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Development of monosomic alien addition lines and introgression of genes from *Oryza australiensis* Domin. to cultivated rice *O. sativa* L.

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Abstract *Oryza australiensis*, a diploid wild relative of cultivated rice, is an important source of resistance to brown planthopper (BPH) and bacterial blight (BB). Interspecific hybrids between three breeding lines of *O. sativa* (2n = 24, AA) and four accessions of *O. australiensis* (2n = 24, EE) were obtained through embryo rescue. The crossability ranged from 0.25% to 0.90%. The mean frequency of bivalents at diakinesis/metaphase I in F_1 hybrids (AE) was 2.29 to 4.85 with a range of 0–8 bivalents. F_1 hybrids were completely male sterile. We did not obtain any BC_1 progenies even after pollinating 20,234 spikelets of AE hybrids with *O. sativa* pollen. We crossed the artificially induced autotetraploid of an elite breeding line (IR31917-45-3-2) with *O. australiensis* (Acc. 100882) and, following embryo rescue, produced six F_1 hybrid plants (AAE). These triploid hybrids were backcrossed to *O. sativa*. The chromosome number of 16 BC_1 plants varied from 28 to 31, and all were male sterile. BC_2 plants had 24–28 chromosomes. Eight monosomic alien addition lines (MAALs) having a 2n chromosome complement of *O. sativa* and one chromosome of *O. australiensis* were selected from the BC_2 F_2 progenies. The MAALs resembled the primary trisomics of *O. sativa* in morphology, and on the basis of this morphological similarity the MAALs were designated as MAAL-1, -4, -5, -7, -9, -10, -11, and -12. The identity of the alien chromosome was verified at the pachytene stage of meiosis. The alien chromosomes paired with the homoeologous pairs to form trivalents at a frequency of 13.2% to 24.0% at diakinesis and 7.5% to 18.5% at metaphase I. The female transmission rates of alien chromosomes varied from 4.2% to 37.2%, whereas three of the eight MAALs transmitted the alien chromosome through the male gametes. BC_2 progenies consisting of disomic and aneuploid plants were examined for the presence of *O. australiensis* traits. Alien introgression was detected for morphological traits, such as long awns, earliness, and *Amp-3* and *Est-2* allozymes. Of the 600 BC_2 F_4 progenies 4 were resistant to BPH and 1 to race 6 of BB. F_3 segregation data suggest that earliness is a recessive trait and that BPH resistance is monogenic recessive in two of the four lines but controlled by a dominant gene in the other two lines.

Key words Rice · *Oryza sativa* · *O. australiensis* · Monosomic alien addition lines (MAALs) · Alien gene transfer · Introgression · Resistance to bacterial blight · Resistance to brown planthopper

Introduction

The genus *Oryza* consists of more than 20 wild and two cultivated species. Both of the cultivated species, *O. sativa* and *O. glaberrima*, are diploid 2n = 24 and have the AA genome. The wild species have 2n = 24 or 2n = 48 chromosomes, and seven genomes (AA, BB, CC, BBCC, CCDD, EE, and FF) have so far been designated for 17 species. The wild species are sources of useful genes for resistance to major diseases and insects and for tolerance for abiotic stresses. *O. australiensis* with the EE genome is resistant to brown planthopper (BPH) and bacterial blight (BB) (Swaminathan 1986). Earlier attempts to transfer useful genes have been confined to closely related wild species with the AA genome, such as *O. nivara* and *O. longistaminata*, through conventional hybridization. Two notable examples include the transfer of a gene for resistance to grassy stunt virus from *O. nivara* (Khush et al. 1977) and the transfer of a gene for resistance to BB from *O. longistaminata* (Khush et al. 1990). More recently, Jena and Khush (1989, 1990) developed monosomic alien addition lines of *O. sativa* having alien chromosomes of *O. officinalis*, and transferred genes for resistance to BPH and whitebacked planthopper from *O. officinalis* to cultivated rice. Amante et al. (1992) also transferred genes for resistance to blast and BB from *O. minuta* to cultivated rice. The study described here was undertaken to explore the possibility of transferring genes for resistance to BPH and BB from *O. australiensis* to *O. sativa*.

