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The wild species of the genus *Oryza* serve as a virtually untapped reservoir of genetic diversity that can be used to improve the world's most important food crop—rice. The genus is composed of two domesticated (*O. sativa* and *O. glaberrima*) and 22 wild species [68] and represents between 15 and 25 million years of evolutionary diversification.

In this chapter we will describe the current status of the genetic and genomic applications of the genus *Oryza* toward the penultimate goal of helping to solve the 9 billion people question—i.e., how can we grow enough food to feed more than 9 billion human inhabitants under 40 years [52]?

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## 1 The Genus *Oryza*: Broadening the Gene Pool of Rice—Exploitation of Diversity of the Wild Species Germplasm

The genus *Oryza* includes two cultivated ( $2n=24$ , AA) and 22 wild species ( $2n=24$ , 48) representing the AA, BB, CC, BBCC, CCDD, EE, FF, GG, KKLL, and HHJJ genome types (Table 2.1). Figures 2.1 and 2.2 show a phylogenetic tree of the genus (inferred from [3, 6, 25, 45]) and a photograph of 12 of these species at the same developmental stage, respectively. These wild *Oryza* species are, in fact, grass-like plants which are phenotypically inferior in agronomic traits—such as poor plant type, low grain yield, poor grain type, and are shattering in nature [1]. The wild species exhibit tremendous diversity in morphological traits, height, tillering, flowering, growth habit, panicle, leaf, culm, and seed characteristics (Fig. 2.2), and adaptation to different habitats and agronomic traits (Table 2.1).

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## 2 Gene Transfer from Wild Species into Rice

The International Rice Research Institute's (IRRI) Rice Gene Bank and The National Institute of Genetics' *Oryza* base, combined, maintain more than 4,000 accessions of wild *Oryza* species and 1,500 accessions of cultivated

**Table 2.1** Chromosome number, genomic composition, and distribution of *Oryza* species, *Oryza*-related genera, and their useful traits

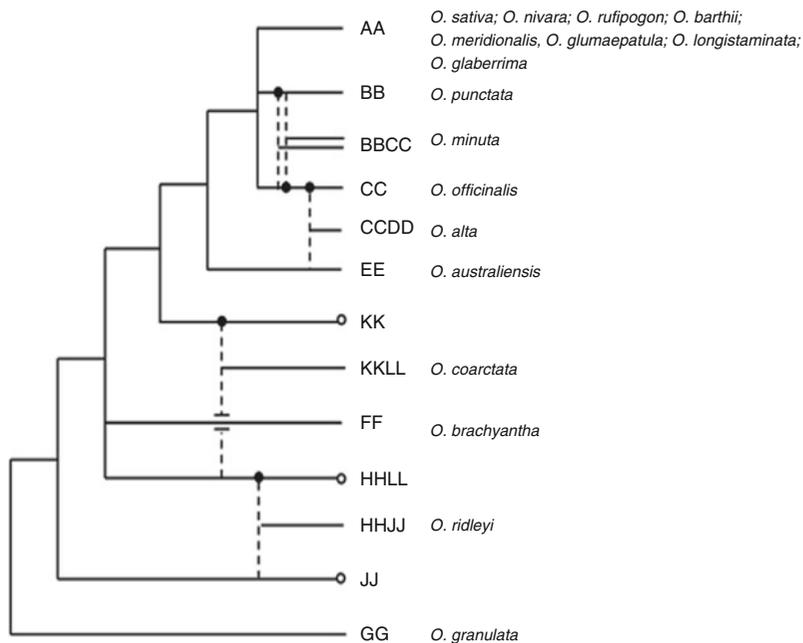
Species	2n	Genome	Number of accessions	Distribution	Useful traits
<i>O. sativa</i> complex					
<i>O. sativa</i> L.	24	AA	96,564	Worldwide	Cultigen, high yielding
<i>O. glaberrima</i> Steud.	24	A <sup>§</sup> A <sup>§</sup>	1,562	West Africa	Cultigen; tolerance to drought, acidity, iron toxicity, P-deficiency; resistance to BB, blast, RYMV, African gall midge, nematodes, weed competitiveness
<i>O. nivara</i> Sharma et Shastry	24	AA	1,260	Tropical and subtropical Asia	Resistance to grassy stunt virus, BB
<i>O. rufipogon</i> Griff.	24	AA	858	Tropical and subtropical Asia, tropical Australia	Resistance to BB, blast, BPH, tungro virus; moderately tolerant to Shb, tolerance to aluminum and soil acidity, increased elongation under deep water; source of CMS and yield-enhancing loci
<i>O. breviligulata</i> A. Chev. et Roehr.	24	A <sup>§</sup> A <sup>§</sup>	218	Africa	Resistance to GLH, BB; drought avoidance; tolerance to heat and drought
<i>O. barthii</i>					
<i>O. longistaminata</i> A. Chev et Roehr	24	A <sup>1</sup> A <sup>1</sup>	203	Africa	Resistance to BB, nematodes, stemborer, drought avoidance
<i>O. meridionalis</i> Ng	24	A <sup>m</sup> A <sup>m</sup>	56	Tropical Australia	Elongation ability; drought avoidance; tolerance to heat and drought
<i>O. glumaepatula</i> Steud.	24	A <sup>SP</sup> A <sup>SP</sup>	54	South and Central America	Elongation ability; source of CMS; tolerance to heat
<i>O. officinalis</i> complex					
<i>O. punctata</i> Kotschy ex Steud.	24, 48	BB, BBCC	71	Africa	Resistance to BPH, BB, zigzag leafhopper; tolerance to heat and drought
<i>O. minuta</i> J.S. Presl. ex C.B. Presl.	48	BBCC	63	Philippines and Papua New Guinea	Resistance to BB, blast, BPH, GLH
<i>O. officinalis</i> Wall ex Watt	24	CC	265	Tropical and subtropical Asia, tropical Australia	Resistance to thrips, BPH, GLH, WPH, BB, stem rot; tolerance to heat
<i>O. rhizomatis</i> Vaughan	24	CC	19	Sri Lanka	Drought avoidance, resistance to blast; tolerance to heat
<i>O. eichingeri</i> A. Peter	24	CC	30	South Asia and East Africa	Resistance to BPH, WBPH, GLH
<i>O. latifolia</i> Desv.	48	CCDD	40	South and Central America	Resistance to BPH, BB, high biomass production
<i>O. alta</i> Swallen	48	CCDD	6	South and Central America	Resistance to striped stemborer; high biomass production
<i>O. grandiglumis</i> (Doell) Prod.	48	CCDD	10	South and Central America	High biomass production
<i>O. australiensis</i> Domin.	24	EE	36	Tropical Australia	Resistance to BPH, BB, blast; drought avoidance; tolerance to heat and drought

