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Introgression of Bcterial Leaf Blight Resistance Gene from Oryza Minuta J.B. Presl. Ex C. B. Presl. into New Rice Type (Oryza sativa L.)

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ABSTRACT

F_1 Hybrids, backcross progenies, advanced introgression lines (2n=24) and monosomic alien addition lines or MAALs (2n=25) were successfully produced following embryo rescue between an elite new plant type (NPT) breeding line of Oryza sativa (2n=24, AA) and a wild species, O. minuta (2n=48, BBCC). F_1 hybrids performance were intermediate between the parents. The F_1 hybrids had 36 chromosomes indicating having 12 chromosome A from O. sativa and 12 B and 12 C from O. minuta. THE BACK CROSS progenies had different chromosome number indicating abnormal meiosis of the hybrids and back cross progenies. Plant with 2n=24 and 25 chromosomes were obtained in BC_4F_1. The hybrids and backcross progenies were susceptible to bacterial leaf blight (BB). However, several of the 2n=24 plants derived resistant plant to bacterial leaf bligkt race 1 of the Philippines races. The gene is different from introgressed gene intio rice from O. longistaminata (Xa21) and from O. Minuta Acc. 101141.

Key words: Oryza minuta, MAALS, Bacterial leaf blight

INTRODUCTION

Rice is important cereal for one third of the world population. During the last three decades, world rice production has doubled from 257 million tons in 1966 to 563 million tons in 1998. More than 90 percent of rice is produced and consumed in Asia. To meet the growing need of an ever increasing human population, 50% more rice is needed by 2025, hence, rice varieties with higher yield potential are needed to meet the global need. To achieve this, IRRI is exploring a new plant type (NPT) of rice which could increase the yield potential by 20% (Khush, 1995). The NPT has the characteristics of few but all productive tillers, large panicles, 200-250 grains per panicles, sturdier stems, deeper root system, thick and dark green leaves and maturity of 115-120 days. Although major increases in rice production have occurred, several biotic and abiotic stresses limit rice production. Moreover, diseases and insect pest are a continued threat, particularly due to changes in insect biotypes and pathotypes. There is, thus, an urgent need to broaden the rice gene pool and identify new genes for resistance to major diseases and insect pests from diverse sources. Wild species are important reservoir of useful genes for rice improvement (Sitch, 1990; Brar and Khush, 1997).

A number of pathogens and insects attack the rice plants. One of the major rice diseases is bacterial blight (BB). Bacterial blight of rice caused by Xanthomonas oryzae pv. Oryzae is one of the important rice production constraints (Mackill, 1986). It reduces grain yield to varying levels depending upon the stage of the crop affected and degree of susceptibility of cultivar. Losses due to bacterial blight in the tropics are higher than in temperate regions (Mizukami and Wakimoto, 1969) because of the prevalence of more virulent populations of the pathogen (Buddenhagen and Reddy, 1972). Xanthomonas oryzae pv. Oryzae (Xoo) produces bacteriocin like substances on solid media (Mew, 1987). The proteins function as signal molecules which affect host-specific plants responses such as cell division, water soaking (i.e.,filling of the cellular spaces in the leaf mesophyll with water instead of air) and the hypersensitive response. The first evidence for pathogenic specialization was reported in 1979 by Vera-Crus and Mew. They recognized four bacterial groups where interaction was confined to a specific cultivar-isolate combination. "Race" was adopted to classify the bacterial isolates. Each race has specific virulence to

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varieties with different resistance genes following a gene-for gene concept in the host pathogen interaction. Race 1 of BB was the most prevalent in the Philippines in the late 1960' s and early 1970's (Khush *et al.*, 1988). Most of the IRRI varieties had Xa4 that convered resistance to BB race 1. Therefore, races 2 and 3 have become more prevalent in the Philippines. In the Philippines, 10 races are currently recognized (Angeles, personal communication). Some genes have same reaction to BB races of the Philippines but different to BB races of Japan (Sakaguchi, 1967; Ogawa, 1987; Yamamoto and Ogawa, 1990).

