

Genetic diversity in *Oryza* species and use of wild species in rice improvement

Kshirod K. Jena*^{1,2}, Darshan S. Brar² and David J. Mackill²

¹ IRRI – Korea Office, NCES-RDA, Suwon 441-100, Republic of Korea

² Plant Breeding, Genetics, and Biochemistry Division, International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines

Introduction

The genus *Oryza* belongs to the subfamily *Oryzoideae* of the family *Poaceae* (*Gramineae*) and has 24 species that grow in a wide range of habitats worldwide, ranging from below sea level to high altitudes. However, the evolutionary transition of wild plants of *Oryza* from the smallest form of *O. granulata* to the tallest form of *O. grandiglumis* has brought under domestication two species, *O. sativa* and *O. glaberrima*, that have pantropical distribution (Vaughan 1989). Of the two cultivated species, *O. sativa* has been more widely grown but *O. glaberrima* was apparently domesticated in Africa and is grown in some small areas of West Africa. This paper reviews the genetic diversity among the species belonging to the genus *Oryza* and describes the research work done for the use of wild *Oryza* species to enrich the genepool of cultivated rice, which would be of great benefit to rice production.

Speciation and genome structure in *Oryza*

The genus *Oryza* has 22 wild species besides two cultivated species. Based on morphological and chromosome pairing studies, the species of *Oryza* have been grouped into four complexes (Morishima and Oka 1960; Vaughan 1989): the *O. sativa* complex, *O. officinalis* complex, *O. meyeriana* complex and *O. ridleyi* complex. The species *O. schlechteri*, *O. brachyantha* and *O. coarctata* remained ungrouped because of their distinctive characteristics. The species of the *O. sativa* complex are all diploid with a chromosome number of 24. However, the species of other complexes are either diploid or allotetraploid. Based on chromosome pairing of F₁ interspecific hybrids as well as total genomic DNA hybridization patterns, the species of *Oryza* belong to ten different genome groups (Khush 1997; Ge et al. 1999). The species of the *O. sativa* complex have a AA genome because of high cross compatibility and homologous chromosome pairing. This group of species belongs to the primary genepool of *Oryza*. The *Oryza officinalis* complex has species with five different genomes, BB, CC, BBCC,

genome. The species with different genomes have a wide range of geographic distribution (Table 1). The species of the *O. officinalis*, *O. meyeriana* and *O. ridleyi* complexes are highly cross incompatible with the species of the *O. sativa* complex and they belong to the secondary genepool of *Oryza*.

Evolution of cultivated rice

The cultivated species *O. sativa* and *O. glaberrima* are thought to have a parallel evolution in their process of domestication (Chang 1976). The wild progenitor of *O. sativa* is the common perennial wild species *O. rufipogon* (formerly *O. perennis*) with an intermediate annual wild species *O. nivara*. However, the African cultivated species *O. glaberrima* evolved from the perennial wild species *O. longistaminata*. *O. glaberrima* is distinguished from *O. sativa* by its short round ligule, panicle lacking secondary branches and glabrous lemma and palea. The area cultivated with *O. glaberrima* is being displaced by cultivars of *O. sativa*.

The annual wild species *O. meridionalis* is the immediate progenitor of the cultivated rice *O. sativa* in Australia. This species often grows with *O. australiensis* in tropical Australia. However, another perennial wild species, *O. glumaepatula*, is the immediate progenitor of cultivated rice in Latin America.

The weedy types of rice have been given various names in different countries. They have special traits that might have originated by mutation of cultivated rice or by hybridization between cultivated and wild species of the AA genome. These weedy rice types are commonly called 'red rice' in the USA or 'Aengmi' in Korea (Heu et al. 1990) and they are a valuable germplasm source in Korea (Cho et al. 1995).

Genetic diversity among *Oryza* species

Genetic diversity among *Oryza* species was analyzed by morphological, cytological and molecular analysis (Morishima and Oka 1960; Jena and Kochert 1991; Aggarwal et al. 1999). The taxonomic status of the species of the genus *Oryza* based on phenotype had poor resolution for determining the interrelationship among the species because of the limited genetic variability. However, advanced molecular approaches have unraveled abundant genetic variability among the species of *Oryza* with extensive genome coverage and these approaches have become valuable tools in the study of

population biology and systematics (Ge et al. 1999; Cai and Morishima 2002; Gao et al. 2002; Mullins and Hilu 2002).

