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# Introgression of genes from *Oryza officinalis* Well ex Watt to cultivated rice, *O. sativa* L.

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**Summary.** Sterile AC hybrids between cultivated *Oryza sativa* (AA) and a distant wild species, *O. officinalis* (CC), were backcross to *O. sativa*. Most of the $BC_1$ progenies were allotriploid (AAC), a few were hypotriploid. AAC progenies were again backcrossed to *O. sativa*. $BC_2$ progenies consisting of disomic or aneuploid individuals were examined for the presence of *O. officinalis* traits. Eleven different traits from *O. officinalis* were identified in these progenies. Segregation data in the subsequent generations suggest that these traits are monogenic in nature. Two of these genes – for resistance to BPH and WBPH – are of value in rice improvement. The extremely low recovery of recombinant progenies is in agreement with the very low amount of pairing between A and C genomes. Because of this restricted recombination, the genotype of the recurrent parent was reconstituted after two backcrosses only. Thus, the $BC_2$ progenies look remarkably similar to *O. sativa*. Most of them are stable and fertile and also interfertile with other *O. sativa* breeding lines. Some of the BPH- and WBPH-resistant progenies are comparable in yield to the best *O. sativa* parents and are being evaluated as varietal possibilities.

**Key words:** Rice – *Oryza sativa* – *O. officinalis* – Monosomic alien addition lines (MAALs) – Recombination – Alien gene transfer

## Introduction

Wild species of *Oryza* are a rich source of useful genes for the improvement of cultivated rice. However, efforts to introgress useful traits from wild species to cultivated rice have been very limited. The only examples are the transfer of a gene for grassy stunt virus resistance from *O. nivara* (Khush et al. 1977) and of a cytoplasm of *O. perennis* to produce cyto-sterile lines for hybrid seed production (Lin and Yuan 1978). In recent years, disease and insect outbreaks on rice have become more intense and germ plasm resources of rice, including wild species, have been evaluated to identify donors for resistance (Khush 1984). Several wild species are highly resistant to all the known biotypes of brown planthopper (BPH), white-backed planthopper (WBPH), and green leafhopper (Heinrichs et al. 1985).

*O. sativa* and its closely related wild species *O. nivara*, *O. perennis*, and *O. longistaminata* share the AA genome. These wild species can easily be crossed with *O. sativa*, and genes from them can be transferred to cultivated rice by conventional crossing and backcrossing procedures. However, wild species with other genomes are difficult to cross and they produce completely male-sterile hybrids. Several workers have investigated the hybrids of *O. sativa* with *O. officinalis*, which has the CC genome. Morinaga et al. (1958), Morinaga and Kuriyama (1959), and Li et al. (1964) observed no bivalent formation in the AC hybrids, but Ramanujam (1937), Nezu et al. (1960), Bouharmont (1962), and Katayama (1965) reported a low frequency of pollen mother cells (PMCs) with one to five rod-shaped bivalents. However, those studies investigated genome homologies and species relationships, not the transfer of useful traits from *O. officinalis* to cultivated rice. In this study we attempted to introgress genes for BPH and WBPH resistance from *O. officinalis* to *O. sativa*.

## Materials and methods

We crossed three breeding lines of *O. sativa* with 18 accessions of *O. officinalis*. The *O. officinalis* accessions are resistant to BPH and WBPH, but the three breeding lines of *O. sativa* are

susceptible to both insects. The accession numbers of the *O. officinalis* ecotypes used were listed by Jena and Khush (1989). A total of 400 $F_1$ hybrids were obtained through the embryo rescue technique (Jena and Khush 1986, 1989). The $F_1$ hybrids were completely sterile and were backcrossed to their respective *O. sativa* parents. Of the 41,437 spikelets of the $F_1$s pollinated, only 1.3% set seed. Although some seeds did not germinate, we obtained 367 $BC_1$ plants. Of that number, 357 were triploid and the rest were hypotriploids, indicating that mostly the unreduced female gametes were functional. These triploids were of AAC constitution and showed a modal chromosome configuration of 12II + 12I's at diakinesis or metaphase I. All 357 triploids were again backcrossed to the recurrent *O. sativa* lines and 94 $BC_2$ plants were obtained. Of those 94, 68 $BC_2F_1$ plants originated from crosses involving IR31917-45-3-2 and four accessions of *O. officinalis*. We obtained 50 $BC_2F_1$ plants from a cross of IR31917-45-3-2 and *O. officinalis* accession 100896 from Thailand (Table 1), 20 from the crosses involving IR25587-109-3-3-3-3 and five *O. officinalis* accessions, and only 6 from the crosses between IR1529-680-3-2 and two *O. officinalis* accessions.

The chromosome number of the $BC_2F_1$ progenies varied from 2n to 2n + 6 (Jena and Khush 1986, 1989). There were 25 2n, 40 2n + 1, 11 2n + 2, 10 2n + 3, 4 2n + 4, 3 2n + 5, and 1 2n + 6 plants. The 2n plants were completely fertile; 2n + 1 and 2n + 2 plants were only partially fertile; and the 2n + 3, 2n + 4, 2n + 5, and 2n + 6 plants were sterile. We grew the $F_2$ populations from the $BC_2F_1$ plants with 2n, 2n + 1, and 2n + 2 chromosomes, but the $BC_2F_1$ plants with a higher number of extra chromosomes were again backcrossed to the respective recurrent parents. The $BC_3F_1$ progenies had one or two extra chromosomes and were partially fertile. We grew the $F_2$ populations from $BC_3$ progenies. We were able to grow $F_2$ progenies from 80 of the 94 $BC_2$ plants.