(continued)

**Table 2.1** (continued)

Species	2n	Genome	Number of accessions	Distribution	Useful traits
<i>O. meyeriana</i> complex					
<i>O. granulata</i> Nees et Arn. ex Watt	24	GG	24	South and South Asia	Shade tolerance, adaptation to aerobic soil
<i>O. meyeriana</i> (Zoll. et (Mor. ex Steud.) Baill.)	24	GG	11	Southeast Asia	Shade tolerance; adaptation to aerobic soil
<i>O. ridleyi</i> complex					
<i>O. longiglumis</i> Jansen	48	HHJJ	6	Irian Jaya, Indonesia, and Papua New Guinea	Resistance to blast, BB
<i>O. ridleyi</i> Hook. F.	48	HHJJ	15	South Asia	Resistance to blast, BB, tungro virus, stem borer, whorl maggot
Unclassified					
<i>O. brachyantha</i> A. Chev. et Roehr	24	FF	19	Africa	Resistance to BB, yellow stemborer, leaf folder, whorl maggot; tolerance to laterite soil
<i>O. schlechteri</i> Pilger	48	KKLL	1	Papua New Guinea	Stoloniferous
<i>O. coarctata</i> Tateoka	48	KKLL	1	Asian Coastal Area	Tolerance to salinity, stoloniferous
<i>Leersia perrieri</i> A. Camus	24	UNKNOWN	1	Africa	Shade tolerance, stoloniferous

*BPB* brown plant hopper, *GLH* green leaf hopper, *WBPH* whitebacked plant hopper, *BB* bacterial blight, *Shb* sheath blight, *CMS* cytoplasmic male sterility, *RYMV* rice yellow mottle virus



**Fig. 2.1** *Oryza* phylogenetic tree. Evolutionary relationships of the *Oryza* genome were inferred from Ammiraju et al. [3, 6], Ge et al. [25], and Lu et al. [46]. Dashed line

indicates origins of allotetraploids; filled circle indicates maternal parents; open circle indicates unidentified diploid species



**Fig. 2.2** The genus *Oryza*: 12 representative species

African rice (*O. glaberrima*). These wild species are reservoirs of many useful genes, particularly for resistance to major biotic and abiotic stresses (Table 2.1). However, these wild species are associated with several weedy traits, such as grain shattering, poor plant type, poor grain characteristics, and low seed yield. Besides, several incompatibility barriers limit the transfer of useful genes from wild species into cultivated species [14, 15]. The major consideration in alien gene transfer is to selectively transfer agronomically important genes from wild species while avoiding linkage drag. To achieve precise transfer of genes from wild species, strategies involving a combination of conventional plant breeding methods with tissue culture and molecular approaches have become important [16, 17]. Advances in tissue culture, molecular marker technology, genomics, and fluorescence in situ hybridization have opened new opportunities to tap alien genetic variability from distant *Oryza* genomes through interspecific hybridization.

### 3 Strategy for Alien Gene Transfer into Cultivated Rice

The strategy used to transfer genes from wild species into rice depends on the nature of the target trait(s), relatedness of the wild species, and

incompatibility barriers. Several protocols are available to overcome such barriers [16]. Some of the steps involved in gene transfer include: (1) *Search for useful genetic variability for target traits*—this involves the screening of wild species to identify specific accession(s) that possess useful genetic variability. (2) *Production of hybrids and alien introgression lines (AIL)*: interspecific hybrids are produced between elite breeding lines with the wild species carrying the desired traits. Such hybrids are produced through direct crosses between rice and AA genome wild species. However, embryo rescue is required to produce hybrids and backcross progenies (introgression lines) between rice and all the wild species of *Oryza* except AA genome species. (3) *Evaluation of introgression lines for transfer of target traits*: AILs generated through backcrossing are evaluated for the transfer of target traits. This involves extensive laboratory, greenhouse, and field testing. (4) *Molecular mapping of genes/QTLs*: molecular markers are developed to track the desired alien trait(s) for marker-assisted selection (MAS).

Following the above strategy, a number of genes have been transferred from wild species into rice (Table 2.2) and varieties have been released for commercial cultivation (Table 2.3). MAS has been practiced and some varieties have become available (Table 2.4).

