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Breeding of Near-Isogenic Lines of Rice with Single Genes for Resistance to Bacterial Blight Pathogen (*Xanthomonas campestris* pv. *oryzae*)

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Introduction

Bacterial blight (BB) caused by *Xanthomonas campestris* pv. *oryzae* is one of the major diseases of rice in the rice-growing countries of Asia.

The pathogenic specialization in the causal bacterium of BB was first reported in Japan by KUHARA *et al.* (1958) and KUSABA *et al.* (1958), and a number of reports have been published on the variability of the pathogenicity of the bacterium and of the resistance. Recent studies on BB have been carried out mostly by Japanese researches and scientists of the International Rice Research Institute (IRRI), Philippines.

Since the rice cultivars and bacterial races used as the differentials in both countries (Japan and Philippines) were different, the two groups of scientists found it difficult to distinguish the resistance gene(s). In order to control the disease, it was important to set up a common base to define the relationship between the virulence of the bacterial races and the resistance of rice cultivars to the races. Thus it was deemed necessary at this stage to compare and analyze the results of the studies conducted in Japan and at IRRI.

Against this background, the collaborative studies on resistance to rice bacterial blight (BB) between Japan and IRRI were initiated in 1979 as a collaborative research project between the Tropical Agriculture Research Center (TARC), Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan and IRRI. Two years later, the MAFF-IRRI joint program for research on resistance to rice bacterial blight was planned and the collaborative program started on 1982. In 1985, the program has been included as part of the Project; a collaborative effort between IRRI and the Government of Japan, concentrating on selective aspects of low-input rice cultivation, technology, sponsored by the Government of Japan.

Breeding of near-isogenic lines for the development of international differentials for resistance to BB

Near-isogenic lines with diverse genes for resistance to major diseases and insects can be powerful tools for identifying races and biotypes of diseases and insects as well as new genes

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for resistance. Moreover, isogenic lines can serve as donors for resistance in breeding programs and be excellent materials for studying the mechanism of resistance.

Regarding rice disease and insects, no near-isogenic lines are avairable which can be used as common differential lines becuase of the difficulty in exchanging materials among rice-growing countries. To establish a common basis of research on resistance to BB, we tried to developd near-isogenic lines with diverse genes for the resistance to BB disease under the above collaboration. As a result, we developed the initial set of near-isogenic lines (OGAWA *et al.* 1988). The seeds have already been distributed to scientists of various Asian countries and biotechnologists, and some of the reports in which the lines were used have been published (INTERNATIONAL RICE RESEARCH INSTITUTE 1989, ZHANG *et al.* 1990, ENDO *et al.* 1991, TAURA *et al.* 1991).

The present paper deals with the breeding procedures adapted for the development of the lines for analysis. Some characteristics of the lines after designation will be described later.

Donors for breeding of near-isogenic lines

The resistant donors for the development of the near-isogenic lines were as follows:

Kogyoku	(Chugoku Natl. Agric. Expt. St.)	for *Xa-1, Xa-12*
Te-Tep	(Chugoku Natl. Agric. Expt. St.)	for *Xa-1, Xa-2*
Chugoku 45	(Chugoku Natl. Agric. Expt. St.)	for *Xa-3*
Java 14	*(Chugoku Natl. Agric. Expt. St.)*	*for Xa-1, Xa-3,* and *Xa-12*
IR8	(HD 151, 1982 IRRI)	for *Xa-11*
IR20	(HD 122, 1982 IRRI)	for *Xa-4*
IR1545-339	(Acc. 32624, IRRI)	for *xa-5*
DV85	(Acc. 8839, IRRI)	for *xa-5, Xa-7*
Zenith	(Acc. 131, IRRI)	for *Xa-3* (*Xa-6*)
Sateng	(Acc. 30193, IRRI)	for *Xa-3* (*xa-9*)
CAS209	(Acc. 15793, IRRI)	for *Xa-10*
PI231129	(Acc. 11114, IRRI)	for *xa-8*

The recurrent parents for the development of the near-isogenic lines were as follows:

IR24	(Natl. Agric. Res. Centr. Japan)	for *indica* background
Toyonishiki	(Natl. Agric. Res. Centr. Japan)	for *japonica* background
Milyang 23	(Natl. Agric. Res. Centr. Japan)	for *indica-japonica* hybrid background

For the selection of the recurrent parents, we reported earlier in detail the reaction to Japanese and Philippine BB races.

