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Transfer of bacterial blight and blast resistance from the tetraploid wild rice *Oryza minuta* to cultivated rice, *Oryza sativa*

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Summary. *Oryza minuta* J.S. Presl ex C.B. Presl is a tetraploid wild rice with resistance to several insects and diseases, including blast (caused by *Pyricularia grisea*) and bacterial blight (caused by *Xanthomonas oryzae* pv. *oryzae*). To transfer resistance from the wild species into the genome of cultivated rice (*Oryza sativa* L.), backcross progeny (BC_1, BC_2, and BC_3) were produced from interspecific hybrids of *O. sativa* cv 'IR31917-45-3-2' (2n = 24, AA genome) and *O. minuta* Acc. 101141 (2n = 48, BBCC genomes) by backcrossing to the *O. sativa* parent followed by embryo rescue. The chromosome numbers ranged from 44 to 47 in the BC_1 progeny and from 24 to 37 in the BC_2 progeny. All F_1 hybrids were resistant to both blast and bacterial blight. One BC_1 plant was moderately susceptible to blast while the rest were resistant. Thirteen of the 16 BC_2 progeny tested were resistant to blast; 1 blast-resistant BC_2, plant 75-1, had 24 chromosomes. A 3 resistant: 1 susceptible segregation ratio, consistent with the action of a major, dominant gene, was observed in the BC_2F_2 and BC_2F_3 generations. Five of the BC_1 plants tested were resistant to bacterial blight. Ten of the 21 BC_2 progeny tested were resistant to Philippine races 2, 3, and 6 of the bacterial blight pathogen. One resistant BC_2, plant 78-1, had 24 chromosomes. The segregation of reactions of the BC_2F_2, BC_2F_3, and BC_2F_4 progenies of plant 78-1 suggested that the same or closely linked gene(s) conferred resistance to races 2, 3, 5, and 6 of the bacterial blight pathogen from the Philippines.

Key word: Disease resistance – *Oryza* species – Interspecific hybridization – *Xanthomonas oryzae* pv. *oryzae* – *Pyricularia grisea*

Introduction

Wild *Oryza* species represent a rich, largely untapped source of resistance to biotic and abiotic stresses, most notably to insect pests (Heinrichs et al. 1985) and diseases (Sitch 1990). Compared to other important cereals such as wheat, alien gene transfer in rice has received limited attention (Brar and Khush 1986). Khush (1977) transferred dominant gene for grassy stunt virus resistance from *O. nivara* to *O. sativa*, and more recently, resistance to brown planthopper and whitebacked planthopper was incorporated from *O. officinalis* into cultivated rice (Jena and Khush 1990).

As part of an effort to broaden the genetic base of cultivated rice through wide hybridization, *O. minuta* J.S. Presl ex C.B. Presl, an allotetraploid species native to Asia (2n = 48), was used as a donor of useful traits. *O. minuta* has a BBCC genome, distinct from that of *O. sativa* (genome AA). Hybrid sterility and a low frequency of chromosome pairing, and hence limited recombination, are strong barriers to gene transfer between the two species (Sitch et al. 1989).

O. minuta is a potential source of resistance to two important rice diseases: rice blast caused by *Pyricularia grisea* Sacc. (synonymous with *P. oryzae* Cavara, Rossman et al. 1990) and bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (ex Ishiyama 1922) nom. rev. or Xoo [synonymous with *X. campestris* pv. *oryzae* (Ishiyama 1922) Dye 1978; Swings et al. 1990]. Twenty-eight *O. minuta* accessions tested with Philippine Xoo

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strains PXO86 (race 2) and PXO99 (race 6) were all found to be resistant (R. Nelson unpublished). Of the 40 *O. minuta* accessions tested in the Blast Nursery at the International Rice Research Institute (IRRI), 39 were resistant and 1 had intermediate resistance to the *P. grisea* population. Of the 37 accessions tested with *P. grisea* strain PO6-6, 34 were resistant and 3 showed intermediate resistance (R. Nelson unpublished).

The objective of this research was to transfer blast and bacterial blight resistance from *O. minuta* into cultivated rice. In this paper, we describe the production of backcross derivatives of *O. sativa* 'IR31917-45-3-2'/*O. minuta* Acc. 101141 F_1 hybrids, and the chromosome numbers, morphology, and fertility of the F_1 hybrids and backcross derivatives. We also describe the reaction of the parents and their interspecific derivatives to blast and bacterial blight.

Materials and methods

Plant materials

The *O. sativa* parent, 'IR31917-45-3-2', is an elite breeding line that is susceptible to strain PO6-6 of the rice blast fungus and to the natural inoculum at the IRRI Blast Nursery. It is also susceptible to race 6 of the bacterial blight pathogen, and is moderately susceptible to susceptible to Xoo races 2, 3, and 4 in the Philippines. However, the *O. sativa* parent is homozygous for a dominant gene, *Xa*-4 (Jena and Khush 1990), that confers resistance to races 1 and 5 of Xoo (Petpisit et al. 1977; IRRI Annual Report 1989).

O. minuta Acc. 101141, the resistance donor, was obtained from the International Rice Germplasm Center. It was shown to exhibit a high level of resistance when exposed to natural inocula of *P. grisea* at the IRRI Blast Nursery and when artificially inoculated with *P. grisea* strain PO6-6. It is also highly resistant to the six Xoo races from the Philippines.

The starting materials consisted of F_1 hybrids and partly fertile plants from cholchicine-treated axillary buds of the F_1 hybrids. These materials were produced by the Wide Hybridization Project, Division of Plant Breeding, Genetics and Biochemistry, IRRI (Sitch et al. 1989). Successive backcrosses to the *O. sativa* parent were made to obtain BC_2 and BC_3 progenies.

