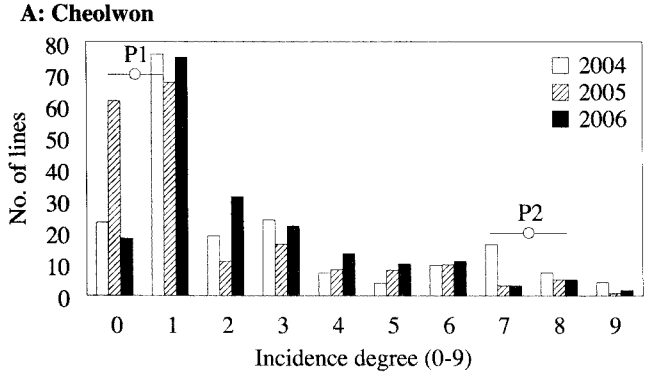


Fig. 1. Frequency distribution of disease incidence degree by screening to four blast races of 190 RILs of the cross between Suweon365 (P1) and Chucheong (P2).



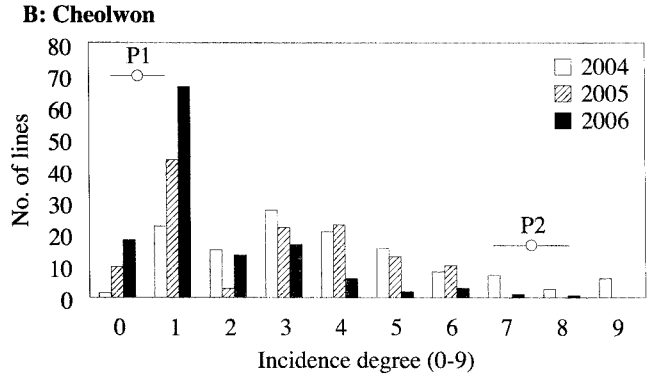


Fig. 2. Frequency distribution of disease incidence degree at blast nursery test for three years of 190 RILs from the cross between Suweon365 (P1) and Chucheong (P2).

tion were given in Fig. 1. The incidence degrees of two parents Suweon365 and Chucheong to four races were 1 to 2, and 7 to 8, respectively. The degree of incidence of race KI-197 showed normal distribution. The degree of incidence of race KJ-301 showed the skewed distributions to degrees 1 to 3. The KI-1117 and KJ-105 races showed skewed distribution with some transgressive segregation. The evaluations for blast nursery were screened at four regions Cheolwon, Suwon, Namyang and Jinbu from 2004 to 2006 (Fig. 2). Two parents, Suweon365 and Chucheong had incidence degrees 1.2±0.61 and 7.3±0.44, respectively in the average of four regions for three years, respectively. The degrees of disease incidence showed the continuous variation skewed to 0 to 2 degree in all regions and years.

Table 1. Putative QTLs for resistance against blast races in *japonica* rice

QTL	Blast race[a]	Chr.	Flanked markers	CIM[b]		
				LOD	R2(%)[c]	AE[d]
qLB1.1	KJ-105	1	RM5638-RM473a	8.22	21.4	-0.95
qLB1.2	KJ-401	1	RM5448-RM1361	3.61	7.5	-0.50
qLB2.1	KJ-301	2	RM213-RM48	4.82	6.9	-0.34
qLB2.2	KJ-301	2	RM138-RM2265	4.47	6.6	-0.33
qLB4.1	KI-197	4	RM5709-OSR15	5.94	9.7	-0.49
qLB4.2	KJ-201	4	OSR15-RM567	5.26	9.3	-0.53
	KI-197		OSR15-RM567	5.83	9.8	-0.49
	KI-409		OSR15-RM567	4.22	6.0	-0.47
	KI-1113		RM349-RM567	7.22	14.7	-0.68
	KI-1117		OSR15-RM567	2.92	5.6	-0.35
qLB6.1	KJ-301	6	RM3132-RM589	3.43	6.2	-4.04
qLB6.2	KJ-401	6	RM5509-RM3509	3.16	9.7	-0.55
qLB7	KJ-301	7	OSR4-RM234	3.62	5.1	-0.43
qLB11.2	KJ-105	11	RM1233-RM144	2.90	5.3	-3.07
qLB12.1	KJ-105	12	OSR20-RM1337	18.56	34.9	-7.88
	KJ-301		OSR20-RM1337	7.46	12.0	-0.62
	KI-313		OSR20-RM1337	6.48	14.1	-0.77
	KI-409		OSR20-RM1337	13.99	25.2	-0.94
qLB12.2	KJ-301	12	RM17-RM1300	3.71	6.7	-4.24