**Table 2.2** Introgression of genes from wild *Oryza* species into rice

Trait	Donor <i>Oryza</i> species		
	Wild species	Gene	Genome
Grassy stunt resistance	<i>O. nivara</i>	<i>GS</i>	AA
Bacterial blight resistance	<i>O. rufipogon</i>	<i>Xa23</i>	AA
	<i>O. longistaminata</i>	<i>Xa21</i>	AA
	<i>O. nivara</i>	<i>Xa38</i>	AA
	<i>O. officinalis</i>	<i>Xa29(t)</i>	CC
	<i>O. minuta</i>	<i>Xa27</i>	BBCC
	<i>O. latifolia</i>	Unknown	CCDD
	<i>O. australiensis</i>	Unknown	EE
	<i>O. brachyantha</i>	Unknown	FF
Blast resistance	<i>O. glaberrima</i> <sup>a</sup>	Unknown	AA
	<i>O. rufipogon</i>	Unknown	AA
	<i>O. minuta</i>	<i>Pi9</i>	BBCC
	<i>O. australiensis</i>	<i>Pi40</i>	EE
Brown planthopper resistance	<i>O. officinalis</i>	<i>bph11, bph12</i>	CC
	<i>O. eichingeri</i>	<i>Bph14, Bph15</i>	CC
	<i>O. minuta</i>	<i>Bph20, Bph21</i>	BBCC
	<i>O. latifolia</i>	Unknown	CCDD
	<i>O. australiensis</i>	<i>Bph10, Bph18</i>	EE
Whitebacked planthopper resistance	<i>O. officinalis</i>	<i>Wbph7(t), Wbph8(t)</i>	CC
	<i>O. latifolia</i>	Unknown	CCDD
Cytoplasmic male sterility	<i>O. sativa</i> f. <i>spontanea</i>	Unknown	AA
	<i>O. perennis</i>	Unknown	AA
	<i>O. glumaepatula</i>	Unknown	AA
	<i>O. rufipogon</i>	Unknown	AA
Tungro tolerance	<i>O. rufipogon</i>	Unknown	AA
Tolerance to iron toxicity	<i>O. rufipogon</i>	Unknown	AA
	<i>O. glaberimma</i> <sup>a</sup>	Unknown	AA
Heat and/or drought-related traits	<i>O. australiensis</i> <sup>b</sup>	Unknown	EE
	<i>O. barthii</i> <sup>b</sup>	Unknown	AA
	<i>O. glaberimma</i> <sup>a</sup>	QTLs	AA
	<i>O. glumaepatula</i> <sup>b</sup>	Unknown	AA
	<i>O. meridionalis</i> <sup>b</sup>	Unknown	AA
	<i>O. officinalis</i> <sup>b</sup>	Unknown	CC
	<i>O. punctata</i> <sup>b</sup>	Unknown	BB
	<i>O. rhizomatis</i> <sup>b</sup>	Unknown	CC
Tolerance to aluminum toxicity	<i>O. rufipogon</i>	QTL	AA
Tolerance to acidic conditions	<i>O. glaberrima</i> <sup>a</sup>	Unknown	AA
	<i>O. rufipogon</i>	Unknown	AA
Tolerance to P-deficiency	<i>O. rufipogon</i>	Unknown	AA
	<i>O. glaberimma</i> <sup>a</sup>	Unknown	AA
Yield-enhancing loci	<i>O. rufipogon</i>	QTL, <i>yld1, yld2</i>	AA
Yellow stemborer (larval mortality)	<i>O. longistaminata</i>	QTL	AA
Increased elongation ability	<i>O. rufipogon</i>	Unknown	AA

<sup>a</sup>*O. glaberrima*—African rice species. Modified from Brar and Khush [17]<sup>b</sup>*O. australiensis, O. barthii, O. glumaepatula, O. meridionalis, O. officinalis, O. punctata, O. rhizomatis*. Sanchez et al. (unpublished data)

**Table 2.3** Rice varieties developed through wide hybridization

Key trait	Wild species	Varieties released	Country
Grassy stunt resistance	<i>O. nivara</i>	Many rice varieties	Rice growing countries in Asia
BPH resistance	<i>O. officinalis</i>	MTL 98, MTL 103 MTL 105, MTL114	Vietnam
Acid sulfate tolerance	<i>O. rufipogon</i>	AS 996	Vietnam
Salinity tolerance	<i>O. rufipogon</i>	BRRIdhan55 (As996)	Bangladesh
Tungro resistance	<i>O. rufipogon</i>	Matatag 9	Philippines
Bacterial blight resistance	<i>O. longistaminata</i>	NSICRc 112	Philippines
Blast resistance	<i>O. rufipogon</i>	Dhanarasi	India
	<i>O. glaberrima</i> <sup>a</sup>	Yun Dao	YAAS, China
High yield, earliness, weed competitive ability, and tolerance to abiotic stresses	<i>O. glaberrima</i> <sup>a</sup>	Many Nerica lines/varieties	African countries
Tolerance to heat	<i>O. meridionalis</i>	Arizona Rice-1 <sup>b</sup>	USA
		Arizona Rice-2 <sup>b</sup>	USA

Modified from Brar and Singh [18] and Sanchez et al. (unpublished data)

<sup>a</sup>*O. glaberrima*—African rice species

<sup>b</sup>Arizona Rice-1 and 2—varieties to be released in 2013, Sanchez et al. (unpublished data)

**Table 2.4** Rice varieties developed through MAS carrying *Xa21* gene from *O. longistaminata* and *Bph18* from *O. australiensis*

Inbreds/hybrids	Year	Resistance gene(s)	Institute/country
NSICRc 142 (Tubigan 7)	2006	<i>Xa4 + Xa21</i> <sup>a</sup>	PhilRice, Philippines
NSICRc 154 (Tubigan 11)	2007	<i>Xa4 + Xa21</i> <sup>a</sup>	PhilRice, Philippines
Improved Sambha Mahsuri	2007	<i>Xa5 + xa13 + Xa21</i> <sup>a</sup>	India
Improved Pusa Basmati 1	2007	<i>Xa5 + xa13 + Xa21</i> <sup>a</sup>	India
Xieyou 218	2002	<i>Xa21</i> <sup>a</sup>	China
Zhongyou 218	2002	<i>Xa21</i> <sup>a</sup>	China
Guodao 1	2002	<i>Xa4 + xa5 + xa13 + Xa21</i> <sup>a</sup>	China
Guodao 3	2004	<i>Xa4 + xa5 + xa13 + Xa21</i> <sup>a</sup>	China
Neizyou	2002	<i>Xa4 + xa5 + xa13 + Xa21</i> <sup>a</sup>	China
Ilyou 8006	2005	<i>Xa4 + xa5 + xa13 + Xa21</i> <sup>a</sup>	China
Ilyou 218	2005	<i>Xa21</i> <sup>a</sup>	China
ZhongbaiYou 1	2006	<i>Xa21</i> <sup>a</sup>	China
Suweon 523	2011	<i>Bph18</i>	Korea

Modified from Brar and Singh [18]

<sup>a</sup>*Xa21* gene has also been transferred into many elite inbreds and parental lines of hybrids by several institutes in India, Philippines, and Thailand