Pathogen cultures

P. grisea strain PO6-6 was used to test for blast resistance in the parents and backcross derivatives of the interspecific hybrids. Fresh cultures of PO6-6 were revived from colonized dry paper disks maintained at 4 °C. To test for bacterial blight resistance, representative isolates of the six Philippine races of Xoo were used: strain PXO61 (race 1), strain PX086 (race 2), strain PXO79 (race 3), strain PXO71 (race 4), strain PXO112 (race 5), and strain PXO99 (race 6). Cultures of these isolates were revived from stock cultures maintained in skim milk or as lyophilized cultures at −20 °C.

Production and culture of backcross progeny

In the backcrosses, 'IR31917-45-3-2' was used as the recurrent male parent. The F_1 hybrids and BC_1 plants were sterile and thus were not emasculated prior to pollination. Partly fertile plants from colchicine-treated axillary buds of the F_1, and the BC_2 plants, were emasculated by clipping the spikelets and removing the anthers by vacuum suction.

To prevent abscission and to aid embryo development, the pollinated panicles were sprayed once daily with a solution of 75 ppm gibberellic acid and 75 ppm naphthalene acetic acid for 14 successive days, starting from the day of pollination. The embryos were rescued 14 days after pollination, following the procedure of Jena and Khush (1984), and were incubated in the dark at 28 °C. When shoots started to develop, they were placed under continuous white fluorescent light. Plantlets were transferred to a hydroponic solution (Yoshida et al. 1976), maintained there for approximately 2 weeks, and then transferred to soil.

Pollen and spikelet fertility

Pollen viability of the derived progeny was determined by staining mature pollen with 1% potassium iodide. Darkly stained pollen grains were considered fertile. At least 500 pollen grains were examined per plant, and the percentage of viable pollen grains was calculated. For each plant, spikelet fertility or seed set, expressed as a percentage of the total number of spikelets, was determined from five fully mature panicles.

Cytology

Root tip squashes were used to determine the chromosome numbers of the F_1 hybrids and backcross derivatives (Islam-Faridi and Sitch 1989). Chromosome pairing at the first meiotic metaphase was examined in the two diploid BC_2 plants, 75-1 (resistant to blast) and 78-1 (resistant to bacterial blight). Squash preparations of the pollen mother cells were stained with acetocarmine.

Disease evaluation

The parents and derived progeny were tested for their reactions to the blast and bacterial blight pathogens. The original *O. minuta* plant used in the hybridization, the F_1 hybrids, and the BC_1 and BC_2 progenies were clonally propagated by separating tillers or by growing axillary buds from nodal cuttings. These clones were used for inoculation tests.

For the evaluation of blast resistance, vegetatively propagated plants at the 3- to 4-leaf stage and 21-day-old seedlings of the *O. sativa* parent line and the BC_2F_2 and BC_2F_3 progenies of the resistant BC_2 plant 75-1 were spray inoculated with a spore suspension of strain PO6-6 or exposed to natural inocula at the IRRI Blast Nursery. The inoculum of strain PO6-6 (ca. 1×10^5 spores/ml) was produced as described by Bonman et al. (1986). After being inoculated with PO6-6, plants were incubated in a dew chamber at 24 °C, 100% relative humidity for 1 day, and then transferred to a mist room at 24 °C under natural light for 5 days. To test their reaction to natural inocula at the IRRI Blast Nursery, plants were placed among five blast-infected *O. sativa* cultivars ('UPLRi-5', 'IR50', 'IR442-2-58', 'IR25587-133-3-2-2-2', and 'Carreon') for 3 days, and then placed in the mist room for another 3 days.

Disease reactions were scored as follows: 0 = no evidence of infection; 1 = with brown specks, no sporulation; 3 = 1–2 mm long, irregularly shaped lesions with brown margins, lesions may coalesce at leaf edges; 5 = less than five 3–7 mm long, typical spindle-shaped lesions per inoculated leaf; 7 = five to many typical lesions per inoculated leaf, lesions often coalesce; 9 = many coalesced lesions infecting 50% or more of inoculated leaf, leaf dies because of lesions. Scores of 0–3 were considered resistant, while scores of 5–9 were considered susceptible (Leung et al. 1988).

Table 1. Production of successive generations of backcross progeny[a] from *O. sativa* 'IR31917-45-3-2'/*O. minuta* Acc. 101141 F_1 hybrids, and the chromosome numbers of the derivatives

Materials backcrossed to IR31917-45-3-2	Spikelets pollinated (*n*)	Seed set (%)	Number of backcross progeny obtained and their chromosome number
F_1	38,000	0.02	2 BC_1 (2n=46)
F_1CT[b]	9,300	0.12	4 BC_1 (2n=44–47 for 3 plants)[c]
BC_1	35,000	0.57	23 BC_2 (2n=32–37 for 18 plants; 2n=24 for 2 plants)[c]
BC_2:			
Plant 75-1 (2n=24)	26	100.0	6 BC_3 (2n=24)
Plant 78-1 (2n=24)	268	22.8	31 BC_3 (2n=24)
Plant 55-1 (2n=35)	1,674	4.4	22 BC_3 (2n=24–29 for 13 plants; 2n=25 for 6 plants; 2n=27 and 29 for 2 plants)[c]
Other BC_2 (2n=32–37)	8,919	0–1.2	6 BC_3 (2n=24 for 2 plants; 2n=25 for 1 plant)[c]

[a] IR31917-45-3-2 is the recurrent male parent
[b] Partially self-fertile F_1 derivatives resulting from colchicine treatment
[c] Chromosome number was not determined for all progeny

For BC_2F_2, BC_2F_3, and BC_2F_4 plants, correlations between reactions of different races were analyzed using the program "Statworks" for the Macintosh computer.

Clones of *O. minuta* and the F_1, BC_1, and BC_2 derivatives were inoculated when several tillers were available. To prepare the inoculum, fresh subcultures of Xoo strains were inoculated to slants of peptone sucrose agar (Goto 1970 in Karganilla et al. 1973) and incubated for 72 h at 30 °C. The growth on one slant was suspended in 10 ml sterile water (ca. 1×10^9 cells/ml).

