Plant Pathol. J. 25(4) : 408-416 (2009)

The Plant Pathology Journal

Evaluation of Bacterial Blight Resistance Using SNP and STS Marker-assisted Selection in Aromatic Rice Germplasm

Jeong Soon Kim, Jae Gyun Gwang, Ki Hun Park and Chang Ki Shim*

National Agrobiodiversity Center, Rural Development Administration, Suwon 441-100, Korea

(Received July on 9, 2009; Accepted on October 5, 2009)

A molecular survey was conducted to identify the presence of the bacterial blight resistance genes (*Xa*1, *Xa*4, *xa*5, *xa*13 and *Xa*21) in 86 accessions of aromatic rice obtained from germplasm. The results revealed that the resistance gene *Xa*4 (32.5%), *Xa*21 (17%), and *xa*5 (16%) were widely observed in tested rice germplasm. Among tested rice germplasm, 49 accessions showed the presence of more than one of five *R* genes, and 37 accessions possessed none of the *R* gene. TALLi and 05-IRRi-M-46 showed the presence of *Xa4*, *xa5*, *xa13* and *Xa21*. Rice race 415×Ir352 exhibited positive amplicon for the *Xa1*, *Xa4*, *xa5* and *Xa21*. Hyangmibyeo1hos, Ir841-85-1-1-2 and Jasmine85 showed the positive amplicon for the *Xa1*, *Xa4* and *xa5* genes. Yekywin Yinkya Hmwe and Khao Dawk Mali105 showed the presence of *Xa1*, *Xa4* and *Xa21* gene. Masino Basmati showed the presence of *xa5*, *xa13*, *Xa21* genes. *Xa1* and *Xa21* genes were noticed in Mihayngbyeo, Tarana Deshi, Mayataung and AZUCENA. Hyangmibyeo2ho, Basmati 6311 and Basmati405 possessed only two R genes such as *Xa4* and *xa5*, and *xa5* and *xa13*, respectively. The evaluation results of bacterial blight resistance genes in aromatic rice germplasm will help in breeding of multi disease resistant varieties.

Keywords : Aromatic rice, bacterial blight resistance, MAS

Bacterial blight (BB) disease is caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and this has been one of the most serious biotic factors limiting rice production in worldwide (Ezuka and Kaku, 2000; Mew, 1987; Shin et al., 1992). The yield losses in severely infected fields generally range from 20 to 30% but may reach up to 80% (Ou, 1985; Singh et al., 1977). It is widespread throughout the world in many countries *viz.*, Nepal (26 accessions), Myanmar (19 accessions), India (17 accessions), Sri Linka (14 accessions), Philippines (10 accessions), China (10 accessions), Japan (6 accessions), Vietnam (6 accessions), Korea (5 accessions) (Adhikari et al., 1999; Fincks and Nelson, 1999; George et al., 1997; Lwin et al., 2007; Noda et al., 1996, 1999, and 2001; Noh et al., 2003; Ochiai et al., 2000; Sing et al., 2003). Recently, three races (K1, K2 and K3) are the most dominant races of *Xoo* in Korea. Pathogen populations of *Xoo* are highly variable, as revealed by virulence and DNA fingerprinting analysis (Adhikari et al., 1995, 1999; Leach et al., 1992; Nelson et al., 1994).

Over 30 resistance (*R*) genes exhibiting host resistance against various strains of *Xoo* have been identified and named from *Xa1* to *Xa28*. Fourteen of the 30 resistance genes have been mapped to chromosomes 4, 5, 6, 7, 8, 11 and 12 (Chen et al., 2002; Ezuka and Kaku, 2000; Gu et al., 2004; Kinoshita, 1995; Lee et al., 2003; Lin et al., 1999; Sakaguchi, 1967; Singh et al., 2002; Wei et al., 2005; Yang et al., 2003; Zhang et al., 1998). The development of donor lines carrying major *R* genes have been incorporated into improved rice cultivars (Khush et al., 1990). Ten of the recessive *R* genes, *xa5*, *xa8*, *xa13*, *xa15*, *xa16*, *xa19*, *xa20*, *xa24*, *xa26*, and *xa28*, have been characterized as encoding different types of proteins and suggested that multiple mechanisms of *R* gene-mediated *Xoo* resistance (Chu et al., 2006; Gu et al., 2005; Lyer and Couch 2004; Song et al., 1995; Sun et al., 2004; Wei et al., 2005; Yoshimura et al., 1998).

The use of resistant cultivars is the most economical and effective method to control the bacterial blight. In modern crop plants breeding programmes, marker assisted selection (MAS) provides a opportunity to circumvent many problems associated with phenotypic selection of crops and increases the efficiency and flexibility of a breeding through selection of marker genotype linked to target genes or quantitative trait loci (QTL) (Melchinger, 1990; Mohan et al., 1997; Ogawa, 1993). The pyramiding of multiple resistance genes into rice varieties is one way to develop durable resistance to bacterial blight (Huang et al., 1997; Kinoshita, 1995; Sanchez et al., 2000; Yoshimura et al., 1995). Singh et al. (2001) reported that a three-gene combination (*xa*5, *xa*13 and *Xa*21) appeared to be the most effective of which *Xa21* is contributing the largest component of resistance. In republic of Korea, the breeding program for resistant rice cultivars to bacterial blight was started in 1965 and japonica type of Seomjinbyeo carrying *Xa1* gene was bred in 1982. Hwayeongbyeo was bred

*Corresponding author.
Phone) +82-31-299-1878, FAX) +82-31-299-1893
E-mail) ckshim@korea.kr

from WAseAikou3 carrying *Xa3* gene (Ezuka et al., 1974) in 1991 and had resistance to K1, K2 and K3 races. The aim of the present study was to get the information about the genetic diversities of bacterial blight resistance genes in aromatic rice germplasm to improve the aromatic rice breeding efficiency using several SNP and STS markers.

Materials and Methods

Plant materials and DNA isolation. Eighty six accessions of aromatic rice were obtained from National Agrobiodiversity Center, Rural Development Administration (RDA), and Republic of Korea and were listed in Table 2. Five rice seeds were imbibed for 4 h in 2% NaOCl further transferred to another container containing tap water for overnight and placed moist filter paper on Petri-dish at 30°C for one-week in light condition. Genomic DNA was extracted from young leaves of one-week-old seedlings by an improved CTAB (hexadecyl trimethyl ammonium bromide) method based on the procedure described by Murray and Thompson (1980). The extracted genomic DNA was examined on 1% agarose gel stained with ethidium bromide. The DNA was quantified by Nano Drop system (Nano Drop, USA), further concentration of DNA was adjusted to 10 ng/μl with sterilized distilled water and stored at 4°C.