#### 4 Examples of Crosses Between Wild AA Genome Species and Cultivated Rice

To date many chromosome segmental substitution lines (CSSLs) or BILs in rice have and are being developed and new varieties are continuously being released at different research stations

around the world (Table 2.3). Crosses between cultivated rice (*O. sativa*,  $2n=24$ , AA) and AA genome wild species can be easily made. Hybrids between *O. sativa* and *O. rufipogon* are partially fertile; however, *O. sativa* × *O. glaberrima* and *O. sativa* × *O. longistaminata* F<sub>1</sub>s are highly sterile. Among the classical examples are the introgression of a gene for grassy stunt virus resistance from *O. nivara* to cultivated rice

varieties [40] and the transfer of a CMS source from wild rice, *O. sativa* f. *spontanea*, to develop CMS lines for commercial hybrid rice production [44]. Other useful genes, such as *Xa21* for BB resistance, were transferred into rice from *O. longistaminata*, and new CMS sources from *O. perennis* and *O. glumaepatula*. Genes for tungro virus tolerance and tolerance to acid sulfate soil conditions have been transferred from *O. rufipogon* into *indica* rice cultivars. Some of the breeding lines with genes introgressed from wild species have been released as varieties (Table 2.3). Ram et al. [55, 56] transferred broad spectrum blast resistance from *O. rufipogon* and also released a variety, Dhanarasi. Some of the alien genes have been tagged with molecular markers and used in MAS (Table 2.4).

At Kyushu University in Japan, Yoshimura et al. [74] developed a series of introgression lines using *O. glaberrima*, *O. glumaepatula*, *O. meridionalis*, *O. nivara*, and *O. rufipogon* accessions as donor parents in *O. sativa* cv. Taichung 65 background [20, 21, 43, 46, 62, 63]. Using these introgression lines, alleles associated with a number of desirable traits were identified, such as awn character [46], days to heading [59] and seed shattering [58], and green leafhopper resistance [23, 24].

In the USA, a University of Arizona, USDA, ARS, and University of Arkansas collaborative study is developing four BIL libraries using several *O. barthii* accessions as donors and *O. sativa* cv. LaGrue and M-202 as the recurrent parents (Eizenga and Sanchez, unpublished). These introgression lines will be tested for heat and drought tolerance in Arizona. At USDA, ARS, Stuttgart, AR, under the RiceCAP program, three BIL libraries are being developed using *O. nivara* and *O. meridionalis* as donors and *O. sativa* cv. Bengal and Lemont as the recipient parents. The introgression lines will be used to map sheath blight and blast resistance genes (Eizenga, personal communication). A collaboration between Cornell University, USDA, ARS Stuttgart, AR, and the University of Arkansas developed introgression lines using three diverse *O. rufipogon/O. nivara* accessions as donors and *O. sativa* cv. IR64 and Cybonnet as recurrent parents [67].

At Huazhong Agricultural University in China, backcrossing programs are underway to develop 14 CSSL/IL libraries using seven AA genome *Oryza* species accessions (6 wild and 1 *O. glaberrima*) that have BAC-end sequence as part of OMAP ([69]; Y. Sibin, personal communication) as donors and *O. sativa* cv. Zhenshan 97B and 93-11 as recurrent parents. The donor wild AA genome accessions include *O. barthii*, *O. glumaepatula*, *O. meridionalis*, *O. nivara*, and *O. rufipogon*.

The CIAT/IRD (International Center for Tropical Agriculture and Institut de Recherche pour le Développement) rice genetics and genomics group lead a Generation Challenge Project (GCP) that is developing four libraries of CSSLs with the wild species *O. barthii*, *O. glumaepatula*, *O. meridionalis*, and *O. rufipogon* as donors, all sharing the same genetic background of the *tropical japonica* cultivar Curinga. The GCP-associated partners with this effort are Cornell University (USA), Fedearroz (Colombia), Embrapa-CNPAP (Brazil), and AfricaRice (Benin) (see [http://www.generationcp.org/arm/ARM06/day\\_2/Lorieux\\_part\\_1.pdf](http://www.generationcp.org/arm/ARM06/day_2/Lorieux_part_1.pdf); [http://www.generationcp.org/arm/ARM06/day\\_2/Lorieux\\_part\\_2.pdf](http://www.generationcp.org/arm/ARM06/day_2/Lorieux_part_2.pdf); [1]). Development of introgression lines from the *O. sativa* × *O. glumaepatula* interspecific cross [57] was also undertaken in the GCP initiative.

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## 5 Introgression from *O. glaberrima* into *O. sativa*

Cultivars of Asian rice *O. sativa* are high yielding, whereas African rice, *O. glaberrima*, is low yielding. However, *O. glaberrima* has several desirable traits, such as resistance to rice yellow mottle virus (RYMV), African gall midge, and nematodes, and tolerance to drought, acidity, and iron toxicity. Another important feature of *O. glaberrima* is its strong weed competitiveness. Thus, interspecific hybridization among Asian and African species offers tremendous potential for combining the high productivity of *O. sativa* with tolerance to biotic and abiotic stresses of *O. glaberrima*. F<sub>1</sub> hybrids between *O. sativa* and

*O. glaberrima*, in spite of complete chromosome pairing, are highly sterile. Backcrossing is used to restore fertility and derive agronomically desirable lines. Molecular analysis has revealed frequent exchange of segments between *O. sativa* and *O. glaberrima*.

Efforts have been made by the Africa Rice Center (ARC) to introgress genes for weed competitiveness from *O. glaberrima* into elite breeding lines of *O. sativa* [39]. At IRRI, a large number of AILs have been produced from crosses between *O. sativa* and several *O. glaberrima* accessions. These progenies are being evaluated in collaborative projects with ARC and NARES for introgression of tolerance to RYMV, African gall midge, and abiotic stresses. Promising lines tolerant to iron toxicity in the genetic background of IR64 (IR75870-5-8-5-B-5-B), IR69502-6-SRN-3-UBN-1-(IR80340-23-B-12-6-B), and IR554230-01 (IR80314-4-B-1-3-B) have been field-tested in Iloilo, Philippines.

Bimpong [8] identified two lines (IR80311-9-B-1-2 and IR80311-2-B-1-2) derived from *O. glaberrima* showing tolerance to nematode, *Meloidogyne graminicola*, based on gall midge rating and Pf/Pi ratio. The CSSL library of MG12, an *O. glaberrima* accession, in the background of the *tropical japonica* cultivar, Caiapo, was used to identify QTLs for rice stripe necrosis virus resistance and yield components [27].