The bacterial cell suspension was applied to the two youngest fully expanded leaves of each tiller by clipping 3–4 cm from the tip of the leaf using a pair of scissors dipped in the inoculum. Lesion length (LL) was measured 14 days after inoculation. Lesion lengths of 0–3.0 cm were scored as resistant (R); LL of 3.1–6.0 cm were scored as moderately resistant (MR); LL of 6.1–9.0 cm were scored as moderately susceptible (MS); and lesion lengths of 9.1 cm or greater were scored as susceptible (S) reactions (Machmud 1978).

For BC_2F_2, BC_2F_3, and BC_2F_4 plants, correlations between reactions of different races were analyzed using the program "Statworks" for the Macintosh computer.

Results

Analysis of the morphology fertility, and cytology of the F_1 hybrids and backcross derivatives

F_1 hybrids. The cross between 'IR31917-45-3-2' and *O. minuta* Acc. 101141 gave 4% seed set from 2,082 spikelets pollinated (Sitch et al. 1989). Eighteen F_1 hybrids were produced after embryo rescue: all of these were triploids (genome ABC) with 36 chromosomes and were completely male sterile. They were vigorous and perennial, tillered profusely, and resembled *O. minuta* in some morphological characters such as descending angle of the flag leaf, long awns, acute ligule shape, and purple stigma color. Characters such as panicle type, panicle exsertion, panicle length, and spikelet width of the F_1 hybrids were intermediate between the two parents.

BC_1 progeny. Extremely low seed set (0.02%) was observed when the F_1 hybrids were backcrossed to the *O. sativa* parent line (Table 1). The partially self-fertile plants from colchicine-treated axillary buds of the F_1 (F_1 CT) gave slightly higher backcross seed set (0.12%) than the original triploid F_1 hybrids. Two BC_1 plants, each with 46 chromosomes, were obtained from backcrosses of the original F_1 hybrids. Four BC_1 plants were obtained by backcrossing the plants resulting from the colchicine treatment. Three of these BC_1 plants had 44–47 chromosomes; the chromosome number of the remaining BC_1 plant was not determined. Thus, 1–4 chromosomes were lost during the first backcross. All BC_1 progeny were completely male sterile.

BC_2 progeny. A 0.57% average seed set was obtained upon backcrossing the BC_1 plants (Table 1). Twenty-three BC_2 plants were obtained from four BC_1 plants that had 46–47 chromosomes. Eighteen BC_2 plants had 32–37 chromosomes, 2 had 24 chromosomes, while the chromosome number of 3 BC_2 plants was not determined. Except for the euploid plant, plant 75-1, all BC_2 plants had morphological features different from those of 'IR31917-45-3-2'. Traits absent in both parents, such as purple basal leaf sheath and apiculus, were observed among the BC_2, presumably resulting from new gene combinations. The euploid plant 78-1 was morphologically distinct from 'IR31917-45-3-2', having a purple leaf sheath base, apiculus, and stigma, and seeds with black hulls. It also had low fertility (21.8% pollen fertility and 16.9% spikelet fertility) and gave only 22.8% seed set upon backcrossing. Plant 75-1, however, was morphologically similar to 'IR31917-45-3-2' and had higher fertility (60.8% pollen fertility and 73.8% spikelet fertility) than plant 78-1. The rest of the BC_2 progeny were completely male sterile.

Table 2. Chromosome pairing at metaphase I in *O. sativa* 'IR31917-45-3-2', and the 'IR31917-45-3-2'/*O. minuta* Acc. 101141 BC_2 derivatives (2n = 24) 75-1 and 78-1

Plant material	Total cells (*n*)	Chromosome association % cells		
		12 II	11 II + 2 I	10 II + 4 I
IR31917-45-3-2	140	55.7 (78)	34.3 (48)[a]	10.0 (14)
78-1	70	95.7 (67)	4.3 (3)	0
75-1	100	69.0 (69)	23.0 (23)	8.0 (8)

[a] Number of cells in parentheses

To determine whether an *O. minuta* chromosome could have been substituted for an *O. sativa* chromosome in the euploid BC_2 plants, chromosome pairing at metaphase I was studied in plants 78-1 and 75-1 (Table 2). In plant 78-1, 12 bivalents were observed in 95.7% of the cells, indicating the presence of 12 homologous pairs of chromosomes. In plant 75-1, 2–4 univalents were observed in 41% of the cells observed, but this was comparable to the frequency of occurrence of cells with univalents in 'IR31917-45-3-2' (44.3%). Thus, no evidence was obtained for single-chromosome substitution in the two euploid *O. sativa*/*O. minuta* derivatives. There would be no substitutions by paired *O. minuta* chromosomes in these plants because they were are products of backcrosses to *O. sativa*.

BC_3 progeny. BC_3 progenies were obtained from the euploid BC_2 plants, 75-1 and 78-1. Plant 75-1 yielded 100% seed set upon backcrossing to 'IR31917-45-3-2' (Table 1); backcrosses of 78-1 gave 22.8% seed set and produced 31 BC_3 plants.

Seed set was low from backcrosses of the BC_2 plants with 32–37 chromosomes. With the exception of BC_2 plant 55-1 (2n = 35), which was relatively female fertile (4.4% seed set), all BC_2 plants gave low seed sets (0–1.2%). Chromosome numbers were determined for 24 of the 28 BC_3 progenies of the aneuploid BC_2 plants: 15 had 24 chromosomes, 7 had 25 chromosomes (putative *O. minuta* monosomic alien addition lines, or MAALs), 1 plant had 27, and another had 29 chromosomes. With the exception of 1 plant, the 13 euploid BC_3 plants examined were morphologically similar to 'IR31917-45-3-2'. Four of the 7 putative *O. minuta* MAALs had features distinct from those of the *O. sativa* parent, and 2 of these resembled particular MAALs from the *O. sativa*/*O. officinalis* cross derived by Jena and Khush (1986, 1989).

