Theor Appl Genet (2003) 107:395–405
DOI 10.1007/s00122-003-1214-3

D. S. Multani · G. S. Khush · B. G. delos Reyes · D. S. Brar

Alien genes introgression and development of monosomic alien addition lines from *Oryza latifolia* Desv. to rice, *Oryza sativa* L.

Received: 19 September 2002 / Accepted: 18 October 2002 / Published online: 23 May 2003

Abstract *Oryza latifolia*, a tetraploid wild relative of cultivated rice is an important source of resistance to bacterial blight (BB), the brown planthopper (BPH) and the whitebacked planthopper (WBPH). Interspecific hybrids were obtained between an elite breeding line (IR31917-45-3-2) of *Oryza sativa* (2n = 24 AA) and *O. latifolia* Acc. No. 100914 (2n = 48 CCDD). The crossability in F_1 was 7.58% and it ranged from 0.11 to 0.62 in backcross generations. The F_1 hybrid showed 2–6 II, 0–2 III, 0–1 IV and 22–32 I; the mean being 3.92 II + 0.11 III + 0.02 IV + 27.30 I per cell at diakinesis. Monosomic alien addition lines (MAALs) having a 2n chromosome complement of *O. sativa* and one chromosome of *O. latifolia* were characterized based on morphology and isozyme banding pattern. The MAALs were designated as MAAL-1, MAAL-2, MAAL-4, MAAL-5, MAAL-6, MAAL-7, MAAL-8, MAAL-9, MAAL-10, MAAL-11 and MAAL-12. The female transmission rates of the alien chromosome varied from 4.4 to 35.5%, whereas 8 of the 11 MAALs transmitted the alien chromosome through the male gamete, the range being 1.7% (MAAL 10) to 11.9% (MAAL 12). Disomic progenies in BC_3 and BC_4 generations had complete resemblance to the *O. sativa* parent. Of the 2,295 disomic BC_3F_3 progenies, 309 showed introgression for resistance to BPH and 188 each for WBPH and BB resistance. Four plant progenies which were resistant to both BPH and WBPH were also resistant to BB race 2 of the Philippines. Nine of the 34 BC_3F_1 plants showed introgression for ten allozymes of *O. latifolia*, such as *Est5*, *Amp1*, *Pgi1*, *Mdh3*, *Pgi2*, *Amp3*, *Pgd2*, *Est9*, *Amp2* and *Sdh1*, located on 8 of the 12 chromosomes. Alien introgression was also detected for morphological traits such as long awns, earliness, black hull, purple stigma and apiculus. Abnormal plants with many wild-species traits suddenly appeared in normal disomic progenies. These plants showing instability and abnormal segregation behaviour are being investigated for the activation of transposons.

Keywords *Oryza sativa* · *Oryza latifolia* · Monosomic alien addition lines (MAALs) · Introgression · Allozyme · Alien gene transfer · Bacterial blight · Brown planthopper · Whitebacked planthopper

Communicated by G. Wenzel

D. S. Multani · G. S. Khush · B. G. delos Reyes · D. S. Brar (✉)
International Rice Research Institute, DAPO Box 7777,
Metro Manila, The Philippines,
e-mail: d.brar@cgiar.org
Tel.: +63-2-845-0563
Fax: +63-2-845-0606

Introduction

The genus *Oryza* to which the cultivated rice (*Oryza sativa* L.) belongs has 22 wild species. These wild species are an important reservoir of useful genes for resistance to biotic and abiotic stresses. Some useful genes from the A-genome wild species have been transferred into rice through conventional hybridization and backcrossing procedures. Notable examples include transfer of a gene for resistance to the grassy stunt virus from *Oryza nivara* (Khush 1977), cytoplasmic male sterility from *O. sativa* f. *spontanea* (Lin and Yuan 1980) and *Xa21*, a gene that imparts resistance to all the six Philippine races of bacterial blight from *Oryza longistaminata* (Khush et al. 1990). Transfer of alien genes from species other than the A genome involves the production of hybrids through embryo rescue followed by backcrossing. During backcrossing, plants with 24 and 25 chromosomes were isolated. Plants having 25 chromosomes, referred to as monosomic alien addition lines (MAALs), carry one extra chromosome of wild species and the normal chromosome complement of *O. sativa*. MAALs have been produced in crosses of *O. sativa* with *Orzya officinalis* (Shin and Katayama 1979; Jena and Khush 1989), *Orzya punctata* (Yasui and Iwata 1991) and *Orzya australiensis* (Multani et al. 1994). These MAALs can be characterized on the basis of morphology, isozyme and RFLP patterns. Recently, useful genes from wild species with other than the A genome have also been transferred to cultivated rice. Examples include transfer of brown planthopper

(BPH) and whitebacked planthopper (WBPH) resistance from *O. officinalis* (Jena and Khush 1990), bacterial blight (BB) and blast resistance from *Orzya minuta* (Amante-Bordeos et al. 1992), BPH and BB resistance from *O. australiensis* (Multani et al. 1994), BPH resistance from *O. minuta* and BB resistance from *Orzya brachyantha* (Brar et al. 1996).

Isozymes are helpful in detecting introgression in the wide-cross-derived progenies. In this investigation we report the results on development and characterization of MAALs from a cross of *O. sativa* and *Orzya latifolia*, and transfer of useful alien genes for BPH and BB resistance including introgression for morphological traits into *O. sativa*.

