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Exploitation of secondary and tertiary gene pool in the genus *Oryza*

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ABSTRACT

The wild species of Oryza are an important reservoir of desirable genes for resistance to major diseases and insect pests, tolerance to several abiotic stresses and also a good source of cytoplasmic male sterility.Even though resistance sources are available in cultivated rice germplasm, the resistant varieties are becoming susceptible to pest and diseases due to change in insect biotypes and pathogen races. In order to create genetic variability and broaden the gene pool of rice there is a need to look for useful genes from alien germplasm sources.*Thus there is an urgent need to broaden the rice gene pool by introgression of alien genes from wild rice species to meet the challenges affecting rice production. Wide hybridization in rice is a cross between wild rice species and the cultivated variety. This is normally difficult to achieve because many wild species of the genus Oryza fail to hybridize with cultivated rice due to differences in chromosome number or genetic constitution. Fertilization may occur but the embryo is aborted. This necessitates the use of embryo rescue and tissue culture techniques to get viable seeds.Details of alien gene introgression have been discussed in this review.*

Key words: *Oryza, gene pool, exploitation, introgression,back cross, interspecific hybrid*

Rice belongs to the family *Poaceae*, tribe *Oryzeae* with eleven genera. The genus *Oryza* has two cultivated and 22 wild species as reported by Brar and Khush, 2002 (Sampath, 1961; Tateoka, 1964; Vaughan et al, 1989 b). The two cultivated species are *Oryza sativa* and *Oryza glaberrima. Oryza sativa* (2n = 24, AA) is the Asian rice grown world wide while *O. glaberrima* (2n = 24, AA),is the African rice cultivated on a limited scale in West Africa. The progenitor of *O.sativa* is a common wild rice *O.rufipogon / O.perennis* (perennial form) as well as *O.nivara* the annual type. The progenitor of *O.glaberrima* is *O.longistaminata* with *O.breviligulata* as the annual form illustrated in Fig. 1 (Chang, 1976).

The wild species have either 24 or 48 chromosomes (Table 1) representing AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ and HHKK genomes in (Brar and Khush, 2002).

The genus *Oryza* has been divided into four species complexes viz. The *Sativa* complex, The *Officinalis* complex, The *Meyeriana* complex, The *Ridleyi* complex.

The wild species *O. brachyantha* (FF) and *O. schlechteri* (HHKK) can not be placed in any of these groups (Vaughan, 1989).

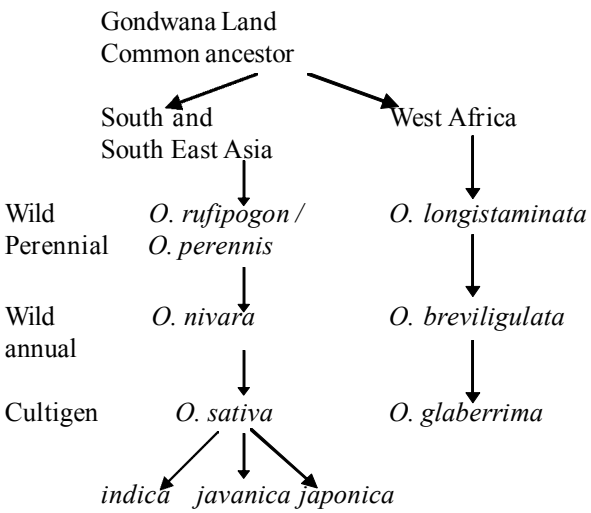

Fig.1. Evolutionary routes of two cultivated rice species (Adapted from Chang, 1976)

Sativa complex consists of two cultivated species and six wild taxa. All are having AA genome and form the primary gene pool. The weedy type species are named as *'fatua'* and '*spontanea*' in Asia and *O.*

stapfii in Africa. These weedy forms usually have 'red rice' and may be more closely related to *O. rufipogon* and *O. nivara* in Asia and *O. longistaminata* or *Oryza breviligulata* in Africa. Another species *O. meridionalis* is distributed in tropical Australia.

Officinalis complex consists of nine species and also known as *Oryza latifolia* complex. The tetraploid species *O. minuta* is sympatric with *O. officinalis* distributed in the Central Philippines. *O. rhizomatis* is a species from Srilanka and another species *O. eichingeri* grows in forest shade of Uganda and Sri Lanka. *O. punctata* is distributed in Africa. *O. latifolia, O. alta* and *O. grandiglumis* are tetraploids. *O. latifolia* is widely distributed in Central and South America and Caribbean islands, whereas *O. australiensis,* a diploid species occurs in Northen Australia.

Meyeriana complex consists of two species *O. granulata* distributed in South and South East and South West China. *O. meyeriana* grows in South East Asia. *O. indoamericana* is a subspecies of *O. granulata* grows in Andaman Islands,in India. These species are with unbranched panicle with small spikelets.