Breeding procedure of the near-isogenic lines

Initially for the development of near-isogenic lines with diverse genes for resistance to BB, the BC_4F_4 lines were bred with each monogenic basis for the resistance. The basic procedure is shown in Fig. 1.

All the crossing work for the development of the near-isogenic lines was continuously carried out at IRRI. After the original crossing between the recurrent parents and each resistant donor and the first backcrosses to the F_1 hybrids were completed, the selection of the

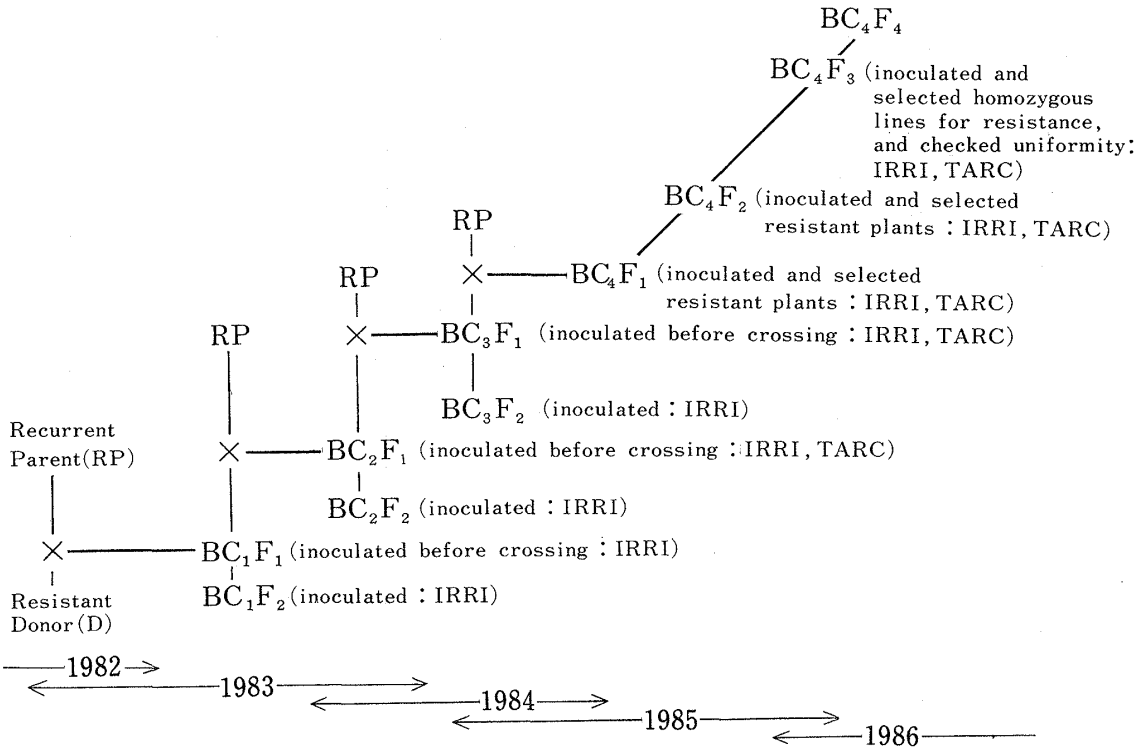

Fig. 1. Procedural steps in breeding for near-isogenic line to bacterial blight resistance

plants carrying dominant resistance genes was started by inoculation using Philippine isolates. For the selection of the plants carrying a recessive gene, the F_2 progenies of the backcrossed hybrids were inoculated with Philippine isolates. From the second backcross, at least 7 plants of each line were crossed with each recurrent parent to obtain a progeny carrying each resistance gene at a probability of more than 99%. This process was repeated until the BC_4F_2 generation of each breeding line which carried the resistance genes to Philippine races.