Materials and methods

Production of the F1 hybrid (O. sativa × O. latifolia)

An elite breeding line, IR31917-45-3-2, of *O. sativa* was pollinated with pollen of an accession of the allotetraploid species *O. latifolia* (Acc. No. 100914) with CCDD genomes received from the International Rice Germplasm Centre of the International Rice Research Institute (IRRI). The elite breeding line has short stature and high yield potential but is susceptible to BPH and BB races. Hybrid plants were produced through embryo rescue following the procedure described by Jena and Khush (1984) and Multani et al. (1994).

Producing backcrosses

Backcross 1 (BC_1) progeny was produced using *O. sativa* as a recurrent parent. Ten BC_1 plants were backcrossed to obtain BC_2 progeny. Similarly BC_3 progeny was produced following the backcrossing of 16 BC_2 plants. In all cases, the backcross progenies were produced through embryo rescue.

Chromosome analysis

Chromosome analysis of the F_1 hybrid, backcross progenies and MAALs were made from meiotic squash preparations. Chromosome associations at diakinesis and metaphase-1 were analyzed in hybrids and MAALs.

Establishment of monosomic alien addition lines

Three hundred and fifty seven F_2 plants were grown. These originated from BC_3 or BC_4 plants which had 2n+1, 2n+2 or 2n+3 chromosomes. F_2 plants with 25 chromosomes were isolated and categorized into 11 different groups on the basis of their morphological and reproductive features. These represented 11 different MAALs. The morphological and reproductive features of MAALs were compared with those of primary trisomics of *O. sativa* cv IR36 developed by Khush et al. (1984). The MAAL resembling triplo 1 was designated MAAL 1, that resembling triplo 2 was designated MAAL 2, and so on. The morphological features of these MAALs were also compared with MAALs of *O. officinalis* developed by Jena and Khush (1989). The pollen stainability, spikelet fertility and transmission rates of the extra alien chromosomes of MAALs were studied.

Characterization of MAALs based on isozyme pattern

The MAALs were characterized on the basis of the allozymes of *O. latifolia*. The white portion of emerging leaves from young plants of parents, F_1 hybrids, MAALs and their disomic sibs were collected and homogenized in 0.1% mercaptoethanol. Samples were subjected to starch-gel electrophoresis and stained for shikimate dehydrogenase (SDH), phosphoglucose isomerase (PGI), phosphogluconate dehydrogenase (PGD), aminopeptidase (AMP), alcohol dehygrogenase (ADH), esterase (EST), endopeptidase (ENP), peroxidase (POX), isocitrate dehygrogenase (ICD) and glutamate oxaloacetate transaminase (GOT) enzyme activities using the procedure described by Glaszmann et al. (1988). Malate dehydrogenase (MDH) enzyme activity was determined using the staining method of Second and Trouslot (1980a,b). Observations were repeated at least three times over two generations.

Detection of alien gene introgression

Besides 2n+1 plants there were 2,295 disomic plants derived from both BC_3F_2 and BC_4F_2 generations. Self seed of these plants was divided into three lots. The first lot was planted in order to raise BC_3F_3 or BC_4F_3 progeny rows. These progenies were inoculated with Philippine races 1, 2 and 3 of BB. In addition, available MAALs were also screened for BB resistance.

The second seed lot of the 2,295 disomic plants was screened for resistance to WBPH. Similarly, the third seed lot along with 11 MAALs was evaluated for resistance to BPH biotype 1 of the Philippines. The observations were repeated from the self-progenies of the resistant plants. The plant progenies resistant to both BPH (biotype 1) and WBPH were also tested for their reaction to BPH biotypes 2 and 3 of the Philippines.

The 2,295 disomic plant progenies were examined for the introgression of qualitative traits from *O. latifolia*, such as purple leaf sheath, purple stigma, purple apiculus, long awns, grain shattering, black hull and red pericarp. Fifty three single-plant selections in F_4 and 1,149 disomic plants derived from the self progenies of 11 MAALs were examined to determine introgression for the above traits. Seven hundred and twenty five lines representing the BC_3F_5 and BC_4F_5 generations were analyzed. A total of 1,189 plant progenies, a few with single or multiple traits of the wild species along with their normal sibs (as checks), were grown in the F_6 to identify lines homozygous for the traits inherited from *O. latifolia*.

Isozyme analysis was conducted on 17 BC_3F_2 and 7 BC_4F_2 disomic plant progenies and 17 plant progenies showing introgression for a qualitative trait(s) in BC_3F_4 and BC_4F_4 and F_5 generations. Leaf samples were examined for SDH, PGI, PGD, AMP, ADH, EST, ENP, POX, ICD, GOT and MDH enzyme activities through starch-gel electrophoresis. The observations on introgression for allozymes of the wild species were repeated at least three times in the same generation. The results were further confirmed from the selfed progenies.

In order to study the inheritance of introgressed traits, crosses were made between the recurrent parent IR31917-45-3-2 and the advanced generation derived-lines having a purple leaf sheath, a purple stigma, a purple apiculus, early maturity and the black hull traits of *O. latifolia*. F_1 and F_2 populations were examined.