Single-plant selections from the $BC_2F_2$ and $BC_3F_2$ populations were made and planted to $F_3$ pedigree nursery. Selected progeny rows were advanced to $F_4$, $F_5$, and $F_6$ generations through single-plant selections. All the progenies at different generations were carefully examined for the presence of monogenic traits from *O. officinalis*. The qualitative traits in which the two species differed are listed in Table 2. The progenies were also evaluated for resistance to BPH, WBPH, and bacterial blight (BB). Advanced generation progenies were evaluated in replicated yield trials.

**Table 1.** Number of $BC_2F_1$ plants obtained from the crosses of three breeding lines of *O. sativa* and different accessions of *O. officinalis*

| *O. sativa* breeding line | *O. officinalis* accession no. | Country of origin of *O. officinalis* | Number of $BC_2F_1$ plants obtained |
|---|---|---|---|
| IR31917-45-3-2 | 100878 | Thailand | 4 |
| IR31917-45-3-2 | 100896 | Thailand | 50 |
| IR31917-45-3-2 | 101150 | Malaysia | 13 |
| IR31917-45-3-2 | 101412 | India | 1 |
| IR25587-109-3-3-3-3 | 100896 | Thailand | 10 |
| IR25587-109-3-3-3-3 | 102385 | Indonesia | 5 |
| IR25587-109-3-3-3-3 | 100947 | India | 2 |
| IR25587-109-3-3-3-3 | 101412 | India | 1 |
| IR25587-109-3-3-3-3 | 101117 | Philippines | 2 |
| IR1529-680-3-2 | 101412 | India | 1 |
| IR1529-680-3-2 | 100896 | Thailand | 5 |

**Table 2.** Monogenic traits in which *O. sativa* and *O. officinalis* differed

| Monogenic trait | *O. sativa* | *O. officinalis* |
|---|---|---|
| Plant height | Dwarf (*sd-1 sd-1*) | Tall (+ +) |
| Pericarp color | White (+ +) | Red (*Rd Rd*) |
| Stigma color | Normal (+ +) | Purple (*Ps Ps*) |
| Apiculus color | Normal (+ +) | Purple (*Pa Pa*) |
| Leaf-sheath color | Normal (+ +) | Purple (*Psh Psh*) |
| Hull color | Straw (+ +) | Black (*Bh Bh*) |
| Growth duration | 130 days (+ +) | 115 days (*ef ef*) |
| BPH reaction | Susceptible (+ +) | Resistant (*Bph Bph*) |
| WBPH reaction | Susceptible (+ +) | Resistant (*Wbph Wbph*) |
| BB reaction | Resistant (*Xa-4 Xa-4*) | Resistant (*Xa Xa*) |

## Results

### *Morphology of backcross progenies*

The 2n plants closely resembled the *O. sativa* parent. However, they differed slightly from each other and from the *O. sativa* parent in leaf length, leaf width, height, and flag-leaf angle. The most notable feature of these progenies was the complete absence of undesirable traits of the wild species, such as grain shattering, spreading growth habit, weak stems, and long awns.

The 2n + 1 plants were monosomic alien addition lines (MAALs) with 24 chromosomes of *O. sativa* and one chromosome of *O. officinalis*. The MAALs resembled the primary trisomics of cultivated rice (Khush et al. 1984). Thus, some 2n + 1 plants resembled triplo 1 and were designated MAAL1, others resembled triplo 2 (MAAL2) and triplo 3 (MAAL3), and so on. All 12 MAALs corresponding to the 12 chromosomes of *O. officinalis* were isolated and characterized (Jena and Khush 1986, 1989).

### *Monogenic traits transferred from O. officinalis*

*Resistance to BPH.* Of the 25 disomic progenies, 6 segregated for resistance to BPH (Table 3). Ten of 39 2n + 1 plants, three of seven 2n + 2, four of seven 2n + 3, and one each with 2n + 5 and 2n + 6 segregated for BPH resistance. Of the 12 MAALs, only MAAL 6 segregated for resistance to BPH. These data suggest that the gene(s) for resistance to BPH is located on chromosome 6. We were able to select BPH-resistant lines from 18 $BC_2$ families. Selections from these families were advanced to $F_7$.