DNA analysis revealed that genetic polymorphism within the species was lower than among the species. The genetic distance between the species of the same genome is about 2.8 times higher than within the species. However, the genetic distance between species of different genomes is five times larger, confirming their diverse evolutionary status. The species belonging to the *O. officinalis* complex were diverse and conserved at the genome level. The genetic distance between the species of the *O. meyeriana* complex is equal but is larger ($D=0.717$) for other species of the genus. *O. brachyantha* of the FF genome was found to be the most distant from the other species in the genus and several accessions of *O. brachyantha* showed high genetic diversity as revealed by AFLP and 10-kDa prolamin polypeptide analysis. Based on sequence polymorphism in the gene (420 bp) that encodes 10-kDa prolamin polypeptide, it has been suggested that *O. brachyantha* is the most basal species, followed by a polytomy of three clades that could be delineated based on genome composition: (1) the GG clade: *O. granulata* and *O. meyeriana*; (2) the EE clade: *O. australiensis*; and (3) the ABCD clade: the remaining *Oryza* species (Mullins and Hilu 2002). This study suggested that the genus *Oryza* has an African origin as opposed to a Euro-Asian origin (Second 1985). Within the Sativa complex, the three geographic forms, African (*O. glaberrima*, *O. barthii* and *O. longistaminata*), American (*O. glumaepatula*) and Asian (*O. sativa*, *O. nivara* and *O. rufipogon*), were distinct and evolved independently. The cultivated species *O. sativa* has been differentiated into two subspecies, indica and japonica, representing two partially isolated genepools. It is believed that the subspecies indica was domesticated primarily from its wild relatives in South or Southeast Asia and the subspecies japonica was a type adaptive to the high altitudes and temperate regions derived from the subspecies indica (Oka 1988).

In a recent phylogenetic analysis using nuclear genes *Adh1* or *Adh2* and the chloroplast gene *matK*, an additional genome type, HHKK, has been recognized for *O. schlechteri* and *Porteresia coarctata*, suggesting that *P. coarctata* is an *Oryza* species (Ge et al. 1999).

Useful genes of *Oryza* species and gene transfer

Rice production and productivity in tropical and temperate countries is severely affected by several biotic and abiotic stresses. Some of these biotic stresses are diseases such as bacterial blight (BB), blast (BI), sheath blight (ShB), tungro virus (RTV), black streak dwarf virus (BSDV) and dwarf virus (DV) and insects such as brown planthopper

(BPH), whitebacked planthopper (WBPH) and stem borer. The major abiotic stresses are drought, cold, submergence, aluminum toxicity and salinity. Cultivated rice germplasm has limited resistance genes to protect rice cultivars under these stress conditions. It is essential to widen the genepool of cultivated rice by incorporating useful genes from diverse genetic sources. The wild species of *Oryza* are a rich source of beneficial genes (Table 1). However, it is difficult to transfer genes from wild species into cultivated rice genotypes because of crossability and recombination barriers. Nevertheless, several beneficial genes have been successfully transferred (Table 2) across crossability and recombination barriers using advanced techniques of tissue culture and chromosome manipulation (Jena and Khush 1989, 1990; Brar and Khush 1997). Recent advances in the development of a highly saturated rice molecular map have made it possible to identify the introgressed genes from wild species on the chromosomes of *O. sativa* (Jena et al. 1992; Ishii et al. 1994; Temnykh et al. 2000; Brondani et al. 2002; Jena et al. 2003; Nguyen et al. 2003).

Molecular identification of gene introgression from wild species

Recent advances in molecular breeding methods hold tremendous potential for the genetic improvement of rice cultivars with beneficial genes from wild species. Near-isogenic lines carrying a dominant BB resistance gene (*Xa21*) introgressed from *O. longistaminata* were traced with DNA markers and one RFLP marker (RG103) located on chromosome 11 was associated with the resistance gene. Subsequently, tightly linked markers were developed and the *Xa21* gene has been cloned. Using molecular approaches, the gene product has been identified as a receptor kinase-like protein (Song et al. 1995). The *Xa21* gene expresses a broad spectrum of resistance to BB races in the rice-growing countries of Asia and contributes to the development of BB-resistant rice cultivars.

Valuable genes from AA genome wild species could be transferred into the cultivated rice genome following the principles of homologous recombination. However, novel beneficial genes present in distantly related species such as *O. officinalis* (CC), *O. australiensis* (EE) and *O. minuta* (BBCC) could be transferred and the possible mechanism was by restricted reciprocal recombination detectable only at the molecular level (Jena et al. 1992). This type of gene transfer is unique in rice, which has a cryptic structurally rare homozygosity at the chromosome level with unrelated genomes, but which is detectable only at the molecular level. This finding has paved the way for rice breeders and geneticists to use the novel beneficial genetic resources of wild species of *Oryza* for cultivar improvement.