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Materials and methods

Production of interspecific hybrids

F_1 hybrids were produced by crossing three elite breeding lines of *O. sativa* – all susceptible to biotypes 1, 2, and 3 of BPH – as female parents with four accessions of *O. australiensis* (all resistant to three BPH biotypes). The pollinated panicles were sprayed twice a day for 5 days with a mixture of the growth hormones gibberellic acid + naphthalene acetic acid and kinetin in the proportion of 100, 25, and 5 mg/l respectively. Ten to 14 days after pollination, hybrid embryos were excised and cultured on 1/4 MS medium. The cultured embryos were incubated in darkness at 25 ± 1 °C until germination and subsequently transferred to light. The young seedlings at the three-leaf stage were transferred to a liquid nutrient solution and were grown in a phytotron. After 10 days, the seedlings were transplanted into soil.

The F_1 hybrids were backcrossed to the respective breeding lines of *O. sativa* as the recurrent parent. An examination of the aborting BC_1 seeds revealed the absence of embryos in the swollen ovaries. Thus, we failed to obtain any BC_1 progenies.

We therefore produced an autotetraploid of one of the breeding lines (IR31917-45-3-2) through colchicine treatment. Cytologically confirmed tetraploid plants were used as the female parent in crosses with *O. australiensis* (Acc. 100882). Triploid hybrids (AAE) were obtained following embryo rescue. These hybrids were backcrossed to the recurrent diploid parent (IR31917-45-3-2) to obtain BC_1 and BC_2 progenies. Embryos from backcrosses were cultured on 1/4 MS medium. BC_2F_2 populations were grown, and plants were classified as diploids or as aneuploids. F_3 and F_4 progenies from diploid and aneuploid plants were grown in the pedigree nursery.

Chromosome analysis

Spikelets at a suitable stage were fixed in a mixture of 1:3 parts acetic acid and 95% ethanol to which traces of ferric chloride had been added. Meiotic chromosome analyses of F_1, BC_1, and BC_2 progenies were carried out at pachytene, diakinesis, and metaphase I following the 1% propionic-carmine squash technique.

Establishment of monosomic alien addition lines (MAALs)

Plants having 25 chromosomes (MAALs) were isolated in BC_2F_2 and studied for their morphological and reproductive features. Their morphology was compared with that of primary trisomics of *O. sativa* cv 'IR36'. The MAAL resembling primary trisomic 1 was tentatively designated MAAL 1, that resembling primary trisomic 4 was designated MAAL 4, and so on. The extra alien chromosome in each MAAL was identified at pachytene. Chromosome pairing in MAALs was also examined at the diakinesis and metaphase I stages of meiosis. Anther length, pollen fertility, seed setting, and transmission rates of alien chromosomes were studied.

Detection of alien gene introgression

Screening for BB resistance

All 600 BC_2F_4 pedigree nursery rows were inoculated with races 1, 2, and 3 of BB in the field. The eight MAALs were also screened for reaction to BB race 6. Disomic plants from selfed progenies of MAAL 12 were also screened with race 6.

Screening for BPH resistance

All of the 600 single-plant selections from BC_2F_4 were screened for resistance to BPH biotype 1 in the greenhouse. Resistant progenies were further evaluated for resistance to biotypes 2 and 3. Eight MAALs and disomics derived from selfed progenies of each MAAL were also evaluated for reaction to BPH.

Introgression of morphological traits

The 600 BC_2F_4 progenies in the pedigree nursery were also examined for the presence of such qualitative traits of *O. australiensis* as long awns, black hull, and growth duration. *O. australiensis* has long awns, whereas the IR parent is devoid of awns, and the derived lines had awns of variable length. Selected progeny rows were advanced to F_5 and F_6 through single-plant selection and bulk seed harvests.

Isozyme analysis

The white portions of emerging leaves from germinated seeds or young seedlings of 18 BC_2F_2 and BC_2F_3 progenies were collected and homogenized in 0.1% mercapto-ethanol. Samples were subjected to starch gel electrophoresis and stained for shikimate dehydrogenase (SDH), phosphoglucose isomerase (PGI), aminopeptidase (AMP), esterase (EST), phosphogluconate dehydrogenase (PGD), alcohol dehydrogenase (ADH), and glutamate oxaloacetate transaminase (GOT) activities using the procedure described by Glaszmann et al. (1988). Malate dehydrogenase (MDH) activity staining was detected using the method of Second and Trouslot (1980).

