
Review

The *Oryza* BAC resource: a genus-wide and genome scale tool for exploring rice genome evolution and leveraging useful genetic diversity from wild relatives

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Rice was the first crop to have a high-quality reference genome sequence and is now at the forefront of intense functional and evolutionary research for two reasons—its central role in world food security, and its status as a model system for grasses. A thorough characterization of the rice genome cannot be accomplished without a deep understanding of its evolutionary history. The genus *Oryza* contains two cultivated and 22 wild rice species that represent 10 distinct genome types embedded within a robust phylogeny spanning a ~15 million year time span. The genus contains an untapped reservoir of agriculturally important traits and a historical record of genomic changes (especially those related to domestication, polyploidy, speciation and adaptation). The two main objectives of the ‘*Oryza* Map Alignment Project’ (OMAP) were to functionally characterize the rice genome from a comparative standpoint and to provide essential tools to leverage the novel genetic diversity from wild relatives for rice improvement. The objective of this review is to summarize our efforts towards developing the most comprehensive genus-wide set of publicly available BAC resources for the genus *Oryza*, the first of its kind among plants (and perhaps higher eukaryotes), and their applications.

Key Words: *Oryza*, BACs, OMAP.

Introduction

Rice (*Oryza sativa* L.), a staple cereal for half of the mankind, is central to the security of our global food supply. To sustain the food security needs of the rapidly expanding world population, current rice productivity must be doubled by the year 2030. The recent food crisis is a renewed call towards this direction (Zeigler and Mohanty 2010). This task is daunting indeed, considering a yield ceiling has been in place for the past 20 years, the projected shrinkage of agricultural resources (land and water), continued emergence of new pests, and increased incidence of abiotic stresses under conditions of rapid global climate change (Auffhammer *et*

al. 2006, Battisti and Naylor 2009, Peng *et al.* 2004, Schmidhuber and Tubiello 2007, Welch *et al.* 2010, Zhang 2007). The rice research community is thus faced with an formidable challenge of developing next generation of rice genotypes (i.e. super green rice varieties (Zhang 2007)), not only with improved productivity and adaptability, but ones that cater to the demands of diverse cultural, social and economic preferences (Zhang 2007). Creation of these next generation genotypes requires an integrated multidisciplinary approach as outlined by Wing *et al.* (2005), Zhang (2007) and Zhang *et al.* (2008) with the rice genome reference sequence serving as a unifying central platform. This approach includes the functional characterization of every rice gene using forward and reverse genomics tools, comparative genomics within *Oryza* and between cereals, and the leveraging of novel genetic diversity from the genus *Oryza* and other grass relatives.

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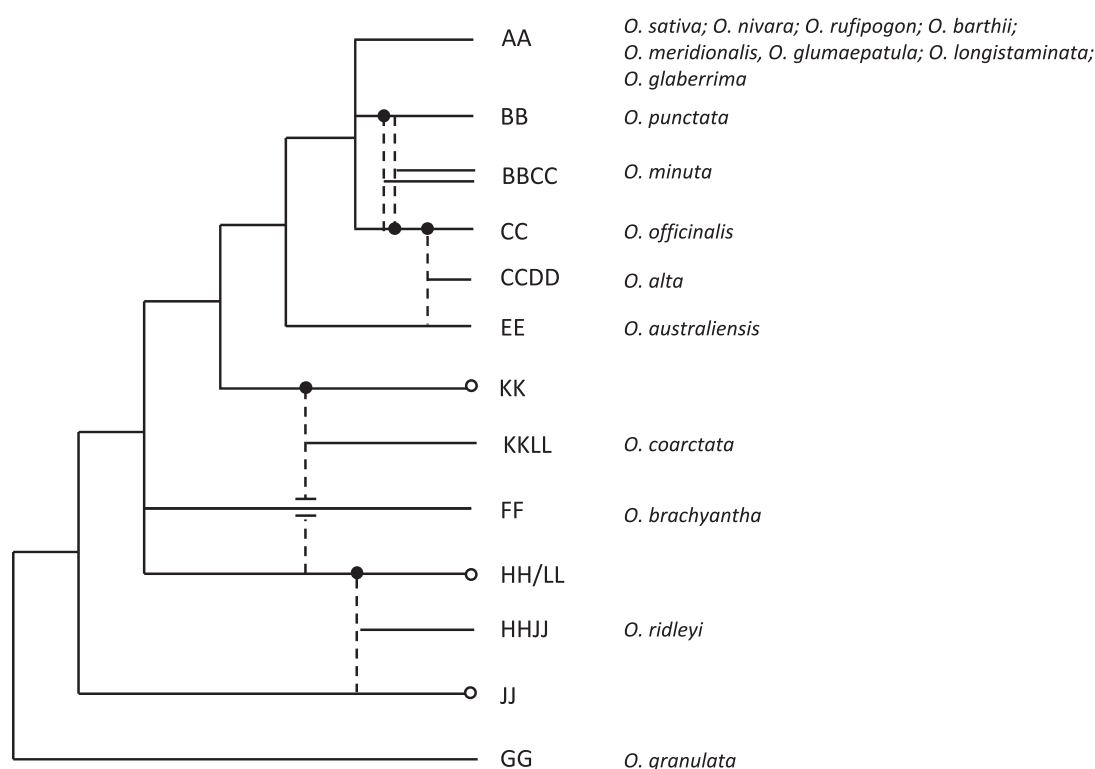