Bacterial Blight (BB) *R* genes specific DNA markers. Previously reported five *R* gene specific DNA markers to develop SNP (*Xa1* and *xa13*) and STS (*Xa4*, *xa5* and *Xa21*) markers for the bacterial blight resistance genes from mapped various differential chromosomes were used for amplification of DNA fragments linked to BB resistant genes (Table 1). All the STS and SNP markers used in this study were obtained from the Oligo Synthesis Facility, Bioneer Co., Korea.

Polymerase chain reaction (PCR) analysis. The PCR analysis was conducted based on procedures described by Bioneer PCR Pre-mix kit manual (Bioneer Co. Ltd, Korea). The PCR reaction mixture contained 50 ng template DNA, 20 pmol of each primer set, 0.25 mM of each dNTPs, 1.5 mM $MgCl_2$, 1X PCR buffer (10 mM Tris-HCl, pH 9.0, 30 mM KCl), and 1 unit of Taq DNA polymerase in a volume of 20 ul. Amplifications were carried out in a MyGenie96 Thermal cycler (Bioneer Co. Ltd, Korea) programmed that template DNA was initially denatured at 94°C for 5 min, followed by 30 cycles of PCR amplifiaction with the following parameters; a 30 sec denaturation at 94°C, 30 sec primer annealing at from 42 to 62°C, and 1 min primer extension at 72°C allowed for completion of primer extension, with final extension at 72°C for 10 min. Initially 5 μl of the amplified products were electrophoretically resolved on a 1.5% agarose gel in 0.5X TAE (Tris-acetate-EDTA) buffer (pH 8.0) and visualized under UV light after staining with 0.1 ug/ml of ethidium bromide. The amplified fragments using STS markers (RM224, RG556 and pTA248) were scored as presence (R) or absence (S) of each gene linked DNA fragment.

PCR-RFLP analysis. The amplified products were digested with restriction enzymes such as *EcoR* V (16PFXa) and *Hinf*I (RG136 linked to the *Xa1* gene). The total volume of the reaction for PCR-RFLP was 20 μl (2.0 μl of 10X restriction enzyme buffer, 0.5 μl of 10 u/μl restriction enzyme (Promega, USA), 7.5 μl of sterile distilled water, 10 μl of amplified products). The reaction mixture was incubated at 37°C for 5 h. The digested DNA fragments were separated through 2% agarose gel electrophoresis and visualized under UV light after staining with 0.1 ug/ml of ethidium bromide. The digested fragments were scored as described previously.

Table 1. Details of single nucleotide polymorphic (SNP) and sequence tagged site (STS) marker used for evaluation of BB resistance and linkage to bacterial blight resistant genes

Linked gene	Located on chromosome	Marker	Primer sequence (5' – 3')	Type of marker	Reference
Xa1	4	16pFXa1	F-ACGGTTCTGAAGGTCGTCAT R-TGCAAGAGCTCCGGTTTAGG	SNP/*EcoR* V	Shin et al., 2006
Xa4	11	RM224	F-ATCGATCGATCTTCACGAGG R-TGCTATAAAAGGCATTCGGG	STS	Chen et al., 1997
xa5	5	RG556	F-TAGCTGCTGCCGTGCTGCGC R-AATATTTCAGTGTGCATCTC	STS	Yoshimura et al., 1995
xa13	8	RG136	F-TCCCAGAAAGCTACTACAGC R-GCAGACTCCAGTTTGACTTC	SNP/*Hinf*I	Zhang et al., 1996
Xa21	11	pTA248	F-AGACGCGGAAGGGTGGTTTCCCGGA R-AGACGCGGTAATCGAAAGATGAAA	STS	Ronald et al., 1992

*Gene symbol with capital and small letter represent dominant and recessive gene, respectively.

Results

MAS of BB resistance accessions of aromatic rice germplasm. Two hundred and sixty accessions of aromatic rice germplasm introduced from 20 origins were preserved in Korean RDA genebank. One hundred and six of Two hundred and sixty accessions of aromatic rice were evaluated with agronomic traits, pshysico-chemical characteristics,

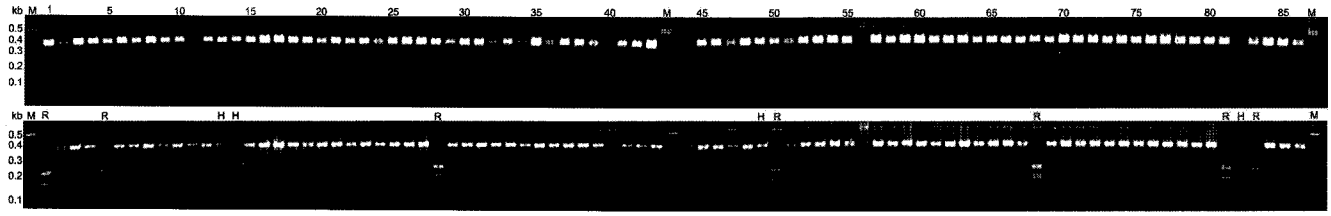

Fig. 1. Agarose gel photograph of 86 aromatic rice germplasm, bacterial blight resistant (R), heterozygous (H) and susceptible (non-marked lanes) accessions amplified with SNP marker 16PFXa (upper), linked to *Xa1* gene and the PCR products were digested by a restriction enzyme *EcoR* V (under). M, molecular marker (Bioneer 100 bp ladder plus); lanes 1-86, 86 accessions of aromatic rice.