We evaluated these selections for BPH resistance during every generation. Some of the progenies consistently showed high resistance to all three Philippine BPH biotypes (Fig. 1). We also evaluated some progenies for resistance to BPH populations in India and Bangladesh. These populations have been variously described as


**Fig. 1.** Screening for resistance to brown planthopper. Some progenies derived from the second backcross of *O. officinalis* to *O. sativa* showed high level of resistance. Some in the front row were susceptible

**Table 3.** Monogenic traits of *O. officinalis* expressed in $BC_2F_1$ plants of the cross of *O. officinalis* and *O. sativa*

| $BC_2$ plants | | Resistant to | | Plants with tall stature | Plants with black hull | Plants with pigmentation in the | | | |
|---|---|---|---|---|---|---|---|---|---|
| Chromosome number | No. of plants | BPH | WBPH | | | pericarp | stigma | apiculus | leaf sheath |
| 24 | 25 | 6 | 12 | 1 | 0 | 0 | 0 | 0 | 0 |
| 25 | 39 | 10 | 10 | 1 | 1 | 3 | 4 | 2 | 2 |
| 26 | 7 | 3 | 5 | 1 | 0 | 1 | 0 | 0 | 0 |
| 27 | 7 | 4 | 0 | 0 | 1 | 0 | 2 | 1 | 1 |
| 28 | 2 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 |
| 29 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

South Asian biotype or biotype 4 (Khush 1984). Some of the progenies were resistant to all the biotypes (Table 4).

Like the *O. sativa* parent, the BPH-resistant progenies have an improved plant type (Fig. 2). They have not inherited any undesirable traits from *O. officinalis*. We have crossed them with several *O. sativa* breeding lines and their $F_1$s and later generation progenies are completely fertile. We thus have new donors for resistance to BPH. We are now studying the allelic relationships of these resistance genes of these progenies with the known genes for resistance to BPH.

*Resistance to WBPH.* Of the 25 disomic progenies, 12 segregated for resistance to WBPH (Table 3). Ten of the 39 2n+1 and five of the 2n+2 progenies segregated for resistance to WBPH. Of the 12 MAALs, only MAAL 6 segregated for resistance to WBPH. These data suggest that gene(s) for WBPH resistance may also be located on chromosome 6. We have selected WBPH-resistant lines from 15 $BC_2$ families. These lines have been advanced to the $F_7$ generation. Like BPH-resistant lines, these progenies are interfertile with other *O. sativa* breeding lines and are excellent donors for WBPH resistance.

*Bacterial blight resistance. O. officinalis* accessions are resistant to six races of BB from the Philippines. The *O. sativa* breeding lines used as parents in the study are homozygous for a dominant gene, *Xa-4*, and are resistant to only races 1 and 5 of the Philippines. $F_3$ progenies derived from two $BC_2$ families segregated for susceptibility to race 1. The appearance of susceptible plants in two families shows that the genes for resistance to BB in *O. officinalis* and *O. sativa* are nonallelic. If the *O. officinalis* gene and *Xa-4* are on the same chromosome, then the susceptible plants could be the result of a single crossover between a chromosome of *O. sativa* and *O. officinalis*. Otherwise, two crossover events would be required for the production of plants susceptible to BB.

**Table 4.** Reaction of selected lines derived from the cross of *O. sativa* × *O. officinalis* to biotypes of BPH from the Philippines, Coimbatore (India), and Joydebpur (Bangladesh)

| Line | Reaction to BPH biotypes[a] | | | | |
|---|---|---|---|---|---|
| | The Philippines | | | Coimbatore | Joydebpur |
| | 1 | 2 | 3 | | |
| IR54741-3-21-22 | S | S | R | S | R |
| IR54742-1-18-12 | R | R | R | R | R |
| IR54742-1-20-10 | R | R | R | R | R |
| IR54742-5-36-4 | R | R | R | R | R |
| IR54742-6-20-3 | R | R | R | R | R |
| IR54742-11-2-8 | R | S | R | R | S |
| IR54742-15-28-38 | S | S | R | R | R |
| IR54742-18-17-20 | R | R | R | R | R |
| IR54742-22-12-15 | S | S | S | S | R |
| IR54742-23-11-19 | R | R | R | R | R |
| IR54742-24-45-22 | S | S | S | S | R |
| IR54742-31-15-20 | R | R | R | R | R |
| IR54742-33-18-20 | R | R | R | S | R |
| IR54742-38-37-16 | R | R | R | R | S |
| IR54742-41-15-30 | R | R | R | R | R |
| IR54745-2-10-17 | R | R | R | R | R |
| IR54746-4-23-3 | R | R | R | S | S |
| IR54751-2-41-10 | R | S | R | S | R |
| IR54751-3-38-10 | R | R | R | R | R |
| IR54751-4-22-10 | R | R | S | R | R |
| IR319817-45-3-2 (check) | S | S | S | S | S |
| *O. officinalis* | R | R | R | R | R |

[a] R = resistant, S = susceptible

**Table 5.** Segregation for plant height in three $BC_2F_3$ progenies from the cross of *O. sativa* × *O. officinalis*

| Family | Plants (no.) | | | $\chi^2$ 3:1 | *P* |
|---|---|---|---|---|---|
| | Tall | Dwarf | Total | | |
| 32-1 | 64 | 26 | 90 | 0.725 | 0.50–0.25 |
| 64-1 | 125 | 35 | 160 | 0.833 | 0.50–0.25 |
| 67-1 | 497 | 139 | 636 | 3.353 | 0.10–0.05 |