Fig. 1. Phylogenetic tree of *Oryza*. Targeted OMAP species are indicated on each phylogenetic branch. Evolutionary relationships of the rice genomes were inferred from Ammiraju *et al.* (2010), Ge *et al.* (1999) and Lu *et al.* (2009). Dashed lines indicate origins of allotetraploids; ● indicate maternal parents. ○ indicate unidentified diploid species.

The Genus *Oryza* and the *Oryza* map alignment project (OMAP)

The genetic base of cultivated rice is narrow owing to intense human selection (domestication) and dramatic agricultural changes brought about by the green revolution (Zeigler and Mohanty 2010). However, a vast amount of genetic variation still remains in wild relatives of rice. The genus *Oryza* is composed of two cultivated (*O. sativa* and *O. glaberrima*) and 22 wild species (<http://www.knowledgebank.irri.org/wildRiceTaxonomy/default.htm>) that have been classified into 10 distinct genome types, [six diploid (AA, BB, CC, EE, FF and GG) and four allotetraploid types (BBCC, CCDD, HHJJ and KKLL)], based on morphological, cytological and molecular divergence analyses (Aggarwal *et al.* 1997, Ammiraju *et al.* 2010, Ge *et al.* 1999, Lu *et al.* 2009, Nayar 1973). Cultivated rice, along with six other diploid wild relatives constitutes the AA genome primary gene pool. The phylogenetic relationships of the 24 *Oryza* species are well resolved (Fig. 1; Inferred from Ammiraju *et al.* 2010, Ge *et al.* 1999, Lu *et al.*, 2009), having rapidly diversified over the last ~15 million years (MY; Ammiraju *et al.* 2008, 2010, Lu *et al.* 2009), and attaining a pan-tropical distribution (Vaughan *et al.* 2003). Within this time frame, genome sizes of *Oryza* have changed over 3.6 fold and polyploidy has originated (Ammiraju *et al.* 2006, 2010, Lu *et al.* 2009). Species of *Oryza* exhibit remarkable variation in phenotypes and adaptation to hostile environments, likely owing to their

contrasting ecological and demographic selection regimes. The wild *Oryza* species offer a largely untapped resource of genes that have the potential to solve many of the world's rice production needs, including yield, drought & salt tolerance, and disease and insect resistance (Brar and Khush 2003). However, significant progress in exploiting genetic variation from wild species of *Oryza* has suffered from the lack of enabling genomic and cross-species translational tools that can speed up the rate of discovery and deployment. In addition, creation of a comprehensive functional toolkit for a thorough characterization of rice genome requires knowledge of its evolutionary history. This knowledge will come from comparative genomics studies of rice and its relatives.

Rice is the central comparative genomics model for all monocots and has been compared to all major cereals at the sequence level, ranging from orthologous genomic segments to whole genomes (reviewed in Bennetzen 2007, Devos 2010, International Brachypodium Initiative 2010, Paterson *et al.* 2009, Schnable *et al.* 2009, Tang *et al.* 2008). These studies characterized the key similarities and differences in the grass genome structure, organization and major underlying mechanisms. However, because of the lack of phylogenetic breadth, these studies were less informative on specifics—for example, why, when and how those similarities and differences may have arisen and their relation to species biology. Comparative phylogenomics of closely related species, such as those within a genus, can yield highly informative insights into these aspects as exemplified by the 12

genomes of *Drosophila* comparative system (*Drosophila* Sequencing Consortium 2007). With representation of a broad ecological selection history, over a short evolutionary period, and an outgroup species at every phylogenetic node, the *Oryza* genus offers similar comparative resolution as that of the *Drosophila* model system among higher eukaryotes. Setting such comparative analyses within *Oryza* allows for the interrogation and discovery of the generalities and specificity of mechanisms affecting genome evolution at the right levels (i.e. population, species, genome group or genus), and context (i.e. adaptation, domestication, ecology, polyploidy and speciation).