Table 1. Genomic composition and agronomically important characters of *Oryza* species

Species	Chromosome no.	Genome	Potentially desired traits
Sativa complex			
O. sativa	24	AA	Cultigen
O. glaberrima	24	A^gA^g	Cultigen, tolerant to drought, acidity and iron toxicity
O. nivara	24	AA	Resistance to grassy stunt virus and BB
O. rufipogon/perennis	24	AA	Resistance to BB, tungro virus, tolerance to aluminium and soil acidity and source of CMS
O. breviligulata	24	A^gA^g	Resistance to GLH and BB
O. longistaminata	24	A^gA^g	Resistance to BB
O. meridionalis	24	A^mA^m	Elongation ability
O. glumaepatula	24	$A^{gp}A^{gp}$	Elongation ability, source of Cytoplasmic Male Sterility
Officinalis complex			
O.punctata	24, 48	BB, BBCC	Resistance to BPH and GLH
O. minuta	48	BBCC	Resistance to ShB, BB, BL,BPH, GLH
O.officinalis	24	CC	Resistance to BPH,WBPH, GLH and thrips
O. rhizomatis	24	CC	Drought resistance
O. eichingeri	24	CC	Resistance to yellow mottle virus, BPH and GLH
O. alta	48	CCDD	Resistance to stem borer, high biomass production
O. grandiglumis	48	CCDD	high biomass production
O. latifolia	48	CCDD	Resistance to BPH and high biomass production
O. australiensis	24	EE	Drought tolerance, resistance to BPH
Meyeriana complex			
O. granulata	24	GG	Shade tolerance, adaptation to aerobic soil
O. meyeriana	24	GG	Adaptation to aerobic soil
Ridleyi complex			
O. longiglumis	48	HHJJ	Resistance to BB, BL
O. ridleyi	48	HHJJ	Resistance to Stem borer, BB and BL
Unclassified			
O. brachyantha	24	FF	Resistance to YSB, leaf folder and whole maggot
O. schlechteri	48	HHKK	
Poteresia coarctata	48	HHKK	Resistance to YSB and salinity

BL- Blast; BB- Bacterial leaf blight; GLH- Green leaf hopper; WBPH- White backed planthopper; ShB: Sheath blight; BPH- Brown plant hopper.

Ridleyi complex has two tetraploid species *O. ridleyi* and *O. longiglumis* which grow in shaded habitats near river, stream or pools. *O. longiglumis* is found along the Koembe river, Irian Java, Indonesia and Papua New Guinea. *O.ridleyi* grows across South East Asia and Papua New Guinea.

O. brachyantha is a diploid species widely distributed in East Africa. *O. schlechteri* a tetraploid species found in North East New Guiana. It is a tufted perennial with 4-5 cm panicles and small awnless spikelets. Besides *Oryza*, the tribe *Oryzae* has ten other genera; viz. *Chikusiochloa, Hygroryza, Leersia, Luziola, Prosphytochoa, Rhynchoryza, Zizania, Zizaniopsis, Porteresia and Potamophila.*

Genomic relationship in *Oryza* : On the basis of chromosome pairing in F_1 hybrids, various researchers have assigned the genome symbol AA for *Sativa* complex, BB,CC, BBCC,CCDD and EE for the *Officinalis* complex and FF for *O. brachyantha* (Table 1). Based on molecular divergence analysis, two new genomes GG to *O. meyeriana* complex and HHJJ to *O. ridleyi* complex have been assigned (Aggarwal *et al.,* 1997). Similarly Aggarwal *et al*. (1996) considering the total genomic DNA hybridization analysis also showed that *O. schlechteri* had a distinct genome. On the basis of nuclear genes i.e. Adh1 and Adh2 and the chloroplast gene (matK) Ge *et al.* (1999) proposed HHKK genome for *O. schlechteri* and *P. coarctata*, which further suggested *P. coarctata* should be treated as an *Oryza* species.

Introgression of useful traits from wild species to *O. sativa*: Wild species are important reservoir of useful traits i.e resistance to major diseases and insect pests, tolerance to several abiotic stresses and also a good source of cytoplasmic male sterility. Even though resistance sources are available in cultivated rice germplasm, the resistant varieties are becoming susceptible to pest and diseases due to change in insect biotypes and pathogen races. In order to create genetic variability and broaden the gene pool of rice there is a need to look for useful genes from alien germplasm sources. The genes for resistance to sheath blight, tungro and yellow stem borer are available in some of the wild *Oryza* species and not available or very limited in cultivated rice germplasm. Therefore, there is an urgent need to broaden the genepool by introgression of agronomically important genes to the cultivated rice gene pool for increasing rice production.

The main objectives of wide hybridization in rice are to widen the gene pool of rice by transferring useful genes for resistance to major diseases and insect pests and abiotic stresses, enhance the grain yield of rice through introgression of useful alleles of wild relatives and precisely determine the mechanism of alien gene transfer with the possibility of enhancing introgression from distant genome for genetic improvement of rice.