Evaluation for blast resistance

The blast disease reaction of the two parents and their F_1 hybrids is shown in Fig. 1. 'IR31917-45-3-2' was susceptible to strain PO6-6 and to the pathogen population at the IRRI Blast Nursery (Fig. 1 A), whereas *O. minuta* Acc. 101141 was highly resistant, with either no lesions or with tiny brown specks forming on the youngest leaves (Fig. 1 B). A hypersensitive resistant reaction was observed among the majority of F_1 plants tested with PO6-6 and with natural inocula at the IRRI Blast Nursery, although the lesions were found to be larger than those observed on *O. minuta* (Fig. 1 C). The intermediate reaction (type 3) was also observed for 21 of 190 F_1 plants (Fig. 1 D). Thus, the F_1 hybrids showed a lower level of blast resistance than did the *O. minuta* parent.

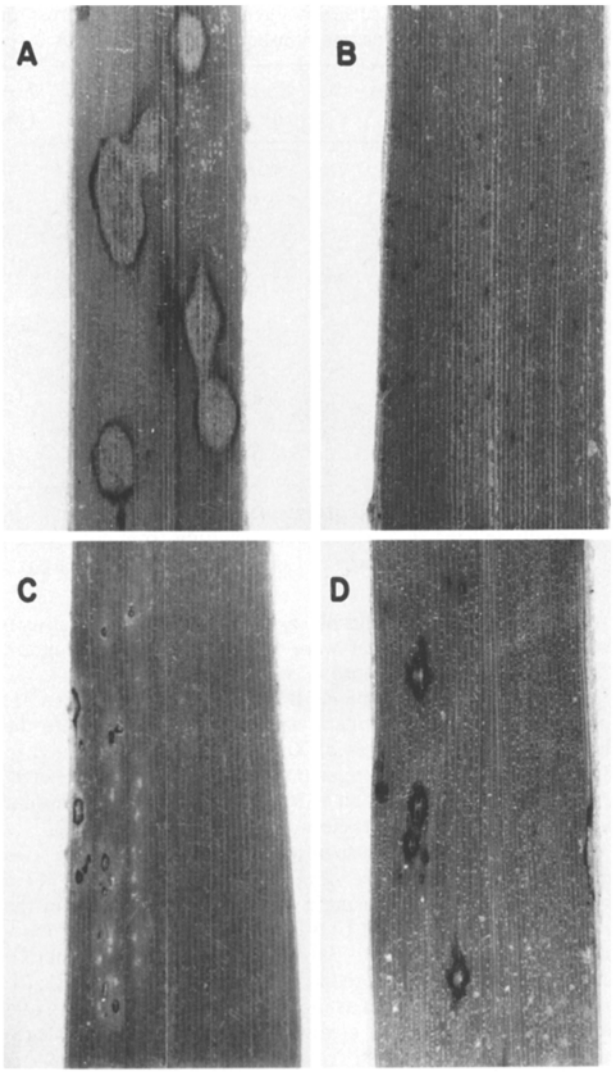

Fig. 1 A–D. Blast lesions on 'IR31917-45-3-2' (**A**), *O. minuta* Acc. 101141 (**B**), and F_1 hybrid (**C, D**)

The reaction of the BC_1 progeny was similar to that of the F_1 hybrid. An exception was BC_1 plant 50-1 (2n = 46), which was more susceptible to PO6-6. The majority of the 50-1 clones (14 out of 20) gave a type 3

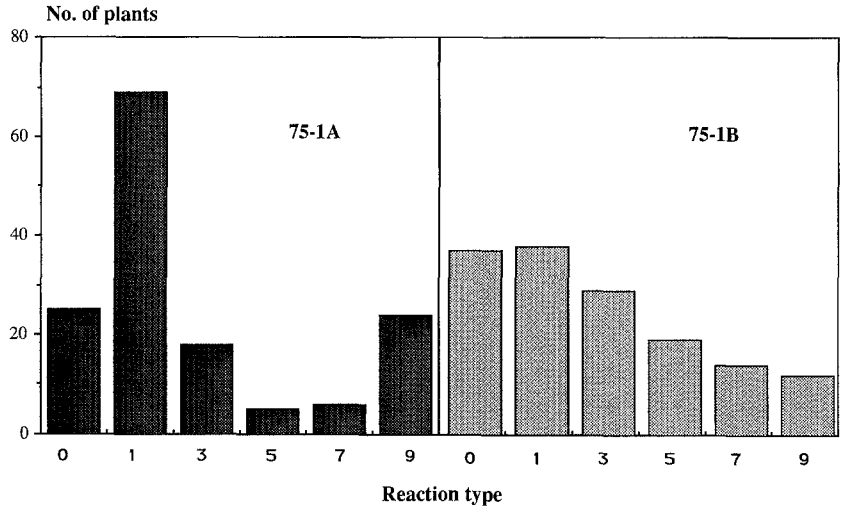

Fig. 2. Two types of frequency distribution of reaction to *Pyricularia grisea* strain PO6-6 observed in BC_2F_2 families of a resistant *O. sativa*/*O. minuta* BC_2 derivative, plant 75-1. Reaction types *0–3*, resistant; reaction types *5–9*, susceptible

Table 3. Lesion lengths (cm) for *O. sativa* 'IR31917-45-3-2', *O. minuta* Acc. 101141 and derivatives after inoculation with representatives of six Philippine races of *Xanthomonas oryzae* pv. *oryzae*