The genus *Oryza* to which cultivated rice belongs has more then 20 wild species. These wild species have 2n = 24 or 48 chromosomes representing nine genomes, AA, BB, BBCC, CC, CCDD, EE, FF, GG, and HHJJ (Vaughan 1994; Aggarwal *et al.*, 1997). Of the two cultivated species, *O.sativa* is grown worldwide whreas *O. glaberrima* is cultivated in limited area of West Africa. One of the wild species, namely, *O. minuta* J.S. Presl. Ex C.B. Presl. (2n = 48, BBCC) possesses genes for resistance to brown planthopper (BPH), whitebacked planthopper (WBPH), bacterial blight (BB), blast and sheath blight. However, several crossability barriers such as high sterility, limited homoeologous chromosome pairing and recombination between the genomes of cultivated and wild species, and hybrid breakdown restrict alien introgression and transfer of useful genes into cultivated plants (Brar and Khush, 1986, 1997). One of approaches involves the use of embryo rescue can be used to produce wide-cross derivatives.

A number of interspecific hybrids, monosomic alien addition lines (MAALs) and advanced introgression lines have been produced from crosses of rice and several wild species (Jena and Khush, 1989; Multani *et al.*, 1994; Brar and Khush, 1997). Some useful genes for resistance to grassy stunt virus, BB, BPH, blast, and cytoplasmic male sterility have been transferred (Khush *et al.*, 1977; Khush *et al.*, 1990; Amante - Bordeos *et al.*, 1992; Jena dan Khush, 1990; Damalcio *et al.*, 1995, 1996; Brar and Khush , 1997). The specific objectives of the investigation were (1) to produce introgression lines (2n=24) from *O. sativa* x *O. minuta* following backcrossing and embryo rescue procedures, and (2) to evaluate introgression lines produced for resistance to bacterial leaf blight.

MATERIALS AND METHODS

Production of Advanced Backcross: Progenies from the Cross of O. sativa x O. minuta

Production of Hybrids and Backcross Progenies

The present investigation was carried out from August 1995 to September 2000 in the Wide Hybridization Laboratory, Plant Breeding, Genetics and Biochemistry (PBGB) Division of the International Rice Research Institute (IRRI), Los Banos, Laguna, Philippines. This Experiment focused on the production of a series of introgression lines (2n=24) from the cross of *O. sativa* x *O. minuta*. Crosses were made between an elite breeding line. NPT (IR65600-81-5-3-2) of rice as female parent with *O. minuta* (Acc. 101089) as male. F_1 plants from seeds obtained were produced through embryo rescue and these were then used to produce advanced backcross progenies using NPT as the recurrent parent.

The standard embryo rescue procedure used in Wide Hybridization Laboratory of IRRI was followed in this experiment. The panicles were sprayed with 75 ppm gibberilic acid (GA_3) solution one day after pollination. Spraying was continued for three successive days. Fourteen days after pollination, spikelets were harvested and surface sterilized in sodium hypochlorite solution (20 % of the commercial grade) supplemented with 2 drops of Tween-20. After washing them in sterilized water, the delicate young embryos were excised and isolated using a stereomicroscope under aseptic conditions under sterile air-flow cabinet. The isolated embryos were cultured in ¼ MS medium and incubated in the dark (25 ^{o}C) until germination. The seedlings were then incubated in a lighted incubation room up to the three-leaf stage. These three leaf stage seedlings were cultured in Yoshida liquid solution (Yoshida *et al.*, 1976) in the IRRI phytotron for 10-15 days. Later, the healthy seedlings with well-developed roots were transplanted to soil in pots.

The fertile plants derived through embryo rescue were selfed to produce introgression lines. Selfed progenies of monosomic alien addition line (MAALs) and introgression lines (2n=24) in the genetic background of NPT were used for evaluation of the transfer of useful genes from *O. minuta* into rice.

Morphological Characteristics of O. sativa x O. minuta Hybrids

Data on morphological characteristics of the parents and hybrids were recorded. Five plants were taken at random and data on plant height, number of tillers, leaf length and width, panicle length, floret

length, awn length, and number of floret per panicle were recorded. Growth habit, grain shattering, and color of leaf, apiculus, stigma, and awns were also observed.