Results

Crossability

The seed set in *O. sativa* × *O. australiensis* crosses ranged from 2.30% to 2.88%. Germination of 10- to 14-day-old hybrid embryos varied from 52.4% to 62.9% (Table 1), and crossability ranged from 0.25 to 0.90%. Of the 101 F_1 hybrids obtained,

Table 1 The crossability of three breeding lines of *O. sativa* and *O. australiensis* accessions

O. sativa (female)	Spikelets pollinated (no.)	Seed Set (%)	Embryos cultured (no.)	Embryos germinated (%)	Hybrid plants obtained (no.)	Cross ability[a] (%)
IR1529-680-3-2	5,350	2.30	97	62.88	48	0.90
IR25587-109-3-3-3-3	3,524	2.30	32	56.25	9	0.25
IR31917-45-3-2	3,072	2.88	42	52.38	13	0.42

[a] $\%\ \text{crossability} = \frac{\text{no. of hybrids}}{\text{no. of spikelets pollinated}} \times 100$ (Wuu et al. 1963)

Table 2 Chromosome associations at diakinesis in the interspecific hybrids between three breeding lines of *O. sativa* and *O. australiensis* accessions

O. sativa line used as female	PMCs studied (no.)	Bivalents/cell (no.)		Univalents / cell (no.)	
		Mean	Range	Mean	Range
IR1529-680-3-2	310	2.29	0–6	20.28	12–24
IR25587-109-3-3-3-3	53	4.85	0–8	17.50	8–24
IR31917-45-3-2	65	2.35	0–5	20.40	14–24

Table 3 Crossability, chromosome number, and fertility of F_1 and backcross progenies from the cross of tetraploid *O. sativa* (AAAA) and diploid *O. australiensis* (EE)

Generation	Crossability (%)	Plants obtained (no.)	Chromosome number	Pollen fertility (%)	Spikelet fertility (%)
F_1	0.26	6	36	0	0
BC_1	3.20	16	28–31	0–0.5	0
BC_2	2.33	35	24–28	0–58.0	0–49.4

70 survived. The F_1 hybrids were morphologically intermediate between the two parents, but were tall and had long awns; they were vigorous but completely male sterile. Backcrosses with the respective recurrent parents gave no viable seed even though 20,234 spikelets were pollinated with *O. sativa* pollen. Hence, an induced autotetraploid (AAAA) of the elite breeding line IR31917-45-3-2 was crossed with *O. australiensis* (Acc. no. 100882). Following embryo rescue, we obtained six F_1 triploid hybrids. The crossability was 0.26%.

Chromosome analysis

Meiotic analysis of F_1 hybrids showed 0–8 bivalents per cell at diakinesis/metaphase I, the range being 2.29 to 4.85 per cell (Table 2). However, the majority of the pollen mother cells (PMCs) showed extensive pairing between the A and E chromosomes at pachytene.

The PMCs of allotriploid hybrids had 12 bivalents, 12 univalents, and only rarely, a trivalent. All of the allotriploid plants were completely male sterile. When these triploid hybrids were backcrossed to the diploid IR31917-45-3-2, a few seeds were produced. Through embryo rescue, 16 BC_1 plants were obtained with chromosome numbers varying from 28 to 31 (Table 3). Only 2 of the BC_1 plants with 28 chromosomes set seed upon backcrossing to IR31917-45-3-2; the rest were completely sterile. We obtained 35 BC_2F_1 plants of which only 20 survived. The chromosome number of these plants varied from 24 to 28: 2 had 24 chromosomes, 10 had 25, 6 had 26, and 1 each had 27 and 28 chromosomes. Most of these BC_2 plants were partially to completely sterile.