*Tall plant stature (sd-1⁺).* Three $BC_2$ plants were tall and the rest were dwarf. Plant 67-1 was 2n, 64-1 was 2n + 1, and 32-1 was 2n + 2. Their $F_2$ and $F_3$ progenies segregated in a ratio of 3 tall : 1 dwarf (Table 5). It is obvious that these families inherited the dominant allele for tall stature (*sd-1⁺*) from *O. officinalis*. This gene is located on chromosome 1 of *O. sativa*. However, MAAL 1, which resembled triplo 1 of *O. sativa*, did not have tall stature. The dominant gene *Rd* for red pericarp is also located on linkage group 1, but MAAL 1 had white pericarp. Families 32-1 and 64-1, which segregated for tall stature, also segregated for red pericarp. Because these traits are on the same chromosome, their transfer was probably the result of a single crossover between a chromosome of

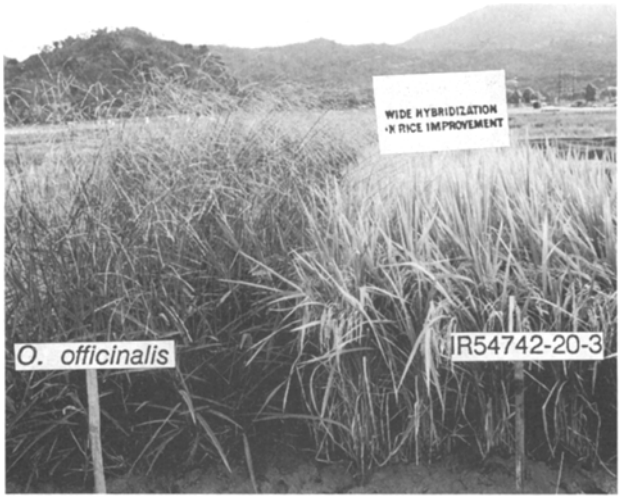


**Fig. 2.** Comparative morphology of *O. officinalis* and a brown planthopper resistant line (IR54742-20-3) derived from the second backcross of *O. officinalis* to *O. sativa*

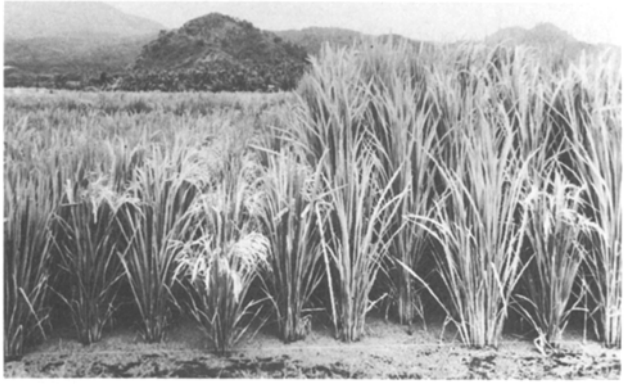

**Fig. 3.** A dwarf line of *O. sativa* and a tall line derived from the second backcross of *O. sativa* to *O. officinalis*

*O. sativa* and *O. officinalis*. The expression of tall stature and red pericarp was independent of the presence of extra chromosomes in families 64-1 and 32-1. The tall plants of family 67-1 (Fig. 3) did not have red pericarp. It seems, therefore, that *sd-1* is distal to *Rd* from the centromere and tall plants were the result of a single crossover between *sd-1* and *Rd*. The position of *Rd* being distal to *sd-1* seems improbable because double crossing-over would be required to produce a gamete with *Sd-1⁺* alone, which seems unlikely because of extremely limited recombination between parental genomes.

*Red pericarp (Rd).* Four $BC_2$ plants (32-1, 64-1, 19-2, 53-1) had red pericarp. Plant 32-1 had 2n + 2 chromosomes and plants 64-1, 19-2, and 53-1 had 2n + 1.

In the $F_2$ population of 32-1, there were ten disomic plants – all of them had red pericarp. Four of these plants were dwarf and six were tall. We grew $F_3$ progenies from

**Table 6.** Segregation of five genes in the $F_3$ progenies of family 64-1, from the cross of *O. sativa* × *O. officinalis*

| Progeny | Number of plants | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Plant height | | Pericarp color | | Stigma color | | Apiculus color | | Leaf sheath color | |
| | Tall | Dwarf | Red | White | Purple | Normal | Purple | Normal | Purple | Normal |
| 1 | 0 | 28 | 28 | 0 | 27 | 0 | 28 | 0 | 0 | 28 |
| 2 | 20 | 6 | 20 | 6 | 0 | 26 | 0 | 26 | 0 | 26 |
| 3 | 19 | 6 | 20 | 5 | 17 | 8 | 21 | 4 | 6 | 19 |
| 4 | 28 | 0 | 20 | 8 | 21 | 7 | 22 | 6 | 8 | 20 |
| 5 | 27 | 0 | 22 | 5 | 22 | 5 | 27 | 0 | 13 | 14 |
| 6 | 20 | 5 | 19 | 6 | 21 | 4 | 23 | 2 | 14 | 11 |
| 7 | 23 | 5 | 24 | 4 | 24 | 4 | 28 | 0 | 20 | 8 |
| 8 | 22 | 6 | 22 | 6 | 28 | 0 | 28 | 0 | 28 | 0 |
| 9 | 21 | 7 | 24 | 4 | 12 | 16 | 18 | 10 | 0 | 28 |

each of these ten plants. Six progenies segregated for red pericarp. Pooled data for six families showed 189 with red pericarp and 64 with white pericarp. These data agree with a 3:1 monogenic ratio.