Table 2. Summarization of PCR assay results for genotyping 86 accessions of aromatic rice germplasm by SNP and STS markers linked to the bacterial blight resistant genes

IT Number[a]	Varieties	Origin	Ecotypes	BB resistant gene				
				Xa1	*Xa4*	*xa5*	*xa13*	*Xa21*
192023	Hyangmibyeo1ho	Korea	Tongil	R[b]	R	R	S	S
191962	Hyangmibyeo2ho	Korea	Tongil	S	R	R	S	S
191971	Hyangnambyeo	Korea	Japonica	S	S	S	S	S
203705	Aranghyangchalbyeo	Korea	Japonica	S	S	S	S	S
196276	Mihyangbyeo	Korea	Japonica	R	S	S	S	R
043511	A-2	Bhutan	Japonica	S	S	S	S	S
113892	A-3, Choh Chang	Bhutan	Japonica	S	S	S	S	S
K037322	Muhyang99-8	China	Japonica	S	S	S	S	S
K037323	Jahyangna861	China	Japonica	S	S	S	S	S
K056007	Jc149	India	Japonica	S	R	S	S	S
K056008	Jc157	India	Japonica	S	S	S	S	S
143478	Iari 7447	India	Japonica	S	S	S	S	S
K141988	Padi danat	Indonesia	Japonica	H	H	S	S	S
K141989	Kemiri	Indonesia	Japonica	H	H	S	S	S
210864	Daebunhyangdo2	Japan	Japonica	S	S	S	S	S
177080	Shiyayuuine	Japan	Japonica	S	S	S	S	S
-	Rasomotrafotsy	Madagascar	Japonica	S	S	S	S	R
136185	TALLI	Nepal	Japonica	S	R	R	R	R
-	Masino Basmati	Nepal	Japonica	S	S	R	R	R
213730	Kalomasino Dhan	Nepal	Japonica	S	R	S	S	S
K046513	Gerdeh	Philippines	Japonica	S	S	S	S	S
212959	KINANDANG PAT	Philippines	Japonica	S	S	S	S	S
219192	Milagrosa MUTANT	Philippines	Japonica	S	R	S	S	S
-	Inaguhu	Philippines	Japonica	S	R	S	S	S
-	Flores	Philippines	Japonica	S	R	S	S	S
-	Kung-ShanWu-Shen-Ken	Taiwan	Japonica	S	S	S	S	S
165761	Daw Dam	Thailand	Japonica	S	S	S	S	S
K016876	415 X Ir352	Vietnam	Japonica	R	R	R	S	R
-	Khau Nua Keo	Vietnam	Japonica	S	S	S	S	S
-	Khau Tan Luong	Vietnam	Japonica	S	S	S	S	S
207636	Goolarath	Australia	Indica	S	S	S	S	S
K055930	Da13	Bangladesh	Indica	S	R	S	S	S
K046496	Basmati 370	Bangladesh	Indica	S	S	S	S	S
900526	Hyanggaengdo	China	Indica	S	S	S	S	S
102310	Seratus Malam	India	Indica	S	S	S	S	R
-	Basmati T3	India	Indica	S	S	S	S	S

Table 2. Continued

IT Number[a]	Varieties	Origin	Ecotypes	BB resistant gene				
				Xa1	Xa4	xa5	xa13	Xa21
K056005	Jc111	India	Indica	S	S	S	S	S
-	Arc 6011	India	Indica	S	R	S	S	S
-	Kaminibhog	India	Indica	S	S	S	S	S
-	Tarana Deshi	India	Indica	S	R	S	S	R
-	Basmati	India	Indica	S	S	S	S	S
213081	Iranbyeopssi	Iran	Indica	S	S	S	S	S
003406	Iranbyeopssi	Iran	Indica	S	S	S	S	S
K056366	Domsiah	Iran	Indica	S	S	S	S	S
-	Mulai	Iran	Indica	S	S	S	S	S
-	Tareme	Iran	Indica	S	S	S	S	S
900724	Hyangdo	Japan	Indica	S	S	S	S	S
009496	Seratus Malam	Malreisia	Indica	S	S	S	S	S
-	Mayataung	Myanmar	Indica	H	R	S	S	R
-	Yekywin Yinkya Hmwe	Myanmar	Indica	R	R	S	S	R
-	Kala Namak	Nepal	Indica	S	S	S	S	S
136257	Masino Basmati	Nepal	Indica	S	R	S	S	S
-	Basmati Dhan	Nepal	Indica	S	S	R	S	S
211194	Basmati	Nepal	Indica	S	S	S	S	S
207665	Basmati370	Pakistan	Indica	S	R	S	S	S
-	Basmati9-93	Pakistan	Indica	S	R	S	S	S
-	Basmati198	Pakistan	Indica	S	R	S	S	S
800806	Basmati370	Pakistan	Indica	S	S	S	S	S
155923	Basmati5836	Pakistan	Indica	S	S	S	S	S
155925	Basmati5854	Pakistan	Indica	S	S	R	S	S
155927	Basmati5875	Pakistan	Indica	S	S	S	R	S
155929	Basmati6113	Pakistan	Indica	S	S	S	S	R
K056304	Basmati1	Pakistan	Indica	S	S	S	S	S
155906	Basmati213 C	Pakistan	Indica	S	S	S	S	R
155910	Basmati372	Pakistan	Indica	S	S	S	S	S
K056308	Chahora 144	Pakistan	Indica	S	S	S	R	S
-	Pakistani Fine	Pakistan	Indica	S	S	S	S	S
122941	Ir841-85-1-1-2	Philippines	Indica	R	R	R	S	S
074744	05-Irri-M-46	Philippines	Indica	S	R	R	R	R
155899	Basmati 107	Philippines	Indica	S	S	S	S	S
155915	Basmati 405	Philippines	Indica	S	S	R	R	S
155924	Basmati 5853	Philippines	Indica	S	S	S	S	S
155926	Basmati 5874	Philippines	Indica	S	S	R	S	S
155930	Basmati 6129	Philippines	Indica	S	R	S	S	S
155932	Basmati 6311	Philippines	Indica	S	R	R	S	S
155933	Basmati 6313	Philippines	Indica	S	S	S	S	S
155934	Basmati 6141	Philippines	Indica	S	S	S	S	S
010205	AZUCENA	Philippines	Indica	S	R	S	S	R
000347	Binicol	Philippines	Indica	S	S	S	S	S
009882	Milfor 6	Philippines	Indica	S	R	S	S	S
123302	Dinorado	Philippines	Indica	R	S	S	S	S
219273	Khao Dawk Mali105	Thailand	Indica	H	R	S	S	R
K037775	Jasmine 85	USA	Indica	R	R	R	S	S
K037773	Dellmont	USA	Indica	S	S	S	S	S
000895	Aroma	USA	Indica	S	R	S	S	S
215237	Ds20	Vietnam	Indica	S	R	R	S	R

[a] Identity number in the Genebank of RDA, Korea. ID number with a prefix "K" is the temporary IT number, –; ID is not available in RDA Genebank.