Results

Interspecific hybrid

The seed set in the *O. sativa* × *O. latifolia* cross was 19.8% and the germination of rescued embryos was 85.5%. Eighty seven F_1 hybrid plants were obtained of which 69 survived (Table 1), the crossability being 7.58%. The F_1 plants showed a predominance of wild-

Table 1 Seed set and number of plants obtained in the *O. sativa* and *O. latifolia* cross, and backcross progenies through embryo rescue

Generation	Spikelets pollinated (no.)	Seed set		Embryos cultured	Germination		Plants obtained (no.)	Crossability[a] (%)
		(No.)	(%)	(No.)	(No.)	(%)		
F_1	910	180	19.8	166	142	85.5	69	7.58
BC_1	10,144	35	0.3	31	20	64.5	11	0.11
BC_2	9,405	75	0.8	65	39	60.9	20	0.21
BC_3	5,451	81	1.5	72	49	68.1	34	0.62

[a] $\%\ \text{Crossability} = \frac{\text{no. of hybrids}}{\text{no. of spikelets pollinated}} \times 100$

Table 2 Chromosome associations in F_1 (2n = 36, ACD) of *O. sativa* × *O. latifolia* at diakinesis (DK) and metaphase-I (MI) of meiosis. Percent values in parenthesis

Meiotic stage	PMCs studied (no.)	No. of cells with chromosome association						
		2_{II} + 32_I	3_{II} + 30_I	4_{II} + 28_I	6_{II} + 24_I	1_{III} + 3_{II} + 27_I	2_{III} + 4_{II} + 22_I	1_{IV} + 2_{II} + 28_I
DK	53	4 (7.5)	6 (11.3)	31 (58.5)	7 (13.2)	2 (3.8)	2 (3.8)	1 (1.9)
MI	57	3 (5.2)	3 (5.2)	39 (68.4)	7 (12.3)	2 (3.5)	2 (3.5)	1 (1.8)

parent traits, such as tall stature, long awns, spikelet shattering, purple leaf sheath, purple stigma, purple apiculus, etc. They were extremely vigorous but completely male-sterile.

Backcross progenies

The seed set upon backcrossing the F_1 hybrid with the recurrent male parent was quite low (0.3%). Eleven BC_1 plants were produced through embryo rescue, crossability being 0.11%. The BC_1 plants were completely sterile and were pollinated with *O. sativa* pollen.

Of the 9,405 spikelets of the BC_1 plants pollinated, only 75 (0.8%) produced seeds (Table 1). Twenty BC_2 plants were obtained from two BC_1 plants. The pollen fertility of BC_2 plants was very low (0.0 to 13.0%) and no self seed-set occurred. The seed set upon backcrossing varied from 0.0 to 3.94% the mean being 1.5%. The culturing of 72 embryos resulted in 41 BC_3 plants. Of the 41, 34 BC_3 plants survived, crossability being 0.62%.

Chromosome analysis

The F_1 hybrid (ACD) showed 2–6 II, 0–2 III and 0–1 IV per cell at diakinesis/metaphase-I (Table 2). Trivalent association was recorded in 7.6%, and a quadrivalent in 1.9%, of the cells. Of the 11 BC_1 plants, one was tetraploid (2n = 48) while the other ten were hypotetraploids (2n = 42 to 47). The chromosome number of 20 BC_2 plants varied from 34 to 44. The chromosome number of 34 BC_3 plants varied from 24 to 30. Thirteen were 2n, four were 2n+1, six 2n+2, nine 2n+3 and one each had 2n+4 and 2n+6 chromosomes. All the 13 disomic (2n = 24) plants showed 12 II.

Development of MAALs

A total of 35 2n+1 plants were isolated in the BC_3F_1, BC_3F_2 and BC_4F_1 generations. Plants with 2n+1 chromosomes having the complete chromosome complement of *O. sativa* and a single chromosome of *O. latifolia* represented MAALs. Eleven different morphological types were identified among 35 2n+1 plants. These MAALs had striking resemblance to the primary trisomics of *O. sativa*. Of the 35 MAALs, six resembled triplo 5; four each of triplo 4, triplo 8 and triplo 9; three each of triplo 11 and triplo 12; two each of triplo 6 and triplo 7; and one each of triplo 1, triplo 2 and triplo 10. The remaining four plants could not be assigned to any group on the basis of morphology. Chromosome analysis showed two plants having a telocentric extra chromosome and other two remained unclassified. The 2n+1 plants had an extra chromosome, either from the C or D genome. The 2n+1 plant resembling triplo 1 in morphology was designated MAAL 1; the plant resembling triplo 2 was designated MAAL 2, and so on. Thus, out of 12 possible MAALs corresponding to the basic chromosome complement of *O. latifolia*, 11 were identified and tentatively designated as MAALs 1, 2, 4, 5, 6, 7, 8, 9 10, 11 and 12.

Characterization of MAALs

All the 11 MAALs differed from each other as well as from the normal disomic sibs in several morphological and reproductive features. All had a slow growth habit as compared to their normal sibs. MAAL 1 had narrow light-green droopy leaves, MAAL 5 possessed twisted leaves and MAAL 8 had narrow dark-green rolled (needle like) leaves. MAALs 2, 4, 6, 7, 9 and 12 were easily distinguishable at the maximum tillering stage. MAALs

Fig. 1 Grains of *O. sativa* (P1), *O. latifolia* (P2) and 11 MAALs. Numbers at the bottom correspond to the respective MAALs

10 and 11 could be distinguished at the flowering stage only. MAAL 2 was quite stunted with short erect leaves. MAAL 4 had a spreading habit and long leaves. MAAL 6 was early in flowering and had a purple leaf sheath, apiculus and stigma. MAAL 7 had narrow inward folding leaves and a red pericarp. MAAL 12 had light-green leaves and degenerated tip florets. MAAL 11 had a gold hull color but was similar to the disomic sib in morphology. MAAL 11 had longest ligule followed by MAAL 12, MAAL 1 had the shortest ligule. MAAL 10 had long slender grains. MAAL 4 had long awns (Fig. 1). Grains of MAALs 6, 7 and 11 also had short awns. Black hull, long awns, purple leaf sheath, purple apiculus, and purple stigma and red pericarp are the dominant traits of *O. latifolia* expressed in MAALs.