Isolation of MAALs

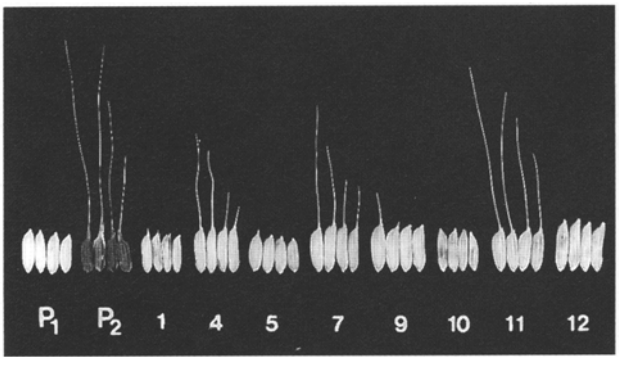
We grew the F_2 populations from BC_2F_1 plants with 24, 25, 26, 27, and 28 chromosomes and obtained a total of 429 BC_2F_2 plants. Of those, 44 plants with 25 chromosomes were selected. These plants had a close resemblance to various primary trisomics of *O. sativa*: 10 plants resembled triplo 12; 8, triplo 5;7, triplo 9; 6, triplo 11; 4 each, triplo 7 and triplo 4; and 1 each, triplo 1 and triplo 10. Three plants had an extra telocentric chromosome. On the basis of their morphological resemblance to the various primary trisomics, these $2n + 1$ plants were tentatively designated as MAAL1, 4, 5, 7, 9, 10, 11, and 12.

Characterization of MAALs

Morphological characteristics

All of the eight MAALs differed from normal disomics as well as from each other in morphological characteristics such as growth habit, height, shape and length of leaves, size of ligule, presence or absence of awns, pollen fertility, seed setting, and shape and size of the seed. All of the MAALs had slower growth rates except MAAL 11, which was as vigorous as disomic sibs. MAAL 1 and MAAL 5 could be easily identified at the seedling stage as they had droopy and twisted leaves, respectively. MAAL 4, 7, 9, and 12 were easily distinguished at the maximum tillering stage. MAAL 10 and MAAL 11 could be identified at the flowering stage. MAAL 10 is stunted and flowers early with long slender grains. MAAL 9 had rolled, thick, erect, dark-green leaves, partially exserted panicles, and bold grains. MAAL 4, 7, and 11 had awned

Fig. 1 Grain of *O. sativa* (P_1), *O. australiensis* (P_2), and eight MAALs. *Numbers* correspond to the respective MAALs



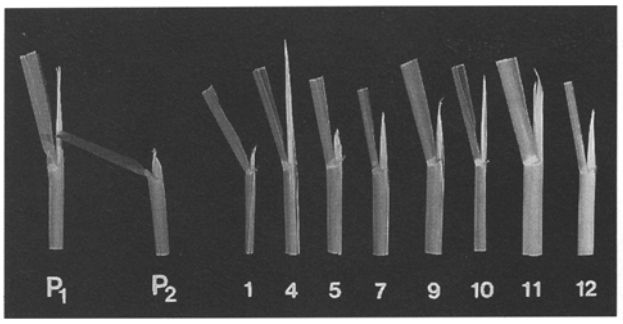


Fig. 2 Segments of uppermost leaves of *O. sativa* (P_1), *O. australiensis* (P_2), and eight MAALs showing differences in ligule length. *Numbers* correspond to the respective MAALs

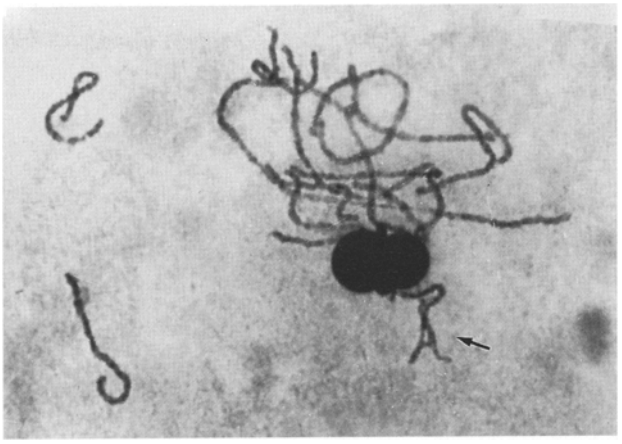


Fig. 3 Pairing between chromosome 9 of *O. sativa* and *O. australiensis* at pachytene showing trivalent configuration (see *arrow*)

spikelets (Fig. 1). MAAL 7 and 11 had gold hulled grains. MAAL 4 had the longest ligule (Fig. 2). The pollen fertility of the MAALs ranged from 16.3% to 90.9%, with the lowest being observed in MAAL 1.