Pollen and Floret Fertility

Pollen fertility was determined from at least 500 pollen grains from each of parents, hybrids, and backcross progenies. The spikelets were collected near anthesis ad immediately stained with 2 % acetocarmine. Stained and round pollen grains were counted as fertile while the unstained, lightly stained and shriveled ones were counted as sterile. Pollen fertility was determined using the following formula :

$$\text{Pollen fertility (\%)} = \frac{\text{Number of fertile pollen grains}}{\text{Total number of pollen grains}} \times 100$$

Floret fertility was determined from five panicles of each plant using similar formula as follows :

$$\text{Florey fertility (\%)} = \frac{\text{Number of filled florets}}{\text{Total number of florets}} \times 100$$

Meiotic Chromosome Analysis

Chromosome analysis was carried out from meiotic pollen mother cells of parents, F_1 hybrids and backcross progenies. Young panicles representing different stages were collected, between 8:00 to 10:00 AM. These panicles were fixed at room temperature in a fresh solution of acetic-alcohol (1 part glacial acetic acid and 3 parts of 95 % ethanol) to which traces of ferric chloride were added. After 24 hours of fixation, panicles were transferred to 70 % ethanol and stored at 4 ^{o}C until used. Squashes were made from anthers in 2 % acetocarmine. Chromosome number of parents, F1, BC_1F_1, BC_2F_1, BC_3F_1, and BC_4F_1, progenies at diakinesis and metaphase I.

Evaluation of Introgression Lines for the Transfer of Alin Genes for Resistance to Biotic Stresses

Alien introgression lines derived from *O. sativa* x *O. minuta* along with parents were evaluated for introgression of resistance to bacterial leaf blight (BB). The parents, hybrids, and backcross progenies were inoculated with six Philippine races (1, 2, 3, 4, 5, 6) of *Xanthomonas oryzae* pv. *Oryzae* (Xoo) while introgression lines were inoculated with three races (1, 6, 9).

Seven Philippine races of Xoo: PXO61 (race 1); PXO86 (race 2); PXO79 (race 3); PXO71 (race 4); PXO112 (race 5); PXO099 (race 6) and PXO339 (race 9) were used for inoculation. The bacteria were transferred to slants of potato semisynthetic agar medium and incubated at 30 ^{o}C for 3 days. Inoculum was prepared by suspending the bacterial mass with sterilized water to a concentration of about 10^9 cells/ml. Leaf blades were inoculated by the clipping technique (Kauffman *et al.*, 1973) at the maximum tillering stage. Evaluation for resistance was done at 14 days after inoculation by lesion length measurement. Plant were classified as resistant, moderately resistant or susceptible based on lesion length: resistant, (R) = 0-6.0 cm; moderately resistant (MR) = 6.1 – 8.0; and susceptible (S) > 8.0 cm.

REULTS AND DISCUSSIONS

Production of Advanced Backcross Progenies from Cross of O. sativa x O. minuta

Production of Hybrids and Backcross Progenies

F_1 hybrids. On the everage, 56.1% seed setting was obtained (906 out of 1616 pollinated florets). However, only 35.4% of the seeds from the cross between NPT of rice and *O. minuta* had embryos (Table 1). After 10 – 15 days of pollination, embryos were excised and cultured on MS medium. Out of the 321 embryos cultured, only 37.7% germinated into F_1 seedlings. In this study, seed setting in hybrids between *O. sativa* and *O. minuta* was much higher than in previous studies. (Sitch *et al.*,1990) reported 4% seed set while (Mariam *et al.*, 1996) obtained 14.8% seed set.

BC_1 progenies. The F_1 hybrids were backcrossed to NPT using the latter as pollinator. On the average, 10.8% florets set seed and majority (94.6%) of the seeds did not have embyo but were only watery. Of the 19 BC_1 F_1 progenies from original F_1 hybrids and colchicine-treated F_1 hybrids. They reported a little higher BC_1 F_1 production from colchicine-treated compared to untreated hybrids, 0.12 and 0.03% (Amante-Bordeos *et al.*, 1992), and 1.2 and 0.63% (Mariam *et al.*, 1996), respectively. Amante-Bordeos et al., (1992) obtained 6 BC_1F_1 plants from 47,300 pollinated florets.