Alien genes introgressed from primary gene pool: The primary gene pool consists of AA genome species which are closely related to *O. sativa*, the cultivated rice. They are cross compatible and have good crossability, shows regular pairing and recombination. Thus gene transfer between these species can be achieved without much difficulty. Several alien genes have been introgressed from AA genome of wild species into *O. sativa*. These are grassy stunt virus from *O. nivara* (Khush ,1977), cytoplasmic male sterility (CMS) from *O. spontanea* (Lin and Yuan 1980), and bacterial leaf blight (BLB) resistance from *O. longistaminata* (Khush *et al*.,1990). The genes for tolerance to tungro disease and tolerance to moderate level of acidity, iron and aluminium toxicity have been transferred from *O.rufipogon* to cultivated rice (Table 2).

Alien genes introgressed from secondary gene pool: It is difficult to produce interspecific hybrids between the cultivated rice and the wild species belonging to secondary gene pool (other than AA genome species) due to very low crossability and degeneration of hybrid embryos during early stages of development, which is the major constraint of the wide crosses. Embryo rescue is essential to develop F_1 hybrids and backcrossing with recurrent *O.sativa* parent is needed till partial fertile plants with normal diploid chromosome complement (2n= 24) or monosomic alien addition lines (MAALs) having (2n+1=25) are produced (Fig.1).The fertile progenies are selfed to develop alien introgression lines with desirable agronomic traits.

Wide hybridization is difficult due to barriers like genome incompatibility, chromosome non homology, low crossability, limited recombination of wild species

Table 2. Introgression of genes from wild *Oryza* to cultivated rice

Genes transferred to *O. sativa*	Donor species	Genome	Introgression method	References
Grassy stunt virus resistance	*O. nivara*	AA	Back crossing	Khush, 1977
Bacterial blight resistance	*O. longistaminata*	AA	Back crossing	Khush *et al.*, 1990
	O. officinalis	CC	Back crossing	Jena and Khush, 1990
	O.minuta	BBCC	Back crossing	Amente-Bordeos *et al.*, 1992
Blast resistance	*O.minuta*	BBCC	Back crossing	Amente-Bordeos *et al.*, 1992
BPH resistance	*O. officinalis*	CC	MAALS	Jena and Khush, 1990
	O. australiensis	EE	MAALS	Multani *et al.*, 1994
WBPH resistance	*O. officinalis*	CC	MAALs	Jena and Khush, 1990
Cytoplasmic male sterility	*O.sativa-spontanea*	AA	Back crossing	Lin and Yuan, 1980
	O.perennis	AA	Back crossing	Dalmacio *et al.*, 1992
Yellow stem borer*	*O. brachyantha*	FF	Back crossing	
	O. ridleyi	HHJJ	Back crossing	
Sheath blight resistance*	*O. minuta*	BBCC	Back crossing	
Tungro tolerance*	*O. rufipogon*	AA	Back crossing	
Tolerance to acidity, iron and aluminium toxicity*	*O.rufipogon*	AA	Back crossing	
	O.glaberrima	AA	Back crossing	

*: Advanced back cross progenies are being produced by IRRI.

and cultivated rice, non viability of fertilized embryo (embryo abortion) and hybrid sterility. Several pre- and post-fertilization barriers are known to limit the production of hybrids during hybridization between cultivated and distantly related species of rice. Several barriers encountered in interspecific and intergeneric crosses, were discussed by Blakslee (1945), Stebbins (1958), Jena & Khush (1986), Sitch and Romero (1990) and Brar & Khush (1997). Biotechnological tools such as embryo rescue and protoplast fusion have become available to overcome crossability (Jena and Khush, 1984).

Pre-fertilization barriers: Pre-fertilization barriers include all factors that hinder effective fertilization due to failure of pollen germination and slow pollen tube growth. Different approaches have been explored to stimulate the pollen germination and pollen tube growth in interspecific and intergeneric crosses with varying degrees of success. A number of substances are known to favour pollen germination such as boric acid, sucrose, calcium and potassium (Zenkteler, 1980; Stewart, 1981; Collins *et al*., 1984 and Vasil, 1987). Sitch and Romero (1989) studied pre-fertilization incompatibility barriers such as pollen germination and pollen tube growth in selected interspecific and intergeneric crosses involving *O. sativa*. They concluded that production of *O. sativa / O. brachyantha* and *O. sativa / Rhynchoryza subulata* hybrid is limited primarily due to failure of pollen tube growth to penetrate the stigma. Germination of *R. subulata* pollen grains was slightly inhibited. Cessation of pollen tube growth before reaching micropyle was observed in crosses of *O. sativa* with *O. officinalis, O. eichingeri, O. minuta, O. alta* and *O. brachyantha*. It was most prevalent in pollination with *O. eichingeri*. The same mechanism may be responsible for the low seed set obtained from crosses with *O. officinalis, O. eichingeri, O. minuta* and may further reduce seed set in crosses with *O. brachyantha*. Sitch and Romero (1989) also studied the pollen germination and pollen tube growth in the crosses of *O. sativa / P. coarctata*. They found that crosses of *O. sativa* with *P. coarctata* were inhibited by strong pre-fertilization barriers. They developed methods to overcome these incompatibility barriers. Possible approaches including mentor pollen technique, application of hormones were utilized to stimulate pollen tube growth and intra ovarian pollination. In 1990 they described pre-fertilization incompatibility in interspecific and intergeric crosses in *O. sativa* and attempts were made to overcome these barriers. They observed that pollen germination was normal in crosses between *O. sativa* and *O. brachyantha*, *O. eichingeri, O. officinalis* and *O. ridleyi* and slightly inhibited in crosses between *O. sativa* and *R. subulata*. Stigmatal penetration of *O. brachyantha* and *R. subulata* pollen tubes was inhibited while in *O. ridleyi* pollen tubes showed both incompatible and weakly compatible