In the $F_2$ population of plant 64-1, there were nine disomic plants, all with red pericarp. One bred true for red pericarp and the other eight segregated for red and white pericarp in a 3:1 ratio (Table 6). Pooled data for the eight segregating families showed 171 with red pericarp and 43 with white pericarp. These data agree with a 3:1 monogenic ratio.

Plant 19-2 resembled MAAL 7 (Jena and Khush 1989). Its $F_2$ population segregated 31 2n plants with white pericarp and 19 2n+1 plants with red pericarp. We grew $F_3$ progeny rows from all 31 disomic plants. All the plants in the 28 progeny rows had white pericarp. In the progenies of the remaining 3 $F_2$ plants consisting of 28 plants each, a single plant with red pericarp appeared. The origin of these three plants is the subject of another paper.

Plant 53-1 resembled MAAL 11. Its $F_2$ population segregated 35 2n plants with white pericarp and 15 2n+1 plants with red pericarp. We grew $F_3$ progeny rows from all the 35 disomic plants. All the plants of 34 progenies had white pericarp, but only one of the 28 plants of one progeny had red pericarp.

These data suggest that chromosomes 7 and 11 of *O. officinalis* may both have a locus for red pericarp.

*Purple stigma (Ps).* Eight $BC_2$ plants had purple stigma. Of these, four were 2n+1, two were 2n+3, and two were 2n+4. We grew $F_2$ progenies from four 2n+1 plants (60-4, 64-1, 69-1, and 27-1). Of the 40 plants in the progeny of 60-4, 29 were 2n. Of these 29, 11 had purple stigma and the rest had normal stigma. We did not grow $F_3$ progenies from these plants.

Of the 60 plants in the $F_2$ populations of 64-1, 39 were 2n. Of those 39, 11 had purple stigma. We grew $F_3$ progenies from nine disomic plants (Table 6). Six segregated for purple stigma and five of them segregated in a ratio of 3 purple:1 normal stigma. Pooled data for the five families showed 106 purple and 30 normal stigma plants. These data agree with a 3:1 monogenic ratio.

Of the 70 plants in the $F_2$ population of 69-1, 13 were 2n+1 and the rest were 2n. All the 2n+1 plants had purple stigma and all the 2n plants had normal stigma. The 2n+1 plants resembled MAAL 3.

Plant 27-1 was also MAAL 3. It had purple stigma. A progeny of 156 plants was grown. Eleven 2n+1 plants had purple stigma and 145 2n plants had normal stigma.

The results show that the *Ps* gene for purple stigma is located on chromosome 3 of *O. officinalis*. Of the three known genes for purple stigma in *O. sativa*, one is known to be located on chromosome 3. Thus, the *Ps* gene of *O. officinalis* may be allelic to the *Ps* locus on chromosome 3 of *O. officinalis*.

Progenies from the other four $BC_2$ plants with purple stigma were not available because of high sterility.

*Purple apiculus (Pa).* Three $BC_2$ plants had purple apiculus. Of these, two were 2n+1 (nos. 60-4 and 64-1) and one (82-2) was 2n+3. We grew $F_2$ populations only from the two 2n+1 plants.

In the $F_2$ population of 64-1, consisting of 60 plants, 39 were 2n and 21 were 2n+1 plants. Eleven of the 39 and 3 of the 21 had purple apiculus. Thus, purple apiculus is independent of the extra chromosome of this plant. We grew $F_3$ progenies from nine of the disomic plants. Three of the families segregated for purple apiculus in a ratio of 3 purple:1 normal (Table 6). Pooled data for the three families showed 66 purple apiculus and 12 normal apiculus. These data agree with a 3:1 monogenic ratio.

In the $F_2$ progeny of plant 60-4, 29 were 2n plants and 11 were 2n+1. Of the 29 2n plants, 10 had purple apiculus and the rest normal apiculus. Of the 11 2n+1

plants, 2 had purple apiculus and the rest normal apiculus. These data show that purple apiculus is independent of the extra chromosome of 60-4.

*Purple leaf sheath (Psh).* Four $BC_2$ plants had purple leaf sheath. Two of these were 2n+1 plants, 1 was 2n+3, and 1 was 2n+4. We grew $F_2$ progenies from the two 2n+1 plants.

The $F_2$ population of 60-4 consisted of 29 2n plants, of which 15 had purple leaf sheath. Of 11 2n+1 plants, 3 had purple leaf sheath. These data show that the purple leaf sheath is independent of the extra chromosome of plant 60-4.

In the $F_2$ population of 64-1, 39 plants were disomic. Of these 39, 13 had purple leaf sheath. Of 21 2n+1, 3 had purple leaf sheath. These data show that purple leaf sheath is independent of the extra chromosome of plant 64-1. We grew $F_3$ progenies from nine $F_2$ plants and six of those had purple leaf sheath. One progeny did not segregate (Table 6). Five progenies segregated, but the segregation did not agree with any particular ratio.

