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Alien introgression in rice

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Abstract

Rice (*Oryza sativa* L.) productivity is affected by several biotic and abiotic stresses. The genetic variability for some of these stresses is limited in the cultivated rice germplasm. Moreover, changes in insect biotypes and disease races are a continuing threat to increased rice production. There is thus an urgent need to broaden the rice gene pool by introgressing genes for such traits from diverse sources. The wild species of *Oryza* representing AA, BB, CC, BBCC, CCDD, EE, FF, GG and HHJJ genomes are an important reservoir of useful genes. However, low crossability and limited recombination between chromosomes of cultivated and wild species limit the transfer of such genes. At IRRI, a series of hybrids and monosomic alien addition lines have been produced through embryo rescue following hybridization between rice and several distantly related species. Cytoplasmic male sterility and genes for resistance to grassy stunt virus and bacterial blight have been transferred from A genome wild species into rice. Similarly, genes for resistance to brown planthopper, bacterial blight and blast have also been introgressed across crossability barriers from distanly related species into rice. Some of the introgressed genes have been mapped via linkage to molecular markers. One of the genes *Xa-21* introgressed from *O. longistaminata* has been cloned and physically mapped on chromosome 11 of rice using BAC library and flourescence *in-situ* hybridization. RFLP analysis revealed introgression from 11 of the 12 chromosomes of C genome species into rice. Introgression has also been obtained from other distant genomes (EE, FF, GG) into rice and in majority of the cases one or two RFLP markers were introgressed. Reciprocal replacement of RFLP alleles of wild species with the alleles of *O. sativa* indicates alien gene transfer through crossing over. The rapid recovery of recurrent phenotypes in BC_2 and BC_3 generations from wide crosses is an indication of limited recombination. Further cytogenetic and molecular investigations are required to determine precisely the mechanism of introgression of small chromosome segments from distant genomes in the face of limited homoeologous chromosome pairing. Future research should focus on enhancing recombination between homoeologous chromosomes. Introgression of QTL from wild species should be attempted to increase the yield potential of rice.

Abbreviations: RFLP, restriction fragment length polymorphism; RAPD, random amplified polymorphic DNA; BAC, bacterial artificial chromosome; MAALs, monosomic alien addition lines; QTL, quantitative trait loci; BPH, brown planthopper; WBPH, whitebacked planthopper; BB, bacterial blight; CMS, cytoplasmic male sterility; WA, wild abortive; cM, centimorgan.

Introduction

Rice (*Oryza sativa* L. 2*n*=24) is an important cereal and a source of calories for more than one third of the world population. During 1995, it was planted to 146 million hectares with a total production of 542 million tonnes of grains. It is grown worldwide under a wide range of agroclimatic conditions. Rice productivity is affected by several biotic (diseases and insects) and abiotic (unfavourable soil, temperature and water conditions) stresses. Some of the major pests affecting rice production include bacterial blight (BB), blast,

sheathblight, tungro, brown planthopper (BPH), and stemborers. Similarly, drought, cold, salinity, acidity, iron toxicity, and submergence under water (flooding) adversely affect rice production. Moreover, changes in insect biotypes and disease races are becoming a continuing threat to increased rice production.

The genetic variability for some traits such as resistance to sheath blight, tungro, yellow stemborer and tolerance to salinity, acid sulfate conditions is limited in the cultivated rice germplasm. There is thus an urgent need to broaden the rice gene pool by introgressing new genes from diverse sources to meet various challenges affecting rice production. Wild species are an important reservoir of useful genes. However, several incompatibility barriers such as low crossability and limited recombination between chromosomes of wild and cultivated species limit the transfer of useful genes [5, 19, 38]. Recent advances in tissue culture have enabled production of wide hybrids and molecular marker technology and *in-situ* hybridization techniques have enabled to precisely detect the introgression of chromosome segments from wild into cultivated species.

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 2*n*=24 and have AA genome, the former is cultivated worldwide whereas the latter is grown on a limited area in Africa. The wild species have either 2*n*=24 or 2*n*=48 chromosomes representing AA, BB, CC, BBCC, CCDD, EE, FF, GG and HHJJ genomes (see Table 2 in 'History, origin, cultivation and variation of rice' by G.S. Khush in this volume). The cultivated rice (*O. sativa*) and its closely related wild species, *O. perennis*, *O. nivara* and *O. longistaminata* share the AA genome. These wild species can be easily crossed with *O. sativa* and genes from them can be transferred to cultivated rice by conventional crossing and backcrossing procedures. However, wild species with genomes other than AA are difficult to cross with *O. sativa* and produce completely male sterile hybrids. Embryo rescue is used to overcome hybrid inviability and to produce interspecific hybrids. Several workers have investigated the hybrids of *O. sativa* with species having BB, BBCC, CC, CCDD, EE and FF genomes [10, 17, 22, 26, 28, 29, 30, 42]. However, these studies investigated genomic homoeologies and species relationships, but did not attempt to transfer useful traits from wild species into cultivated rice. At International Rice Research Institute (IRRI), a series of hybrids and monosomic alien addition lines (MAALs) have been produced through embryo rescue following hybridization between elite breeding of lines of rice and several distantly related species of *Oryza* and useful genes for resistance to BPH, BB and blast have been successfully transferred into rice [2, 3, 4, 6, 14, 27, 38].