BC_2F_1 progenies. BC_1F_1 plants of crosses between NPT and *O. minuta* Acc. 101089 were pollinated with tha recurrent panent to produce BC2F1. Of 16,682 florets pollinated, 14.5% set seed (Table 1). Less than 1% of the BC_2 F_1 seed had embryos. Only 8 plants could be produed from 16,862 pollinated florets in BC_2 F_1. The BC_2 F_1 plant were produced from a BC_1 F_1 plant, IS 14531. Jena and Khush (1989) reported that seed set in *O. sativa x O. minuta officinalis* crosses ranged from 8.8 to 17.3%, while Multani *et al.* (1996), 0.57 and 2.4%, respectively. Twenty-three BC_2 F_1 plant were produced from 35,000 pollinated florets (Amante-Berdeos *et al.*,1992).

Tabel 1. Production of hybrids and backcross progenies from a cross between new plant type (NPT) rice female, *O. sativa* (IR65600-81-5-3-2) and *O. minuta*.

Cross combination	Female parent		Florest Pollinated (No)	Seed Set		Seed With Embryo		Embryo Produced Seedling	
	Gene-ration	Chromo-some no. (2n)		(No.)	(%)	(No.)	(%)	(No.)	(%)
F1 hybrid :									
NPT x *O. minuta* (101089)	Parent	24	1616	906	56.1	321	35.43	121	37.7
BC_1F_1 :									
(NPT x 101089) x NPT	F_1	36	3297	356	10.8	19	5.34	6	31.6
BC_2F_1 :									
- WHDIS1453-1 x NPT	BC_1	35	9642	1509	15.6	24	1.59	8	33.3
- WHDIS1525-1 x NPT	BC_1	40	7220	1096	15.2	0.0	0.00	0	0.0
	Total		16862	2605	14.5	24	0.92	8	33.3
BC_3F_1 :									
- WHDIS1668-1 x NPT	BC_2	33	5304	528	9.9	26	4.92	3	11.5
- WHDIS1669-1 x NPT	BC_2	34	3746	1375	36.7	57	4.15	8	14.0
- WHDIS1963-1 x NPT	BC_2	36	3790	781	20.6	2	0.30	0	0.0
	Total		18945	2799	14.8	37	1.38	11	21.6
BC_4F_1 :									
- WHDIS1874-1 x NPT	BC_3	26	257	77	30.0	27	35.06	21	77.8
- WHDIS1875-1 x NPT	BC_3	31	134	0	0.0	0	0.00	0	0.0
- WHDIS1878-1 x NPT	BC_3	24	316	186	58.9	2	1.08	0	0.0
- WHDIS1878-2 x NPT	BC_3	26	438	222	50.7	15	51.80	54	47.0
- WHDIS1881-1 x NPT	BC_3	29	469	15	3.2	0	0.00	0	0.0
- WHDIS1881-2 x NPT	BC_3	-	411	59	14.4	0	0.00	0	0.0
	Total		2025	559	27.6	44	2579	75	52.1

Table 2. Morphological characteristics of parents and hybrids of a cross between NPT rice, *O. sativa* (IR65600-81-5-3-2) and *O. minuta* (Acc.101089)

Trait	*O.sativa*	F_1 Hybrids	*O.* Minuta
Plant height (cm)	100.94	96.26	97.28
No. of tiller	9.80	28.20	112.40
Leaf length (cm)	62.38	37.32	17.78
Leaf width (cm)	2.12	1.88	1.48
Panicle length (cm)	25.16	23.56	17.28
Floret length (mm)	6.00	5.80	4.60
Awn length	awnless	2.52	2.30
No. of floret/panicle	264.60	116.60	98.80
Leaf color	dark green	dark green	dark green
Apiculus color	purple	purple	purple
Stigma color	purple	purple	purple
Awn color	awnless	colorless	colorless
Grain shattering	not	shattering	shattering
Growth habit	hattering erect	intermediate	spreading

BC_3 F_1 progenies. The BC_2 F_1 plants from the cross of NPT x *O. minuta* 101089 were backcrossed with the recurrent parent to produce BC_3 F_1. Out of 18,945 pollinated florets, 14.8 % set seeds (Table 1). However, majority of the seeds was without embryos (1.4 % of seeds obtained). In BC_3F_1, only 11 plants were produced from 18,945 pollinated florets. The plants were produced from 2 plants out of 3 plants used in backcrossing. In BC_3, Amante-Bordeos *et al.* (1992) reported 22.8 and 100 % seedset from two BC_2F_1 (2n=24) plants, however, only 0 to 4.4 % seedset from BC_2F_1 plants.