*Black hull (Bh).* Two $BC_2$ plants had black hulls. Plant no. 45-1 was 2n+1 and resembled MAAL 12. Plant 70-7 had 2n+3 chromosomes. We grew $F_2$ population from both plants. The $F_2$ population of plant 45-1 consisted of 50 plants, of which 46 were 2n and the rest 2n+1. Only two of the 46 2n plants were with black hulls, but all four 2n+1 plants had black hulls. These data suggest that *Bh* is located on chromosome 12. *Bh* in *O. sativa* is also located on chromosome 12. We grew $F_3$ progenies of two disomic black hull plants. One family had 28 black hull plants and six were normal. All 36 plants of the second family had black hulls. The segregation data of the first family suggest that black hull is a monogenic, dominant trait.

We grew an $F_2$ population consisting of 44 plants from plant 70-7. Thirty-four of these were 2n, the rest were 2n+1. Of the 34 2n plants, 2 had black hulls. All the 2n+1 plants had black hulls.

It is obvious that even though we did not obtain any 2n plants with black hulls in the $BC_2$ progenies, it was possible to transfer the *Bh* gene from the alien chromosome through recombination in the $F_3$ progeny of MAAL 12.

*Awns (An).* Six $BC_2$ plants had long awns and all of them were 2n+1. We grew $F_2$ progenies of these six plants. None of the 2n plants had long awns. A few 2n plants with short awns and small-tip awns were present; some of them bred true in the later generations. Long awns in *O. sativa* are due to the combined action of three dominant genes – *An-1, An-2,* and *An-3*. It is obvious that none of the progenies of this cross inherited all three genes. However, the presence of short awns and tip awns in some progenies suggests that these progenies inherited either one or two genes for this trait from *O. officinalis*. However, we did not follow the segregation of the genes for awns in our progenies.

**Table 7.** Performance of selected breeding lines derived from the $BC_2$ progenies of *O. sativa* × *O. officinalis* in replicated yield trials at IRRI, Los Banos

| Breeding line | Growth duration (days) | Yield (tons/ha) | | | | |
|---|---|---|---|---|---|---|
| | | 1987 | | 1988 | | 1989 |
| | | DS[a] | WS[b] | DS | WB | DS |
| IR54742-5-36-4 | 115 | 6.4 | 4.7 | 5.5 | 3.8 | 5.7 |
| IR54742-6-20-3 | 130 | 6.4 | 4.0 | 5.2 | 3.8 | 5.8 |
| IR54742-11-2-8 | 112 | 5.6 | 3.7 | 4.3 | 3.6 | 5.4 |
| IR54742-18-17-20-15 | 125 | – | 5.4 | 5.2 | 4.5 | 5.6 |
| IR54742-23-11-19-6 | 130 | – | 4.9 | 5.3 | 3.7 | 5.5 |
| IR54742-31-9-26 | 125 | 5.6 | 4.6 | 4.7 | 4.2 | 5.6 |
| IR54742-31-16-25 | 125 | 6.1 | 4.5 | 5.6 | 3.9 | 6.4 |
| IR42 (check) | 130 | 6.1 | 3.3 | 3.6 | 3.1 | 5.5 |
| IR36 (check) | 112 | 5.5 | 3.5 | 4.4 | 3.2 | 5.4 |

[a] DS = dry season (January–May)
[b] WS = wet season (July–November)

*Short growth duration (ef).* We observed segregation for days to flowering in four $F_2$ populations. These populations originated from $BC_2$ plants 29-1 (=2n+2), 29-2 (=2n+2), 53-1 (=2n+1), and 53-2 (=2n). We did not count the number of plants with different growth durations in the $F_2$ populations. However, some of the $F_3$ and later generation progenies flowered as much as 20–30 days earlier than *O. sativa* parent. Our records do not permit the analysis of segregation pattern for earliness in these progenies. However, earliness is a monogenic, recessive trait and has been transferred from *O. officinalis* genome to that of *O. sativa*.

### *Yield potential of BPH- and WBPH-resistant lines*

We made 1,395 single-plant selections from the $F_2$ populations of different $BC_2$ plants, grew them in an $F_3$ pedigree nursery, and evaluated them for various agronomic traits such as height, maturity, lodging resistance, grain quality, and resistance to BPH, WBPH, and BB. There was variation in all these traits among the lines. However, as mentioned earlier, none of the progenies inherited undesirable traits such as grain shattering, weak stems, and spreading growth habit. In fact, all the progenies, surprisingly, resembled the *O. sativa* parents, which are improved breeding lines. We made 793 plant selections from the $F_3$ pedigree nursery and planted them in an $F_4$ pedigree nursery. Superior-looking progenies were advanced through $F_5$ and $F_6$ pedigree rows. Selections with BPH and WBPH resistance were evaluated in repli-

cated yield trials for two seasons in 1987 and 1988 and one season in 1989. Most of the lines have excellent yield potential; some outyielded the check varieties by a small margin (Table 7).