Introgression from AA genome wild species

The first example of transfer of a useful gene from wild species is the introgression of a gene for grassy stunt virus resistance from *O. nivara* to cultivated rice varieties [20], and transfer of a cytoplasmic male sterility source (CMS) from wild rice, *O. sativa f. spontanea* to develop CMS lines for commercial hybrid rice production [23]. More recently, *Xa-21* for BB resistance was transferred to rice from *O. longistaminata* [18] and new CMS sources from *O. perennis* and *O. glumaepatula* into rice [7, 8].

Introgression of gene(s) for resistance to grassy stunt virus

During 1970s, epidemics of grassy stunt virus were reported in several countries. The grassy stunt virus is transmitted by BPH. The diseased rice plants either produce no panicles or produce only small panicles with deformed grains. Severe yield losses or even total losses may occur under epidemic conditions. Of the 6723 accessions of cultivated rice and several wild species of *Oryza* screened for resistance, only one accession of *O. nivara* (Acc 101508) was found to be resistant [24]. Crosses were made between improved rice varieties IR8, IR20, IR24 and *O. nivara*. Following three backcrosses with improved varieties, the gene for grassy stunt resistance was transferred into cultivated germplasm. The first grassy stunt resistant varieties, IR28, IR29 and IR30 were released for cultivation in 1974. Subsequently, other grassy stunt resistant varieties, IR32, IR34, IR36 were released. IR2071-625-3, a sister selection of IR36 was released in Kerala, India where a serious epidemic of grassy stunt occurred in 1973–1974. Since then, grassy stunt resistance gene has been incorporated into numerous varieties developed at IRRI as well as by the national rice improvement programs.

Introgression of gene(s) for resistance to bacterial blight

The BB caused by *Xanthomonas oryzae pv. oryzae* is one of the most destructive diseases of rice in Asia. A dominant gene for resistance to BB was transferred from *O. longistaminata* by backcrossing to IR24 as the recurrent parent and was designated as *Xa-21* [18]. This gene has a very wide spectrum of resistance and conveys resistance to all races of BB in the Philippines [21]. lkeda *et al.* [11] also studied the inheritance of resistance in BC_4F_3 lines from the cross of IR24 and *O. longistaminata* using Philippine races 1, 2, 4 and 6 of BB and confirmed that the same gene conveys resistance to all these races.

Incorporation of CMS sources from wild species

The A genome wild species have been an important source of CMS, the major tool used to breed commercial rice hybrids. Lin and Yuan [23] reported the development of male sterile line having cytoplasm of wild species (*O. sativa* L. f. *spontanea*) and nuclear genome of rice. This wild species was found growing in Hainan Islands, China. The cytoplasmic source has been designated as wild abortive (WA), which refers to a male sterile wild rice plant having abortive pollen. About 95% of the male sterile lines used in commercial rice hybrids grown in China and other countries have WA type of cytoplasm [45]. To diversify the CMS sources, attempts have been made to identify and transfer new CMS sources from other A genome wild species.

Recently, a new CMS source from *O. perennis* was transferred into indica rice [7]. Crosses of 46 accessions of A genome wild species were made as female parents with *O. sativa* cv IR64. In successive backcrosses, highly sterile plants were selected and used in backcrosses. One cross involving accession 104823 of *O. perennis* yielded completely sterile plants. This newly developed CMS line has been designated as IR66707A. It has the cytoplasm of *O. perennis* and the nuclear genome of IR64. Genetic studies show that male sterility source of IR66707A is different from that of WA. Southern hybridization of IR66707A, *O. perennis*, IR66707B (maintainer) and V20A (WA cytoplasm) using mitochondrial DNA specific probes (5 endonucleases $\times$ 8 probes) showed identical banding pattern between IR66707A (recipient) and *O. perennis* (donor). It appears CMS may not be caused by any major rearrangement or modification of mtDNA. We have developed another CMS line (IR69700A) having cytoplasm of *O. glumaepatula* (A genome species) and nuclear genome of IR64 [8]. Both IR66707A and IR69700A are completely stable for male sterility. Search for restorers of these CMS sources is underway.

We are now evaluating advance progenies derived from crosses of elite breeding lines of rice with different A genome species for the possible transfer of resistance to tungro virus disease, increased elongation ability, water submergence and tolerance to acid sulfate conditions (Table 1).

Introgression from distantly related genomes

Hybrids between cultivated rice and A genome wild species can be produced through normal procedures. Hybrids between rice and distantly related wild species are usually difficult to produce. Low crossability and abortion of hybrid embryos are the common features of such crosses. These hybrids are completely male sterile. Subsequent backcrosses are made with the recurrent rice parent to produce disomic progenies ($2n$=24). Embryo rescue is used to produce F_1 and backcross progenies until fertile plants with normal diploid chromosome complement ($2n$=24) or $2n$=25 (monosomic alien addition lines) become available (Figure 1). The fertile progenies are selfed to produce advanced introgression lines and evaluated for transfer of useful traits.