Most of the MAALs resembled the MAALs of *O. officinalis*, developed by Jena and Khush (1989). However, for each of the MAALs 6, 8 and 9 we isolated an additional variant type showing variation from typical corresponding MAALs of *O. officinalis*. Similarly, the variant type in MAAL 8 had broad erect leaves and was vigorous, and variant MAAL 9 had gold hull grains. In MAAL 6 the variant type is devoid of pigmentation of the leaf sheath, apiculus and stigma, but had striking resemblance with triplo 6 and MAAL 6 of *O. officinalis* in its morphological and reproductive features.

Characterization of the alien chromosome in MAALs through isozyme analysis

Polymorphism was detected between *O. sativa* and *O. latifolia* for 16 of the 21 isozyme loci studied (Table 3). The F_1 hybrid (ACD) showed the presence of both parental bands for 13 isozyme loci. For *Amp3*, *Est2* and *Est9* loci, F_1 had a band of intermediate mobility between two parents. MAAL 2 showed the *O. latifolia*-type allozyme for *Got3* and *Amp1*, whereas for the rest of the 14 isozyme loci it had a banding pattern similar to that

Table 3 Identification of extra alien chromosome in MAALs by using isozymes as chromosome markers

Plant material	Isozyme locus															Alien chromosome characterized in MAAL
	Est5	*Got1*	*Got3*	*Amp1*	*Pgi1*	*Mdh3*	*Pgd2*	*Amp3*	*Est2*	*Pgi2*	*Est9*	*Enp1*	*Amp2*	*Amp4*	*Pgd1*	*Sdh1*	
O. sativa cv																	
IR31917-45-3-2-2	F[a]	F	F	S[b]	S	F	S	S	F	S	F	S	F	F	S	F	–
O. latifolia																	
(Acc. 100914)	S	FS	S	F	F	S	F	F	S	FS	S	F	S	S	F	S	–
F_1	FS	FS	FS	FS	FS	FS	FS	S*[c]	S*	FS	S*	FS	FS	FS	FS	FS	–
MAAL 1	F	F	F	S	S	F	S	S	F	S	F	S	F	F	S	F	–
MAAL 2	F	F	FS	FS	S	F	S	S	F	S	F	S	F	F	S	F	Chromosome 2
MAAL 4	F	F	F	S	S	F	S	S	F	S	F	S	F	F	S	F	–
MAAL 5	F	F	F	S	S	FS	S	S	F	S	F	S	F	F	S	F	Chromosome 5
MAAL 6	F	F	F	S	S	F	FS	S*	S*	FS	F	S	F	F	S	F	Chromosome 6
MAAL 7	F	F	F	S	S	F	S	S	F	S	S*	S*	F	F	S	F	–
MAAL 8	F	F	F	S	S	F	S	S	F	S	F	S	FS	FS	S	F	Chromosome 8
MAAL 9	F	F	F	S	S	F	S	S	F	S	F	S	F	F	S	F	–
MAAL 10	F	F	F	S	S	F	S	S	F	S	F	S	F	F	S	F	–
MAAL 11	F	F	F	S	S	F	S	S	F	S	F	S	F	F	FS	F	Chromosome 11
MAAL 12	F	F	F	S	S	F	S	S	F	S	F	S	F	F	S	FS	Chromosome 12

[a] F = fast
[b] S = slow
[c] S* = modified *sativa* band

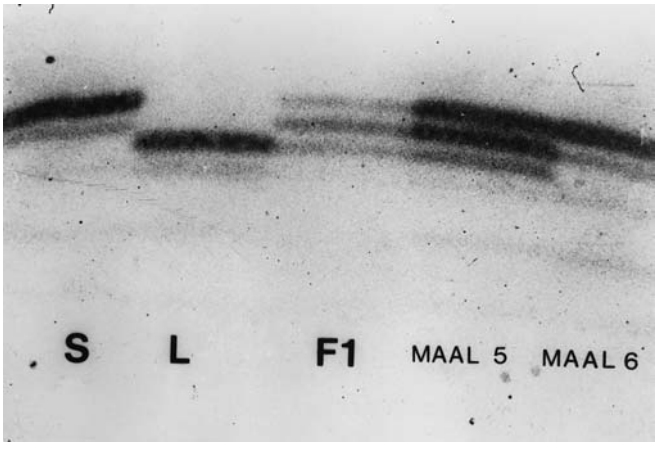

Fig. 2 Zymogram showing gene dosage for *Mdh-3* in a critical MAAL5 of *O. latifolia*. *S* = *O. sativa*, *L* = *O. latifolia*

of the recurrent *sativa* parent (Table 3). The allozyme patterns of *O. latifolia* were observed as *Got3* and *Amp1* for MAAL 2, for *Mdh3* in MAAL 5, for *Pgd2* in MAAL 6, for *Amp2* and *Amp4* in MAAL 8, for *Pgd1* in MAAL 11 and for *Sdh1* in MAAL 12. We also observed modified *sativa* zymograms for *Amp3* and *Est2* in MAAL 6, but it showed the presence of both parental bands for the *Pgd2* and *Pgi2* loci (Table 3). MAAL 1 did not show the *O. latifolia* allele for *Est5* and *Got1*, as expected, indicating that the extra chromosome in this MAAL might be the modified chromosome 1 of *O. latifolia*. We could not identify extra alien chromosomes in MAALs 4, 9, 10 through isozyme analysis.