Conclusions

Rice is the staple food for most of the human population in tropical and temperate countries of Asia. Rice production has increased in many regions of the rice-growing countries because of the Green Revolution but many regions still have limitations to increasing rice production because of biotic and abiotic stresses. The narrow genetic base of modern rice cultivars has to be widened by incorporating beneficial genes from the wild species of the AA genome or other genomes distantly related to cultivated rice. A clear understanding of the evolutionary relationship of *Oryza* species from several studies has now led us to search for beneficial genes and widen the genepool of both tropical and temperate rice. Developments in tissue culture techniques, understanding chromosome homoeology between the genomes and the availability of a highly saturated molecular map of the rice genome have made it possible to transfer novel genes for biotic and abiotic stress resistance from several wild species as well as detect the molecular mechanism of gene transfer. Furthermore, with the complete genome sequence of rice opened recently to the public domain, it should be possible to use many beneficial genes from wild species of *Oryza* to improve the yield potential, grain quality and stress resistance of rice cultivars.

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References

- Aggarwal RK, Brar DS, Nandi S, Huang N, Khush GS (1999) Phylogenetic relationships among *Oryza* species revealed by AFLP markers. *Theor Appl Genet* 98:1320-1328
- Brar DS, Khush GS (1997) Alien introgression in rice. *Plant Mol Biol* 35:35-47
- Brondani C, Rangel PHN, Brondani RPV, Ferreira ME (2002) QTL mapping and introgression of yield related traits from *Oryza glumaepatula* to cultivated rice (*Oryza sativa*) using microsatellite markers. *Theor Appl Genet* 104:1192-1203
- Cai HW, Morishima H (2002) QTL clusters reflect character associations in wild and cultivated rice. *Theor Appl Genet* 104:1217-1228
- Chang TT (1976) The origin, evolution, cultivation, dissemination and diversification of Asian and African rices. *Euphytica* 25:435-444

- Cho YC, Chung TY, Suh HS (1995) Genetic characteristics of Korean weedy rice (*Oryza sativa* L.) by RFLP analysis. *Euphytica* 86:103-110
- Gao LZ, Schaal BA, Zhang CH, Jia JZ, Dong YS (2002) Assessment of population genetic structure in common wild rice *Oryza rufipogon* Griff. using microsatellite and allozyme markers. *Theor Appl Genet* 106:173-180
- Ge S, Sang T, Lu BR, Hong DY (1999) Phylogeny of rice genomes with emphasis on origins of allotetraploid species. *Proc Natl Acad Sci USA* 96:14400-14405
- Heu MH, Cho YC, Suh HS (1990) Cross affinity of Korean weedy rice to the cultivars. *Korean J Crop Sci* 35:233-238
- Ishii T, Brar DS, Multani DS, Khush GS (1994) Molecular tagging of genes for brown planthopper resistance and earliness introduced from *Oryza australiensis* into cultivated rice, *O. sativa*. *Genome* 37:217-221
- Jena KK, Khush GS (1989) Monosomic alien addition lines of rice: production, morphology, cytology, and breeding behavior. *Genome* 32:449-455
- Jena KK, Khush GS (1990) Introgression of genes from *Oryza officinalis* Well ex Watt to cultivated rice, *O. sativa* L. *Theor Appl Genet* 80:737-745
- Jena KK, Kochert G (1991) Restriction fragment length polymorphism analysis of CCDD genome species of the genus *Oryza* L. *Plant Mol Biol* 5:109-118
- Jena KK, Khush GS, Kochert G (1992) RFLP analysis of rice (*Oryza sativa* L.) introgression lines. *Theor Appl Genet* 84:608-616
- Jena KK, Pasalu IC, RAO YK, Varalaxmi Y, Krishnaiah K, Khush GS, Kochert G (2003) Molecular tagging of a gene for resistance to brown planthopper in rice (*Oryza sativa* L.). *Euphytica* 129:81-88
- Khush GS (1997) Origin, dispersal, cultivation and variation of rice. *Plant Mol Biol* 35: 25-34
- Morishima H, Oka HI (1960) The pattern of interspecific variation in the genus *Oryza*: its quantitative representation by statistical methods. *Evolution* 14:153-165
- Mullins IM, Hilu KW (2002) Sequence variation in the gene encoding the 10-kDA prolamin in *Oryza* (*Poaceae*). I. Phylogenetic implications. *Theor Appl Genet* 105:841-846
- Nguyen BD, Brar DS, Bui BC, Nguyen TV, Pham LN, Nguyen HT (2003) Identification and mapping of the QTL for aluminum tolerance introgressed from the new source, *Oryza rufipogon* Griff. into indica rice (*O. sativa* L.). *Theor Appl Genet* 106:583-593
- Oka HI (1988) Origin of cultivated rice. Japan Sci Soc. Press, Tokyo. 25 p
- Second G (1985) Evolutionary relationships in the *Sativa* group of *Oryza* based on

isozyme data. *Genet Sel Evol* 17:89-114

Song WY, Wang GL, Chen LL, Kim HS, Pi YL, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauquet C, Ronald P (1995). A receptor kinase like protein encoded by the rice disease resistance gene, *Xa-21*. *Science* 270:1804-1806