[a]KJ-, Korean blast races compatible to *japonica* rice; KI-, Korean blast races compatible to *japonica* and *indica* rices.
[b]CIM: Composite interval mapping
[c]R^2(%): Phenotypic variation explained by each QTL
[d]AE: Additive effect of Suweon365 allele of resistance to blast races

QTLs for blast races

Twelve QTLs associated with resistance to nine blast races were detected on chromosmes 1, 2, 4, 6, 7, 11 and 12 (Table 1, Fig. 3). Two QTLs *qLB4.2* and *qLB12.1* were identified against five and four blast races, respectively, and the rest QTLs were against the single race. The phenotypic variation explained by each QTL ranged from 5.1% to 34.9%. The phenotypic variation explained by two QTLs, *qLB4.2* and *qLB12.1* which were identified against five and four blast races, ranged from 5.6% to 14.7% and from 12.0% to 34.9%, respectively. Ten QTLs against single races KJ-401, KI-197, KJ-105 and KJ-301, respectively explained to 5.1-21.4% of total phenotypic variation.

QTLs for blast nursery

Eight QTLs identified by the alleles of Suweon365 for blast resistance in the blast nursery test across four regions for three years were located on chromosmes 1, 2, 4, 11 and 12 (Table 2, Fig. 3). Two QTLs, *qLB1.1*, and *qLB11.1* were identified against the only races in one region for one year. *qBL2* was identified only for each one year in three regions, and explained 5.2% to 5.5% of phenotypic variation. Two QTLs, *qLB4.2* and *qLB11.2* were identified against three and two regions, respectively. They explained 6.7-18.8% and 9.7-14.6% of total phenotypic variation, and were reduced 0.64-1.00 and 0.82-1.01 degrees of disease incidence by the allele effect of Suweon365. Three QTLs *qLB4.1*, *qLB12.1* and *qLB12.2* were identified across four regions for three years. It explained 6.9-16.2%, 4.3-10.9% and 11.8-37.7% of total phenotypic variation, respectively. Three QTLs, *qLB4.1*, *qLB4.2*, and *qLB12.2* were identified against