Bimpong [9] and Bimpong et al. [10] analyzed backcross progeny derived from crosses of two *indica* rice varieties (IR64 and IR55423-01) with *O. glaberrima*. The progenies were evaluated under drought stress and a number of drought-related QTLs were identified. *O. glaberrima* contributed 50–67 % of the alleles to the newly identified QTL. Two QTLs for grain yield per plant (*ypp2.1* and *ypp4.2*) were new and two others (*yld1.1* and *yld8.1*) were common in two locations. In IR55423-01 × *O. glaberrima*, 11 new QTLs for biomass were identified, of which one QTL (*bm8.1*) was common in two locations. Nine QTLs for yield, of which two were new (*ypp3.1* and *ypp8.2*), were identified. Three QTLs (*bm2*, *dth2*, and *dth4*) were common in two populations derived from IR64 and IR55423-01. QTLs *ypp6.1*, *bm6.1*, *hi6.1*, and *ps6.1* associated with an increase in

grain yield were identified in the same region on chromosome 6 at locus RM275.

The University of Arizona, USDA, ARS, and the University of Arkansas collaborative project also used *O. glaberrima* as a donor to develop introgression lines with two US rice cultivars, LaGrue, a long grain *tropical japonica*, adapted to the southern region of the USA, and M-202, a medium grain *temperate japonica* adapted to California, as recurrent parents (Eizenga and Sanchez, unpublished).

## 6 Identification and Introgression of Yield-Enhancing Loci/QTLs

As discussed, the wild relatives of rice are phenotypically inferior to cultivated rice, with respect to agronomic traits. However, the detection of transgressive segregation for yield in crosses between cultivated and wild species suggests that, despite their inferior phenotypes, the wild relatives of rice contain genes that can improve quantitative traits, such as yield.

Using advanced introgression lines, alleles from *O. rufipogon* (IRGC 105491) were simultaneously identified, mapped, and introgressed into the genetic background of several adapted cultivars [47]. Yield-enhancing traits from this *O. rufipogon* accession were incorporated into the genetic background of IR64 [19, 60], Jefferson [65], V20B maintainer line of CMS line “V20A” [71], and Hwaengbyeo [72]. Alleles associated with the yield-related traits, such as spikelet number, grain weight, and panicle length, were identified in this *O. rufipogon* accession using a BIL population derived from a cross with Zhenshan 97B [1]. Similarly, alleles associated with yield-related traits from two other *O. rufipogon* accessions were introgressed into TeQing [64] and Guichao 2 [66]. Yield trials of selected Jefferson/*O. rufipogon* NILs revealed yield-enhancing QTLs when compared to the donor parent [42].

QTLs from wild AA genome species for increased yield have also been identified by Xiao et al. [70]. *O. rufipogon* alleles at two marker

loci, RM5 (*yld1-1*) on chromosome 1 and RG256 on chromosome 2 (*yld2-1*), were associated with enhanced yield. In another experiment, Xiao et al. [71] identified 68 QTLs. Of these, 35 (51 %) had trait-improving alleles derived from the wild species, 19 of which had no deleterious effects on other characters.

Moncada et al. [48] and Septiningsih et al. [60] also reported QTL, “wild species alleles,” as having beneficial effects for yield and yield components. Yoon et al. [73] also mapped QTL for yield components introgressed from *O. grandiglumis*. Imai et al. [32] evaluated advanced backcross progenies (BC<sub>3</sub>, BC<sub>4</sub>) in field trials in Arkansas and showed an average yield enhancement of 23 % compared to the recurrent parent “Jefferson.” McCouch et al. [47] summarized the results of various studies on transgressive segregation and QTL responsible for increased yield from crosses of *O. sativa* × *O. rufipogon* supporting that yield-enhancing loci from wild species could increase yield potential of both inbred and hybrid rice varieties. Future research should focus on the identification of QTL from wild species and introgression into high yielding elite breeding lines.

Results at IRRI of advanced backcross progeny derived from the crosses of an elite breeding line of new plant type (NPT) rice, with *O. longistaminata* and IR64 × *O. rufipogon*, also support transgressive segregation for yield and yield components. These findings show that genes from wild *Oryza* species can increase the yield of elite rice lines, even though wild species are phenotypically inferior to cultivated rice. Yield-enhancing QTL, “wild species alleles,” identified need to be transferred into high yielding genotypes and validated in well-designed field experiments.

## 7 Introgression for Tolerance to Abiotic Stresses

Little or no work has been done on the transfer of genes for tolerance to abiotic stresses from wild *Oryza* species into rice. Recently, the Arizona Genomics Institute (AGI) evaluated the

performance of wild rice species under natural conditions in Arizona. Representative accessions of two AA genome wild rice species, *O. meridionalis* and *O. barthii*, and an EE genome *O. australiensis* were chosen from eight wild rice species grown under natural environmental conditions. The Arizona environment is characterized by limited precipitation of less than 2 mm and varying extreme temperatures (35–43 °C) during summer, where rice can be grown. Hence, Arizona is a perfect place for heat and drought studies. The three species tested showed medium to tall plant height and high tillering ability. These traits are important for developing heat- and drought-tolerant rice varieties because height and tillering of cultivated rice have been shown to decrease by at least 30 % and 20 %, respectively, when subjected to heat and drought (Sanchez and Wing, unpublished).

Two heat-tolerant varieties, *Arizona Rice-1* and *Arizona Rice-2*, were developed from a cross between one of the best accessions of *O. meridionalis* and *O. sativa* cv. M-202. These varieties were selected from advanced backcross inbred lines that resemble the *O. sativa* phenotypic traits. These varieties are currently being evaluated for heat and drought in the field. At least five additional *O. meridionalis* accessions from a total of 18 are currently crossed to *O. sativa* in order to develop more heat- and drought-tolerant varieties.

Crosses were made between *O. barthii*, *O. glaberrima*, and US cultivars to develop CSSLs and BILs. Populations generated from these crosses will be used to develop new varieties with other abiotic stress-related traits (Eizenga and Sanchez, unpublished). Most of the parental donors and recurrent parents used in developing ILs for abiotic stress have been re-sequenced using second-generation sequencing technology (Wing and Sanchez, unpublished).