Temnykh S, Park WD, Ayres N, Cartinhour S, Hauk N, McCouch SR (2000) Mapping and genome organization of microsatellite sequences in rice (*O. sativa* L.). *Theor Appl Genet* 100:697-712

Vaughan DA (1989) The genus *Oryza sativa* L.: current status of taxonomy. IRRI Res Paper Ser No. 138. pp 1-21

Table 1: Genome composition, ploidy level and useful traits of *Oryza* species

	Species	Genome	2n	Distribution	Useful traits*
(A)	<i>O. sativa</i> complex				
	<i>O. sativa</i>	AA	24	Worldwide	Cultigen
	<i>O. nivara</i>	AA	24	Tropics & subtropics	Resistance to GSV, BI
	<i>O. rufipogon</i>	AA	24	Tropics & subtropics	Resistance to BB, RTV; AI tolerance and CMS
	<i>O. glaberrima</i>	AA	24	West Africa	Cultigen
	<i>O. barthii</i>	AA	24	Africa	Resistance to BB & GLH
	<i>O. longistaminata</i>	AA	24	Africa	Resistance to BB
	<i>O. meridionalis</i>	AA	24	Tropical Australia	Elongation ability
	<i>O. glumaepatula</i>	AA	24	South & Central America	Elongation ability & yield traits
(B)	<i>O. officinalis</i> complex				
	<i>O. punctata</i>	BB, BBCC	24, 48	Africa	Resistance to BPH
	<i>O. officinalis</i>	CC	24	Tropical & subtropical Asia	Resistance to BPH, WBPH & BB
	<i>O. rhizomatis</i>	CC	24	Sri Lanka	Drought tolerance
	<i>O. eichingeri</i>	CC	24	South Asia & East Africa	Resistance to yellow mottle virus, BPH & WBPH
	<i>O. minuta</i>	BBCC	48	Philippines & Papua New Guinea	Resistance to sheath blight, BI, BB & BPH
	<i>O. latifolia</i>	CCDD	48	South & Central America	Resistance to BPH, high biomass
	<i>O. alta</i>	CCDD	48	South & Central America	Resistance to BPH, high biomass
	<i>O. grandiglumis</i>	CCDD	48	South & Central America	Resistance to BPH
	<i>O. australiensis</i>	EE	24	Tropical Australia	Drought avoidance
(C)	<i>O. meyeriana</i> complex				
	<i>O. granulata</i>	GG	24	Southeast Asia	Adaptation to aerobic soil
	<i>O. meyeriana</i>	GG	24	South & Southeast Asia	Adaptation to aerobic soil
(D)	<i>O. ridleyi</i> complex				
	<i>O. ridleyi</i>	HHJJ	48	Southeast Asia	Resistance to stem borer, BI & BB
	<i>O. longiglumis</i>	HHJJ	48	Indonesia, Papua New Guinea	Resistance to blast, BB
(E)	Outgroup complex				
	<i>O. brachyantha</i>	FF	24	Africa	Resistance to yellow stem borer, leaf folder & tolerance of laterite soil
	<i>O. schlechteri</i>	HHKK	48	New Guinea	
	<i>O. coarctata</i>	HHKK	48	Eastern India	Tolerance of salinity

*BPH = brown planthopper; WBPH = whitebacked planthopper; BB = bacterial blight, BI = blast; GSV = grassy stunt virus; RTV = rice tungro virus; GLH = green leafhopper; CMS = cytoplasmic male sterility

Table 2: Useful genes, associated DNA markers with chromosome location and mechanism of gene transfer from wild species of *Oryza* into *O. sativa*

Gene	Wild species	DNA marker	Chromosome	Transfer mechanism
Grassy stunt virus (GSV) resistance	<i>O. nivara</i>	-	-	Homologous recombination
Bacterial blight resistance (<i>Xa21</i>)	<i>O. longistaminata</i>	RG103	11	Homologous recombination
Blast resistance (<i>Pi-9t</i>)	<i>O. minuta</i>	-	-	Rare recombination
BPH resistance (<i>Bph-10t</i>)	<i>O. australiensis</i>	RG457	12	Rare recombination
BPH resistance (<i>Bph-6t</i>)	<i>O. officinalis</i>	OPA16 ₉₃₈	11	Rare recombination
Tungro virus resistance (RTV)	<i>O. rufipogon</i>	-	-	Homologous recombination
Aluminum tolerance (QAIr3.1, QAIr1.1, QalRr9.1)	<i>O. rufipogon</i>	RG391, RZ252, RM201	1, 3, 9	Homologous recombination