Elite lines selected from these trials are now being evaluated as varietal possibilities. Several hundred breeding lines have been distributed to other countries in Asia where BPH and WBPH are a serious threat to rice production. We have also used these lines in our crossing program as donors for resistance to BPH and WBPH. They are fully interfertile with other *O. sativa* parents.

## Discussion

### *Monogenic traits from O. officinalis*

In this first study of this kind in genus *Oryza,* we have transferred at least 11 monogenic traits from *O. officinalis* to cultivated rice. All the genes, with the possible exception of *ef* for earliness, are dominant. Genes for three traits – resistance to BPH, WBPH, and BB – are of great interest and value in rice improvement. We are investigating the allelic relationships of these genes with known genes for resistance. Meanwhile, we have started incorporating these genes into our breeding materials.

### *Chromosomal location of monogenic traits*

The segregation data clearly show that *Ps* for purple stigma and *Bh* for blackhull are located on chromosomes 3 and 12, respectively, of *O. officinalis*. At least one of the three loci for purple stigma is located on chromosome 3 of *O. sativa* and the only known locus for *Bh* in *O. sativa* is on chromosome 12. Thus, the chromosomal location of these two genes corresponds in the two species. However, we need to test the allelic relationships of these genes from two sources.

Because *Rd* and *sd-1* are on chromosome 1, MAAL 1 should have displayed red pericarp and tall stature. However, the presumed MAAL 1 was dwarf and had white pericarp. MAAL 7 and MAAL 11, on the other hand, had red pericarp. These results suggest that either the identification of MAALs was erroneous or the loci for *Rd* and *sd-1* are not on chromosome 1 of *O. officinalis*. The two crossover events that resulted in the transfer of *Rd* from *O. officinalis* to *O. sativa* genome also involved the transfer of *sd-1*$^+$, suggesting that those two genes may be on the same chromosome of *O. officinalis*. Allele tests between the red-pericarp progenies derived from this cross and the red pericarp gene of *O. sativa* should clarify the problem. It is possible that, due to chromosomal repatterning during the evolutionary history, the location of certain loci in two species had been altered. However, alterations, if any, must be of a minor nature. Kurata and Omura (1984) have shown that chromosomes of *O. officinalis* and *O. sativa* are strinkingly similar in gross morphology. Our data do not permit the localization of other traits to specific chromosomes, although there is strong evidence that at least one of the genes for BPH resistance is located on chromosome 6.

### *Recombination or substitution*

The appearance of disomic progeny with *O. officinalis* traits may be explained theoretically by either genetic recombination between the chromosomes of *O. sativa* and *O. officinalis* or substitution of whole *O. officinalis* chromosomes for those of *O. sativa*. We consider substitution unlikely because the extra alien chromosome is usually unpaired and we did not observe any trivalents in the MAALs (Jena and Khush 1989). This allows the separation of two *O. sativa* chromosomes to opposite poles and the alien chromosome as extra to one of the daughter cells. Moreover, the alien substitution heterozygotes should exhibit pairing disruption, resulting in pollen and seed sterility. We have examined numerous lines with monogenic traits from *O. officinalis,* but did not observe any pairing disruption or partial sterility. Whole-chromosome substitutions would result in drastic modifications of morphology, but no deviations from the morphology of the *O. sativa* parent were observed. All the evidence, therefore, points to the genetic recombination between the chromosomes of two species as the cause of gene transfer from *O. officinalis* to *O. sativa*.

### *Extent of recombination*

When we first examined the disomic $BC_2$ progenies, we noted their striking resemblance to the *O. sativa* parent. Reports on the rapid recovery of the phenotype of the recurrent parent after only two backcrosses are rare in literature. This suggests only a limited amount of recombination between the genomes of two species. Of the 25 disomic progeny, only 1 segregated for tall stature. Six segregated for resistance to BPH and 12 for resistance to WBPH (Table 3). The appearance of a fairly large number of progenies with resistance to BPH and WBPH may be due to the involvement of several loci for resistance. Several of the recombinant progenies also inherited extra alien chromosomes. The segregation of the recombinant monogenic traits, such as red pericarp and tall stature in plant 32-1, and the extra chromosomes was independent. Some of the traits, such as *Bh,* did not recombine with the *O. sativa* chromosome in the $BC_2$ progenies, but disomic recombinants were obtained in the progeny of the MAAL 12.

Most of the recombinant progenies appeared to be the result of a single crossover. Thus, BPH- and WBPH-resistant progenies did not have any other monogenic traits from *O. officinalis*. A few $BC_2$ progenies, however, must be the result of multiple crossovers. Plant 32-1, for