IRRI evaluated several introgression lines derived from crosses between *O. sativa* × *O. rufipogon* and *O. sativa* × *O. glaberrima* at hotspots under field conditions for tolerance to abiotic stresses at Iloilo, the Philippines. Elite breeding lines with good agronomic traits and moderate tolerance to iron toxicity, aluminum toxicity, and

acid sulfate conditions have been identified. One of the wild species (*O. rufipogon*) that grows under natural conditions in the acid sulfate soils of Vietnam was used in crosses with IR64. Three promising lines were selected and tested through the yield-testing network of the Cuu Long Delta Rice Research Institute (CLRRI), Vietnam. Of the three breeding lines, IR73678-6-9-B has been released as a variety (AS996) for commercial cultivation in the Mekong Delta, Vietnam. This variety has become popular and occupies 100,000 ha (Bui Chi Buu, personal communication). It is a short-duration (95–100 days) semi-dwarf variety with good plant type suitable for moderately acid sulfate soils and is tolerant to BPH and blast. Nguyen et al. [51] mapped QTL for aluminum toxicity tolerance introgressed from *O. rufipogon* into rice.

A set of advanced introgression lines derived from *O. rufipogon* have been tested for elongation ability under deep water conditions in the Philippines and India. One of the lines in the All India Coordinated Rice Improvement Project has shown good promise.

## 8 Introgression of Genes from Distantly Related Genomes

Introgression lines have been produced from crosses of *O. sativa* with distantly related species with CC, BBCC, CCDD, EE, FF, GG, and HHJJ genomes. However, gene transfer has been achieved only from the CC, BBCC, CCDD, EE, and FF genomes (Table 2.2). So far, no introgression could be achieved from GG and HHJJ genomes.

Jena and Khush [37] produced several introgression lines from an *O. sativa* × *O. officinalis* cross. One of the most successful examples of the transfer of genes from the C genome wild species rice is that of brown planthopper (BPH). Four genes, *Bph10*, *Bph18*, *bph11*, and *bph12*, have been transferred from *O. officinalis* to rice. Four breeding lines have been released as varieties (MTL95, MTL98, MTL103, and MTL110) for commercial cultivation in the Mekong Delta, Vietnam. Hirabayashi et al. [28] mapped *bph11* and *bph12* introgressed from wild species

on chromosomes 3 and 4. Huang et al. [30] also transferred BPH resistance from *O. officinalis* into Zhensheng 97B.

## 9 Introgression from the BBCC Genome Species

An advanced set of introgression lines was produced and resistance to BB and blast was transferred from *O. minuta* [2]. The introgressed blast resistance gene has been designated *Pi9(t)* and has resistance to several isolates of blast. Introgression lines were produced from a NPT × *O. minuta* cross and evaluated for resistance to 10 Philippine races of bacterial blight, where the NPT parent was susceptible to each of the 10 races. One of the families, WHDIS 1958-19, was found to have a broad spectrum of resistance to all the 10 races tested. The genes introgressed from *O. minuta* seem to have a wide spectrum of resistance, and also the number of genes introgressed could be more than one. Similarly, BPH resistance from *O. minuta* has been transferred to rice [11]. These lines have shown a wide spectrum for resistance to BPH in the Philippines and Korea.

Rahman et al. [54] conducted genetic analysis of BPH resistance using an F<sub>2</sub> population derived from a cross between an introgression line, “IR71033-121-15,” from *O. minuta* (accession number 101141) and a susceptible Korean *japonica* variety, “Junambyeo.” Two major QTLs have been designated as *Bph20(t)* on chromosome 4 and *Bph21(t)* on chromosome 12.

## 10 Introgression from the CCDD Genome Species

A number of breeders have produced hybrids between rice and CCDD genome species [12, 61]. Several introgression lines derived from *O. sativa* × *O. latifolia* have been evaluated for introgression of useful traits [50]. Genes for resistance to BPH and BB have been introgressed from *O. latifolia*. Yoon et al. [73] reported yield-enhancing QTLs from *O. grandiglumis*.

## 11 Introgression from the EE Genome Species

Multani et al. [50] produced hybrids between colchicine-induced autotetraploids of rice and *O. australiensis* ( $2n=24$  EE). Introgression was detected for morphological traits, such as long awns, earliness, and the *Amp-3* and *Est-2* allozymes. Of 600 BC<sub>2</sub>F<sub>4</sub> progenies, four were resistant to BPH, two (IR65482-4-136 and IR65482-7-216) of which have proven to be resistant to a Korean BPH population. One of the lines (IR65782-4-136-2-2) carried the *Bph10* gene located on chromosome 12 [34].

A major resistance gene *Bph18(t)* has been identified in an introgression line (IR65482-7-216-1-2) that has inherited the gene from *O. australiensis*. A marker allele of 1,078 bp completely co-segregated with the BPH resistance phenotype. STS marker 7312.T4A was validated from two temperate *japonica* backgrounds [36]. *Bph18* has been transferred through MAS, and a *japonica* variety, Suweon 523, has been released (KK Jena, personal communication).

Jeung et al. [38] identified a new gene in the introgression line IR65482-4-136-2-2 that inherited a resistance gene from *O. australiensis* (Acc. 100882). Molecular analysis localized a major resistance gene, *Pi40(t)*, on the short arm of chromosome 6. Following association analysis and detailed haplotyping, a DNA marker, 9871.T7E2b, was identified and found to be linked to the *Pi40(t)* gene at the 70 kb chromosomal region and differentiated the *Pi40(t)* gene from the LTH monogenic differential lines possessing genes *Piz*, *Piz-5*, *Piz-t*, and *Pi-9*. *Pi40(t)* was validated using the most virulent isolates from Korea as well as the Philippines, suggesting a broad spectrum for the resistance gene.

## 12 Introgression from the FF Genome Species

A series of introgression lines have been derived from a cross between *O. sativa* (cv. IR56) and *O. brachyantha* ( $2n=24$  FF). IR56 is susceptible to

bacterial blight races 1, 4, and 6 from the Philippines, whereas *O. brachyantha* is resistant. Of the 149 backcross progeny analyzed, 27 showed introgression for resistance to bacterial blight races 1, 4, and 6 [11]. Introgression for awning and growth duration has also been achieved.