On the other hand, for the breeding lines carrying resistance genes to the Japanese races, the crossed progenies between recurrent parents and resistant donors for the development of near-isogenic lines were inoculated mostly from the BC_3F_1 advanced generations with Japanese isolates at TARC, because there is only one rice cropping season per year in Japan. For identifying the lines with resistance genes among the backcrossed progenies, more than seven crossed seeds per cross were sent to TARC and grown for inoculation.

By the above-mentioned process, the backcrossed progenies carrying each resistance gene were advanced until the BC_4F_2 generation. The BC_4F_2 plants that were resistant to the BB isolates and in which a recessive gene, such as *xa-5* in IRI545-339, had been introduced were considered to be homozygous for the recessive gene. On the other hand, the homozygosity of the breeding lines in which a dominant gene had been introduced was tested in the BC_4F_3 lines. From the the BC_4F_3 lines, each line was inoculated with a suitable BB isolate and the morphology as compared with that of the recurrent parent. The uniformity regarding plant type was also examined within each line.

Designation of the near-isogenic lines

In the BC_4F_4 generation, or in more advanced generations, each breeeding line was given the name of IR-BB as a near-isogenic line for resistance to BB. The first and second figures of the name refer to the gene number assigned to each resistance gene to BB and the third to the recurrent parent used, that is, IR24 to none, Toyonishiki to 1 and Milyang 23 to 2. For example, the near-isogenic line carrying *Xa-3* gene and backcrossed to Toyonishiki was designated as IR-BB 103 (Table 1).

Table 1. Near-isogenic lines for resistance to bacterial blight of rice developed in 1987 under Japan-IRRI collaboration

R-gene Designation	Generation	Line No.	Cross
Xa-1 (*Xa-12*)[1)]			
IR-BB 1	BC_4F_4	IS630(BLB4674-2-2)	IR24*5/Kogyoku
IR-BB 101	BC_4F_4	IS638(BLB4523-2-4)	Toyonishiki*5/Kogyoku
IR-BB 201	BC_4F_4	IS634(BLB4570-1-2)	Milyang 23*5/Kogyoku
Xa-2 (*Xa-1*)[1)]			
IR-BB 2	BC_4F_5	B 174(BLB4974-3-8-2)	IR24*5/Te-tep
IR-BB 102	BC_4F_5	B 205(BLB5065-41-7-5)	Toyonishiki*5/Te-tep
IR-BB 202	BG_4F_5	B 221(BLB5099-67-1-4)	Milyang 23*5/Te-tep
Xa-3			
IR-BB 3	BC_4F_6	IS 22(BLB3727-3-11-10-15)	IR24*5/Chugoku 45
IR-BB 103	BC_4F_6	IS103(BLB4083-11-1-4-9)	Toyonishiki*5/Chugoku 45
IR-BB 203	BC_4F_6	IS 13(BLB3854-7-7-7-25)	Milyang 23*5/Chugoku 45
IR-BB 3J	BC_4F_6	IS 40(BLB3791-5-12-22-7)	IR24*5/Java 14
IR-BB 103J	BC_4F_6	IS 27(BLB4165-12-13-16-3)	Toyonishiki*5/Java 14
IR-BB 203J	BC_4F_6	IS 37(BLB4023-6-9-6-12-18)	Milyang 23*5/Java 14
IR-BB 3Z	BC_4F_6	IS 74(BLB3769-1-4-11-19)	IR24*5/Zenith
IR-BB 103Z	BC_4F_6	IS283(BLB4111-9-14-18-2)	Toyonishiki*5/Zenith
IR-BB 203Z	BC_4F_6	IS 68(BLB3954-1-5-19-11-12)	Milyang 23*5/Zenith
IR-BB 3S	BC_4F_6	IS 91(BLB3736-5-12-10-11)	IR24*5/Sateng
IR-BB 103S	BC_4F_6	IS 79(BLB4101-1-12-10-10)	Toyonishiki*5/Sateng
IR-BB 203S	BC_4F_6	IS341(BLB3876-6-4-10-12)	Milyang 23*5/Sateng
Xa-4			
IR-BB 4	BC_4F_6	IS110(BLB3759-3-13-26-15)	IR24*5/IR20
IR-BB 104	BC_4F_6	IS 98(BLB4123-1-7-16-7)	Toyonishiki*5IR20
IR-BB 204	BC_4F_6	IS104(BLB3929-2-10-26-8)	Milyang 23*5/IR20