	PXO61 (race 1)		PX086 (race 2)		PXO79 (race 3)		PXO71 (race 4)		PXO112 (race 5)		PXO99 (race 6)	
IR31917-45-3-2[a]	3.37		10.8		11.9		11.9		3.5		18.6	
Oryza minuta (acc. 101141)[b]	0.58		0.46		0.56		0.83		0.49		0.43	
Derivatives[c]	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
F_1 hybrids ($n=14$)	0.29	0.94	0.45	0.99	0.47	1.37	0.38	0.92	0.34	0.73	0.45	1.26
BC_1 ($n=5$)	0.4	1.1	0.7	1.4	1.4	2	1.4	2.4	1.2	1.8	1.2	2.1
BC_2 ($n=14-21$)	0.3	10.4	0.7	15.4	0.7	17.6	1.2	14.7	0.6	12.1	0.7	20.0
BC_2 78-1												
Derivatives[c]												
BC_2F_2 ($n=11-21$)	1.4	4.6	2.6	28.5	1.5	26.3	6.5	20.8	1.0	7.9	2.3	32.3
BC_2F_3 ($n=157-162$)	0.5	10.8	0.5	31.2	0	28.4	2.5	35.3	0.5	11.5	n.d.	n.d.
BC_2F_4 ($n=82-146$)	1.3	17.5	1.2	33.8	1.8	32.0	5.3	30.0	0.4	15.1	1.9	45.6

[a] Mean of means from three independent experiments
[b] Mean of means from two independent experiments
[c] Ranges of means from several inoculated leaves per plant; *n* refers to the number of plants tested; min. refers to average lesion length of most resistant plant; max. refers to average lesion length of most susceptible plant

disease reaction to PO6-6, and a few susceptible lesions were detected on 4 clones.

Thirteen of the 16 BC_2 plants tested were as resistant as the F_1 and BC_1 plants to strain PO6-6. A resistant BC_2 plant, 75-1, had 24 chromosomes, suggesting that blast resistance had been introgressed from *O. minuta* into *O. sativa*.

A 3 resistant: 1 susceptible ratio ($\chi^2=0.17$, $0.5<P<0.7$) was obtained when the BC_2F_2 progeny of plant 75-1 were evaluated at the IRRI Blast Nursery, indicating the action of a single dominant gene. However, two inoculation tests with strain PO6-6 gave inconsistent results: a 3 R:1 S ratio ($\chi^2=0$, $P=1.0$) was observed in one trial; in the other, where poor seedling survival was a problem, a 1 R:1 S ratio was observed.

Fifteen BC_2F_3 progeny of each of 98 BC_2F_2 of plant 75-1 were tested for their reaction to PO6-6. The results of this progeny testing confirmed results of the BC_2F_2 tests in 95 of the 98 families tested. Of the 98 BC_2F_2 plants for which F_3 progeny were analyzed, 26 were inferred to be homozygous resistant, 20 were inferred to be homozygous susceptible, and 52 were inferred to be heterozygous. The results are consistent with the hypothesis that resistance is conditioned by a single dominant gene ($\chi^2=1.10$, $0.5<P<0.7$).

Of the 52 heterozygous BC_2F_2 families, 48 showed a 3 R:1 S bimodal distribution, and 4 showed a greater proportion of intermediate reaction types. To further confirm the action of a single dominant gene, we tested a larger number of progeny of 2 heterozygous BC_2F_2

plants. Family 75-1A was selected for its distinct bimodal distribution of reaction types, while family 75-1B was chosen to represent those with more intermediate types (Fig. 2). Of the 147 BC_2F_3 plants of family 75-1A tested, 112 were found to be resistant while 35 were found to be susceptible ($\chi^2 = 0.06$; $0.07 < P < 0.9$). The 3 R:1 S segregation ratio was thus confirmed in BC_2F_2 family 75-1A. For family 75-1B, a 104 R:45 S ratio was observed ($\chi^2 = 1.88$; $0.1 < P < 0.2$). Based on the chi-square test, this segregation ratio was also consistent with the action of a single resistance gene. However, because the distribution of reaction types in this family was not bimodal, it is unlikely that resistance was conditioned only by a single major gene (Fig. 2).

Evaluation for bacterial blight resistance

The *O. minuta* parent showed a high level of resistance to representatives of the six Philippine races of Xoo. Lesions were very short (<1.0 cm) and necrotic 14 days after inoculation (Fig. 3 A). 'IR31917-45-3-2' was susceptible to race 6 and moderately susceptible to susceptible to races 2, 3, and 4, but was resistant to races 1 and 5 at maximum tillering stage (Fig. 3 B). The resistance of 'IR31917-45-3-2' to races 1 and 5 is due to the presence of the *Xa-4* gene for resistance to bacterial blight.

All F_1 hybrids and BC_1 progeny were resistant to the six races (Table 3). Ten of 21 BC_2 plants were resistant or moderately resistant to Xoo races 2, 3, 4, and 6.

BC_2 plant 78-1 ($2n = 24$) was resistant to races 1, 2, 3, 5, and 6 and moderately resistant to race 4. The BC_2F_2, BC_2F_3, and BC_2F_4 progeny of plant 78-1 were evaluated for their reaction to the bacterial blight pathogen. For race 1, the 13 BC_2F_2 plants tested showed R/MR reactions. For race 5, 9 of the 11 BC_2F_2 plants tested were R/MR, while 2 were MS. For races 2, 3, and 6, reactions ranged from R to S. For race 4, only MS to S reactions were observed.

To determine whether the same or closely linked gene(s) conditioned resistance to different races, correlation coefficients were calculated based on lesion lengths for each pairwise combination of races within each family. The lesion lengths for races 2 and 3 were closely correlated for the 20 BC_2F_2 individuals tested with both races ($r = 0.96^{**}$). For races 2 and 6 and races 3 and 6, lesion lengths were correlated, but not as closely ($r = 0.80^{**}$, $n = 20$ for race 2 versus race 6; $r = 0.84^{**}$, $n = 21$ for race 3 versus race 6).