[b] R, resistance; H, heterozygous; S, susceptible reactions linked to the bacterial blight resistant gene.

and analysis of aromatic compounds in 2007 (Kim et al., 2008a and Kim et al., 2008b).

Marker-assisted selection (MAS) was conducted to identify the presence of the bacterial blight resistance genes (*Xa1*, *Xa4*, *xa5*, *xa13* and *Xa21*) in 86 accessions of aromatic rice obtained from germplasm. Bacterial blight resistance gene *Xa4* (32.5%), *Xa21* (17%) and *xa5* (16%) is widely detected in tested rice accessions. Out of 86 accessions of aromatic rice germplasm, forty nine accessions showed the presence of more than one of bacterial blight resistance gene. Thirty seven accessions did not possess with anyone of the resistance gene (Table 2).

Genetic diversity of *Xa1* gene. Detection of gene carrying of aromatic rice germplasm by SNP marker 16PFXa (Shin et al., 2006) showed the 350 bp fragment. Seven out of 86 accessions showed the BB resistant double fragments as 180 bp and 210 bp digested with restriction enzyme, *EcoR* V which corresponds to *Xa1* gene (Fig. 1). Among 11 *Xa1* positive rice races, 2 accessions such as Hyangmibyeo1ho (Choi et al., 1995) and Mihyangbyeo (Ha et al., 2003), were bred through domestic breeding program. Two japonica (Mihyangbyeo and 415×Ir352) and 3 accessions of indica type rice (Kemiri, Padi danat and Mayataung; originated from Indonesia and Myanmar) possessed *Xa1* gene (Fig. 1, Table 2). The BB susceptible (S) fragments were not digested with restriction enzyme, *EcoR* V for overnight.

Genetic diversity of *Xa4* gene. Amplification of *Xa4* gene carrying of aromatic rice germplasm by STS marker RM224 (Chen et al., 1997) showed the BB resistant (R) fragment as 150 bp and the BB susceptible (S) fragments as 120 bp (Fig. 2). Thirty two of eighty six accessions of aromatic rice showed the appropriate amplification for BB resistant fragments of *Xa4* gene (Fig. 2). Of which two tongil type rice accessions such as Hyangmibyeo1ho and Hyangmibyeo2ho were bred domestic breeding program (Moon et al., 1998) (Table 2). Out of 28 accessions of japonica type aromatic rice, seven accessions (Flores, Inaguhu, JC149, Kalo Masino Dhan, Milagrosa mutant, TALLI, and 415XIr352) showed the BB resistant fragments linked to *Xa4* gene. Positive amplicon (150 bp) for *Xa4* gene was observed in 19 of 56 races of indica type aromatic rice (Arc6011, Aroma, AZUCENA, Basmati370, Basmati 9-93, Basmati198, Basmati 6129, Basmati6311, Da13, DS20, Jasmine85, Ir841-85-1-1-2, Khao Dawk Mali105, Tarana Deshi, Mayataung, Milfor6, Yekywin Yinkya Hmwe, and 05-Irri-M-46) (Fig. 2, Table 2).

Genetic diversity of *xa5* gene. Presence of *xa5* gene in aromatic rice germplasm was amplified by STS marker RG556 (Yoshimura et al., 1995) which showed the BB resistant (R) fragment as 500 bp and the BB susceptible (S) double fragments as 500 bp and 480 bp (Fig. 3). The results revealed that only 14 of 86 accessions possessed *xa5* gene. Of which two accessions of tongil type rice such as Hyangmibyeo1ho and Hyangmibyeo2ho were bred domestic breeding program possessed *xa5* gene (Fig. 3, Table 2). Three of twenty eight accessions of japonica type aromatic rice (Masino Basmati, TALLI, and 415×Ir352) showed the BB resistant fragments linked to *xa5* gene. Nine of firty six accessions of indica type aromatic rice (Basmati Dhan, Basmati405, Basmati5874, Basmati6311, Basmati5854, DS20, Ir841-85-1-1-2, Jasmine85, and 05-Irri-M-46) showed the positive amplification for *xa5* gene (Fig. 3, Table 2).

Genetic diversity of *xa13* gene. Existence of *xa13* gene in aromatic rice germplasm was amplified through SNP marker (RG136) (Zhang et al., 1996) and showed the 1 kb positive fragment. None of the tongil type rice which was bred on domestic bred program showed the presence of *xa13* gene (Fig. 4). Two accessions of japonica type aromatic rice (TALLI and Masino Basmati) and four accessions of indica type aromatic rice (Basmati405, Basmati5875, Chahora 144, and 05-Irri-M-46) showed the presence of *xa13* gene (Fig. 4, Table 2).

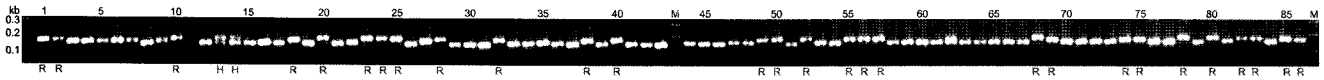

Fig. 2. Agarose gel photograph of 86 aromatic rice germplasm, bacterial blight resistant (R) as 150 bp DNA fragment, susceptible (non-marked laned) as 120 bp DNA fragment, and bacterial blight heterozygous (H) as double fragment with 150 bp and 120 bp amplified with STS marker RM224 (Chen et al., 1997), linked to *Xa4* gene. M, molecular marker (Bioneer 100 bp ladder plus); lanes 1-86, 86 accessions of aromatic rice.

Fig. 3. Agarose gel photograph of 86 aromatic rice germplasm, bacterial blight resistant (R) accessions as 500 bp of single fragment and susceptible (non-marked lanes) accessions as 500 bp and 480 bp of double fragments amplified with STS marker RG556 (Yoshimura et al., 1995), linked to *xa5* gene. M, molecular marker (Bioneer 100 bp ladder plus); lanes 1-86, 86 accessions of aromatic rice.