Introgression from BB genome

Interspecific hybrids have been produced between auto-tetraploids of japonica cultivar Nipponbare, and *O. punctata* ($2n$=24 BB). From this cross, MAALs and disomic progenies ($2n$=24) have been produced [44]. However, none of the lines have been analyzed for introgression of traits from *O. punctata.*

Introgression from CC genome

Following embryo rescue, interspecific hybrids have been produced between cultivated rice and three wild species with CC genome [4, 13, 37]. Jena and Khush [14] produced several introgression lines from the cross of *O. sativa* $\times$ *O. officinalis.* Useful genes for resistance to BPH, whitebacked planthopper (WBPH) and BB have been transferred from *O. officinalis* into an elite breeding line of rice. Of the 25 BC_2F_1 disomic progenies, 6 segregated for resistance to BPH and 12 segregated for resistance to WBPH. The recurrent

Table 1. Introgression of genes from wild *Oryza* species into cultivated rice.

Trait transferred to *O. sativa* (AA genome)	Donor *Oryza* species		
	Wild species	Genome	Accession number
Grassy stunt resistance	*O. nivara*	AA	101508
Bacterial blight resistance	*O. longistaminata*	AA	–
	O. officinalis	CC	100896
	O. minuta	BBCC	101141
	O. latifolia	CCDD	100914
	O. australiensis	EE	100882
	O. brachyantha	FF	101232
Blast resistance	*O. minuta*	BBCC	101141
Brown planthopper resistance	*O. officinalis*	CC	100896
	O. minuta	BBCC	101141
	O. latifolia	CCDD	100914
	O. australiensis	EE	100882
	O. granulata[a]	GG	100879
Whitebacked planthopper resistance	*O. officinalis*	CC	100896
Cytoplasmic male sterility	*O. sativa* f. spontanea	AA	–
	O. perennis	AA	104823
	O. glumaepatula	AA	100969
Yellow stemborer resistance	*O. brachyantha*[a]	FF	101232
	O. ridleyi[b]	HHJJ	100821
Sheath blight resistance	*O. minuta*[a]	BBCC	101141
Tungro tolerance	*O. rufipogon*[a]	AA	105908
	O. rufipogon[a]	AA	105909
	O. officinalis[b]	CC	105220
Increased elongation ability	*O. rufipogon*[a]	AA	CB751
Tolerance to acid sulfate soils	*O. rufipogon*[a]	AA	106412
	O. rufipogon[a]	AA	106423

[a]Material under test.
[b]Advance backcross progenies (introgression lines) being produced.

rice parent (IR31917-45-3-2) is susceptible to all three Philippine BPH biotypes whereas *O. officinalis* (Acc 100896) is resistant to these biotypes. Several introgression lines resistant to three BPH biotypes have been identified. These lines were also evaluated for resistance to BPH populations in India and Bangladesh. Several progenies were found to be resistant to BPH in three countries.

Some of the BPH resistant progenies were evaluated in replicated yield trials. Most of the lines had excellent yield potential and some outyielded the check varieties by a small margin. The selected progenies were free of undesirable weedy traits such as grain shattering, weak stems and spreading growth habit. A few of the BPH resistant lines were also resistant to BPH population in Vietnam. Three breeding lines have been released as varieties for commercial cultivation in Mekong Delta of Vietnam. IR54751-2-44-15-24-3 was named as MTL98, IR54751-2-34-10-6-2 as MTL103 and 1R54751-2-41-10-5-1 as MTL105.

The recurrent rice parent has *Xa-4* gene and is resistant to BB races 1 and 5 whereas *O. officinalis* is resistant to all 6 races prevalent in the Philippines. F_3 progenies from two BC_2 families segregated for sus-

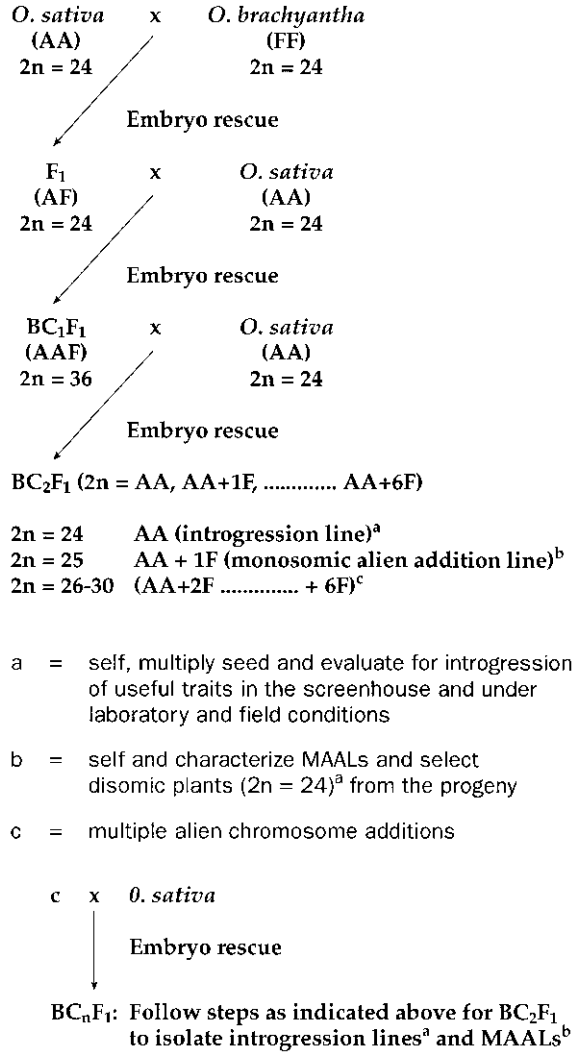

Figure 1. Scheme showing production of monosomic alien addition lines (2*n*=25) and introgression lines (2*n*=24) from crosses of rice and distantly related wild species of *Oryza.*

ceptibility to race 1. The appearance of susceptible plants in two families shows that the gene(s) for resistance to BB in *O. officinalis* and *O. sativa* are non-allelic to X*a-4*. Besides introgression of gene(s) for resistance to BPH and WBPH, introgression for other plant traits such as hull colour, pigmented pericarp, stigma, apiculus and leaf sheath from *O. officinalis* into rice was also detected.