## 13 Introgression from the GG and KKLL Genome Species

Hybrids have been produced from a cross between *O. sativa* and *O. granulata* [12]. Advanced progenies have also been produced; however, none of the lines tested have shown introgression of traits from *O. granulata* into rice.

The tetraploid *ridleyi* complex comprises two species: *O. ridleyi* and *O. longiglumis*. *O. ridleyi* shows strong resistance to all the 10 Philippine races of BB. Hybrids between rice cv. IR56 and *O. ridleyi* (accession 100821) have been produced; however, the cross shows a strong necrosis phenotype. Thus, only a few introgression lines (BC<sub>3</sub>F<sub>3</sub>) from this cross have been produced, but no introgression could be detected.

Intergeneric hybrids between *O. sativa* and *O. coarctata* (KKLL) have been produced through both sexual crosses following embryo rescue [13] and protoplast fusion [35]. The hybrid ( $2n=36$ ) is sterile and shows no chromosome elimination of either parent. Due to strong incompatibility barriers, no backcross progenies could be obtained.

BC<sub>2</sub> progenies derived from crosses of *O. sativa* with *O. officinalis* (CC), *O. australiensis* (EE), *O. brachyantha* (FF), and *O. granulata* (GG) resembled the recurrent rice parent for most morphological traits. This suggested limited recombination between the A genome of *O. sativa* and the C, E, F, and G genomes of the wild species. Progenies recovered in BC<sub>2</sub> of *O. sativa* × *O. officinalis* were so similar in morphology to *O. sativa* that they were evaluated in field trials and released as varieties for commercial cultivation in Vietnam. Molecular analysis also supports introgression of small segments, limited recombination between rice and wild species chromosomes as the possible cause for the rapid recovery of the recurrent parent phenotype.

Some of the genes (e.g., *Xa21* and *Bph18*) introgressed from wild species have been used in MAS and varieties have been released (see for review [17, 18]).

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#### 14 Utilization of Wild Species for Rice Improvement: Future Priorities

- Search for new genes from diverse sources with wide spectrum of resistance in the wild species germplasm.
- Identify resistance to major biotic (sheath blight, stem borer, false smut, neck blast) and abiotic stresses (drought, heat, etc.) where there is limited variability for the target traits in the cultivated species. Transfer such genes into high yielding rice genotypes.
- Allele mining to identify novel genes/QTL with different mechanisms of resistance and pyramid such genes/QTL to enhance tolerance to major biotic and abiotic stresses.
- Identification and introgression of yield-enhancing loci “wild species alleles” into elite breeding lines to further increase the diversity and yield potential of *indica* and *japonica* rice cultivars.
- Develop CSSLs from different wild species for mapping genes/QTL and use in functional genomics; BAC libraries developed under OMAP could be used advantageously in genomics and breeding research.
- Search for genes controlling homologous pairing to promote recombination and transfer genes from distant genomes of wild species into rice.
- Intensify exploratory research on C4ness in wild species.
- Exploratory research to identify endophytes in wild species as novel source for nitrogen fixation is emphasized.
- Explore the production of haploids particularly in *indica* rice through wide hybridization similar to the existing chromosome elimination system in wheat and barley.
- Explore the production of biofuels from wild rice with high biomass yield.

- Collect new *Oryza* germplasm accessions from different countries, with an emphasis on *Oryza* hotspots to enhance genetic diversity in rice through wide hybridization.

Wild species are an important genetic resource to broaden the gene pool of rice for tolerance to biotic and abiotic stresses. The extinction of wild species is a threat to genetic diversity and international efforts are needed to overcome the trend in the loss of biodiversity. Integration of the *Oryza* Map Alignment and *Oryza* Genome Evolution projects (OMAP, OGEP) with the wide hybridization research is emphasized to develop improved high yielding varieties with multiple resistances to biotic and abiotic stresses. With the advances in tissue culture, molecular markers, and genomics, the scope for utilization of wild species in genetic enhancement of rice seems more promising than before.

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#### 15 The Genomics of the Genus *Oryza*

As described above, tremendous progress has been made toward the introgression of important genes from the wild relatives of rice into cultivated rice using classical breeding approaches. However, in order to fully utilize and understand the genetic diversity hidden within the genus a more systematic approach must be taken using the tools of genomics.

Since 2003, the *Oryza* Map Alignment and *Oryza* Genome Evolution projects (OMAP, OGEP) have led to the establishment of a genus-level comparative genomics platform to be able to fully interrogate the genus *Oryza*. This work has led to the creation of large array of publicly available genomic resources most notably a set of manually edited BAC-based physical maps (i.e., 18 deep-coverage BAC libraries—finger-printed, end-sequenced, and FPC assembled) representing 18 of the 24 recognized *Oryza* species, covering all 8 AA genome species and one each of the other 9 genome types (BB, CC, BBCC, CCDD, EE, FF, GG, KKLL, HHJJ) [3, 5, 6, 41]; and a set of chromosome 3 short arm sequences from all 8 AA genome species, as

well as the BB, CC, BBCC, FF, GG, and *Leersia perrieri*, an *Oryza* outgroup species. All of these data and resources are accessible through the [www.Gramene.org](http://www.Gramene.org) and [www.genome.arizona](http://www.genome.arizona) web sites, respectively.

Analysis of these data sets revealed the following key points: (1) LTR Retro-transposable element amplifications dramatically increased the size of both the *O. australiensis* [EE] and *O. granulata* [GG] by as much as 400 and 200 Mb, respectively [7, 53]; (2) the AA genomes of *O. nivara*, *O. rufipogon* (the putative progenitor species of *O. sativa*), and *O. glaberrima* have expanded/contracted by at least 40 Mb (>10 % of their genome sizes) relative to the IRGSP RefSeq [31]; and (3) analysis of the *Adh1* region (~100–200 kb) across the entire *Oryza* phylogeny (diploid and polyploidy) showed significant perturbations of synteny including dynamic evolution of gene families, transposable element-mediated gene movement, mutations, genome size changes, and large scale physical rearrangements [3, 4, 6].