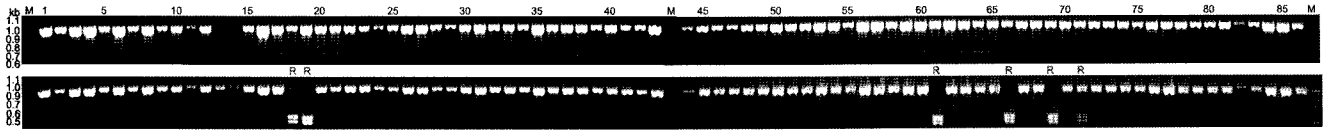

Fig. 4. Agarose gel photograph of 86 aromatic rice germplasm, bacterial blight resistant (R) and susceptible (non-marked lanes) accessions amplified with SNP marker RG136 (upper), linked to *xa13* gene and the PCR products were digested by restriction enzyme *Hinf*I (under). M, molecular marker (Bioneer 100 bp ladder plus); lanes 1-86, 86 accessions of aromatic rice.

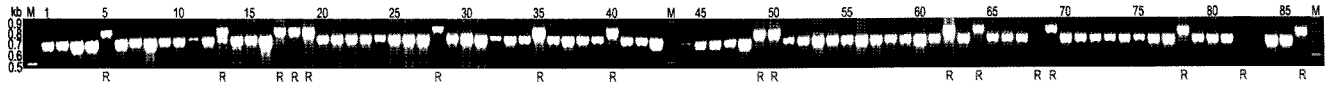

Fig. 5. Agarose gel photograph of 86 aromatic rice germplasm, bacterial blight resistant (R) accessions as 800 bp DNA fragment and susceptible (non-marked lanes) accessions as 700 bp DNA fragment amplified with STS marker pTA248 (Ronald et al., 1992), linked to *Xa21* gene. M, molecular marker (Bioneer 100 bp ladder plus); lanes 1-86, 86 accessions of aromatic rice.

Genetic diversity of *Xa21* gene. Examination of *Xa21* gene carrying aromatic rice by STS marker pTA248 (Ronald et al., 1992) showed the BB resistant (R) fragment as 800 bp and the BB susceptible (S) fragments as 700 bp (Fig. 5). *Xa21* was detected on five accessions of japonica type aromatic rice (Masino Basmati, Mihyangbyeo, TALLI, and 415XIr352) and 10 accessions of indica type aromatic rice (AZUCENA, Basmati6113, Basmati213C, DS20, Khao Dawk Mali105, Mayataung, Seratus Malam, Tarana Deshi, Yekywin Yinkya Hmwe, and 05-Irri-M-46) (Fig. 5, Table 2).

Discussion

Numerous studies have been carried out on the rice-*Xanthomonas oryzae* pv. *oryzae* pathosystem, resulting in powerful advancement in the understanding of molecular basis of interaction (Nayak et al., 2008; Shen and Ronald, 2002). Due to continuous evolution of pathogenic races, breakdown of resistance has occurred in many improved varieties. Incorporation of resistance genes is very difficult using conventional methods of breeding due to epitasis or masking effects of other genes (Mew, 1987 and Mew et al., 1992). In Korea, seven varieties of aromatic rice, Hyangmibyeo1ho (IR841-76-1×Suwon334), Hyangnambyeo (Iri389 ×Dohoku144), Hyangmibyeo2ho (IR841-76-1×Suwon334), Aranghyangchalbyeo (Sinseonchalbyeo×Dohoku144), Mihyangbyeo (Seomjinbyeo×Dohoku144), Seolhyangchalbyeo (Miyagaori×Suwon357), and Heughyang (Sanghaehyanghyeolla, γ-300Gy) were bred from 1993 to 2000 (Choi et al., 1995; Ha et al., 2003; Ha et al., 2006; Moon et al., 2003; Shin et al., 2001).

In this study, eight of eighty six accessions of aromatic rice showed the presence of BB resistance gene linked to *Xa1* and four accessions of aromatic rice (Kemiri, Khao Dawk Mali 105, Mayataung, and Padi danat) exhibited the presence the heterozygous BB resistance gene linked to *Xa1*. Bacterial blight resistance gene *Xa1* was first reported by Sakaguchi (1967) and mapped it on rice chromosome 4 and cloned recently by a map-based cloning strategy (Yoshimura, et al., 1998). The *Xa1* gene has widely been used in Japanese rice breeding program for BB resistance, since, race1 of *Xoo* is the most dominant race in Japan. In the current study, *R* gene linked to *Xa1* was observed in Hyangmibyeo1ho and Mihyangbyeo. Shin et al. (2007) reported the same result using SNP marker 16PFXa linked to *Xa1* gene. It is interesting to note that most of the rice varieties bred in South Korea showed resistant to BB Korean race K1.

BB resistance gene *Xa4* is one of the most widely exploited resistance gene in many Asian rice breeding programs and conferred durable resistance in many commercial rice cultivars (Mew et al., 1992). *Xa4* gene offers resistance to bacterial blight at all stages of plant growth (Khush, 1981; Mew et al., 1992). In the present study also among 86 accessions of aromatic rice, thirty two accessions were positive for *Xa4* gene.

*Xa*21 was reported to confer resistance to pathotype from the Philippines and India at post-seedling growth stages (Ikeda et al., 1990). Besides, this has been reported as the single most effective gene against 17 isolates of *Xoo* from Punjab (Singh et al., 2001). The *Xoo-Xa21* interaction provides a model system in which to study the molecular basis for disease resistance (Song et al., 1995). *Xa21* gene is the only known resistance gene that encode three structural features (leucine-rich repeats (LRR) in the putative extracellular domain, a single-pass transmembrane domain whereas other resistance gene products possess cytoplasmic serine/threonine kinase (STK) intracellular domain) found in various combinations in other resistance gene products (Song et al., 1995).

The presence of two major types of disease resistance to plant pathogens, vertical resistance (complete whole-life resistance) and horizontal resistance (quantitative resistance), have been recognized in interactions between rice and *Xoo*

(Zhang and Mew, 1985). In this study, two accessions of aromatic rice (TALLi and 05-IRRi-M-46) showed the presence of four multi bacterial blight resistance gene such as *Xa4*, *xa5*, *xa13* and *Xa21*. The multiple resistance genes *Xa1*, *Xa4*, *xa5* were detected on single rice accessions such as Hyangmibyeo1ho, Ir841-85-1-1-2, Jasmine85, Khao Dawk Mali105, and Yekywin Yinkya Hmwe.