BC_4F_1 progenies. Six BC3F1 plants having 24 to 36 chromosomes were backcrossed to recurrent parent (Tabel 1). Of the 2025 pollinated florets, 75 BC_4 F_1 plants could be obtained. These plants (WHDIS 1874-1, WHDIS 1878-2) each possessing 2n=26 chromosomes. A BC_3 F_1 plant (WHDIS 1875-1) with 2n=31 chromosomes did not set seed upon backcrossing with NPT. One of the plants (WHDIS 1878-1) with 2n=24 was also found to be highly sterile and did not produce any BC_4 F_1 progeny. Another plant (WHDIS 1881-1) with 29 chromosomes set only 3.02 % seed and all seeds were abortive and lack viable embryos. In this study, introgression lines (2n=24) and MAALs (2n=25) of *O. sativa* x *O. minuta* were obtained in BC_4 F_1 progenies. Procedure in producing the interspecific hybrid *O. sativa* (IR65600-81-5-3-2) x *O. minuta* (101089) is shown in Figure 1.

Morphological Characteristics Of O. sativa x O. minuta Hybrids

The hybrids were intermediate between their parents in many characteristics, e. g., plant height, leaf length and width, panicle length, and growth habit (Tabel 2). The key distinguishing characteristics of *O. minuta* included narrower and smaller leaves, heavy seed shattering, presence of awn, spreading growth habit, and reduced floret size. Mariam *et al.* (1996) reported that hybrids of *O. sativa* x *O. minuta* were similar to the wild parent, *O. minuta.*

Chromosome number, and pollen and floret fertility

Chromosome number of parents, F_1 hybrids and backcross derivatives was studied. Chromosome analysis showed 2n=24 chromosomes in *O. sativa* and 2n=48 in *O. minuta.* The F_1 hybrids had 36 chromosomes; indicating meiosis was normal in the two parents with *O. sativa* and *O. minuta* respectively. In back cross progeny, six BC_1F_1s had 35 and 40 chromosomes, eight BC_2F_1s had 33 to 36, 11 BC_3F_1s had 24 to 26, and 75 BC_4F_1s had 24 -26; indicating that meiosis in F_1s and backcross progenies was not normal. The seventy-five BC_4F_1 plants, of these 23 plants had 2n=24 chromosomes with 12 bivalents, while 51 had 2n=25 with 12 bivalents and two univalents. Therefore, they were designated as introgression lines, monosomic alien addition lines (MAALs) and was double monosomic alien addition lines (DMAALs), respectively. In the cross between *O. sativa* and *O. minuta*, plants with 2n=24 chromosomes were obtained in BC_2F_1 progenies derived from colchicine-treated F_1 hybrids (Amante-Bordeos *et al,*. 1992; Mariam *et al.,* 1996). Amante-Bordeos *et al.* (1992) produced 15 plants with 24 chromosomes, 7 with 25, and two others with 27 and 29. MAALs were also obtained from the cross of *O. sativa* and *O. australiensis* (Multani *et al.*, 1994).

Data on pollen fertility as determined by stainability in 2% acetocarmine and spikelet fertility were recorded. On spikelet fertility average, *O. sativa* and *O. minuta* showed 93% and 88.5% pollen fertility, and 89.6% and 85.4%, respectively. The hybrids showed very low pollen fertility (0.4 to 2.2%) (Tabel 3) and so did the BC_1F_1s (0 to 1.64 %). Pollen fertility in BC_2F_1 ranged from 1.42 to 4.06% (Table 3). The BC_3F_1 plants with 2n=26 chromosomes showed 0 to 31.9% pollen fertility. Relatively high pollen fertility was found in BC_4F_1 (12.7-97.5%), but some of them are sterile. All hybrids and early back cross progenies had less than 40% pollen fertility were sterile (0% floret fertility) while the others were partly sterile to sterile. Complete male sterility (0% pollen fertility) of hybrids between *O. sativa* and *O. australiensis* was reported by Multani *et al.* (1994). High pollen sterility (99.57 to 99.37% unstained pollen) of hybrids between *O. sativa* and *O. minuta* was also reported by Mariam *et al.* (1996).