The overriding conclusion from these studies, and many others, indicates that a SINGLE reference genome for the genus *Oryza* (i.e., IRGSP RefSeq) is insufficient to capture and understand the allelic diversity/natural variation hidden with the genus to help solve the 9BPQ.

## 16 The International *Oryza* Map Alignment Project

To address this resource/knowledge gap the International *Oryza* Map Alignment Project (I-OMAP) was organized and has held five grand challenge meetings (Japan 07, Korea 08, Philippines 09, Brazil 10, Taipei 11) in conjunction with the annual International Symposia on Rice Functional Genomics (ISRFG). The three primary focus areas of I-OMAP are to: (1) generate RefSeqs & Transcriptome data sets for all eight AA genome species and a representative species of the nine other genome types; (2) generate, map, and phenotype advanced ABC, CSSL, RIL populations for the AA genome species for functional and breeding studies; and (3) identify

**Table 2.5** *Oryza* reference genome sequencing project

<i>O. sativa</i> ssp. <i>japonica</i> [AA]	IRGSP, completed
<i>O. sativa</i> ssp. <i>indica</i> [AA]	BGI, completed
<i>O. nivara</i> [AA]	Y. Hsing, Taiwan, completed, unpublished
<i>O. rufipogon</i> [AA]	B. Han, China, completed, unpublished
<i>O. glaberrima</i> [AA]	R. Wing, USA, completed, unpublished
<i>O. barthii</i> [AA]	R. Wing, USA, completed, unpublished
<i>O. longistaminata</i> [AA]	W. Wang, BGI, PRC, completed, unpublished
<i>O. punctata</i> [BB]	R. Wing, USA, completed, unpublished
<i>O. brachyantha</i> [FF]	M. Chen, BGI, China, completed, unpublished
<i>Leersia perrieri</i>	R. Wing, USA, completed, unpublished
<i>O. glumaepatula</i> [AA]	A. Oliveria, Brazil, in progress
<i>O. meridionalis</i> [AA]	R. Henry, Australia, O. Panaud, France, in progress
<i>O. officinalis</i> [CC]	N. Kurata, Japan, in progress
<i>O. eichingeri</i> [CC]	N. Kurata, Japan, in progress
<i>O. rhizomatis</i> [CC]	N. Kurata, Japan, in progress
<i>O. australiensis</i> [EE]	O. Panaud, France, in progress
<i>O. granulata</i> [GG]	L. Gao, China, in progress

IRGSP International Rice Genome Sequencing Project, BGI Beijing Genomics Institute

collections of naturally occurring populations of the wild *Oryza* species for diversity, conservation, population, and evolutionary analyses.

As focus Area 2 has already been discussed in detail (above), this section will focus on Area 1: the generation of RefSeqs & Transcriptome data sets for all eight AA genome species, and a representative species of the nine other genome types. Table 2.5 lists the status of each *Oryza* genome project as of October 2012. Sixteen of the 23 genome sequencing project are in progress or have been completed, and include all the diploid *Oryza* species. Draft sequences of two subspecies of *O. sativa* were published a decade [26, 75] ago followed by the IRGSP “gold standard” RefSeq of *O. sativa* ssp. *japonica* (cv. Nipponbare) in 2005 [33]. Significant progress has been achieved

over the past ~2 years with completion of the *O. glaberrima* [AA], *O. barthii* [AA], *O. longistaminata* [AA], *O. punctata* [BB], and *O. brachyantha* [FF] genomes (all unpublished but in Genbank). Assembly is currently progress for the *O. nivara* [AA], *O. rufipogon* [AA], and the *O. glumaepatula* [AA] genomes, and sequencing is underway for a majority of the remaining diploid species.

It should be noted that the I-OMAP project has a huge advantage over other next generation genome sequencing projects (e.g., *Drosophila* 12 genomes; [22]) in that physical maps are available for all AA genome species as well as representatives of all other nine genome type. Such resources facilitate the assembly of more complete genome sequences vs. ones that rely solely on next generation short-read sequence data and assembly algorithms, the so-called gene space assemblies.

These genomes are presently being comparatively annotated using a common annotation platform “MAKER” [29] and a publication is planned for the summer of 2013.

Once the I-OMAP consortia has completed the *Oryza* RefSeq project, these sequences can be used as references upon which resequencing data from the majority of all *Oryza* accessions can be mapped in order to capture the majority of allelic diversity that is present within the genus. This data in turn can be used: (1) to accelerate MAS of agriculturally important traits as described above; (2) to identify and clone new wild alleles that can be integrated into elite rice lines via genetic engineering; and (3) to conduct a plethora of evolutionary tests aimed at the identification and testing of genes that are under adaptive selection that will open new opportunities for growing rice in nontraditional rice growing areas.

Such genetic diversity work is already underway for cultivated rice but is sorely lacking for the wild relatives of rice.

hungry world. Rice scientists are unified in their goal to develop a wave of new designer rice varieties that are “greener,” in terms of environmental impact (i.e., less water, fertilizer, pesticides, herbicides), and significant increases in yield. As outlined in this chapter, the wild relatives of rice will play an important role in the generation of the new “green super rice” varieties [76] that are required to help solve the 9BPQ.

With the advent of next generation sequencing information combined with genetic maps and molecular markers (SSRs, SNPs, SNVs), it is now possible to rapidly map and identify regions of the genome associated with specific components of a phenotype and determine which parental line contributes the favorable allele(s) at a particular locus. These are helpful tools that provide information for selecting which genes or components of quantitative trait variation to introduce from the wild gene pool into elite cultivars.

Traditional introgression approaches have been very successful in the transfer of numerous traits into cultivated rice, but have been limited due to sterility and wide crossing barriers. Using the tools of genomics we anticipate a full array of 16 reference quality *Oryza* genome sequences by the summer of 2013 at the latest. Such a data set will facilitate rapid gene discovery from the wild relatives of rice and provide the evolutionary insights needed to feed the future.

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## 17 Conclusion

As the world approaches the 9 billion people mark by the middle of this century, the rice community must do everything it can to help feed a

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