The pyramiding of *Xa4* and other bacterial blight resistance genes offered a broad range of resistance to *Xoo* strains besides exhibited a higher level of resistance than the single resistance gene (Huang et al., 1997; Yoshima et al., 1995; Zheng et al., 1998). It could be concluded that the presence of multiple resistance genes in a single accessions of aromatic rice probably useful for gene pyramiding. The advent of molecular markers tagged to different resistant genes enabled convergence breeding and pyramiding of more than two different genes into an agronomic variety. Sanchez et al. (2000) reported that recessive genes such as *xa5* and *xa13* are difficult to select through conventional breeding in the presence of a dominant gene such as *Xa21*. *Xa21* gene was governing resistance to all known races of *X. oryzae* pv. *oryzae*. It is impossible to distinguish between plants having *Xa21* alone and those having *Xa21* and other genes due to masking effects of former (Huang et al., 1997; Singh et al., 2001)

Similarly, a molecular survey was conducted by Ramalingam et al. (2001) to examine the presence of BB resistance genes such as *xa5*, *xa13* and *Xa21* in Chinese rice germplasm. Although conventional approach for the identification of different resistance genes in rice germplasm is also being used (Kihupi et al., 2001; Lee et al., 2003), however, it is time consuming and need artificial inoculation of all the lines with different pathotypes of the *Xoo*.

When single gene resistant varieties were continuously cultivated in wide area, the resistance to the specific BB race would be broken down through pathogen's variation (Bai et al., 2000; Mew et al., 1992; Noh et al., 2003). A MAS has been advocated as a highly efficient breeding method, because it can offer rapid and precise selection of target gene. The present study demonstrated the presence of multiple bacterial blight resistance genes in aromatic rice germplasm stored at National Agro-biodiversity center of Republic of Korea. In future, the results of the present study will be exploited for breeding programs to develop a multi disease resistant aromatic rice varieties against *Xantomaonas oryzae* pv. *oryzae*.

Acknowledgements

This research was supported by a grant program (200901-FHTO2060950301) funded by Rural Development Administration (RDA), Republic Korea in 2009.

References

Adhikari, T. B., Mew, T. W. and Leach, J. E. 1999. Genotypic and pathotypic diversity in *Xanthomonas oryzae* pv. *oryzae* in Nepal. *Phytopathology* 89:687-694.

Adhikari, T. B., Vera Cruz, C. M., Zhang, Q., Nelson, R. J., Skinner, D. Z., Mew, T. W. and Leach, J. E. 1995. Genetic diversity of *Xanthomonas oryzae* pv. *oryzae* in Asia. *Appl. Environ Microbiol.* 61:966-971.

Bai, J., Choi, S. H., Ponciano, G., Leung, H. and Leach, J. E. 2000. *Xanthomonas oryzae* pv. *oryzae* avirulence genes contribute differently and specifically to pathogen aggressiveness. *Mol. Plant-Microbe Interact.* 13:1322-1329.

Chen, H., Wang, S. and Zhang, Q. 2002. New gene for bacterial blight resistance in rice located on chromosome 12 identified from Minghui 63, an elite restorer line. *Phytopathology* 92: 750-754.

Chen, X., Temnykh, S., Xu, Y., Cho, G. and McCouch, S. R. 1997. Development of microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 95:553-567.

Choi, Y. G., Kim, M. K., Jung, K. H., Cho, S. Y., Moon, H. P., Jun B. T., Choi, H. C., Park, N. G., Kim, G. W., Hwang, K. H., Kim, Y. S., Park, R. K. and Cho, J. Y. 1995. An
aromatic semi-dwarf lodging resistant rice variety "Hyangmibyeo1ho". *Agricultural Science Reports of RDA, Korea.* 37:67-74.

Chu, Z., Fu, B., Li, Z., Zhang, Q. and Wang, S. 2006. Targeting recessive rice gene, *xa13*, for bacterial blight resistance to a 14.8-kb DNA fragment. *Theor. Appl. Genet.* 112:455-461.

Ezuka, A., Horino, O., Toriyama, K., Shinoda, H. and Morinaka, T. 1974. Inheritance of resistance of rice variety WaseAikoku3 to *Xanthomonas oryzae* pv. *oryzae*. *Bull. Tokai-kinki, Nat. Agr. Exp. Sta.* 28:124-130.

Ezuka, A. and Kaku, H. 2000. A historical review of bacterial blight resistance in rice. *Bull. Natl. Inst. Agr. Resour.* 15:1-207.

Finckh, M. R. and Nelson, R. J. 1999. Phylogenetic and pathotypic analysis of rice bacterial blight race 3. *Eur. J. Plant Pathol.* 105:743-751.

George, M. L. C., Bustamam, M., Cruz, W. T., Leach, J. E. and Nelson, R. J. 1997. Movement of *Xanthomonas oryzae* pv. *oryzae* in southeast Asia detected using PCR-based DNA fingerprinting, *Phytopathology* 87:302-309.

Gu, K., Tian, D., Yang, F., Wu, L., Sreekala, C., Wang, D., Wang, G. L. and Yin, Z. 2004. High-resolution genetic mapping of *Xa27*(t), a new bacterial blight resistance gene in rice, *Oryzae sativa* L. *Theor. Appl. Genet.* 108:800-807.

Gu, K., Yang, B., Tian, D., Wu, L., Wang, D., Sreekala, C., Yang, F., Chu, Z., Wang, G. L., White, F. F. and Yin, Z. 2005. *R* gene expression induced by type-III effector triggers disease resistance in rice. *Nature* 435:1122-1125.

Ha, K. Y., Lee, J. K., Shin, H. T., Lee, S. Y., Yang, B. G., Kim, B. K., Joung, J. I., Kim, Y. D., Shin, M. S., Ko, J. K., Lee, K. S., Kim, C. H. and Cho, S. Y. 2006. A new aromatic and good grain quality Japonica rice variety "Hyangnambyeo". *Agricultural Science Reports of RDA, Korea.* 38:54-60.

Ha, K. Y., Kim, Y. D., Lee, J. K., Shin, H. T., Noh, T. H., Choung, J. I., Shin, M. S., Ko, J. K., Kim, B. K., Lee, Y. T., Lee, S. Y., Kim, H. S., Kang, H. J., Lee, K. S. and Lee, S. Y. 2003. A new aromatic and high-yielding Japonica rice cultivar "Mihyang-byeo". *Korean J. Breed.* 35(4): 251-252.

Huang, N., Angeles, E. R., Domingo, J., Magpantay, G., Singh, S., Zhang, G., Kumaravadivel, N., Bennett, J. and Khush, G. S. 1997. Pyramiding of bacterial blight resistance genes in rice: Marker-assisted selection using RFLP and PCR. *Theor. Appl. Genet.* 95:313-320.