xa-5			
IR-BB 5	BC_4F_5	IS133(BLB3813-2-22-1)	IR24*5/IR1545-339
IR-BB 105	BC_4F_4	IS118(BLB4183-6-1)	Toyonishiki*5/FIR1545-339
IR-BB 205	BC_4F_5	IS299(BLB4047-12-14-4)	Milyang 23*5/IR1545-339
Xa-7			
IR-BB 7	BC_4F_7	IS165(BLB4796-8-7-14-9-3)	IR24*5/DV85
IR-BB 107	BC_4F_4	IS499(BLB7793-12-1)	fToyonishiki*5/DV85
IR-BB 207	BC_4F_5	IS491(BLB7859-5-10-4)	Milyang 23*5/DV85
xa-8			
IR-BB 8	BC_4F_5	IS513(BLB6196-2-20-7)	IR24*5/PI231129
IR-BB 108	BC_4F_5	IS831(BLB6355-3-5-3)	Toyonishiki*5/PI231129
IR-BB 208	BC_4F_5	IS818(BLB6927-5-10-1)	Milyang 23*5/PI231129
Xa-10			
IR-BB 10	BC_4F_6	IS154(BLB3722-1-6-17-9)	IR24*5/CAS209
IR-BB 110	BC_4F_5	IS140(BLB5028-6-4-3)	Toyonishiki*5/CAS209
IR-BB 210	BC_4F_6	IS150(BLB2994-6-4-26-15)	Milyang 23*5/CAS209
Xa-11			
IR-BB 11	BC_4F_4	IS618(BLB4677-2-3)	IR24*5/IR8
IR-BB 111	BC_4F_4	IS627(BLB4513-1-2)	Toyonishiki*5/IR8
IR-BB 211	BC_4F_4	IS624(BLB4538-1-4)	Milyang 23*5/IR8

[1] not segregated.

Reaction of the near-isogenic lines to BB pathogen

The reaction of the development near-isogenic lines to the Japanese and Philippine races of BB pathogen is shown in Table 2.

It should be noticed that only two Myanmor isolates among more than 500 isolates of our collection from Asian countries (YAMAMOTO and OGAWA 1988) were nor virulent to the recurrent parents while they were virulent to a few BB differentials. In addition, one Japanese isolate was nor virulent to IR24 and Milyang 23 while it was virulent to Toyonishiki (YAMAMOTO and OGAWA 1991). We are now developing susceptible lines with the genetic background of the recurrent parents to the three isolates mentioned above.

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Table 2. Reaction[1] of near-isogenic lines to Japanese and Philippine races of bacterial blight pathogen

Near-isogenic lines	Japanese race[2]						Philippine race					
	IA	IB	II	IIIA	IIIB	IV	1	2	3	4	5	6
IR-BB 1	HR	M	S	S	S	S	S	S	S	S	S	S
IR-BB 2	HR	HR	S	S	S	S	S	S	S	S	S	S
IR-BB 3	R^B	R^B	R^B	R^B	R^B	S	R^B	R^B	R^B	R^B	R^B	S
IR-BB 4	R	R	R	R	R	R	R	S	S	M	R	S
IR-BB 5	R	R	R	R	R	R	R	R	R	M	R	S
IR-BB 7	HR	HR	HR	HR	HR	HR	R	HR	HR	S	HR	S
IR-BB 8	R	HR	HR	HR	R	R	R	R	R	M	R	M
IR-BB 10	S	S	S	S	S	S	S	HR	S	S	HR	S
IR-BB 11	S	R	R	R	S	S	S	S	S	S	S	S

[1] Reaction at booting stage, HR: highly resistant, R^B: resistant with browning margin, R: resistant, M: moderately susceptible to moderately resistant, S: susceptible.