Chromosome location of isozyme loci by using MAALs

The MAALs were used to locate the isozyme loci on specific chromosomes. Analysis of 11 MAALs showed that the allozyme of the wild species for *Mdh3* was present only in MAAL 5 (Fig. 2), indicating the possible location of *Mdh3* on chromosome 5 of *O. latifolia*. MAAL 5 of *O. officinalis* had also shown a critical banding pattern for *Mdh3* (Khush et al. 1991).

Isozyme analyses of 11 MAALs of *O. latifolia* for *Pgd2* showed the allozyme pattern of wild species only in MAAL 6. Similarly from the isozyme analysis of ten *O. officinalis* MAALs, *Pgd2* was located on chromosome 6. These results confirm the location of *Pgd2* on chromosome 6 of these two wild species.

Meiotic behavior of MAALs

The chromosome pairing in MAALs was examined at the diakinesis and metaphase stages of meiosis. The model chromosome association in all MAALs was 12 II + 1 I. However, a trivalent (11 II + 1 III) configuration was also observed in MAALs 6, 8 and 12 in 6.2%, 3.3% and 11.8% of the cells, respectively. One plant each in the progenies of MAALs 6 and 12 had 26 chromosomes (13 II) indicating male transmission of the extra chromosome, resulting in the occurrence of disomic alien addition lines.

Transmission rates of the alien chromosomes

Transmission of extra chromosome through the female in 11 MAALs ranged from 4.4% in MAAL 1 to 35.5% in MAAL 12 (Table 4). Transmission through male gametes was observed in 8 of the 11 MAALs, the range being 1.7% (MAAL 10) to 11.9% (MAAL 12).

Alien gene introgression

Disomic plants recovered in BC_3 or later-selfed generations resembled the *O. sativa* parent. However, some of them differed slightly from the *sativa* parent with respect to height, growth duration and spikelet, and grain characteristics. In some progenies undesirable traits, such as spreading growth habit, tall stature, long awns, black hulls, red pericarp and grain shattering of *O. latifolia*, were associated with the transfer of desirable traits (Table 5). Introgression for several useful traits was achieved particularly for resistance to BPH, WBPH and BB, and for ten isozyme loci from *O. latifolia* to *O. sativa*:

Table 4 Female and male transmission rates of the extra chromosome in the MAALs of *O. latifolia*

MAAL	(2n + 1) × 2n				2n × (2n × 1)			
	Total	2n (no.)	2n+1 (no.)	2n+1 (%)	Total	2n (no.)	2n+1 (no.)	2n+1 (%)
1	46	44	2	4.4	12	12	0	0.0
2	79	68	11	13.9	43	43	0	0.0
4	120	87	33	27.5	91	83	8	8.8
5	132	109	23	17.4	67	65	2	3.0
6	48	34	14	29.2	108	104	4	3.7
7	44	35	9	20.5	87	81	6	6.9
8	192	154	38	19.8	117	111	6	5.1
9	108	85	23	21.3	49	49	0	0.0
10	48	36	12	25.0	58	57	1	1.7
11	96	74	22	22.9	72	68	4	5.6
12	180	116	64	35.5	117	103	14	11.9

Table 5 Traits of *O. latifolia* expressed in BC_3F_1 plants from the cross of *O. sativa* × *O. latifolia*

BC3 plants		Resistant to			Plants With					Plants with pigmentation in the				Plants with wild-type isozyme	Plants with multiple traits
Chromo-some #	No. of plants	BPH	WBPH	BB	Tall stature	Early maturing	Spreading growth habit	Black hull	Awns	Grain shattering	Stigma	Apiculus	Leaf sheath	Pericarp		
24	13	4	6	6	2	1	0	2	2	2	2	2	2	2	3	2
25	4	1	1	1	2	1	0	2	2	1	2	2	2	2	3	1
26	6	4	5	5	2	0	0	0	2	1	0	0	0	0	1	0
27	9	3	4	4	2	3	1	0	2	1	1	1	1	0	2	0
28	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	34	12	16	16	8	5	1	4	8	5	5	5	5	4	9	3

Resistance to BPH. Three hundred and nine of the 2,295 disomic BC_3F_3 progenies were segregating for resistance to the BPH biotype 1 of the Philippines. These progenies originated from 12 of the 34 BC_3F_1 plants. Progenies which showed resistance to BPH biotype 1 and WBPH were also tested for resistance to BPH biotypes 2 and 3 of the Philippines. All the BPH resistant progenies were like the recurrent *sativa* parent in plant type and did not carry any undesirable traits of *O. latifolia*. Some of the progenies showing consistently multiple resistance were advanced to the F_6 generation.

Resistance to WBPH. As many as 188 of the 2,295 BC_3F_3 progenies were found to be resistant to WBPH. These progenies originated from 16 BC_3F_1 plants. Some of the WBPH progenies were also resistant to BPH.