The reactions of 15 BC_2F_3 progeny of each of 11 BC_2F_2 derivatives of plant 78-1 were tested. For races 2 and 3, a wide range of lesion lengths was observed, and there was a strong correlation between lesion lengths for the two races among the 157 BC_2F_3 progeny tested with both races ($r = 0.94^{**}$). Although resistance to race 4 was not seen in the BC_2F_2 generation, this resistance ap-

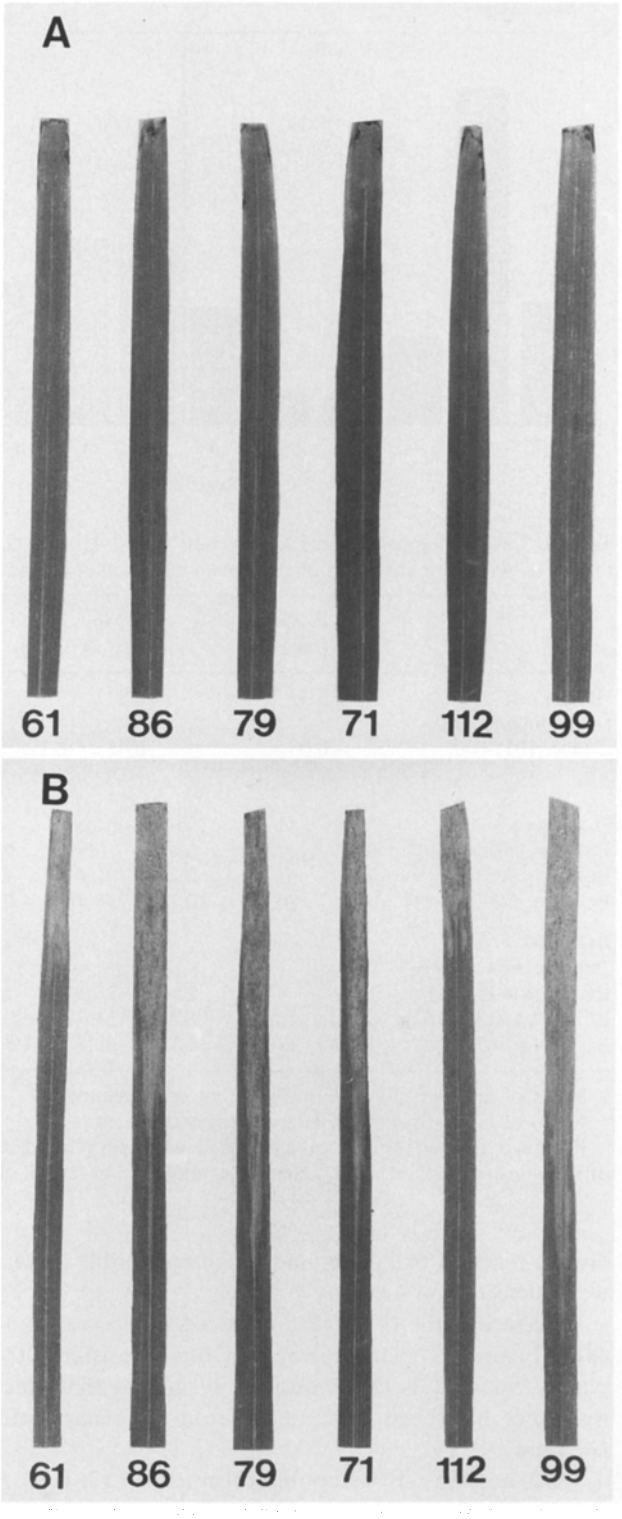

Fig. 3 A, B. Reaction of *O. minuta* Acc. 101141 (**A**) and *O. sativa* 'IR31917-45-3-2' (**B**) to six races of *Xanthomonas oryzae* pv. *oryzae* in the Philippines