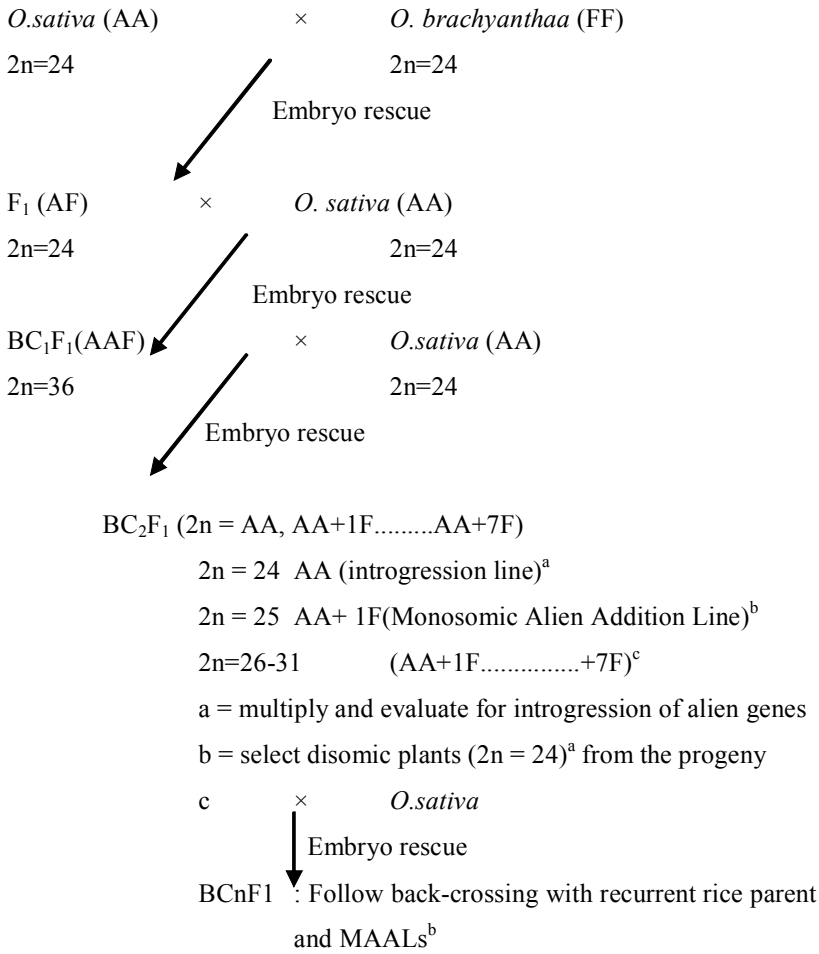

Fig.2. Schemeatic diagram showing production of monosomic alien addition lines (2n=25) and introgression lines (2n=24) from crosses of rice with distantly related wild species

reactions. Pollen tubes of *O. eichingeri*, *O. officinalis* and *O. ridleyi* penetrated into the stigma but growth was frequently inhibited in the style or ovary wall, particularly in *O. eichingeri* crosses. Post-pollination application of boric acid, the immunosuppressant epsilon- amino carpic acid (EACA) and gibberlic acid (GA_3) enhanced pollen germination in *O. brachyantha* and boric acid and EACA inhibited pollen tube growth in *O. brachyantha* , *O. eichingeri*, and *O. ridleyi*. Pollen tube growth in the control and GA_3 treatments was similar. Temperature (29 and 35^{0}C) had no effect on pollen germination and pollen tube growth was stimulated in *O. ridleyi* crosses at 35^{0}C only.

Domingo in 1995, reported the incompatibility barriers in crosses of *O. sativa P. coarctata* and *O. ridleyi O. brachyantha*. He found that the major incompatibility barriers in these crosses were the slow pollen tube growth and lack of effective fertilization. To overcome the barriers, various approaches, which involves the use of growth hormones for pollen recognition, and immuno-suppressants were made and eventually produced a hybrid subsequently to transfer the genes for salinity tolerance from *P. coarctata* to cultivated rice and determined the genomic relationship of *O. brachyantha* and *O. ridleyi*.