Introgression from BBCC genome parents

Interspecific hybrids have been produced between *O. sativa* and the tetraploid wild species *O. minuta* (2*n*=48, BBCC) [38]. Following backcrossing and embryo rescue, advanced lines have been produced from the cross of *O. sativa* (IR31917-45-3-2) and *O. minuta* (Acc 101141). Amante-Bordeos et al. [2] evaluated advanced progenies for resistance to BB and blast. Two introgression lines were resistant; one to race 6 of BB and another to race P06-6 of blast. Brar *et al.* [3] evaluated introgression lines derived from *O. sativa* × *O. minuta*. Of the 96 backcross progenies screened, 10 were found to be segregating for resistance to BPH biotype 1 of the Philippines.

Introgression from CCDD genome parents

A number of workers have produced hybrids between rice and CCDD genome species [4, 38]. Of the three CCDD species, advanced lines derived from the cross of *O. sativa* × *O. latifolia* have been investigated. Introgression from *O. latifolia* for resistance to BPH, WBPH and BB including other traits such as growth duration and purple pigmentation has been obtained (Multani *et al.*, unpublished).

Introgression from EE genome

Multani *et al.* [27] produced hybrids between colchicine induced autotraploids of rice with *O. australiensis* (2*n*=24 EE). The BC_2 progenies consisting of disomic and aneuploid plants were examined for the presence of *O. australiensis* traits. Introgression was detected for morphological traits such as long awns, earliness and *Amp-3* and *Est-2* allozymes. Of the 600 BC_2F_4 progenies, 4 were resistant to BPH (Figure 2) and 1 to race 6 of BB. BPH resistance was found to be controlled by a recessive gene in two of the four lines but was controlled by a dominant gene in the other two lines.

Introgression from FF genome

A series of introgression lines have been derived from the cross of *O. sativa* cv. IR56 and the wild species, *O. brachyantha* (2*n*=24 FF). IR56 is susceptible to BB races 1, 2, 3, 4 and 6 from the Philippines, whereas *O. brachyantha* is resistant. Of the 149 backcross progenies analyzed, 27 showed introgression for resistance to BB races 1, 2, 3, 4 and 6 [3].

BC_2 progenies derived from the crosses of *O. sativa* with *O. officinalis* (CC), *O. australiensis* (EE) and *O. brachyantha* (FF) resembled the recurrent rice parent in most of the morphological traits, suggesting limited recombination between A genome of *O. sativa* and C, E and F genomes of wild species.

Introgression from GG genome species

All three species (*O. meyeriana, O. granulata, O. indandamanica*) in the diploid *O. meyeriana* complex have been found to be highly divergent based on total genomic DNA hybridization analysis and thus a new genome (GG) has been proposed for *meyeriana* complex [1]. Hybrids have been produced from the cross of *O. sativa* (IR31917-45-3-2) and *O. granulata* (Acc 100879) [4]. Advanced progenies have been produced [9] and are being evaluated for introgression of traits from *O. granulata.*

Introgression from HHJJ genome parents

Hybrids between rice cv. IR56 and *O. ridleyi* (Acc 100821) have been produced. The tetraploid *ridleyi* complex comprises of two species; *O. ridleyi* and *O. longiglumis.* Based on total genomic DNA hybridization analysis, *O. ridleyi* complex is also highly divergent from all other species of *Oryza.* Hence new genomes (HHJJ) have been proposed for this complex [1]. Only a few introgression lines from this cross have been produced and no introgression has been detected.

Molecular mapping of alien genes introgressed from wild species into rice

Traits introgressed from different wild species into rice are listed in Table 1. Some of the introgressed genes have been mapped via linkage to molecular markers.

Mapping of Xa-21 *gene for BB resistance*

Ronald *et al.* [34] and Ronald and Tanksley [35] analyzed near isogenic lines (NILs) of rice cultivar IR24 carrying *Xa-21* gene. One of the markers (RG103) on chromosome 11 detected polymorphism between the NILs that co-segregated with *Xa-21.* All other DNA markers of chromosome 11 tested were monomorphic between the NILs. These results localized the *Xa-21* to an 8.3 cM interval on chromosome 11. Two random amplified polymorphic DNA (RAPD) markers (RAPD 818, RAPD 248) also co-segregated with the resistance locus, *Xa-21.* The results from the pulsed field gel electrophoresis showed that the three *Xa-21* linked markers were physically close to each other, with one copy of the RAPD 818 sequences located within the 60 Kb of RAPD 248 and the other copy within 270 kb of RG 103.