The morphological characters of all BC_4F_1 plants were similar to rice. Most of the plants with 24 chromosomes were NPT type in appearance. Pollen grains of most BC_4F_1 plants observed were fertile to partially sterile (12.7-97.5% fertility) (Table 3). Thus they were selfed and the BC_4F_2 seeds were used for screening for introgression genes for resistance of diseases and insects.

Table 3. Chromosome number and pollen fertility in the parents, hybrid, and backcross progenies of a cross between NPT rice, *O. sativa* (IR65600-18-5-3-2) and *O. minuta* (101089).

Line	Number of Plant Observed	Chromosome Number (2n)	Fertility (%)[1]	
			Pollen (Range)	Floret (Range)
IR65600-18-5-3-2	1	24	93.0	89.6
O. minuta (101089)	1	48	88.5	85.4
F1	25	36	0.4 – 2.2	0.0
BC_1F_1	3	35-40	0.0 – 1.6	0.0
BC_2F_1	4	33-36	0.0 – 4.1	0.0
BC_3F_1	11	24-29	0.0 – 31.9	0.0
BC_4F_1	75	24-26	12.7 – 97.5	0.0 – 91.5

Table 4. Reaction of BC_4F_2 plants derived from a cross between NPT rice, *O. savita* (IR65600-81-5-3-2) and *O. minuta* 101089) to race 1 of bacterial blight, 14 days after inoculation.

Material	Gene-ration	Chromo-some No. (2n)	BB LESION (14 DAI)[1]		Morphological Traits
			Cm	Score	
Parent, F_1 and BC_1					
IR65600-81-5-3-2	Parent	24	44.34	R	NPT
O. minuta (101089)	Parent	48	1.4	R	spreading, small tillers, narrow, short leaves
O. minuta (101141)	Parent	48	0.02	R	spreading, small tillers, medium, leaves medium
HWDIS1270-7	F_1	36	13.6	S	spreading, intermediate leaf
HWDIS1453-1	BC_1	35	22.3	S	spreading, long and wide leaves
Introgression lines					
HWDIS1958-5-1	BC_4F_2	24	5.94	R	NPT type, medium dense panicle
HWDIS1958-5-8	BC_4F_2	24	5.10	R	NPT type, late maturity
HWDIS1958-9-1	BC_4F_2	24	4.54	R	NPT type
HWDIS1958-9-3	BC_4F_2	24	4.62	R	NPT type
HWDIS1958-9-10	BC_4F_2	24	4.72	R	NPT type party fertile
HWDIS1958- 9-11	BC_4F_2	24	3.64	R	NPT type, awned, sterile
HWDIS1958-10-4	BC_4F_2	24	5.56	R	NPT type, late maturity
HWDIS1958-10 -5	BC_4F_2	24	5.24	R	NPT type, late maturity
HWDIS1958-10-6	BC_4F_2	24	4.76	R	NPT type, late maturity
HWDIS1958-10-9	BC_4F_2	24	5.02	R	NPT type, late maturity
HWDIS1958-19-3	BC_4F_2	24	4.34	R	NPT type, late maturity
HWDIS1958-19-6	BC_4F_2	24	4.72	R	NPT type, late maturity
HWDIS1958-19-7	BC_4F_2	24	2.28	R	NPT type, medium densed psnicle
HWDIS1958-19-12	BC_4F_2	24	5.28	R	NPT type
HWDIS1958-22-2	BC_4F_2	24	4.70	R	NPT type, late maturity
HWDIS1958-22-3	BC_4F_2	24	5.30	R	NPT type, short
HWDIS1958-29-2	BC_4F_2	24	2.40	R	NPT type, very late maturity
HWDIS1958-29-5	BC_4F_2	24	4.56	R	NPT type
HWDIS1958-29-9	BC_4F_2	24	1.50	R	NPT type, awned
HWDIS1958-34-4	BC_4F_2	24	2.38	R	NPT type, very late maturity
HWDIS1958-34-5	BC_4F_2	24	4.16	R	NPT type
HWDIS1958-4-3	BC_4F_2	24	3.88	R	NPT type,late, dense panicle
HWDIS1957-17-4	BC_4F_2	24	4.02	R	NPT type, medium densed panicle
HWDIS1959-5-2	BC_4F_2	24	4.96	R	NPT type, very late maturity
HWDIS1959-5-3	BC_4F_2	24	5.67	R	NPT type, medium densed panicle
HWDIS1959-5-11	BC_4F_2	24	4.96	R	IR type, late maturity
IR 54 (R control)	-	24	2.20	R	semi dwarf variety
IR24 (S control)	-	24	14.06	S	semi dwarf variety