Post-fertilization barriers: Post-fertilization barriers hinder or retard the development of the zygote after fertilization and normal development of the seed is affected. These barriers may be due to chromosome elimination, hybrid inviability or weakness and hybrid sterility.

In vitro culture of hybrid embryos is useful in cross combination where the fertilized embryo begins to develop but abort before reaching the maturity due to the inability of the endosperm to nourish the developing

embryo or the maternal tissue being antagonistic to the development of the embryos (Collins *et al*., 1984). Hybrid embryos are rescued aseptically by isolating them prior to their abortion and culturing them directly into an artificial media (Stewart, 1981; Collins *et al*., 1984, Sastri and Mallikarjune, 1985 and Brar and Khush 1986). This technique is referred as "embryo rescue".

Techniques for overcoming crossability barriers has been described by Brar and Khush (1986). By the use of exogenous growth substances and immunosuppressants chances of producing wide hybrids have improved considerably. Gibberllic acid (GA_3) and other growth substances have been used successfully before and after pollination. Solution (A) containing Sucrose 5 g/l (Dissolved in DW) and NAA 25 mg/l (Dissolve in KOH) can be applied before pollination. Solution (B containing Gibberllic acid (GA3) 100 mg/l, Napthalene acetic acid 25 mg/l and Kinetin 5 mg /l can be applied after pollination.

Before pollination, hormonal solution A is sprayed with an atomizer on the emasculated spikelets for adhering the pollens and to help in the pollen germination. Mature anthers were collected from the desired donor parents and kept on a thin paper in a petri dish. The ripe anthers held with fine forceps are bursted on the stigma of the emasculated spikelets on the same day between 8 a.m to 11.00 a.m. After pollination the sapikelets are covered with butter paper bags to avoid the outcrossing. After 24 hours of pollination, hormonal solution B is sprayed on the spikelets twice daily for five days to arrest shattering of spikelets.

Embryo rescue: Recent studies have pointed out the potential application of embryos, ovules and ovary culture to obtain fertile hybrids among the incompatible species or genera. Successful production of interspecific hybrids from an incompatible cross involving interspecific hybrids of *Linum* has been demonstrated for the first time through embryo rescue technique (Laibach, 1929). Several workers employed the same technique and obtained F_1 hybrids in various crops. In the genus *Oryza*, embryo culture proved to be successful to raise the hybrids from crosses between distantly related species with cultigen in early fifties (Niles 1951). Iyar and Govila in 1964 reported successful recovery of hybrids from 27 interspecific crosses of *Oryza* using embryo rescue technique. They found that young embryos showed better growth on Nitsch's medium when supplemented with 10 percent coconut milk. Jena and Khush in 1984 successfully produced interspecific hybrids by isolating 12 to 14 days old embryos and culturing them on 1/4th MS medium devoid of auxin.

Eloran *et al*. (1992) produced successfully interspecific hybrids through embryo rescue technique in crosses between *O. sativa* and *O. officinalis*, *O. latifolia* and *O. ridleyi* to widen the rice gene pool and to transfer the desirable alien genes resistance for diseases and insect pests.The seed set ranged from 0.24 to 29.37%. Suptitada *et al*. (1994) also developed interspecific hybrids employing embryo rescue technique in rice.

With the help of embryo rescue technique, Farooq *et al*. (1991) attempted wide hybridization between *O. punctata* and collar grass (*Leptochola fusua*) to transfer salt tolerant genes to cultivated rice. Presently most of the rice scientists use embryo rescue technique in wide hybridization. Except AA genome, all the genomes are incompatible with *O. sativa*. Therefore, the embryo rescue is very much essential for developing interspecific hybrids using species of distant genomes.

Procedure for embryo rescue technique: Immature fertilized spikelets are collected from the panicles of interspecific crosses after 10-14 days of pollination followed by washing of the spikelets with doubled distilled water, surface sterilization with 70% ethanol for 2-3 minutes and removal of the excess alcohol with the help of absorbent cotton or tissue paper are to be done. The spikelets are then transferred to 4% sodium hypochlorite (Commercial bleach) for 15 minutes inside the laminar air flow chamber followed by washing with sterilized distilled water for 2-3 times. After that spikletes are treated with 0.1% $HgCl_2$ solution for 2-3 minutes followed by washing with sterilized distilled water for 2-3 times. To excise the embryo the lemma and paleas of the spikelets are opened by fine forceps under stereomicroscope (50 to 100 X) in an asceptic condition and endosperm with embryo are taken out from the spikelet. Then the young hard, globular embryo from the watery endosperm is excised by sterilized needle. The excised embryo is to be inoculated in 1/4th MS media (Murashige and Skoog, 1962) by inoculating needle and incubated in dark at 25 ± 1^{0}C until

germination. Subsequently they are transferred to illuminating incubation room and allowed to grow up three leaves stage. Then the young seedlings are transferred to rooting nutrient solution medium. After 15-20days when seedlings are fully grown with roots and leaves they are transplanted in the earthen pot with sterilized soil.