Wang *et al.* [41] constructed bacterial artificial chromosome (BAC) library in rice consisting of 11 000 clones with an average DNA insert size of 125 kb. Twelve clones were isolated that hybridized with the three DNA markers linked to the *Xa-21* locus. Song *et al.* [39] isolated *Xa-21* gene by positional cloning and used this gene in rice transformation. The transgenic plants carrying the cloned *Xa-21* showed high level of resistance to BB pathogen. Jiang *et al.* [16] used

Figure 2. Reaction to brown planthopper of introgression lines derived from the cross of *O. sativa* (AA) and *O. australiensis* (EE). Note resistant reaction of introgression lines (WC1, WC2, WC5, WC6); *O. australiensis* (donor parent) and Rathu Heenati (check): susceptible reaction of introgression lines (WC3, WC4); recurrent rice parent (IR31917-45-3-2) and TN-1 (check), reproduced from [27].

BAC clones and flourescence *in-situ* hybridization and physically mapped *Xa-21* locus on chromosome 11 of rice.

Mapping of Bph-10(t) for BPH resistance

A gene conferring resistance to three BPH biotypes from Philippines was introgressed from *O. australiensis* into rice [27]. The recurrent parent (IR31917-45-3-2) is susceptible to all three biotypes whereas *O. australiensis* is resistant. The introgression line (IR65482-4-136-2-2) derived from this cross was also resistant to three biotypes of BPH. The F_1, of IR65482-4-136-2-2 and IR31917-45-3-2 was resistant to BPH biotype 1, and the segregation data showed that a single dominant gene confers BPH resistance. Monosomic alien addition line analysis showed that the gene for BPH resistance is located on chromosome 12 of *O. australiensis.*

Table 2. Cosegregation for BPH resistance (biotype 1) and RG457 in F2 derived from the cross of recurrent parent IR31917-45-3-2 (BPH susceptible) with introgression line IR65482-4-136-2-2 (BPH resistant).

RG457 genotype[b]	No. of plants with BPH reaction[a]			Total
	Resistant (RR)	Resistant (R/S)	Susceptible (SS)	
11	0	4	23	27
12	2	57	0	59
22	23	2	0	25
Total	25	63	23	111

[a]F2, genotype determined from BPH reaction of F3 progenies, reproduced from [12].
[b]1, allele from recurrent parent; 2, allele from introgression line.

Using the probes of chromosome 12, RFLP survey was carried out with the recurrent parent, wild species, and the introgression line (Figure 3). All the 14