Resistance to BB. The elite breeding line, IR31917-45-3-2, of *O. sativa*, used as a recurrent parent, is homozygous for the dominant gene, *Xa4*, and is resistant to only races 1 and 5 of the Philippines. The *O. latifolia* parent is resistant to races 1, 2 and 3 of BB. As many as 188 of the 2,295 progenies showed resistance to race 2. None of these progenies was found to be resistant to race 3 of BB. Four plant progenies which were resistant to both BPH and WBPH were also resistant to BB race 2.

Introgression for qualitative traits

Tall plant stature. Eight BC_3F_2 plants were tall. Two each originated from 2n, 2n+1, 2n+2 and 2n+3 BC_3F_1 plants. Of these, three tall plants had multiple traits of the wild parent. Two progenies had long awns and grain shattering, traits of the wild parent. Progenies raised from all the eight tall-stature plants segregated in a ratio of 3 tall:1 dwarf, showing introgression of the dominant allele for tall stature from *O. latifolia*. Wild-type allozyme loci were also detected in four of these progenies.

Early growth duration. Five BC_3F_2 progenies segregated for days to flowering. These progenies originated one each from 2n and 2n+1, and three from 2n+3, BC_3F_1 plants. One early maturing plant progeny derived from the 2n+1 plant also had a purple leaf sheath, apiculus and stigma. This 2n+1 plant was MAAL 6. The F_1 hybrid between the *sativa* parent and this line was early in growth duration, like the derived line. In the F_2 population 201 plants were of short growth duration and 69 were like the recurrent parent. F_2 segregation fits the 3:1 ratio for early maturity, and normal-indicating-earliness in this line is controlled by a single dominant gene. One early maturing progeny also inherited long awns.

Spreading growth habit. The spreading growth habit is an undesirable trait. One BC_3F_2 plant from a 2n+3 BC_3F_1 plant segregated for the spreading growth habit. This trait was associated with narrow erect leaves. Selfed progenies

of spreading and erect leaved plants were true breeding while some of the normal sibs showed segregation for this trait in the following generation.

Black hull. Three out of 26 plants in a BC_3F_4 progeny had black-hulled grains. This was the selfed progeny of a disomic plant which originated from the 2n+1 BC_3F_2 plant, and which also had a black hull and awned grains. The F_1s between the *sativa* parent and a black hull plant also had black hulls, indicating that black hull is a dominant trait. The F_2 population segregated into 176 black-hull and 68 normal-hull color, thus showing that the black-hull trait of *O. latifolia* is controlled by a single dominant gene.

Two of the three plants of the BC_3F_4 progeny with black-hull grains and awns showed unusual behaviour in their self progenies. We grew self progenies of 61 F_5 plants over two generations. The chimeric nature with respect to black-hull grains and awns was recorded in some of the self progenies and black-hulled plants appeared as only 20% of the total population (318/1558).

Awns. Segregation for awning was observed in eight plants, two each from the 2n, 2n+1, 2n+2 and 2n+3 BC_3F_1. Four of these had very long awns (Fig. 3). Some of them bred true for long awns in the later generations. In three progenies long awns were also associated with grain shattering and black-hull traits.

Grain shattering. Five BC_3F_2 plants had shattering grains. Two of these originated from 2n and one each from the 2n+1, 2n+2 and 2n+3 BC_3F_1 plants. All of them were of tall stature. Progeny of a plant having a grain-shattering trait segregated into the normal and shattering habit, indicating that this trait in *O. latifolia* is controlled by a dominant gene(s).

Purple stigma and apiculus. Four BC_3F_2 plants had a purple stigma and a purple apiculus. Two of these originated from 2n and one each from 2n+1 and 2n+3 BC_3F_1 plants. The first three families had other traits from the wild parent along with a purple stigma and a purple apiculus. Of the 11 MAALs, MAAL 6 had a purple stigma and a purple apiculus. This indicates that genes controlling these traits are located on chromosome 6. The F_1 hybrids between a breeding line with a normal leaf sheath, stigma and apiculus, and a derived breeding line with a purple leaf sheath, stigma and apiculus, also had pigmentation in these plant parts. The F_2 population segregated in a ratio of 3 purple stigma:1 normal stigma. All the plants with a purple stigma also had a purple leaf sheath and a purple apiculus, indicating that the same gene is responsible for pigmentation in three plant parts, and was inherited from *O. latifolia*.

Red pericarp. Four BC_3F_2 plants had a red pericarp. Of these, two originated from disomic plants and other two from 2n+1 BC_3F_1 plants. One plant from the latter group had a weak stem, short pale-yellow leaves and was 10–15 cm taller in height. The self progeny of this plant was true breeding for the red pericarp in the BC_3F_4 generation. Other three progenies had several traits from the wild parent including a red pericarp.