Ikeda, R., Kush, G. S., Tabien, R. E. and Ogawa, T. 1990. A new resistance gene to bacterial blight from *Oryza longistaminata*. Jpn. *J. Breed.* (Suppl. 1). 40:280-281.

Iyer, A. S. and McCouch, S. R. 2004. The rice bacterial blight resistance gene *xa5* encodes a novel form of disease resistance. *Mol. Plant Microbe. Interact.* 17:1348-1354.

Khush, G. S. 1981. Breeding rice for multiple disease and insect resistance. p 220-237. In Rice improvement in China and other Asian countries. international Rice Research Institute, Los Banos, Philippines.

Khush, G. S., Bacalangco, E. and Ogawa, T. 1990. A new gene for resistance to bacterial blight from *O. Longistaminata*. *Rice Genet. Newsl.* 7:121-122.

Kim, J. S., Ahn, S. N., Kang, H. K., Cho, Y. H., Gwag, J. G. and Lee, S. Y. 2008a. Estimation of Physico-chemical Characteristics of domestic aroma rice and foreign aroma rice. *Korean J. Crop Sci.* 53:203-216

Kim, J. S., Ahn, S. N., Cho, Y. H., Gwag, J. G., Kim, T. S., Lee, J. R. and Lee, S. Y. 2008b. Estimation of agronomic characteristics of domestic aromatic rice germplasm and foreign aromatic rice germplasm in RDA Genebank, Korea. *Korean J. Crop Sci.* 53:261-272.

Kihupi, A. N., Angeles, E. R. and Khush, G. S. 2001. Genetic analysis of resistance to bacterial blight, *Xanthomonas oryzae* pv. *oryzae*, in rice, *Oryza sativa* L. *Euphytica.* 117:39-46.

Kinoshita, T. 1995. Report of committee on gene symbolization, nomenclature and linkage groups. Rice Genet. Newsl. 12:9-153.

Leach, J. E., Rhoads, M. L., Vera Cruz, C. M., White, F. F., Mew, T. W. and Leung, H. 1992. Assessment of genetic diversity and population structure of *Xanthomonas oryzae* pv. *oryzae* with repetitive DNA element. *Appl. Environ. Microbiol.* 58:2188-2195.

Lee, K. S., Rasabandith S., Angeles, E. R. and Khush, G. S. 2003. Inheritance of resistance to bacterial blight in 21 cultivars of rice. *Phytopathology.* 93:147-152.

Lin, X. H., Luo, L. J., Mei, H. W., Paterson, A. H., Zhao, X. H., Zhong, D. B., Wang, Y. P., Yu, X. Q., Zhu, L., Tabien, R., Stansel, J. W. and Ying, C. S. 1999. A "defeated" rice resistance gene acts as a QTL against a virulent strain of *Xanthomonas oryzae* pv. *oryzae*. *Mol. Gen. Genet.* 261:58-63.

Lwin, T., Myint, Y. Y., Nwe, K. T. and Thet, K. M. 2007. Evaluation of Myanmar rice germplasms for resistance to bacterial blight. International training-workshop. *The conservation and utilization of tropical / subtropical plant genetic resources*. pp. 73-89.

Melchinger, A. E. 1990. Use of molecular markers in breeding for oligogenic disease resistance. *Plant Breed.* 104:1-19.

Mew, T. W. 1987. Current status and future prospects of research on bacterial blight of rice. *Ann. Rev. Phytopathol.* 25:359-382.

Mew, T. W., Vera Cruz, C. M. and Medalla, E. S. 1992. Changes in race frequency of *Xanthomonas oryzae* pv. *oryzae* in response to rice cultivars planted in Philippines. *Plant Dis.* 76:1029-1032.

Mohan, M., Nair, S., Bhagwat, A., Krishna, T. G., Tano, M., Bhatia, C. R. and Sasaki, T. 1997. Genome mapping, molecular markers and marker-assisted selection in crop plants. *Mol. Breed.* 3:87-103.

Moon, H. P., Choi, Y. G., Jeong, G. H., Hwang, G. H., Lee, J. H., Kim, M. G., Cho, S. Y., Jeon, B. T., Kim, J. I., Hwang, H. G., Choi, H. C., Park, R. G. and Kim, Y. S. 1998. A new, multi disease resistant and semi-dwarf aromatic rice cultivar "Hangmibyeo 2ho". *Agricultural Science Reports of RDA, Korea.* 40:34-40.

Moon, H. P., Jung, K. K., Choi, Y. G., Lee, J. H., Hwang, H. G., Kim, M. K., Yang, S. J., Hwang, K. H., Song, M. T., Hong, H. C., Shin, Y. S., Kim, H. Y., Ryu, H. Y., Choi, H. C. and Chung, M. N. 2003. A new aromatic and glutinous rice cultivar "Seolhyangchalbyeo". *Korean J. Breed.* 35:241-242.

Nayak, D., Bose, L. K., Singh, S. and Nayak, P. 2008. Additive main effects and multiplicative interaction analysis of host-pathogen relationship in rice-bacterial blight pathosystem. *Plant Pathol. J.* 24:337-351.

Nelson, R. J., Baraoidan, M. R., Vera Cruz, C. M., Yap, I. V., Leach, J. E., Mew, T. W. and Leung, H. 1994. Relationship bewteen phylogeny and pathotype for the bacterial blight pathogen of rice. *Appl. Environ. Microbiol.* 60:3275-3283.

Noda, T., Du, P. V., Van, E. L., Dinh, H. D. and Kaku, H. 1999. Pathogenicity of *Xanthomonas oryzae* pv. *oryzae* in Vietnam. *Ann. Phytopathol. Soc. Jpn.* 65:293-296.

Noda, T., Li, C., Li, J., Ochiai, H., Ise, K. and Kaku, H. 2001. Pathogenic diversity of *Xanthomonas oryzae* pv. *oryzae* strain from Yunnam province, China. *JARQ.* 35:97-103.

Noda, T., Yamamoto, T., Kaku, H. and Horino, O. 1996. Geographical destribution of pathogenic races of *Xanthomonas oryzae* pv. *oryzae* in Japan in 1991 and 1993. *Ann. Phytopathol. Soc. Jpn.* 65:549-553.