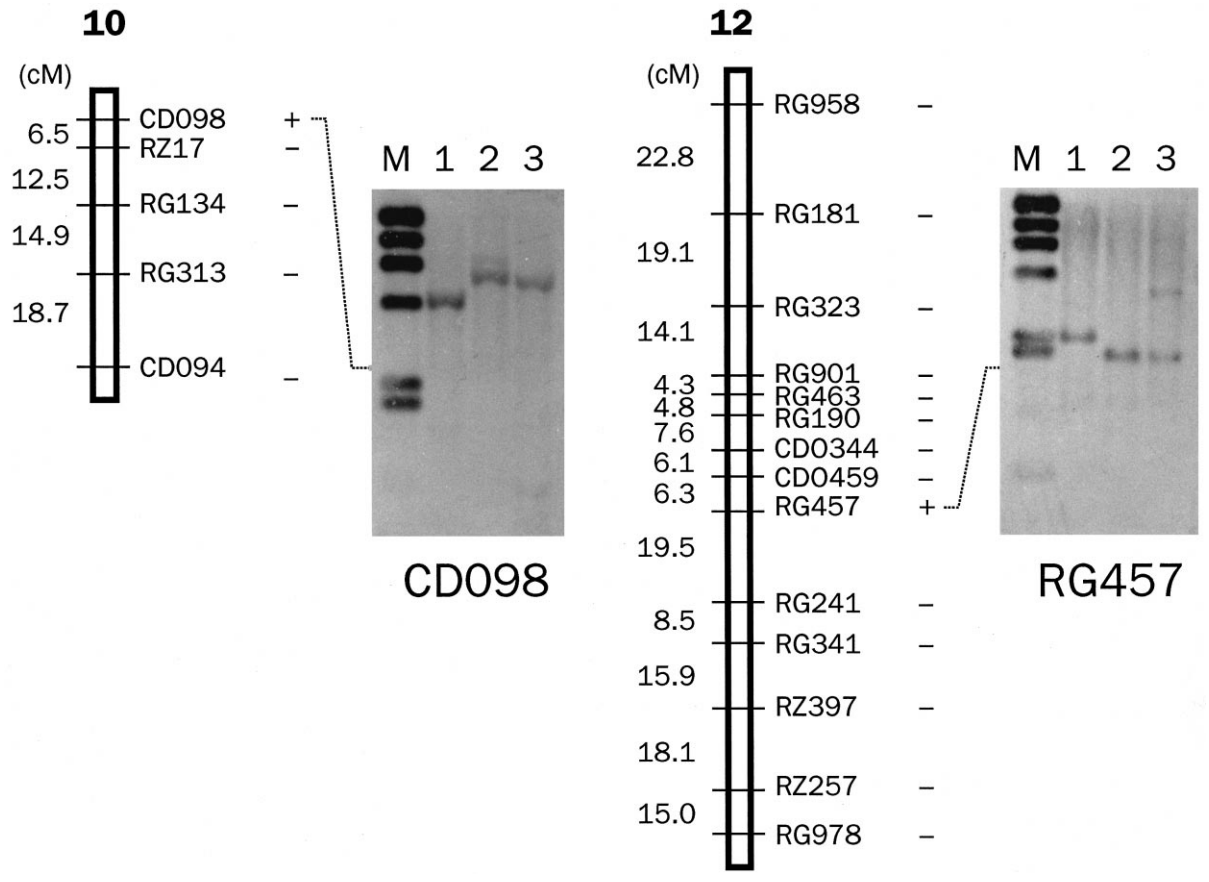

Figure 3. Parental survey to detect introgressed segments from *O. australiensis* into BPH resistant introgression line. Total DNA was digested with *Eco*RI or *Hin*dIII and probed with molecular markers on chromosomes 10 and 12. Numbers to the left and right of the chromosome represent distance and clone designation, respectively, McCouch and Tanksley [25]. Last column indicates whether introgression is found (+) or not (–). M, lambda DNA digested with *Hin*dIII; 1, recurrent parent (IR31917-45-3-2); 2, introgression line (IR65482-4-136-2-2); 3, *O. australiensis* (accession 100882), reproduced from [12].

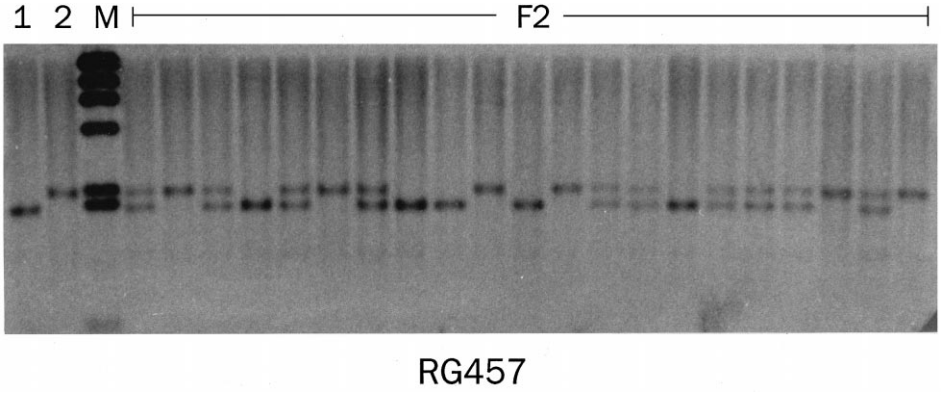

Figure 4. RFLP pattern in F_2 population of a cross of BPH resistant introgression line (IR65482-4-136-2-2) with the recurrent parent (IR31917-45-3-2). Total DNA was digested with *Eco*RI and probed with RG457 on chromosome 12. M, lambda DNA digested with *Hin*dIII, 1; introgression line (IR65482-4-136-2-2); 2, recurrent parent (IR31917-45-3-2), reproduced from [12].

probes were polymorphic in recurrent parent and wild species; however, only RG457 detected introgression from *O. australiensis* to the introgression line. DNA from 111 F_2 plants was hybridized with RG457 (Figure 4). Cosegregation for BPH reaction and RG457 was determined from the F_2 data (Table 2). The results showed that the gene for BPH resistance is linked with RG457 with a distance of 3.68 ± 1.29 cM [12]. Such close linkage is useful in practicing marker-based selection while transferring BPH resistance from the introgression line into any elite breeding line and in monitoring alien gene introgression.

Mapping of gene for earliness

The introgression line (IR65482-4-136-2-2) derived from the cross of IR31917-45-3-2 and *O. australiensis* was crossed with recurrent parent (IR31917-45-3-2). The F_1 was not early and the F_2 segregated into early and late plants in a ratio of 1:3 (x^2 = 0.004), indicating that the introgressed gene for earliness is recessive. Since the gene for earliness is located on chromosome 10 [36], probes from chromosome 10 were hybridized with the DNA of recurrent parent, wild species, and the introgression line (Figure 3). All the five probes were polymorphic between recurrent parent and wild species. However, only CDO98 detected introgression from *O. australiensis.* Cosegregation between CDO98 and days to flowering in F_2 showed that the gene for earliness is situated at a distance of 9.96 ± 3.28 cM from CDO98, thus indicating that this recessive gene for earliness is also located on chromosome 10.

*Mapping of gene for blast (*Pi-9t*) resistance*

A gene for blast resistance (*Pi-9t*) was introgressed from *O. minuta* (BBCC) into rice [2]. The introgression line (WHD75-1-127) was surveyed using 103 polymorphic RFLP markers located at an average distance of 20 cM intervals in the rice genome. However, no linkage could be established between any markers and *Pi-9^t*. In another experiment, a backcross population produced by crossing the introgression line and the susceptible parent IR31917-45-3-2 was anaiyzed. Three RAPD markers were found to be linked to *Pi-9^t* (R. Nelson personal communication).