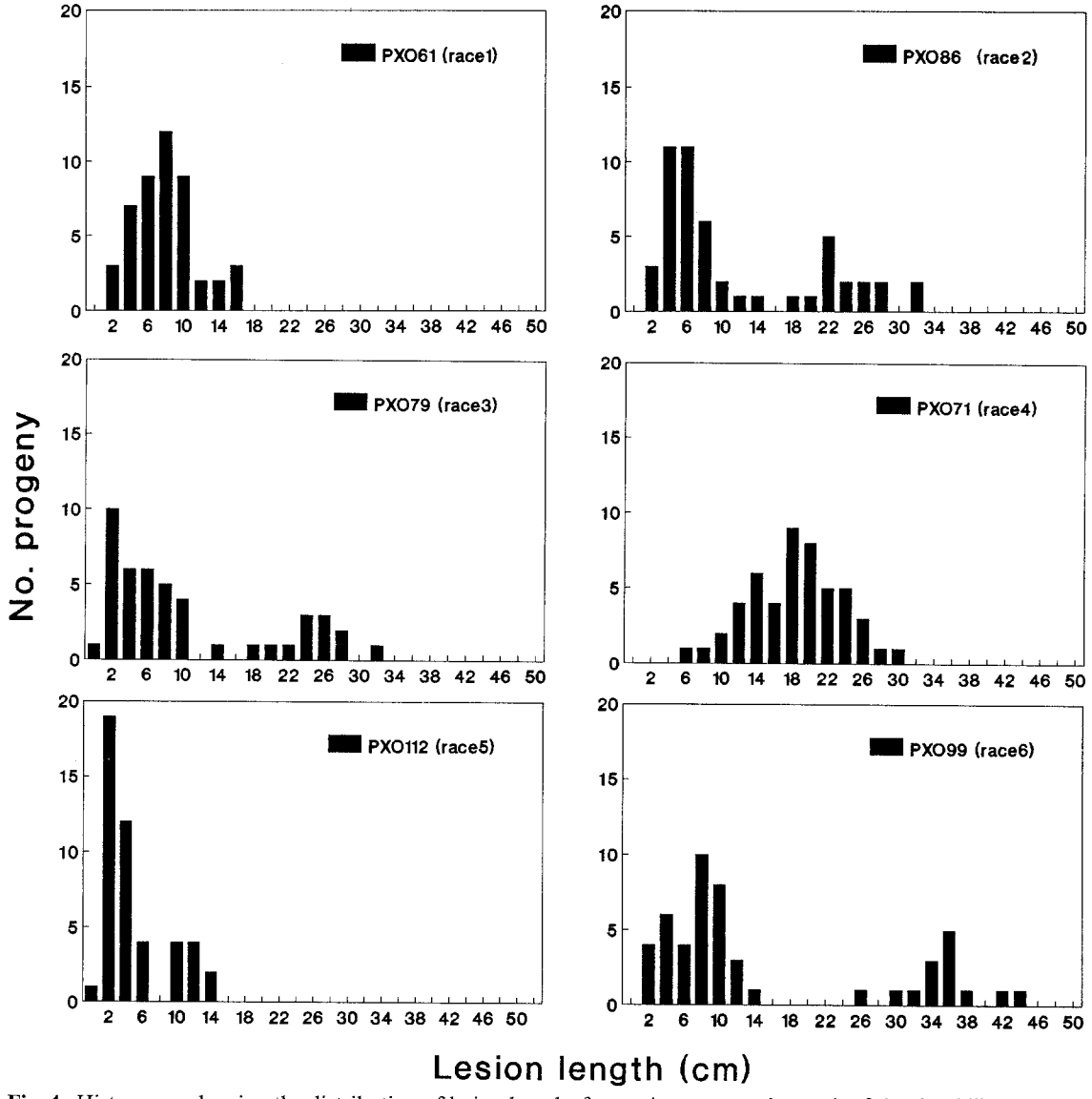

Fig. 4. Histograms showing the distribution of lesion lengths for strains representing each of the six Philippines races *Xanthomonas oryzae* pv. *oryzae* in the BC_2F_3 family 78-1P. Correlations among the reactions to the different races are discussed in the text

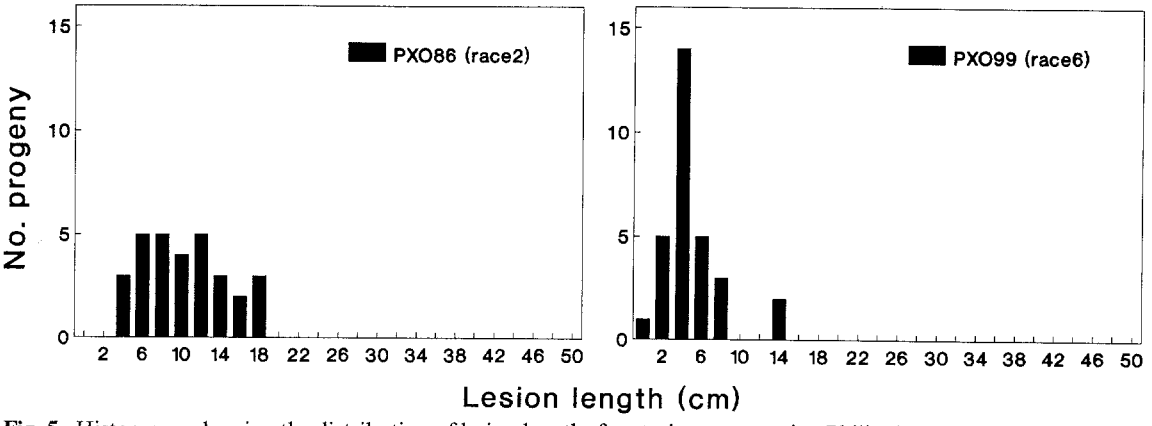

Fig. 5. Histograms showing the distribution of lesion lengths for strains representing Philippine races 2 and 6 of *Xanthomonas oryzae* pv. *oryzae* in the BC_2F_3 family 78-1Q. The distribution of lesion lengths for race 2 is different from that seen in 78-1P (Fig. 4)

Table 4. Presence or absence of markers (morphological, disease reaction, isozyme) among euploid backcross derivatives of 'IR31917-45-3-2'/*O. minuta* Acc. 101141 (2n=24)

Euploid plants	Generation	Blast reaction[a,b]	Bacterial blight reaction[a]	Morphological characters unlike *O. sativa* parent	Isozyme systems from *O. minuta* (7 tested)[c]
75-1	BC_2	R	S	0	0
78-1	BC_2	S	R	Many	4
69-4	BC_3	S	S	0	0
69-8	BC_3	ND	MS/S	0	2
69-9	BC_3	ND	S	0	0
69-10	BC_3	S	S	0	0
69-11	BC_3	S	MS/S	0	0
71-3	BC_3	ND	MS/S	Many	0
81-2	BC_3	ND	ND	0	0
69-2	BC_3	S	ND	0	0
69-6	BC_3	S	MS/S	0	0
71-1	BC_3	S	S	0	0
71-4	BC_3	S	MS/S	0	0
71-7	BC_3	S	MS/S	0	0
80-1	BC_3	S	ND	0	0

[a] R, Resistant; MS, moderately susceptible; S, susceptible; ND, not determined
[b] Based on the reaction of selfed progeny, except for the BC_2 75-1
[c] Romero (1989)

peared among the BC_2F_3 derivatives. Among the 11 families tested, 4 had 1–3 individuals each showing resistant or moderately resistant reactions to race 4.

Reactions to races 1 and 5 were not perfectly correlated among the 153 BC_2F_3 plants tested ($r=0.74$**) since 3 of the plants were more susceptible to race 1 than to race 5. In the previous (BC_2F_2) generation, 1 of the 21 plants was resistant to race 1 but moderately susceptible to race 5. The reactions of the BC_2F_3 progeny to race 6 were not tested.

The progeny of 5 BC_2F_3 plants with resistance to races 1, 2, 3, and 5 were tested with strains representing the six Philippines races of Xoo. Different patterns of resistance were observed among progenies in 4 of the 5 families. One BC_2F_3 family, 78-1P, was particularly informative because of the wide range of reactions seen for races 1, 2, 3, 5, and 6 (Fig. 4). Lesion lengths for race 6 varied from 3.4 cm to 45.6 cm among the 50 plants tested. Lesion lengths for race 6 were strongly correlated with lesion lengths for race 2 ($r=0.92$**, $n=49$), race 3 ($r=0.90$**; $n=45$), and race 5 ($r=0.90$**, $n=46$). These data suggested that the same gene or closely linked genes derived from *O. minuta* were responsible for resistance to races 2, 3, 5, and 6. In another BC_2F_3 family, 78-1Q, a greater range of lesion lengths was observed for race 2 than for race 6 (Fig. 5). The uncoupling of resistance to races 6 and 2 in this family suggests that either the resistance factor is different from that in BC_2F_3 family 78-1P, or that modifier genes were affecting the expression of resistance to race 2.