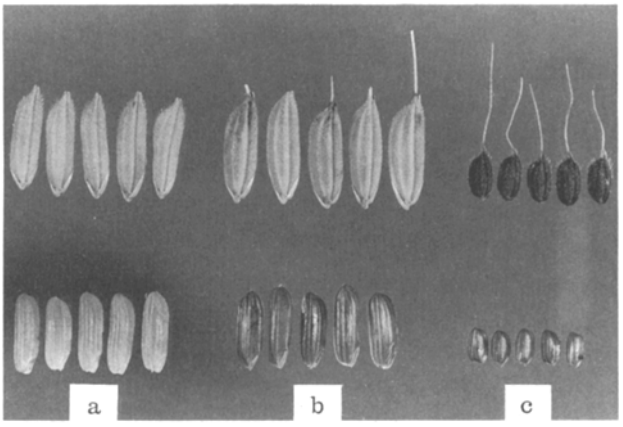


**Fig. 4.** Grains (*top row*) and kernels (*bottom row*) of: *a* IR31917-45-3-2, *b* a recombinant line from the second backcross of *O. officinalis* to *O. sativa*, and *c O. officinalis*. IR31917-45-3-2 has white pericarp, the recombinant line and *O. officinalis* have red pericarp

example, segregated for red pericarp and tall stature (both the result of a same crossover event), as well as for susceptibility to BB, which resulted from either a single or two crossover events as discussed earlier. Thus, the female gamete that gave rise to 32-1 must be the result of at least two crossover events. The grains of tall segregants with red pericarp were somewhat bolder (Fig. 4) as compared with the *O. sativa* parent. Minor genes for grain boldness may be located on the crossover segments of *O. officinalis*. Plant 67-1 segregated only for tall stature. Since this plant did not segregate for the linked marker *Rd*, it may be the result of a single crossover if *Rd* is between the centromere and *Sd-1*, or of a double crossover if *Rd* is distal to *Sd-1* from the centromere. The latter possibility seems unlikely.

Only one $BC_2$ plant (64-1) segregated for five monogenic traits. This plant had an unidentified extra chromosome that segregated independently of the monogenic traits. Of the five genes, two (*Rd* and *sd-1*) belong to the same linkage group and the others (*Ps*, *Pa*, and *Psh*) are on different chromosomes. The female gamete that gave rise to this plant must be the result of at least four crossover events. This plant had a few other quantitative traits from *O. officinalis*, such as shorter and bolder grains and finer stems and foliage.

Besides the monogenic traits, the progenies differed from each other in some quantitative traits such as height (within the dwarf group), leaf length, leaf width, straw strength, etc. Thus, some recombination for the quantitative traits must also have occurred.

The restricted recombination observed in this cross agrees with the observations on chromosome pairing in the $F_1$ hybrid. For example, only one to five rod-shaped bivalents were observed and some of the products of recombination are probably inviable. One advantage of this restricted recombination is the rapid recovery of the recurrent parent genotype. Progenies recovered after only two backcrosses are so similar to *O. sativa* that we are evaluating them as varietal possibilities. Single-gene transfers such as those for resistance to BPH and WBPH have not been accompanied by any undesirable traits from *O. officinalis*. Rapid recovery of the recurrent parent genotypes in the backcross progenies of wide crosses has been reported in *Gossypium* by Stephens (1949) and *Lycopersicon* by Rick (1963, 1969, 1971). However, in *Gossypium* and *Lycopersicon* a higher number of backcrosses were required to reconstitute the recurrent genotype.

### *Stability of derived progenies*

We observed some novel mutants in certain $F_3$ progenies and later generations of the derived lines. We also observed plants with partial sterility and chromosomal abnormalities in the $F_4$ and $F_5$ generations of the progenies that had no such abnormalities in the $F_2$ or $F_3$ generations. Tsujimoto and Tsunewaki (1985) and Kota and Dvorak (1988) observed such instability in wheat derivatives having alien chromatin. Similarly, Mangelsdorf (1974) reported higher mutation rates in corn progenies derived from crosses with its wild relative, teosinte. The occurrence and analysis of this instability are the subject of our next paper in the series. Nevertheless, we have obtained stable and true breeding progenies of great value in rice improvement.

### *Prospects of rice improvement through wide hybridization*

Many situations arise where plant breeders must resort to interspecific crosses for transferring useful genes from a wild to a cultivated species. In many interspecific crosses, the first barrier encountered is the failure to obtain $F_1$ plants because of embryo abortion. This barrier can now be overcome in most cases. The second barrier, the complete male sterility of the $F_1$s, can be overcome by producing allotriploid hybrids having two genomes of the cultivated parent and one genome of the wild species. Two methods are commonly used for producing allotriploids. (1) The $F_1$ is backcrossed to the cultivated parent. Occasional unreduced gametes produced by the $F_1$, when ferilized with an *n* gamete, result in a desired allotriploid. (2) Production of an autotetraploid of the cultivated species and its hybridization with the wild species. The resulting hybrids are allotriploids. The first method is preferred, because in the diploid hybrid, there is more likelihood of pairing and recombination between the parental genomes. In the second method, there is preferential pairing between the homologous chromosomes, and the chances for homoeologous pairing and recombination are minimized.

Triploid hybrids have also been successfully used in transferring genes from *Secale cereale* to cultivated wheat (O'Mara 1940), from *Nicotiana glutinosa* to *Nicotiana tabacum* (Mann et al. 1963), from *Beta procumbens* to *Beta vulgaris* (Savitsky 1975), and from *Solanum lycopersicoides* to *Lycopersicon esculentum* (Rick 1988; Chetelat et al. 1989). At IRRI we are using the technique to explore the possibility of transferring useful genes from other wild species, such as *O. australiensis, O. latifolia, O. minuta, O. brachyantha,* and *O. ridleyi* to cultivated rice.

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