Somatic cell hybridization: It is an important approach to produce hybrid between sexually incompatible species. It involves the fusion of protoplast of different species followed by regeneration of somatic hybrids Hayashi *et al*.(1988) produced 250 somatic hybrids through electrofusion of protoplast of rice with four wild species, viz. *O. officinalis, O. eichingeri, O. brachyantha* and *O. perrieri*. Somatic hybrid plants have also been produced through electrofusion of protoplast of *O. sativa* and *P. coaractata*, a salt tolerant species (Jelodar *et al*., 1999).

A series of hybrids and MAALs representing 7-12 chromosomes of six wild species and introgression lines have been produced at the International Rice Research Institute, through direct crosses as well as employing embryo rescue following hybridization. Most of these crosses have been used for transferring useful genes for genetic improvement of cultivated rice.

Alien gene introgression from the CC genome : Interspecific hybrids have been produced by employing embryo rescue method between *O. sativa* and wild species with CC genome. Jena and Khush (1990) produced several introgressed lines from the wide cross *O. sativa/O. officinalis* for transferring BPH, WBPH and BB resistant genes into cultivated rice. Three breeding lines have been released as varieties viz., MTL 98, MTL 103 and MTL 105. Hirabayashi and Ogawa (1999) analyzed recombinant inbred lines (RILs) from the cross between Hinohikari (susceptible Japonica) with IR 54742-1-11-17 Indica introgression line derived from the cross of *O. sativa / O. officinalis*. Two genes bph 11(t) and bph 12 (t) were identified and mapped to chromosome 3 and 4 of rice.

Alien gene introgression from the BBCC genome: Sitch and Romero (1990) produced interspecific hybrids between *O. sativa and O. minuta* (BBCC). Following backcross and embryo rescue, advanced introgressed lines have been produced and evaluated against bacterial blight and blast (Amante – Bordeos *et al*., 1992). Two lines were found resistant – one to race 6 of BB and another to race P06-6 of blast [Pi-9 (t)].

Alien gene introgression from the CCDD genome: Several workers have produced hybrids between *O.sativa* and CCDD genome species (Sitch and Romero, 1990; Brar *et al.,* 1991). Out of three CCDD species, the advanced progenies derived from the cross *O. sativa / O. latifolia* have investigated and introgression of resistance gene to BPH, WBPH, BB and other traits like growth duration and purple pigmentation have been obtained (unpublished).

Alien gene introgression from the EE genome: Multani *et al.* (1994) produced hybrid between colchicines induced autotetraploid of *O. sativa* and *O. australiensis* (2n = 24, EE) employing embryo rescue technique. Introgression was observed for morphological traits such as long awn and earliness and for Amp-3 and Est-2 allozymes. Four backcross progenies resistant to BPH and one race 6 of BB. BPH resistance was found to be controlled by a recessive gene in two lines and controlled by a dominant gene in other two lines.

Alien gene introgression from the FF genome: A large number of introgression lines have been derived from the cross of *O. sativa* cv. IR 56 and the wild species *O. brachyantha* (2n = 24, FF). Out of 149 back cross progenies analyzed, 27 showed resistance to bacterial blight races 1-4 and 6. The backcross introgressed progenies are being evaluated for resistance to Yellow stem borer (YSB) (Brar *et al.,* 1996).

Alien gene introgression from the GG genome: Hybrids have been produced from the cross of *O.sativa and O.granulata* (Brar *et al.,* 1991). Backcrossed advanced progenies have also been produced; but none of these lines has shown introgression of traits from *O. granulata* into cultivated rice. Backcross progenies derived from the crosses of *O. sativa* with *O. officinalis* (CC), *O. australiensis* (EE), *O. brachyantha* (FF) and *O. granulata* (GG) resembled the recurrent parent in all most all morphological traits. This indicated that only a limited recombination between a genome of *O. sativa* and C, E, F and G genomes wild species occurred.

Alien gene introgression from the HHJJ genome: Hybrid between *O. sativa* cv. IR 56 and *O. ridleyi* have been produced. The tetraploid *ridleyi* complex

includes species viz., *O. ridleyi* and *O. longiglumis*. BC_2F_1 of this cross have been produced. However, no introgression could be detected.

Alien gene introgression from the HHKK genome: Interspecific hybrids between *O. sativa* and *P. coarctata* have been produced both through sexual crosses following embryo rescue (Jena, 1994; Brar *et al*., 1997) and through protoplast fusion (Jelodar *et al*., 1999). Due to strong incompatibility barrier no backcross progenies could be obtained.