[2] Standard isolates for each race are as follows:
T7174 for race IA, T7156 for race IB, T7147 for race II, T7133 for race IIIA, Q6803 for race IIIB, H75373 for race IV, PX061 for race 1, PX086 for race 2, PX079 for race 3, PX071 for race 4, PX0112 for race 5, PX099 for race 6.
The reaction to Japanese race V(H75304) is not included in this Table due to the low virulence.

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白葉枯病抵抗性遺伝子に関するイネの準同質遺伝子系統の育成

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イネ白葉枯病抵抗性に関する研究は主に日本と国際稲研究所(IRRI)，フィリピンで行われてきたが，植物防疫上病菌の相互交換が行えなかったため，両国のみならず各国のイネ白葉枯病抵抗性に関する研究結果は相互に比較検討出来なかった．このため，日本農林水産省とIRRIはイネ白葉枯病抵抗性に関する研究の相互比較を行うと共にその共通基盤を作成するため，1982年に共同研究を開始した．すなわち，日本とIRRIの判別品種をフィリピン産及び日本産白葉枯病菌レースを用いて分析し，抵抗性遺伝子を一つずつもつ準同質遺伝子系統の育成をして，イネ白葉枯病菌レースの国際判別品種を確立することとした．

その結果，1987年に準同質遺伝子系統の一組が育成され（OGAWA *et al.* 1988)，最近その準同質遺伝子系統を供試した研究結果も公表され始めた．このため，その準同質遺伝子系統の育成経過とその育成主体を明らかにするため本報告を行った．

準同質遺伝子系統育成のための反復親としては，日本品種のトヨニシキ，IRRI品種のIR 24及び韓国品種の密陽23号を用いた（OGAWA・YAMAMOTO 1987)．また，抵抗性親としては，日本とIRRIの判別品種及び両判別品種が持たない遺伝子をもつ品種，すなわち白葉枯病抵抗性遺伝子 *Xa-1* から *Xa-12* をもつ品種を供試した．

準同質遺伝子系統育成のための交配は全てIRRIで行った（Fig. 1)．抵抗性遺伝子を次代に導入するため第2回目の戻交配からは7個体以上に房交配を実施し，同じ組み合わせの交配種子をIRRIと日本に二分した．フィリピン産白葉枯病菌レースを使っての抵抗性個体の選抜はBC_1F_1からIRRIで開始したが，日本では年一回しか稲の栽培ができなかったことから，BC_3F_1から日本産白葉枯病菌レースを使っての選抜を行った．劣性遺伝子(*xa-5*，*xa-8*)の導入のためには戻交配したF_2を接種検定して抵抗性遺伝子を含むF_1を同定した．4回の戻交配の後，自家授精によって世代を進めた．優性の抵抗性遺伝子の場合にはBC_4F_2で抵抗性個体の選抜をした後，20系統のBC_4F_3を養成し，接種検定することによって，ホモの抵抗性系統を同定した．劣性遺伝子の場合はBC_4F_2の抵抗性個体を直ちに抵抗性系統として展開した．形態上の選抜と系統の均一性はBC_4F_3から実施した．形態上反復親と類似し，均一性にも問題ないと判断された系統は，BC_4F_4から，準同質遺伝子系統として，命名を開始した．

準同質遺伝子系統にはすべてIR-BBの名を付し，最初の2桁の数字は遺伝子番号を，百の位の数字は反復親を意味するようにした「なし：IR 24，1：トヨニシキ，2：密陽23号)．結果として，36系統を準同質遺伝子系統として命名した(Table 1)．IR 24を反復親とした準同質遺伝子系統は国際判別品種候補として配布されている．これらの系統の日本産及びフィリピン産白葉枯病菌レースに対する反応をTable 2に示めした．なお，供試した反復親はアジア各国からの500以上の白葉枯病菌株に感受性を示したが，ミャンマー産の2菌株及び日本産の1菌株には抵抗性を示めした．また，黄玉及びジャワNo.14の *Xa-12* とTe-tepの *Xa-1* は数千のレベルの分離集団では単独にもつ分離個体は出現しなかった．

この準同質遺伝子系統の育成による国際判別品種の設定はイネの病虫害抵抗性に関しては最初の成果である．