RFLP analysis of alien introgression

The molecular markers provide unique opportunity to determine the extent and process of alien introgression. RFLP analysis was carried out using 52 markers located on 6 of the 12 rice chromosomes. Analysis of 29 derivatives of *O. sativa* × *O. brachyantha* and 40 derivatives of *O. sativa* × *O. granulata* showed extensive polymorphism between rice and wild species. Of the 6 chromosomes analyzed, no introgression was detected from chromosomes 7, 9, 10 and 12 of *O. granulata* and chromosome 10 and 12 of *O. brachyantha* [3]. For each of the remaining chromosomes, 1 to 2 RFLP markers showed introgression in some of the derived lines (Figure 5). Although the level of introgression was low but the results show possibilities of introgressing chromosome segments even from distantly related genomes into cultivated rice and thus feasibility of transferring useful genes from distant *Oryza* species.

Jena et al. [15] analyzed 52 introgression lines derived from crosses of *O. sativa* × *O. officinalis.* Of the 174 informative RFLP markers, 28 identified putative *O. officinalis* introgressed segments in one or more of the introgression lines. Introgressed segments were found on 11 of the 12 rice chromosomes (Figure 6). In majority of the cases, *O. sativa* alleles were replaced by *O. officinalis* alleles. Introgressed segments were smaller in size and some non-conventional mechanism of recombination may be involved in the transfer of such small chromosome segments from *O. officinalis* chromosomes to those of *O. sativa.*

Mechanism of alien introgression

Cytogenetic and RFLP analysis of introgression lines derived from *O. sativa* and distantly related *Oryza* species did not show any evidence of alien chromosome substitution. The results indicate genetic recombination between chromosomes of cultivated and wild species as the cause of alien gene transfer. RFLP analysis of introgression lines showing reciprocal replacement of alleles of *O. officinalis* and *O. australiensis* with the alleles of *O. sativa* further supports alien gene transfer through crossing over rather than the substitution of a complete or an arm of chromosomes of wild species [12, 15]. The rapid recovery of recurrent parent phenotypes in BC_2 and BC_3 of *O. sativa* × *O. officinalis, O. sativa* × *O. australiensis, O. sativa* × *O. brachyantha,* and *O. sativa* × *O. granulata* is an indication of limited recombination between A genome on one

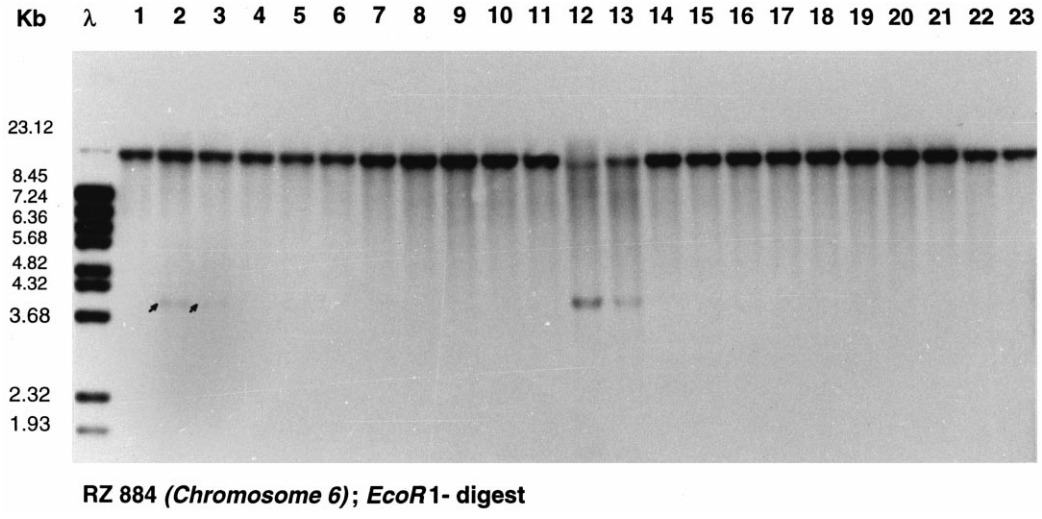

Figure 5. *Eco*RI – RFLP patterns of *O. sativa* × *O. granulata* derivatives after hybridization with RZ884 (chromosome 6). Lanes: λ- molecular weight marker; 1–10 and 14–23 different derivatives; 11 *O. sativa* IR31917-45-3-2; 12 *O. granulata* (Acc. 100879); 13 BC_1 (Arrow indicates introgression of *O. granulata* allele in lanes 2 and 3).