Work on the introgression of alien genes, wide hybridization between *O.sativa* and wild species of different genomes were also carried out at Central Rice Research Institute, Cuttack under the Projects. Morpho-cytological characterization of 108 accessions of different wild rice species namely, *O. officinalis, O. eichingeri, O. punctata, O. australiensis, O. malampuzhaensis, O. minuta, O. grandiglumis, O. alta, O. latifolia, O. longistaminata, O. brachyantha, O. ridley*i, *O. longiglumis* and *P. coarctata* were done for 16 key characters. Observations on different qualitative and quantitative traits revealed enormous variability among different species. Of the wild gene pool, 69 accessions were screened against three biotic stresses such as bacterial leaf blight, sheath blight and brown plant hopper (biotype-1) and out of these, two accessions of *O. longistaminata,* one accession of *O. eichingeri* and two accessions of *O. officinalis*, two accessions of *O.minuta* and one accession of *Porteresia coarctata* were observed to be resistant to 14 isolates of BLB collected from all over India. For sheath blight, however, no resistant accessions could be identified. For BPH-1, resistant sources could be identified in *O. longistaminata, O. eichingeri, O. latifolia, O. minuta and O. malampuzhaensis* (Mishra *et al.,* 1991; Mohapatra *et al.,* 1993, 1994; Bose, 2005).

With an objective of transferring alien genes into elite cultivated rice, wide crosses were effected employing hormonal treatment before and after pollination and rescuing embryos, 10-14 days after pollination. *Oryza sativa* was crossed with the following wild species viz. *O. officinalis, O. eichingeri, O. punctata, O. australiensis, O. malampuzhaensis, O, minuta, O. grandiglumis, O. alta, O. latifolia, O. longistaminata, O. brachyantha, O. ridley*i and *O. longiglumis* (Bose, 1997 and Panda, 2006). Most of these hybrids were sterile and backcrossing to recurrent *sativa* parent was done in some selected crosses to transfer alien genes for bacterial blight resistance from *O. malampuzhaensis, O. eichingeri* and *O. longistaminata*. For the introgression of alien genes for biotic stress tolerance, specially for bacterial blight (BB), the interspecific hybrids of *O.sativa* cv. Jaya and Swarnaprava and *O. longistaminata* were produced. The backcross hybrid derivatives were evaluated against bacterial leaf blight. Out of 102 lines, two lines i.e., CR 2212-64 and CR 2212-72 were identified as resistant donors with yield level of 3.0-4.0 t/ha (Sen *et al*., 2007). Backcrossing with F_1 hybrid involving AA genome species was fairly easy but with F_1 hybrids involving distant genomes, a large number of pollinations were made with hormonal treatments in order to obtain fertile embryos. The fertilized embryos were rescued and two BC_1F_1 plants in *O. sativa*/*O. eichingeri* and one in *O. sativa/O. malampuzhaensis* were obtained. Cytological studies of F_1 interspecific hybrid (*O. sativa/ O. malampuzhaensis*) involving three different *sativa* parents revealed that mostly there are 36 univalents. However, 15-30% progenies revealed the presence of 2-3 bivalents at metaphase I (Bose, 1997 and Mishra *et al.,* 1992).

Yellow stem borer (YSB) is a major insect pest which damages the unprotected rice crop more than 40 percent. Resistance to stem borer is almost lacking in the cultivated varieties and also in primary gene pool (AA) of rice. Evaluation of wild rice species for YSB resistance, confirmed that the Accession No. 1052 and 1050 of *O. officinalis* and Acc. No. 1059 of *O. ridleyi* were resistant to YSB both at vegetative and reproductive stages and *O. brachyantha* (Acc No. 1086) was resistant and moderately resistant to YSB at vegetative and reproductive stages, respectively. Antibiosis study showed that *O. ridleyi* was resistant to YSB (Padhi and Sen, 2002; Sen *et al.,* 2002).

Wide hybridization was made using these wild rice species as donor parents and high yielding cultivars as recipient parents with hormonal treatments. Interspecific F_1 hybrids were successfully developed from all the possible cross combinations by rescuing fertilized immature embryos (10-14 days old) in $1/4^{th}$ MS medium supplemented with IAA (0.5 mg/l), KIN (1.0 mg/l), 7.5 g/l sugar and 7.5 g/l agar. The seed setting and crossability were highest in *O. sativa* cv Ratna / *O. officinalis* and was least in case of *O. sativa* cv

Ratna/*O. ridleyi*.

The F_1 interspecific hybrids were morphologically intermediate between the two parents with preponderance of few wild characters. In the cytological behaviour at metaphase I, all the F_1 interspecific hybrids showed the presence of univalents. Only in *O. sativa* cv. Ratna/*O. officinalis* one bivalent was observed in 1.2 to 11.1 % of PMCs. But all the F_1 interspecific hybrids were completely sterile. The F_1 hybrids viz. *O. sativa* cv. Ratna/*O. officinalis* (Wide Hybrid 2), *O. sativa* cv. Savitri/*O. brachyantha* and Swarna/*O. brachyantha* were found resistant, whereas *O. sativa* cv Ratna/*O. officinalis* (Wide Hybrid 1), Swarna/*O. ridleyi* and Udaya/*O. ridleyi* were moderately resistant. In order to enhance the fertility and alien gene recombination of the resistant F_1 hybrids, somaclones were developed by culturing panicle primordia (1.0 and 1.5 cm length) of F_1 interspecific hybrids in MS medium supplemented with 2,4-D (2.0 mg/l), KIN (1.0 mg/l), 3 % sucrose and 7.5 g/l agar. The pollen fertility of the somaclones of *O. sativa* cv Ratna/*O. officinalis* increased upto 10% as compared to its F_1 hybrids (2.8 %). Chromosome paring in the somaclones was also enhanced resulting in formation of 1-3 IIs in 53.85 to 67.65 % of PMCs (Panda, 2006).