Transgressive segregation was seen in the BC_2F_2, BC_2F_3, and BC_2F_4 generations. Greater susceptibility was observed among the progeny for each of the races than was observed in either parent (Table 3).

Discussion

Alien gene transfer in O. sativa/O. minuta euploid derivatives

We have demonstrated that useful traits from a tetraploid wild rice *O. minuta* (BBCC) can be transferred to cultivated rice *O. sativa* (AA). Jena and Khush (1990) have successfully transferred resistance to brown planthopper and whitebacked planthopper from *O. officinalis* (CC).

Based on the expectation that there would be minimal recombination between the A and the BC genomes, the original design of this study involved the production of monosomic alien addition lines and the induction of translocations between *O. sativa* and *O. minuta* chromosomes. Putative *O. minuta* MAALs were obtained in the BC_3 generation. However, two euploid derivatives with resistance to either blast or bacterial blight were obtained after the second backcross. Alien gene transfer in these two euploids may have occurred by recombination, translocation, and/or chromosome substitution. The characteristics of these euploid plants, and of others derived in the BC_3 generation, are summarized in Table 4.

The blast-resistant euploid BC_2 plant 75-1 and its selfed progenies were fertile and showed no apparent morphological traits inherited from *O. minuta*. This implies that only a relatively small amount of genetic material was transferred from the wild parent. The bacterial

blight-resistant euploid BC_2 plant 78-1 had low fertility and showed a number of characters apparently inherited from *O. minuta*. In addition, plant 78-1 had seven *O. minuta*-specific isozyme bands (Romero 1989; L.A. Sitch unpublished). It appears that a greater amount of introgression had occurred in plant 78-1 than in plant 75-1. However, chromosome pairing in both euploids was apparently normal and provided no evidence for substitution.

Low levels of pairing were observed during meiosis in the F_1 hybrids. Pollen mother cells of the hybrids used in this study showed a mean of 3.80 bivalents at pachytene, 2.15 bivalents at diakinesis, and 2.25 bivalents at metaphase I (Amante-Bordeos et al. 1990). It has been suggested that the observed chromosome pairing in *O. sativa*/*O. minuta* hybrids are between the A genome and the B or C genomes (Nezu et al. 1960; Kihara et al. 1961; Katayama 1966). This implies that gene transfer from the *O. minuta* genome into the *O. sativa* genome could be due to recombination.

The seven *O. minuta*-specific isozyme bands detected in BC_2 plant 78-1 were inferred to be homoeoalleles of the *Pgd-2*, *Sdh-1*, *Est-2*, and *Est-5* loci in *O. sativa* (Romero 1989; L.A. Sitch unpublished). The different chromosomal locations of the homoeologous *O. sativa* loci suggest that recombination events involving more than one *O. minuta* chromosome may have occurred in plant 78-1. Molecular analysis of the 2 resistant euploids is in progress to clarify the mechanism of introgression.

Disease resistance

O. minuta is highly resistant to strain PO6-6 of the rice blast fungus and to the presumably diverse races of the fungus at the IRRI Blast Nursery. A lower level of resistance was seen in the F_1 hybrids than in the *O. minuta* parent, suggesting that resistance in *O. minuta* was either monogenic but incompletely dominant, or was conditioned by polygenes. BC_1 plant 50-1 showed a moderately resistant to moderately susceptible reaction. This BC_1 plant gave rise to plant 75-1, from which progeny showing reactions ranging from highly resistant to highly susceptible were derived.

Segregation ratios among the BC_2F_2 and BC_2F_3 progenies of plant 75-1 were consistent with the action of a single major gene. In most of the heterozygous BC_2F_3 lines derived from 75-1, resistant and susceptible reactions were very distinct. In a few families, however, a continuous range of reactions was observed, as shown by the different distributions of reaction types in two BC_2F_3 families. Apparently, plant 75-1 contained a single major gene conditioning resistance as well as other loci that modify the degree of resistance expressed.

Resistance to bacterial blight was also apparently transferred from *O. minuta* to *O. sativa*. The euploid BC_2 plant, 78-1, was resistant to races 1, 2, 3, 5, and 6 and moderately resistant to race 4 of Xoo. Many of the derivatives of 78-1 were more susceptible to Xoo than either parent. Apparently recombination between the chromosomes of the two parents resulted in gametes that did not inherit the resistance genes from either parent.

Because the *O. sativa* parent was susceptible to race 6, the resistant reaction of some of the derived lines to the race 6 strain PXO99 indicates that resistance to the bacterial blight pathogen was transferred from *O. minuta*. Analysis of the BC_2F_2 and BC_2F_3 progenies of plant 78-1 suggested that the same or closely linked gene(s) conferred resistance to races 2 and 3. Similarly, analysis of the disease reactions of BC_2F_4 plants to the six Philippine races suggested that a gene or closely-linked genes conferred resistance to races 2, 3, 5, and 6. The correlation between resistance to race 6 and resistance to other races suggested that the gene or closely linked genes from the wild species confer(s) resistance to multiple races of the pathogen.

An imperfect correlation between the reactions to races 1 and 5 was observed in the BC_2F_2, BC_2F_3, and BC_2F_4 generations. This would not be expected if resistance to races 1 and 5 in these progenies were conferred only by the *Xa-4* gene inherited from the *O. sativa* parent because *Xa-4* confers resistance to both races 1 and 5. To clarify the identity and characteristics of the resistance gene(s) present in the derivatives of plant 78-1, crosses are being made to transfer the gene(s) to susceptible var 'IR24'.

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