[1])DAI = days after inoculation; 0 – 6 cm = R (resistant); 6.1 – 8.0 cm = MR (moderately resistant); > 8.1 = S (susceptible).

Table 5. Reaction of BC4F3 lines derived from a cross between NPT rice, *O.savita* (IR65600-81-5-3-2) and *O. minuta* (101089) to race 1 of bacterial blight, 14 days after inoculation.

Material	Gene-ration	Chromo-some No. (2n)	BB LESION (14 DAI)[1]		Morphological Traits
			Cm	Score	
Parent, F_1 and BC_1					
IR65600-81-5-3-2	Parent	24	28.15	S	NPT line, wide, long thick leaves
O. minuta (101089)	Parent	48	1.2	R	spreading, small tillers, narrow, short leaves
O. minuta (101141)	Parent	48	0.01	R	spreading, small tillers, medium, short leaves
HWDIS1270-7	F_1	36	12.4	S	spreading, intermediate leaf
HWDIS1453-1	BC_1F1	35	20.1	S	spreading, long and wide leaves
HWDIS1958-19	BC_4F_1	24	10.9	S	NPT type,
Introgression lines					NPT type,
HWDIS1958-5-1	BC_4F_3	24	11.0	S	NPT type,
HWDIS1958-9-1	BC_4F_3	24	11.9	S	NPT type,
HWDIS1958-9-3	BC_4F_3	24	12.9	S	NPT type,
HWDIS1958-9-10	BC_4F_3	24	8.8	S	NPT type,
HWDIS1958-10-5	BC_4F_3	24	16.7	S	NPT type,
HWDIS1958-10-6	BC_4F_3	24	10.5	S	NPT type,
HWDIS1958-10-9	BC_4F_3	24	13.0	S	NPT type,
HWDIS1958-19-3	BC_4F_3	24	4.5	R	NPT type,
HWDIS1958-19-6	BC_4F_3	24	15.0	S	NPT type,
HWDIS1958-19-7	BC_4F_3	24	4.9	R	NPT type,
HWDIS1958-19-12	BC_4F_3	24	5.9	R	NPT type,
HWDIS1958-22-2	BC_4F_3	24	11.2	S	NPT type,
HWDIS1958-22-3	BC_4F_3	24	17.3	S	NPT type,
HWDIS1958-22-22	BC_4F_3	24	5.8	R	NPT type,
HWDIS1958-29-5	BC_4F_3	24	7.9	MR	NPT type,
HWDIS1958-29-8	BC_4F_3	24	22.3	S	NPT type,
HWDIS1958-29-9	BC_4F_3	24	9.2	S	NPT type,
HWDIS1958-29-31	BC_4F_3	24	7.1	MR	NPT type,
HWDIS1959-5-2	BC_4F_3	24	9.6	S	NPT type,
HWDIS1959-5-3	BC_4F_3	24	13.5	S	NPT type,
HWDIS1959-5-11	BC_4F_3	24	13.1	S	NPT type,
HWDIS1957-4-3	BC_4F_3	24	5.7	R	NPT type,
HWDIS1958-17-4	BC_4F_3	24	8.0	MR	NPT type,
IR54 (R control)	-	24	2.4	R	semi dwarf variety
IR24 (S control)	-	24	26.0	S	semi dwarf variety

[1] DAI = days after inoculation; 0 – 6 cm = R (resistant); 6.1 – 8.0 cm = MR (moderately resistant); > 8.1 = S (susceptible).