The resistant lines of F_1 hybrids and the somaclones were backcrossed with recurrent parents for development of backcross lines with higher fertility. The crossability was remarkably less than that of F_1 hybrids. Thus, BC_1F_1 hybrids of *O. sativa* cv Ratna/ *O. officinalis* and *O. sativa* cv Savitri /*O. brachyantha* were developed employing embryo rescue technique. In case of BC_1F_1 of *O. sativa* cv Ratna/*O. officinalis* the pollen fertility increased to 5.96% as against 2.85 % recorded in F_1 hybrids. The BC_1F_1 progenies resembled more towards the *sativa* parent and were allotriploid in nature. The cytological behaviour revealed that 40.18 % of PMCs had 12 IIs + 12 Is per cell and 29.46 % of PMCs had 1-3 IIIs. In case of *O. sativa* cv Savitri/*O. brachyantha//Savitri* , the BC_1F_1 hybrid also resembled more towards the *sativa* parent. The BC_1F_1 progenies showed an improvement of pollen fertility over the F_1 hybrids. In these BC_1F_1 progenies 37.79 % of PMCs showed 12 IIs + 12 Is per cell and 22.05 % of PMCs showed 1-2 IIIs per PMC, indicating the allotriploid nature of hybrid (Panda 2006).

The introgressed lines of backcross hybrid (BC_1F_1) of *O. sativa* cv Savitri/*O. brachyantha* and somaclones (SC_1F_1) of *O. sativa* cv Ratna/*O. officinalis* were moderately resistant to YSB. The

Table 3. Interspecific breeding programme at Central Rice Research Institute

Cross Combinations	Hybrids developed through	Genes resistance for	Present status of the interspecific hybrids
O. sativa x*O. longistaminata* (cv. Jaya,Swarnaprava,Swarna)	Direct hybridization	BB	Back cross hybrid derivatives developed
Swarnaprava x *O. punctata*	Embryo rescue	BPH and GLH	F_1
Swarnaprava x *O. minuta* Neela x *O. minuta*	Embryo rescue	ShB, BLB, BL, BPH, GLH	F_1
Swarnaprava x *O. eichingeri*	Embryo rescue	BPH and WBPH	SC_1F_1
Neela x *O. latifolia* TR-600x *O. latifolia* Swarnaprava x *O. latifolia* CR 112-32x *O. latifolia*	Embryo rescue	BPH and high biomass production	F_1
Ratna x *O. officinalis* Savitri x *O. brachyantha*	Embryo rescue	BPH and YSB	$SC_1F_1BC_1F_1BC_1F_2$
Swarna x *O. brachyantha* Swarna x *O. ridleyi*	Embryo rescue	YSB	$BC_1F_1BC_2F_1$ (Chromosome variants)F_1
Udaya x *O. ridleyi*	Embryo rescue	YSB	F_1F_1

SCF- Somaclonal of F_1 BCF-Backcross 1

resistant BC_1F_1 hybrids of *O. sativa* cv Savitri/*O. brachyantha* were again backcrossed with recurrent parent Savitri for the development of chromosome variants. The rescued BC_2F_1 progenies showed higher crossability and germination percentage than F_1 and BC_1F_1 hybrids (Panda 2006; Sen *et al*., 2004 and 2006; Behura, 2007). The BC_2F_1s (chromosome variants) were studied morpho-cytologically and different chromosome addition lines (1, 2, 3 and more than 3) were found. Out of them eight plants with addition of single chromosome were identified and they were grouped into six MAALs according to their morphological peculiarity and resemblance with morphology of trisomics as grassy, dwarf, sterile, erect, bushy and pseudonormal (Behura, 2009). Interspecific breeding programme has been taken up at Central Rice Research Institute is given in (Table 3).

The alien traits from the wild species were successfully transferred to cultivated rice and derivatives are developed with introgressed genes resistance to specific stresses. Backcross derivatives of *O. sativa/O. longistaminata* resistance to BB are developed and identified as a donor with yield potential of 3.5 t/h and good grain quality. The MAALs and chromosome variants can be utilized to develop a complete set of 12 MAALs which can be used in breeding programme to develop introgressed lines resistant to YSB, the deadly pest of rice. Hence, the rice productivity and production will be enhanced.

Research should be focused on overcoming of the key barriers in the transfer of useful genes from distantly related wild species by enhancing recombination among the homologous chromosomes. With the advancement of tissue culture, molecular markers, *in situ* hybridization and genomics, the future outlook should be for broadening the gene pool of rice through precise transfer of useful genes from wild species to cultivated rice.

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