Noh, T. H., Lee, D. K., Kang, M. H., Shin, M. S., Shim, H. K. and Na S. Y. 2003. Identification of new of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) in Korea. *Phytopathology* 93:S66.

Ogawa, T. 1993. Methods and strategy for monitoring race distribution and identification of resistance genes to bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*) in rice. *JARQ* 27:71-80.

Ou, S. H. 1985. Rice disease (2nd edition). Common wealth Mycological Institute 380p.

Ramlingam, J., Basharat, H. S. and Zhang, G. 2001. Polymorphism of DNA markers linked to bacterial blight resistance genes in useful rice germplasm. *IRRN.* 26:23-24.

Ronald, P. C., Albano, B., Tabien, R., Abenes, L., Wu, K., McCouch, S. and Tanksley, S. 1992. Genetic and physical analysis of the rice bacterial blight disease resistance locus,

Xa21. Mol. Gen. Genet. 236:113-120.

Sakaguchi, S. 1967. Linkage studies on the resistance to bacterial leaf blight, *Xanthomonas oryzae* (Uyeda et Ishihama) Dowson, in Rice. *Bull. Natl. Inst. Agr. Sci. Ser.* (in Japanese with English summary)

Sanchez, A. C., Brar, D. S., Huang, N., Li, Z. and Khush, G. S. 2000. Sequence tagged site marker-assisted selection for three bacterial blight resistance genes in rice. *Crop Science* 40:792-797.

Sanchez, A. C., Ilag, L. L., Yang, D., Brar, D. S., Ausubel, F., Khush, G. S., Yano, M., Sasaki, T., Li, Z. and Huang, N. 1999. Genetic and physical mapping of *Xa-13* a recessive bacterial blight resistance genes in rice. *Theor. Appl. Genet.* 98:1022-1028

Shen, Y. and Ronald, P. 2002. Molecular determinations of disease and resistance in interactions of *Xanthomonas oryzae* pv. *oryzae* and rice. *Microbes Infect.* 4:1361-1367.

Shin, I. C., Lim, Y. T., Ahn, C. B. and Lee, S. B. 2001. An early-maturing, aromatic pigmented and glutinous rice variety "Heugseongchal". *Korean J. Breed.* 32:135-136.

Shin, M. S., Noh, T. H., Kim, K. Y., Shin, S. H., Ko, J. K. and Lee, J. K. 2005. Reaction of Korean rice varieties to bacterial blight race, K3a. *Korean J. Crop Sci.* 50:151-155.

Shin, M. S., Shin, H. T., Jun, B. T. and Choi, B. S. 1992. Effects of inoculation of compatible and incompatible bacterial blight races on grain yield and quality of two rice cultivars. *Korean J. Breed.* 24:264-267.

Shin, S. H., Kim, K. Y., Shin, M. S., Ko, J. K. and Kim, C. K. 2007. Identification of rice varieties containing bacterial blight resistance gene *Xa1* by SNP marker (16PFXa). *Korean J. Breed. Sci.* 39:63-69.

Shin, W. C., Baek, S. H., Seo, C. S., Kang, H. J., Kim, C. K., Shin, M. S., Lee, G. S., Hahn, J. H. and Kim, H. S. 2006. Improvement of selection efficiency for bacterial blight resistance using SNP marker in rice. *J. Plant Biotechnol.* 33:309-313.

Singh, G. P., Srivastara, M. K., Singh, R. V. and Singh, R. M. 1977. Variation in quantitative and qualitative losses caused by bacterial blight on rice varieties. *Indian Phytopathol.* 30:180-185.

Singh, K., Vikal, Y., Singh, S., Leung, H., Dhaliwal, H. S. and Khush, G. S. 2002. Mapping of bacterial blight resistance gene *xa8* using microsatellite markers. *Rice Genet. Newsl.* 19:94-97.

Singh, S. 2001. Pyramiding three bacterial blight resistance genes (*xa5, xa13, Xa21*) using marker assisted selection into indica rice cultivar PR106. *Theor. Appl. Genet.* 102:1011-1015.

Song, W. Y., Wang, G. L., Chen, L. L., Kim, H. S., Pi, L. Y., Holsten, T., Gardner, J., Wang, B., Zhai, W. X., Zhu, L. H., Fauquet, C. and Ronald, P. 1995. A receptor kinase-like protein encode by the rice disease resistance gene, *Xa21. Science.* 270:1804-1806.

Song, W. Y., Wang, G. L., Chen, L., Kim, H. S., Holsten, T., Zhai, W. X., Zhu, L., Fauquet, C. and Ronald, P. 1995. The rice disease resistance gene *Xa21*, endodes a receptor kinase-like protein. *Science.* 270:1804-1806.

Sun, X., Cao, Y., Yang., Z., Xu., C., Li, X., Wang, S. and Zhang, Q. 2004. Xa26, a gene conferring resistance to *Xanthomonas oryzae* pv. *oryzae* in rice, encodes an LRR receptor kinase-like protein. *Plant J.* 37:517-527.

Wei, M. L., Lu, H. Y. and Lu, C. T. 2005. Bioinfomatics collection and statictics of rice disease/pest resistance genomics research. *Crop. Environment & Bioinfomatics.* 2:296-306 (in Chinese with English summary).

Yang, Z., Sun, X., Wang S. and Zhang, Q. 2003. Genetic and physical mapping of a new gene for bacterial blight resistance in rice. *Theor. Appl. Genet.* 106:1467-1472.

Yoshimura, S., Yamanouchi, U., Katayose, Y., Toki, S., Wang, Z. X., Kono, I., Kurata, N., Yano, M., Iwata, N. and Sasaki, T. 1998. Expression of *Xa1*, a bacterial blight resistance gene in rice, is induced by bacterial inoculation. *Proceedings of the National Academy of Science, USA.* 95:1663-1668.

Yoshimura, S., Yoshimura, A., Iwata, N., McCouch, S. R., Abebes, M. L., Baraoidan, Mew, T. W. and Nelson, R. J. 1995. Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers. *Mol. Breed.* 1:375-387.

Zhang, Q. and Mew, T. W. 1985. Adult resistance of rice to bacterial blight. *Plant Diseases.* 69:896-898.

Zhang, Q., Lin, S. C. and Zhao, B. Y. 1998. Identification and tagging a new gene for resistance to bacterial blight, *Xanthomonas oryzae* pv. *oryzae* from *O. rufipogon. Rice Genet. Newsl.* 15:138-142.