Evaluation of Introgression Lines for the Transfer of Alien Genes for Resistance to Bacterial Leaf Blight

The parents, *O.sativa* (NPT), IR 65600-81-5-3-2 and *O. minuta* (Acc. 101089) F_1 hybrids, and BC progenies were inoculated with six Philippine races of bacterial leaf blight (BB). The NPT was found to be susceptible to all the six races while *O. minuta* was resistant. The hybrids were found to be susceptible to all six races. Similarly, all BC_1F_1 to BC_4F_1 plants of were also susceptible to 6 BB races. Forty-four lines of BC_4F_2 derived from BC_4F_1 plants with 24 and 25 chromosomes were trasplanted in the screenhouse. The plants were inoculated with race 1 and race 9. The NPT, *O. minuta*, and BC_4 plants were resistant to race 9. The NPT parent was susceptible to race 1 while *O. minuta* was resistant. Twenty-six plants from 11 of the 44 lines tested showed resistance. These plants had lesion length of 1.50 to 5.96 cm 2 weeks after inoculation (Table 4).

The BC_4F_3 progenies derived from resistant BC_4F_2 plants were inoculated with races 1 and 6 BB in the screenhouse. Although BC_4F_2 lines were not tested for resistance to race 6, BC_4F_3 lines derived from plants reistant to BB race 1 were tested for resistance to race 6. All BC_4F_3 lines were found susceptible to race 6. Five lines showed resistance to race 1 (lesion length less than 6.0 cm), three, moderate resistance (7.1 and 8.0 cm); and 20, susceptibility (11.2- 22.3 cm) (Table 5). Amante-Bordeos et al. (1992) reported that all hybrids of *O.sativa* x *O. minuta* Acc. 101141 were resistant to race 1 to 6 of BB of the Philippines. However, a BC_2 plant with 24 chromosomes was only resistant against races 2, 3, 5, and 6 of BB of the Philippines. The rice parent, IR31917-45-3-2, was susceptible to race 6, moderately susceptible to races 2, 3, and 4, and resistant to races 1 and 5.

The rice parent had Xa4 gene for BB and, therefore, was resistant to races 1 and 5. Similarly, Mariam et al. (1996) found that all hybrids of *O.sativa* x *O. minuta* Acc.101141 were resistant to five Malaysian isolates of BB. However, a BC_2 plant with 24 chromosomes was found resistant to isolates XO66, XO99, XO257, and XO319 and moderately resistant to XO100, in this study, all hybrids, BC_1F_1, BC_2F_1, BC_3F_1 and BC_4F_1 progenies of O.sativa (NPT) x O. minuta (Acc.101089) were susceptible to races 1 to 6 of BB the Philippines. Out of 44 BC_4F_2 tested for reaction to BB races 1 and 6, 26 plants of 11 lines were found resistant to race 1. No plants were resistant to race 6. in BC_4F_3 generation, of the 26 plants resistant to race 1, five lines showed resistance, 3 moderate resistance, 3 moderate susceptibility and the others susceptibility to race 1. These results show that a gene for resistance to BB has been transferred from *O. minuta* (Acc.101089) into O.sativa (NPT). However, this gene differs from the gene transferred in the previous studies (Amante-Bordeos *et al.*, 1992; Mariam *et al.*, 1996). Since all F_1 hybrid and first backcross progenies were susceptible to BB race 1 and the resistant plants were found in the segregating population (BC_4F_2), the gene for resistance to BB race 1 is, therefore, a recessive one. The introgression lines were resistant to BB race 1, but susceptible to race 6, further supporting the conclusion that the gene introgressed for BB resistance is different from previous genes transferred from wild species, such as Xa21 from *O.longistaminata* which is resistant to races 1 to 6 (Khush et al., 1990) as well as transferred from *O. minuta* Acc.101141) which is resistant to race 2, 3, 5 and 6 (Amante-Bordeos *et al.*, 1992). The results suggest that the gene introgressed from *O. minuta* Acc.101089 into NPT is new.

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