Fig. 3 Two panicles each of *O. sativa* (left), introgression line (center) and *O. latifolia* (right)

Appearance of plants with multiple traits from wild parent in later generations

Two BC_3F_2 and one BC_3F_3 plant progenies had abnormal, tall-statured plants (Fig. 4) in low frequencies (usually two to three amongst 26 plant progenies). Parent plants in the previous generation were diploid, short and normal looking. The aberrant plants had multiple traits from the wild parent, such as tall stature, a purple leaf sheath, a purple stigma, a purple apiculus, grain shattering, long awns, a black hull and a red pericarp. Self progenies of all these plants also segregated for the wild traits in the following generation. Segregation data of 11 representative plant progenies in the F_5 generation of a plant which appeared in BC_3F_3 are given in Table 6. Segregation for

Table 6 Segregation of seven traits in 11 BC_3F_5 representative progenies of a disomic plant which appeared in the BC_3F_3 generation of *O. sativa* × *O. latifolia*

Progeny number	Number of plants												
	Plant height		Stigma color		Apiculus color		Leaf sheath color		Awns		Shattering grain		Hull color
	Tall	Dwarf	Red	Normal	Purple	Normal	Purple	Normal	Present	Absent	Shattering	Normal	Black	Straw
1	20	6	17	9	17	9	17	9	25	1	21	5	3	23
2	7	16	2	21	2	21	2	21	13	10	15	8	17	6
3	18	7	2	23	2	23	2	23	22	3	18	7	2	23
4	18	6	22	2	22	2	22	2	24	0	21	3	18	6
5	18	7	0	25	0	25	0	25	1	24	0	25	0	25
6	13	11	17	7	17	7	17	7	18	6	17	7	14	10
7	16	6	16	6	16	6	16	6	16	6	16	6	10	12
8	22	3	19	6	19	6	19	6	17	8	18	7	17	8
9	19	2	18	3	18	3	18	3	19	2	18	3	18	3
10	11	6	13	11	13	11	13	11	13	11	13	11	13	11
11	25	0	25	0	25	0	25	0	9	16	9	16	8	17

Fig. 4 Abnormal plants in the progeny of introgression lines of *O. latifolia*

Fig. 5 Introgression for the *Est5* allozyme in disomic progenies derived from the cross of *O. sativa* × *O. latifolia*; *S* = *sativa* allele, *L* = *latifolia* allele, + = introgression, – = no introgression, *H* = heterozygote

height, plant pigmentation, awns, grain shattering and hull color was abnormal in several families.

Introgression for isozyme loci

Disomic plants obtained in the BC_3F_1 and BC_4F_2 generations were analyzed for 15 isozyme loci. Nine of the 34 BC_3F_1 plants had wild-type allozymes. Introgression was detected for ten allozymes of *O. latifolia*, such as *Est5*, *Amp1*, *Pgi1*, *Mdh3*, *Pgi2*, *Amp3*, *Pgd2*, *Est9*, *Amp2* and *Sdh1*, located on 8 of the 12 chromosomes (Table 7). The *Amp3*, *Est9* and *Sdh1* allozymes showed a modified

Table 7 Introgression of isozyme loci from *O. latifolia* into elite breeding lines of rice

Isozyme locus	Chromosome	Allozyme	BC_3 progenies (no.) with wild allele
Est5	1	L[a]	1
Amp1	2	L	8
Pgi1	3	L	6
Mdh3	5	L	2
Pgi2	6	L	5
Pgd2	6	L	3
Amp3	6	*[b]	6
Est9	7	*	4
Amp2	8	L	3
Sdh1	12	*	3

[a] L = *O. latifolia* allele
[b] * = Modified expression

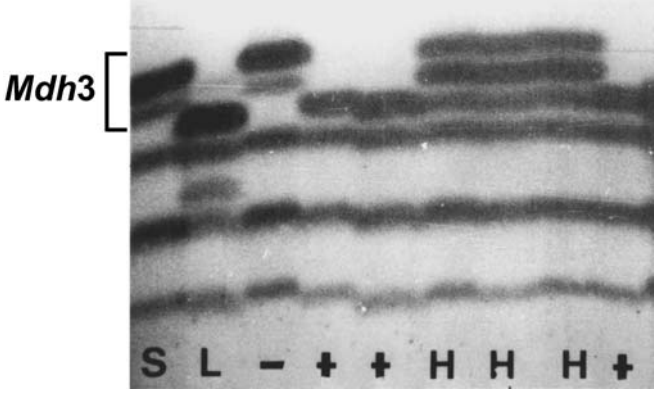

Fig. 6 Introgression for the *Mdh3* allozyme in disomic progenies derived from the cross of *O. sativa* × *O. latifolia*; *S* = *sativa* allele, *L* = *latifolia* allele, + = introgression, – = no introgression, *H* = heterozygote

expression, whereas the rest of the seven allozymes in the derived lines had a similar expression as in *O. latifolia*.

The maximum number of eight BC_3 progenies had introgression for the *Amp1* allele located on chromosome 2, followed by six progenies each for the *Pgi1* and *Amp3* allozymes belonging to chromosomes 3 and 6, respectively (Table 7). Only one progeny derived from the 2n+1 BC_3 plant had the *Est5* allozyme of *O. latifolia* (Fig. 5), and two of the 13 disomics in BC_3 showed introgression for the *Mdh3* allele of *O. latifolia* (Fig. 6). Introgression for the *Pgd2* allele located on chromosome 6, was detected in three progenies. One of these progenies also had introgression for the *Amp3* allele and other two had introgression for the *Pgi2* allozyme of the wild parent. A plant progeny with a less number of tillers and with tall stature, originated from the 2n+1 BC_3 plant and had the *Pgi1* allele of the wild parent. Three progenies, one from 2n+3 and two from 2n+1 BC_3 plants, associated with late growth duration, showed introgression for the *Amp1* allele of the wild parent. A progeny segregating for tall stature, long awns and grain shattering traits of the wild parent, also had introgression for the *Amp1* and *Amp3* alleles. Introgression was detected for the *Amp3* and *Sdh1* alleles belonging to chromosomes 6 and 12, respectively, in a progeny having a spreading growth habit with narrow leaves. In a black-hulled plant, introgression for the *Pgi1*, *Amp2* and *Amp3* allozymes of *O. latifolia* was detected. One progeny with pigmentation in the stigma, apiculus and the leaf sheath showed introgression for the *Est9* allele of the wild parent belonging to chromosome 7. Introgression for the *Est9* allele was also detected in the progeny of a diminutive BC_3 plant. A red pericarp plant progeny was associated with introgression for the *Amp1*, *Pgi2* and *Est5* allozymes of *O. latifolia*. Most of the plants showing introgression for isozyme alleles were heterozygous and segregated in a 1:2:1 ratio in self progenies.