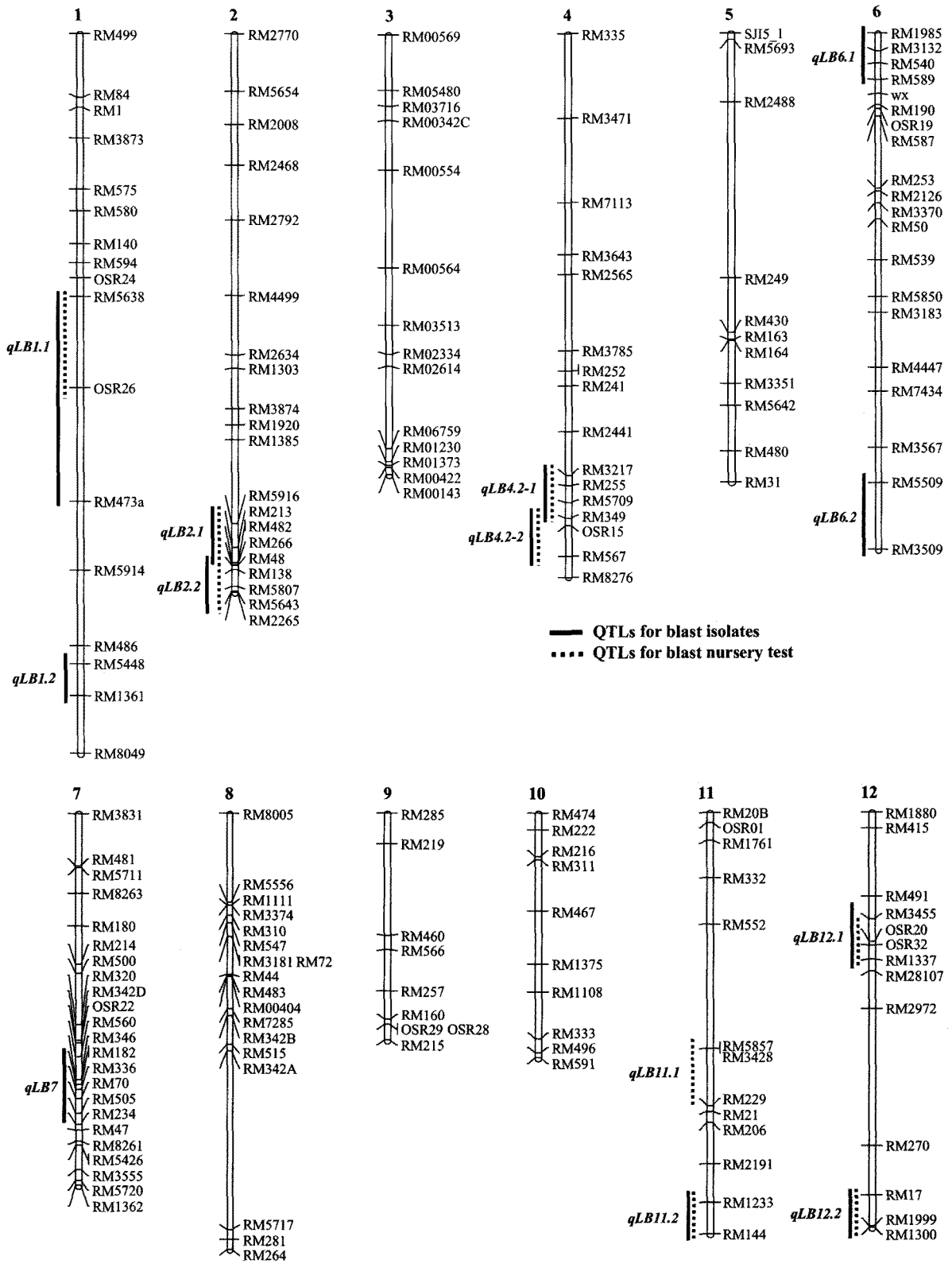


Fig. 3. QTLs mapping using an RIL population from the cross between Suweon365 and Chucheong of *japonica* rice.

three years in Cheolwon and explained to 7.5-10.1%, 6.7-13.3% and 28-37.7% of the total phenotypic variation, respectively. Two QTLs, *qLB12.1* and *qLB12.2* on chromosome 12 identified against the disease incidence for two and three years in Suwon and explained to 4.8-5.2% and 20.6-25.2% of the total phenotypic variation, respectively. The *qLB12.2* was the major QTL explaining 11.8-37.7% of total phenotypic variation and was reduced to 0.69-1.44 of the degrees of disease incidence by the allele effect of Suweon365 against all regions and years.

Table 2. Putative QTLs for resistance against leaf blast in *japonica* rice

QTL	Region[a]	Year	Chr.	Flanked markers	CIM[b]		
					LOD	R^2(%)[c]	AE[d]
qLB1.1	CW	2005	1	RM5638-OSR26	2.66	5.7	-0.63
qLB2	CW	2005	2	RM213-RM2265	4.25	5.2	-0.58
	SW	2005	2	RM213-RM2265	4.40	5.5	-0.54
	NY	2004	2	RM213-RM2265	4.11	5.2	-0.47
qLB4.1	CW	2004	4	RM255-RM349	5.49	7.5	-0.66
		2005	4	RM255-RM349	6.87	10.1	-0.83
		2006	4	RM255-RM349	5.71	9.1	-0.70
	SW	2005	4	RM255-RM349	9.53	14.4	-0.92
	NY	2004	4	RM255-RM349	5.01	6.9	-0.56
	JB	2005	4	RM255-RM349	10.13	16.2	-0.82
qLB4.2	CW	2004	4	OSR15-RM567	4.49	6.7	-0.64
		2005	4	RM349-RM567	8.52	13.3	-0.95
		2006	4	RM349-RM567	6.20	10.1	-0.74
	SW	2005	4	RM349-RM567	11.53	17.4	-1.00
	JB	2005	4	RM349-RM567	10.12	18.8	-0.89
qLB11.1	NY	2004	11	RM5857-RM229	4.14	6.4	-0.59
qLB11.2	CW	2005	11	RM1233-RM144	6.03	12.1	-0.91
		2006	11	RM1233-RM144	8.49	14.6	-1.01
	SW	2006	11	RM1233-RM144	5.90	9.7	-0.82
qLB12.1	CW	2004	12	RM3455-OSR32	4.14	6.4	-0.59
	SW	2005	12	OSR20-RM1377	3.23	5.2	-0.53
		2006	12	RM3455-RM1377	2.91	4.8	-0.41
	NY	2004	12	RM3455-RM1377	7.22	10.9	-0.67
		2006	12	RM3455-RM1377	4.10	7.8	-0.56
	JB	2005	12	OSR20-RM1377	2.77	4.3	-0.41
qLB12.2	CW	2004	12	RM17-RM1300	21.50	37.7	-1.44
		2005	12	RM17-RM1300	15.83	29.3	-1.38
		2006	12	RM17-RM1300	14.92	28.0	-1.18
	SW	2004	12	RM17-RM1300	12.10	20.6	-0.55
		2005	12	RM17-RM1300	12.25	21.0	-1.07
		2006	12	RM17-RM1300	11.66	25.2	-0.97
	NY	2004	12	RM17-RM1300	10.39	18.5	-0.89
		2006	12	RM17-RM1300	13.40	28.2	-1.09
	JB	2005	12	RM17-RM1300	6.32	11.8	-0.69