The anthers of *O. australiensis* at the time of anthesis are 4.1 ± 0.01 mm long and are twice as long as those of IR31917-45-3-2. MAAL 12 had the longest anthers (3.0 ± 0.02mm). The anthers of the seven other MAALs varied in length from 1.4 ± 0.02mm to 2.5 ± 0.01mm as compared with 2.0 ± 0.01mm of the recurrent parent. The data indicate that the gene(s) for long anther is located on chromosome 12 of the *O. australiensis* genome. Seeds of *O. australiensis* are black hulled. However, none of the eight MAALs showed the black hull trait, indicating that the gene for black hull is not present on any of chromosomes 1, 4, 5, 7, 9, 10, 11, and 12 of *O. australiensis*.

Pachytene analysis

The somatic chromosomes of *O. australiensis* are indistinguishable in size and other cytological features from those of *O. sativa*. Therefore the extra chromosomes of the MAALs were examined at the pachytene stage of meiosis. Trivalent configurations resulting from pairing between *O. australiensis* and homoeologous *O. sativa* chromosomes were examined. Chromosome identification at the pachytene stage is easier and by examining trivalent configuration, we were able to identify the extra alien chromosome of all MAALs. For example, the trivalent in Fig. 3 (see arrow) is clearly for chromosome 9, which is attached to the nucleolus. This MAAL closely resembles the primary trisomic for chromosome 9. In this way, morphological identification of all the MAALs was confirmed through pachytene analysis.

Chromosomal associations at diakinesis and metaphase

Chromosome pairing in MAALs was also examined at diakinesis and metaphase I. The MAALs showed trivalent formation ($11_{II} + 1_{III}$) in 7.5% to 24.0% of the cells (Table 4). The maximum and minimum frequencies showing a trivalent at diakinesis was in MAAL 11 and MAAL 4, respectively.

Table 4 Chromosome associations in monosomic alien addition lines (MAALs) at diakinesis (*DK*) and metaphase I (*MI*) of meiosis

MAAL	PMCs observed (no.)	Meiotic stage	Number of cells with chromosome association			% cells showing $11_{II} + 1_{III}$
			$12_{II} + 1_{I}$	$11_{II} + 1_{III}$	$11_{II} + 3_{I}$	
1	91	DK	68	19	4	20.9
4	189	DK	156	25	8	13.2
	119	MI	103	9	7	7.5
5	193	DK	153	34	6	17.6
	87	MI	75	8	4	9.2
7	189	DK	149	40	0	21.7
	288	MI	229	42	17	14.6
9	205	DK	154	41	10	20.0
	105	MI	89	11	5	10.4
10	318	DK	249	61	8	19.2
	198	MI	152	31	15	15.6
11	275	DK	180	66	29	24.0
	220	MI	177	23	20	10.5
12	195	DK	153	39	3	20.0
	248	MI	191	46	11	18.5

Table 5 Female and male transmission rates of the extra chromosome in the MAALs of *O. australiensis*

MAAL	(2n + 1) × 2n[a]				2n × (2n + 1)			
	Total	2n	2n + 1	% (2n + 1)	Total	2n	2n + 1	% (2n + 1)
1	24	23	1	4.2	43	43	0	0.0
4	102	80	22	21.6	81	81	0	0.0
5	92	81	11	12.0	170	168	2	1.2
7	181	149	32	17.7	119	119	0	0.0
9	120	100	20	16.7	124	120	4	3.2
10	96	78	18	18.8	56	56	0	0.0
11	97	78	19	19.6	74	74	0	0.0
12	218	137	81	37.2	83	81	2	2.4

[a] 2n parent was IR31917-45-3-2

However, the modal chromosome association in the MAALs was $12_{II} + 1_{I}$. The chromosome configuration of $11_{II} + 3_{I}$ was also observed in a few cells of most of the MAALs.