Discussion

The F_1 hybrids of *O. sativa* and *O. latifolia* were studied by Morinaga (1941). He reported a crossability of 14.0%. In this investigation the crossability was 7.58%. The crossability of the F_1 hybrid when backcrossed with the *O. sativa* parent was extremely low (0.11%). This was probably due to high proportion of non-functional female gametes produced by the triploid F_1 hybrid. Morinaga (1941) and Nezu et al. (1960) did not observe any bivalent formation in the F_1 hybrid of *O. sativa* and *O. latifolia*. However, Li et al. (1962) reported 1–8 bivalents per cell with a mean of 4.64%. In few cells they observed two trivalents and/or a quadrivalent(s). The results of this study are in agreement with those of Li et al. (1962). As a result of this chromosome pairing and recombination, the transfer of useful genes from *O. latifolia* to *sativa* could be obtained.

Identification of alien chromosomes

MAALs have been studied in several species such as wheat, oats and tobacco (Khush 1973). MAALs of *O. sativa* having a single alien chromosome of *O. officinalis* were investigated by Jena and Khush (1989). Yasui and Iwata (1991) examined the MAALs of *O. sativa* with the alien chromosomes of *O. punctata*. Similarly, Multani et al. (1994) studied the MAALs of *O. sativa* with the alien chromosomes of *O. australiensis*. In this study, 11 of the possible 12 MAALs of *O. sativa* with single alien chromosomes of *O. latifolia* were obtained. In all of these studies, MAALs closely resembled the respective primary trisomics of *O. sativa*. These results show that the gene contents of chromosomes in the wild species are similar to each other, as well as to that of cultivated rice.

Extent of recombination

Most of the 2,295 disomic progenies obtained in BC_3 and BC_4 generations, had complete resemblance to the *O. sativa* parent. Similar results were obtained in the cross of *O. sativa* and *O. officinalis* (Jena and Khush 1990), and

the cross of *O. sativa* and *O. australiensis* (Multani et al. 1994), where disomic progenies derived after only two backcrosses resembled the *O. sativa* parent. This rapid recovery of the recurrent parent phenotype after only two or three backcrosses is the result of extremely limited recombination between the genome of cultivated rice on the one hand and the genomes of wild species on the other. Rapid recovery of the recurrent parent genotypes in the backcross progenies of wide crosses has also been reported in *Gossypium* by Stephens (1949) and *Lycopersicon* by Rick (1963, 1969, 1971).

Gene introgression from *O. latifolia*

Several single gene transfers from *O. latifolia* to *O. sativa* were obtained in this study. Genes of economic importance, such as those for resistance to BPH, WBPH and BB, were transferred. These results show the value of wild germplasm in rice improvement. Similar results were obtained in wide crosses between *O. sativa* and *O. officinalis* (Jena and Khush 1990), and *O. sativa* and *O. australiensis* (Multani et al. 1994). In addition, introgression was obtained for the allozyme of ten isozymes of *O. latifolia* and for several morphological traits, such as plant height, black hull, red pericarp, shattering grain, long awns, plant pigmentation and shorter growth duration.

Instability and abnormal segregation

Two black-hulled plants derived from a BC_3F_4 progeny showed distorted segregation. Selfed progenies of 61 F_5 plants grown over two generations segregated for only 20% (318/1558) of the plants instead of the 75% expected for a monogenic dominant trait. Moreover, hulls of several plants were chimeras for hull color. The most-interesting feature of this study was the appearance of plants with dominant mutants in the progenies of normal plants. For example, a BC_3F_3 plant was diploid, dwarf and normal-looking like any other plant of *O. sativa*. Plants with mutant traits, e.g. tall stature, purple stigma, apiculus and leaf sheath, awns, shattering grains and black hull, appeared in the F_4 progeny. We progeny tested 11 F_4 plants with mutant traits. As the data of Table 6 show, several progenies showed distorted segregation. For example, abnormal segregation was obtained for awns and hull color in progeny 1; for stature and plant pigmentation in progeny 2; for plant pigmentation and hull color in progeny 3; for plant pigmentation and grain shattering in progeny 4; for plant pigmentation, awns, grain shattering and hull color in progeny 5; for plant height in progeny 8; for all traits in progeny 9; and for awns, grain shattering and hull color in progeny 11. Absence of dominant mutants for plant pigmentation, shattering grains and hull color was unusual. Activation of transposons may be responsible for instability and abnormal segregation behaviour. Novel mutants have been observed in the later generations of these progenies which are under investigation.

Acknowledgements The financial support from the Rockefeller Foundation is gratefully acknowledged.

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