[a] Region: CW, Cheolwon; SW, Suwon; NY: Namyang; JB, Jinbu
[b] CIM: Composite interval mapping
[c] R^2(%): Phenotypic variation explained by each QTL
[d] AE: Additive effect of Suweon365 allele of resistance to field blast

Confirmation of QTL effect to field resistance

Two desirable RILs (RIL4 and RIL93) carrying QTL segments were backcrossed to the susceptible parent Chucheong to verify the effect of Suweon365 alleles for blast resistance. Three chromosome segment substitution lines (CSSLs) CSSL4-1, CSSL4-2 and CSSL93 which were advanced by marker-assisted selection to BC_2F_6 generations had *qLB6.2* and *qLB7*, *qLB11.2*, and *qLB12.1*, respectively (Fig. 4). Three CSSLs and the susceptible parent, Chucheong were screened to blast nursery test in two regions for two years (Table 3). A susceptible parent Chucheong showed 7-8 degrees of disease incidence. The line CSSL4-1 having two QTLs *qLB6.2* and *qLB7* showed 6-7 degrees of disease incidence to blast. Two lines CSSL4-2 and CSSL93 showed moderate resistance score of 3-4 degrees against the races in blast nursery test.

Table 3. Field resistance effect of QTLs to blast nursery in three chromosome segment substitution lines (CSSL) of BC_2F_6

Region	Blast nursery test							
	Chucheong		CSSL 4-1		CSSL 4-2		CSSL 93	
	2007	2008	2007	2008	2007	2008	2007	2008
Suwon	8*	8	6	7	3	3	3	4
Cheolwon	7	8	7	7	4	4	4	4

* Incidence degrees are from 0 (no lesion) to 9 (necrosis of all leaves and sheaths).

Discussion

A *japonica* rice cultivar Suweon365 could be a potential donor for the resistance to blast races in the field. The present study was carried out to identify chromosomal regions for stable field resistance to blast races in Korean *japonica* cultivars using an RILs from the cross between Suweon365 and Chucheong. QTLs identified for this resistance were transferred into a susceptible parent Chucheong, by backcross method and later developed three CSSLs of BC_2F_6.

Among 190 RILs, the scores for disease severity in the blast nursery test ranged from 0 to 9. Some lines had a higher resistance compared to Suweon365 and other lines were more susceptible than Chueheong. This suggests multi-genic inheritance of QTLs for resistance to races in the field confirmed by QTL analysis. The Suweon365 allele increased the resistance attributed to all QTLs identified for races and nursery test to blast. In this study, three QTLs *qLB1.2*, *qLB7*, and *qLB12.2* were newly identified. Two QTLs *qLB1.2* and *qLB7* were effective against only single race KJ-401 and KJ-301, respectively, and a QTL *qLB12.2* was significant against blast nursery as well as single race KJ-301 (Table 1 and 2). One candidates of resistance gene analog (RGA) ORFs of LRR family protein type in one Nipponbare BAC clones, OSJNBa0035N13, was identified in the counterpart of *qLB12.2* between markers RM17 and RM1300 in the TIGR Pseudomolecule assembly release 5 (http://www.gramene.org). This QTL may be needed to clarify allele effect as a new QTL from Suweon365 using chromosome segment substitution line in future.