Transmission rates of the alien chromosomes

Transmission rates of alien addition chromosomes through the female gametes were fairly high (Table 5). MAAL 12 showed the highest transmission rate (37.2%) and MAAL 1 the lowest (4.2%). Transmission of alien chromosomes through the male gametes was recorded only in MAALs 5, 9, and 12 at low frequencies (1.2, 3.2, and 2.4%, respectively).

Alien gene transfer

The diploid (2n) plants that appeared in the BC_2F_1 and later generations closely resembled the *O. sativa* parent. However, some of the progenies differed from the *O. sativa* parent in plant height, leaf length and width, leaf angle, and growth duration. Undesirable traits of the wild species such as grain shattering and spreading growth habit were not recorded in any of the diploid progenies. However, introgression for the following monogenic traits was observed in some of the progenies.

Awns. Of the 20 BC_2F_1 plants, 6 showed awns (Fig. 4). Of these awned plants 1 was 2n; 3, 2n + 1; and 2 were 2n + 2 (Table 6). The BC_2F_2 progenies of these plants segregated for awned and awnless plants. Awn length varied from progeny to progeny.

Fig. 4 Three panicles each of *O. australiensis* (*left*), introgression line (*centre*), and *O. sativa* (*right*)

Growth duration. Four BC_2F_3 plants progenies segregated for days to flowering. Two of the lines originated from 2n + 1 and one each from 2n + 3 and 2n + 4 BC_2 plants. In BC_2F_4 these progenies flowered in 69–78 days compared with 97 days for the *O. sativa* parent.

When the early-maturing plants were crossed with the *O. sativa* parent, the growth duration of F_1 progenies was similar to that of the *O. sativa* parent, indicating that earliness is a recessive trait. The F_2 populations segregated in a ratio of 1:3 for earliness (Table 7), confirming the monogenic recessive control of earliness in these progenies.

Table 6 Traits of *O. australiensis* expressed in BC_2F_1 of the cross of *O. sativa* × *O. australiensis*

Chromosome number of BC_2F_1 plants	Number of plants studied	Number of BC_2F_1 plants segregated for				
		Long awns	Early maturity	Resistance to		Isozyme allele of wild parent
				BPH	BB	
2n	2	1	0	0	0	1
2n + 1	10	3	2	2	1	1
2n + 2	6	2	0	0	0	0
2n + 3	1	0	1	1	0	0
2n + 4	1	0	1	1	0	0

Table 7 Segregation pattern for early-maturity and BPH reaction of four early maturing and BPH-resistant introgressed lines in crosses with *O. sativa*

Cross combination	Growth duration of F_1 and F_2 plants					Reaction[a] of F_1 and F_3 progenies to BPH biotype 1					
	F_1	Early (no.)	Normal (no.)	Total (no.)	χ^2 (1:3)	F_1	R (no.)	R/S (no.)	S (no.)	Total (no.)	χ^2 (1:2:1)
O. sativa/IR65482-4-136	Normal	72	264	336	2.28	R	89	178	69	336	3.57
O. sativa/IR65482-7-216	Normal	59	229	288	3.13	S	60	153	75	288	2.69
O. sativa/IR65482-17-511	Normal	55	209	264	2.44	R					
O. sativa/IR65482-18-539	Normal	27	117	144	3.00	S					

[a] R, Resistant; S, susceptible; R/S, segregating

Three of the BC_2F_4 progenies had longer growth duration than the *O. sativa* parent. They flowered 15 days later.

Resistance to BPH. Two of the 10 2n + 1 plants, and 1 each of 2n + 3 and 2n + 4 BC_2 plants segregated for resistance to BPH (Table 6). Of the eight MAALs, only MAAL 12 segregated for resistance to BPH. We also tested a total of 491 disomic plants from the selfed progenies of MAALs in the BC_2F_4 generation. Out of 120 2n plants of MAAL 12, 1 segregated for resistance to BPH. Similarly, 1 of the 48 disomic plants in the selfed progeny of MAAL 12 in the BC_2F_5 generation was found to be resistant to BPH. The data suggest that the gene(s) for resistance to BPH is located on chromosome 12 of *O. australiensis*. BPH resistant lines selected from different generations were purified and tested for resistance in subsequent generations (Fig. 5). Of the 4 BPH-resistant progenies (IR65482-4-136) 1 was resistant to all the three biotypes of BPH, 1 was resistant to biotypes 1 and 3 (IR65482-17-511), another was resistant to biotypes 1 and 2 (IR65482-7-216), and the 4th (IR65482-18-539) was resistant to biotype 1 only.