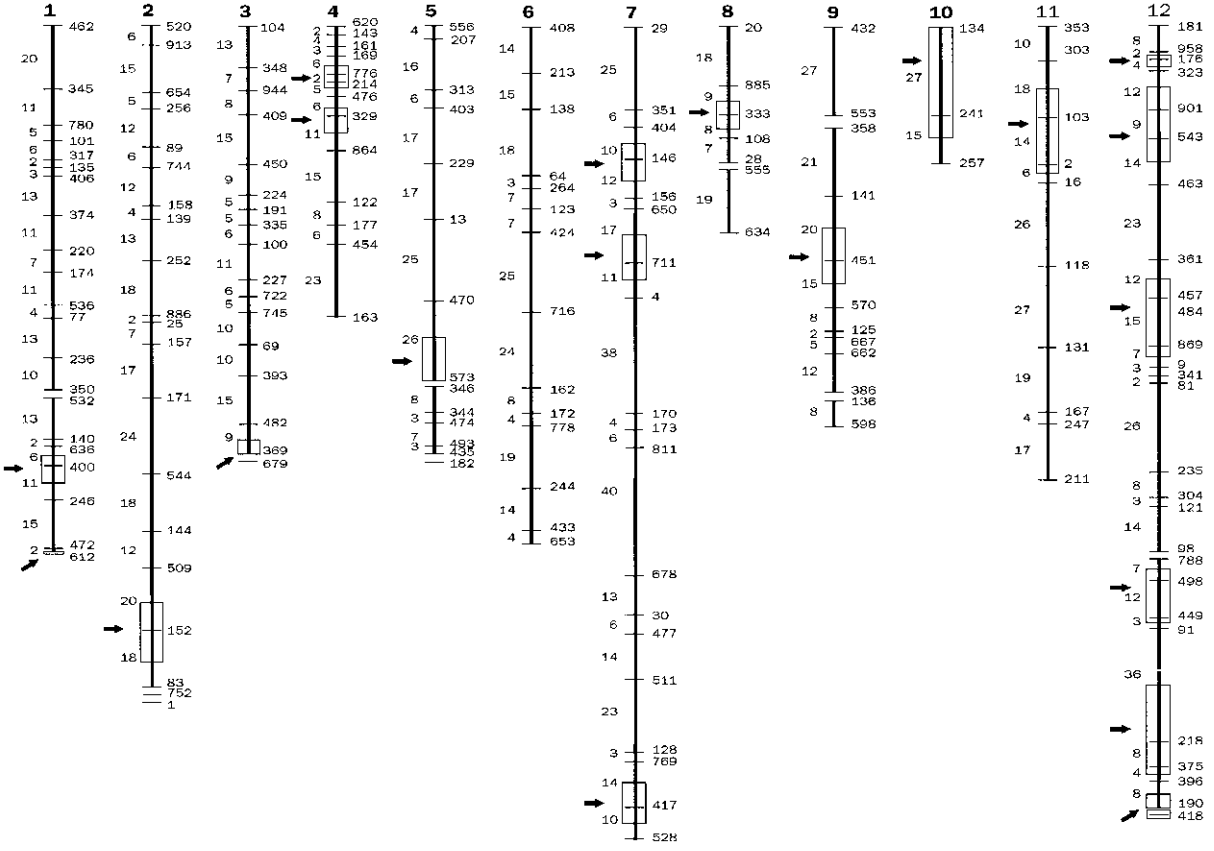

Figure 6. RFLP map showing markers used in analysis of introgression lines derived from *Oryza sativa* × *O. officinalis*. Segments introgressed from *O. officinalis* are identified by boxes and arrows, reproduced from [15].

hand and C, E, F and G genomes on the other. Progenies recovered in BC_2 of *O. sativa* × *O. officinalis* were so similar to *O. sativa* that these were evaluated in field trials and released as varieties for commercial cultivation in Vietnam.

Most introgressed segments were detected by single RFLP markers and the flanking markers were negative for introgression. This also supports the conclusion regarding limited recombination and the possible cause for rapid recovery of recurrent parent phenotype. Rapid recovery of the recurrent parent phenotypes in the backcross progenies of wide crosses has been reported in *Gossypium* by Stephens [40] and *Lycopersicon* by Rick [31, 32, 33] although a higher number of backcrosses were required to reconstitute the recurrent phenotypes.

In some of the introgression lines, non-parental bands were detected. This could result from genomic interactions of cultivated and wild species or an activation of some transposable elements resulting into novel bands.

Future outlook on alien introgression

Rice productivity is affected by various biotic and abiotic stresses. There is thus an urgent need to widen the rice gene pool by incorporating genes for such traits from diverse sources. Wild species are an important reservoir of useful genes and offer great potential to incorporate such genes into commercial rice cultivars for resistance to major diseases, insects and tolerance to various abiotic stresses including new source of CMS and apomixis. Moreover, many of the useful alien genes are different from these of the cultivated species and are thus useful in broadening the sources of resistance to various stresses. Recently, QTL have been identified in *O. rufipogon* which may enhance yield potential when transferred to cultivated rice [43]. However, several crossability barriers limit the transfer of genes from wild species into rice. One of the key barriers is the limited recombination among the homoeologous chromosomes of cultivated and wild species and the mechanism of alien introgression is poorly understood. Future research should focus on enhancing recombination between homologous chromosomes. One strategy should aim at identifying gene(s) controlling homoeologous chromosome pairing in *Oryza.* Alien introgression could also be enhanced through tissue culture of wide-hybrids (F_1s, BC_1 and MAALs) resulting from chromosomal exchanges between genomes of cultivated and wild species.

There is a need to precisely understand the process of introgression. Our results on RFLP analysis of introgression lines derived from crosses of *O. sativa* and wild species suggest that introgression from C, E, F and G genomes occurs for 1 or 2 RFLP markers and the introgressed segments are quite small. Introgression of such segments requires double-crossovers. These results are contrary to expectation based on the extremely low chromosome pairing observed at metaphase I in F_1 hybrids of cultivated and wild species. It is also possible that chromosomes of cultivated and wild species show strong desynapsis at metaphase I. Hence, there is a need to re-investigate chromosome pairing in F_1 hybrids of *O. sativa* and distantly related species at earlier stages of meiosis such as pachytene. The other possibilities is that larger alien introgressed segments resulting from single cross overs may not be transmitted through the male and or female gametes, hence introgression lines only with short alien segments are recovered. Such possibilities need further cytogenetic and molecular investigations.

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