Ten QTLs, *qLB1.1*, *qLB2.1*, *qLB2.2*, *qLB4.1*, *qLB4.2*, qLB*6.1*, *qLB6.2*, *qLB11.1*, *qLB11.2* and *qLB12.1* were identified in the same chromosome locations with *R* genes and/or QTLs similar to previous reports (Wang et al. 1994 ; Bryan et al. 2000; Fujii et al. 2000; Tabien et al. 2000; Zenbayashi et al. 2002; Xu et al. 2008). A major QTL *qLB1.1* explaining 24.1% of phenotypic variation for KJ-105 race on chromosome 1 was identified in the regions *Pitp*(t) (Barman et al. 2004). The QTL between markers RM213 and RM2265 in the telomere region on the chromosome 2 was divided into two QTLs *qLB2.1* and *qLB2.2* against the blast races. These QTLs were from the blast race KJ-301. However, the QTL against blast nursery showed single peak between markers RM213 and RM2265 at Namyang in 2004 and at Cheolwon and Suwon in 2005. This region is known to have located two *R* genes *Pib* and *Pitq-5* (Wang et al. 1999; Tabien et al. 2000). The resistant parent, Suweon365, had been identified to hold the *Pib* gene (Cho et al. 2007). As a result, further analysis is needed to clarify allelic relationship between two QTLs,

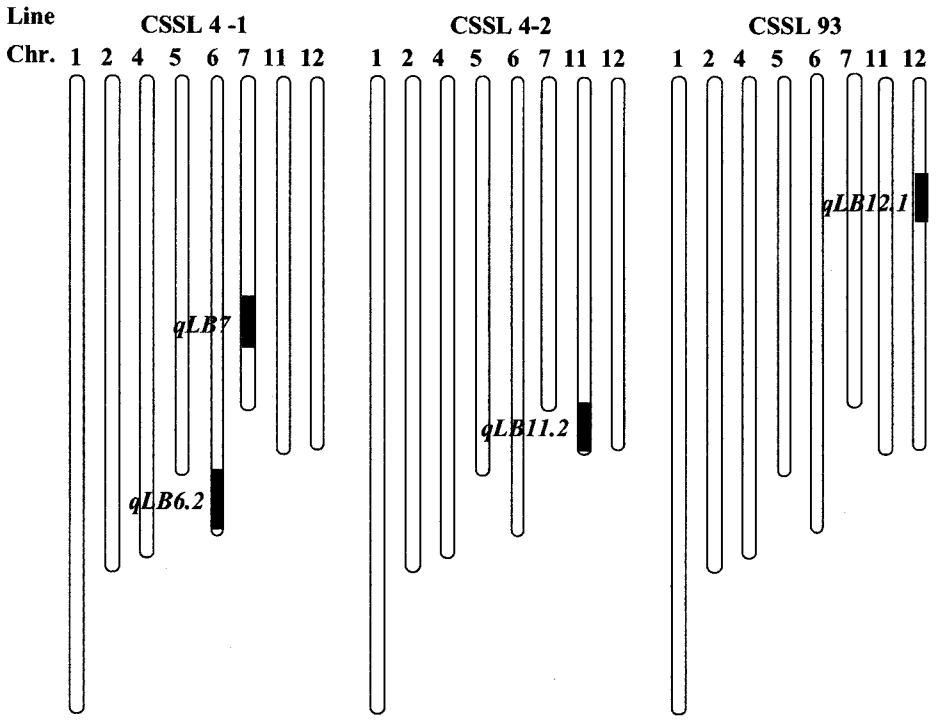


Fig. 4. Diagram of genotypes for four QTL regions to blast resistance in eight chromosomes in three chromosome segment substitution lines of BC_2F_6.

qLB2.1 and *qLB2.2* as well as between two genes *Pib* and *Pitq-5*. The QTL peaks *qLB4.1* and *qLB4.2* were identified between RM255 and RM567 on chromosome 4. The *qLB4.1* is detected against one race KI-197, and qBL4.2 has been identified against four races KJ-201, KI-197, KI-1113 and KI-1117. These two QTLs (*qLB4.1* and *qLB4.2*) were detected in blast nursery test for three years in Cheolwon, and one year in other regions. The presense of seven candidates of resistance gene analog (RGA) ORFs of NBS-LRR type in two Nipponbare BAC clones, OSJNBa0058K23 and OSJNBb0085C12, were also identified in the counterpart of *qLB4.1* between markers RM255 and RM349, but was not present in *qLB4.2* in the TIGR Pseudomolecule assembly release 5 (http://www.gramene.org). Xu et al. (2008) reported that a strong QTL of field resistance against rice blast, *Pikahei(t)*, from upland rice Kahei was mapped within 300kb at 31.2-31.5Mb on the chromosome 4. In the future, there is a need to clarify the relationship between two QTLs *qLB4.1* and *qLB4.2*.