We crossed the BPH resistant lines with IR31917-45-3-2. The F_1s involving IR65482-4-136 and IR65482-17-511 were resistant to BPH, indicating that resistance in these lines is dominant. The F_3 families segregated in a 1:2:1 ratio for homozygous resistant, segregating (R/S), and homozygous susceptible, respectively (Table 7). The data indicate that BPH resistance in these 2 is governed by a dominant gene. F_1s involving the two other lines were susceptible (Table 7), and the F_3 progenies segregated in 1:2:1 ratio for homozygous resistant, segregating (R/S), and homozygous susceptible. Thus, resistance to BPH in these lines is monogenic recessive.

Fig. 5 Reaction of check varieties and WC progenies (introgression lines) to brown planthopper. 'Rathu Heenati', *O. australiensis*, and WC1, WC2, WC5 and WC6 are resistant, 'IR31917-45-3-2', TN1, WC3, and WC4 are susceptible

Resistance to BB. IR31917-45-3-2, the recurrent parent used in this study, is homozygous for *Xa-4* and is resistant to Philippine races 1 and 5 of BB, whereas *O. australiensis* is resistant to all the six races. One out of 600 BC_2F_4 progenies was susceptible to race 1. This line originated from MAAL 12. Furthermore, this line was also found to be resistant to BPH and had short growth duration. The crossover event that transferred BPH resistance from the alien chromosome 12 to the homoeologous *O. sativa* chromosome presumably involved the transfer of the *Xa-4* allele from chromosome 12 of *O. sativa* to homoeologous chromosome of *O. australiensis*.

Race 6 of BB is most virulent and IR31917-45-3-2 is highly susceptible to it. Of the eight MAALs, only MAAL 12 was found to be resistant to race 6. Disomic plants derived from the selfed progeny of this MAAL were inoculated with race 6, and 4 were found to be resistant. The progenies of these plants segregated for resistance, thus indicating that resistance is dominant. Allelic tests are presently being made with other genes for resistance to race 6 to determine if this is a new gene.

Allozyme patterns. Polymorphism was detected between *O. sativa* and *O. australiensis* for 12 of the 15 isozyme loci examined (Table 8). One progeny from a 2n BC_2F_1 plants was found to be heterozygous for *Amp-3* (*sativa* and modified *O. australiensis* bands). Similarly, another 2n + 1 BC_2F_1 plant was heterozygous for *australiensis* alleles of the *Amp-3* and *Est-2* loci. A disomic progeny with introgression for the *Amp-3* allele of *O. australiensis* is shown in Fig. 6. The BC_2F_2 progeny of 2n plants segregated in a 1:2:1 ratio for homozygous (*O. sativa* allele), heterozygous, and homozygous (*O. australiensis* alleles), respectively.

Discussion

Extent of recombination

There are several reports on hybrids between *O. sativa* and *O. australiensis*. Most of these crosses were made to study the genomic relationships of the two species. Morinaga and Kuriyama (1960) and Nezu et al. (1960) did not observe any pairing between the chromosomes of the two species at diakin-

Table 8 Isozyme polymorphism between *O. sativa* (IR31917-45-3-2) and *O. australiensis* (Acc. 100882)

Parent	Isozyme[a]											
	Sdh-1	*Pgi-1*	*Amp-3*	*Amp-4*	*Pgd-1*	*Pgd-2*	*Adh-1*	*Mdh-3*	*Got-1*	*Got-3*	*Enp-1*	*Pox-5*
O. sativa	F	S	S	F	F	S	S	F	S	F	F	F
O. australiensis	S	F	F	S	S	F	F	S	F	S	S	S