Two QTLs *qLB6.1* and *qLB6.2* were identified to two blast races KJ-301 and KJ-401 at the telomeres of short and long arms of chromosme 6, respectively (Table 1, Fig. 3). The regions of *qLB6.1* and *qLB6.2* are in fact the regions of QTLs for lesion number and DLA (Wang et al. 1994 ; Tabien et al. 2002), and the major genes *Pitq-1* and *qBLASTads-6* (Tabien et al. 2000, 2002), respectively. A QTL *qLB11.1* identifed between markers the RM5857 and RM229 showed resistance explaining 6.9% of phenotypic variation against blast nursery test at Namyang in 2004. This region has been previously reported as *Pi34(t)* and *Pi44* to leaf blast, and *Pb1* to neck blast (Chen et al. 1999 ; Fujii et al. 2000). Two QTLs, *qLB11.2* and *qLB12.1* identified in this study are associated with the regions of a rice blast *R*-gene clusters of *Pik* on chromosome 11 and *Pita* on chromosome 12, respectively. The resistance parent Suweon365 was reported to have *Pi18* gene on chromosome 11 against KI-313 race (Kwon et al. 2002). As a result, the resistance effect of *qLB11.2* against a specific blast race and in blast nursery test may be related to *Pi18* gene. Moreover, the cluster region of *R* genes, *Pik*-multiple alleles, *Pi1* and *Pi7* was also located in this region (Inukai et al. 1994; Wang et al. 1994). A QTL *qLB12.1* against four blast races and in blast nursery at four regions by the allele of Suweon365 was identified in the flanking segment of RM3455-RM1337 in the centromere region of chromosome 12. The resistant parent Suweon365 was reported to have a blast *R*-gene *Pi25(t)* against KI-197 race (Kwon et al. 2002). Also, it was identified to have *Pita-2* gene using *R*-gene specific DNA markers in this region (Cho et al. 2007). This chromosome region was identified to locate a blast *R*-gene rich cluster of *Pi4a*, *Pita*, *Pitq-6*, *Pi12*, *Pi19*, and *Pi157* and a few QTLs (Inukai et al. 1994; Wang et al. 1994; Niqvi and Chattoo 1996; Rybka et al. 1997; Hayashi and Imbe 1998; Tabien et al. 2000). As a result, the resistance effect of *qBL12.1* by the allele of Suweon365 might be related to the blast *R*-gene *Pi25(t)* and/or *Pita-2*.

Three CSSLs, CSSL4-1, CSSL4-2, and CSSL93 have been developed to confirm the resistance effect of four QTLs, *qLB6.2*, *qLB7*, *qLB11.2*, and *qLB12.1* to leaf blast. They were evaluated to determine resistance in blast nursery test in two regions for two years (Table 3). A CSSL4-1 having chromosome segments for two QTLs *qLB6.2* and *qLB7* showed incidence degrees of 6 to 7. However, two lines, CSSL4-2 and CSSL93 having chromosome segments for *qLB11.2* and *qLB12.1*, respectively showed disease incidence of 3 to 4 degrees indicating moderately resistance. The QTLs identified against only blast races did not have the resistance effect enhanced highly in blast nursery test, but the QTLs detected against blast races and nursery test showed the enhanced resistance in nursery test. The *R* gene *Pita* (*Pita-2*) showed resistance effect across regions and years by the analysis of haplotype for the DNA markers linked to the *R*-genes (Cho et al. 2008). The resistance effect of *qLB12.1* might be related to *Pita* (*Pita-2*) gene present in Korean japonica rice cultivars. As a result, it is the most effective way of pyramiding genes/QTLs showing resistance to leaf blast in the field as well as blast races and, thus, developing enhanced and stable resistance cultivars in rice breeding. Interestingly, the two QTLs, *qLB7* and *qLB11.2*

were related to the QTLs for spikelet number and grain yield by the alleles of Suweon365 (Kwon et al. 2008), and would be useful to increase yield potential as well as to enhance effective resistance to blast.

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