[a] F, Fast mobility; S, slow mobility

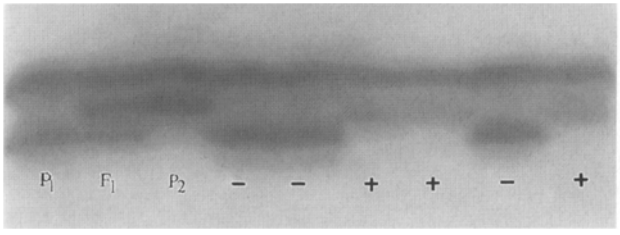


Fig. 6 Zymogram showing introgression for Amp-3 in progenies derived from the cross of *O. sativa* (P_1) and *O. australiensis* (P_2). + Introgression with modified band, – no introgression

esis or metaphase. A limited amount of pairing has been observed at diakinesis (0–8 bivalents) by other authors (Gopalakrishnan 1959; Shastry and Ranga Rao 1961; Li et al. 1963; Watanabe and Wakasa 1973). The data on metaphase pairing reported in this paper is in conformity with the earlier reports as we also observed 0–8 bivalents with a mean of 2.29 to 4.85 per cell. Although complete pairing was observed at pachytene in many cells there was only limited pairing at later stages, indicating that most of this pairing was achiasmatic.

These observations are in agreement with the rapid recovery of the phenotype of the recurrent parent after only two backcrosses. Diploid BC_2F_1 plants and diploid plants derived from the MAALs in the BC_2F_2 and later generations resembled the recurrent parent closely, and none of the undesirable traits of the wild species such as shattering spikelets, spreading growth habit, and weak stems were inherited by these progenies. This rapid recovery of the recurrent parent phenotype after only two backcrosses is the result of restricted recombination between the parental genomes. Similar observations were made by Jena and Khush (1990) in the cross between *O. sativa* and *O. officinalis* where because of restricted recombination, the phenotype of the recurrent parent was reconstituted only after two backcrosses. 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. australiensis*

Several single gene transfers from *O. australiensis* have been obtained in this study. At least two genes for BPH resistance (one dominant and one recessive), a dominant gene for BB resistance, a recessive gene for earliness, and at least two dominant genes for awning have been transferred to different progenies. In addition, introgression was obtained for *O. australiensis* alleles of *Amp-3* and *Est-2*. The genes for BPH and BB resistance are very useful in the improvement of rice and are being incorporated into elite breeding lines. The allelic relationships of these genes with known genes are being investigated. Molecular analysis of the introgression lines has shown that only small chromosome segments of the wild species have been transferred (Ishii et al. 1994).

Of the eight MAALs, only MAAL 12 is resistant to BPH and BB, indicating that the genes for resistance are located on chromosome 12 of *O. australiensis*. Diploid plants with resistance to race 6 of BB were obtained in the selfed progenies of MAAL 12, thus indicating the usefulness of maintaining MAALs as sources of useful genes. It should be noted that of the twelve MAALs of *O. officinalis*, only MAAL 12 was resistant to BPH (Jena and Khush 1990).

The alien chromosomes in the MAALs in this study paired with the homoeologous chromosome pair to form trivalents in about 20% of the cells at diakinesis (Table 4). In contrast, trivalents have been observed only rarely in the MAALs of *O. officinalis* (Jena and Khush 1989) and those of *O. minuta* and *O. brachyantha* (Brar et al. unpublished). These observations indicate that there is greater homology between chromosomes of *O. sativa* and *O. australiensis* than between chromosomes of *O. sativa* and other wild species such as *O. officinalis*, *O. minuta*, and *O. brachyantha*.

The usefulness of triploid hybrids in transferring genes from wild to cultivated species is demonstrated in this study. The diploid hybrids produced were completely male and female sterile, and no seeds were obtained upon backcrossing. We therefore produced a triploid hybrid by crossing an artificial tetraploid of *O. sativa* and normal diploid *O. australiensis*. The resulting F_1 was partially fertile because of regular pairing between the two sets of *O. sativa* chromosomes and gave some seed set upon backcrossing with the diploid *O. sativa*. This allowed us to carry on the further breeding work to transfer genes from *O. australiensis* to cultivated rice.

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