The *Oryza* MAP Alignment Project (OMAP) has evolved from the critical need of the international rice research community to create a genus-level experimental 'omics' platform to address the two complementary needs described above. The broad objectives of OMAP are: a) to create species specific comparative genomics resources along the *Oryza* phylogeny at two different resolutions—macro (chromosomal level) and micro (sequence level); b) to apply these resources to derive knowledge from many facets of rice biology—genome biology, evolution, ecology, genetics and breeding; and c) to integrate this knowledge to model the impact of genome diversity on phenotypic plasticity, and intelligent deployment in genomics assisted rice breeding.

The objective of this review is to summarize the comparative genomics tools and resources, in particular the BAC resources, that are already available, or being developed for *Oryza* as part of OMAP, and their potential uses for various applications.

***Oryza* BAC library resource**

Because of their salient attributes such as single copy number, large insert capacity (up to 300 Kb), low rates of chimerism, and ease of conventional plasmid based DNA manipulation for a plethora of downstream applications, bacterial artificial chromosome (BAC) and P1-derived artificial chromosome (PAC) libraries have become fundamental tools for genomics research. BAC/PACs have been used as the key reagents for decoding several plant genomes (*Arabidopsis* and maize) including *O. sativa* ssp. *japonica* genome (Baba *et al.* 2000, Chen *et al.* 2002, IRGSP 2005). A primary resource for the OMAP project is a set of deep-coverage and high-quality BAC libraries for different species spanning all 10 genome types of *Oryza*. The choice of species and particular genotypes targeted for BAC resource development was based on criteria developed from consultations with breeders and basic researchers across the world, and include: (1) a stratified sampling of each phylogenetic branch and each known genome type of *Oryza* to establish a 'phylogenomics system' that captures the entire range of evolutionary distances across the *Oryza*; (2) a strategic sampling based on their degree of relatedness to *O. sativa* (e.g. we targeted the entire primary gene pool of cultivated rice (representatives of several AA genome wild species) where

transfer of traits to cultivated rice is routine through conventional hybridization, and for the remaining genome types, a single representative species was initially chosen); (3) the presence of useful traits and the likelihood of discovering new genes, pathways, regulatory elements and insights into important phenomenon (i.e. the ability of each indexed species to serve as a model by itself for a biological process—speciation, polyploidy and adaptations to extreme environments); (4) the tractability of chosen species for genetic and evolutionary investigations (i.e. availability of segregating populations/introgression lines/wild populations); and (5) accessibility of genetic material, seed or vegetative forms for community distribution.

Currently there are about 5,000 and 1,750 accessions each of wild *Oryza* species archived at the International Rice Research Institute (IRRI) Genebank (www.irri.org/GRC/GRChome/Home.htm) and the National Institute of Genetics (NIG), Mishima, Japan, respectively. Deploying all these lines in breeding programs is impractical, therefore IRRI and NIG geneticists have, assembled 'core collections' that capture the broad genetic diversity of each *Oryza* species. The majority of the wild species accessions selected for OMAP belongs to the 'Rank1' core collection of NIG (<http://www.shigen.nig.ac.jp/rice/oryzabase/top/top.jsp>) and have been in use intensively at IRRI and NIG as donors in breeding programs. Resistance to more than seven different pathogens/pests has been identified and introgressed from these accessions (Brar and Khush 1997) (Table 1), with some lines already advanced into commercial cultivation. Selected lines exhibit broad geographic and habitat distribution, show wide phenotypic variation and differ in life history traits (annual vs. perennial), photosynthetic efficiency, biomass accumulation, reproductive barriers, wide compatibility genes, weed competitiveness, cytoplasmic male sterility (major trait for hybrid rice production), soil acidity, salt tolerance, that are present only in the wild gene pool of *Oryza* (Fig. 2 and Table 1). A dedicated voucher plot maintains all OMAP accessions vegetatively at IRRI and the University of Arizona. Plant material in the form of seeds or vegetative form can be obtained from IRRI or NIG for interested researchers.

We have generated 18 BAC libraries for 16 different species spanning all 10 genome types of *Oryza* (Ammiraju *et al.* 2006, Song *et al.* unpublished; Table 2). All libraries were generated using the restriction enzyme *Hind* III. The total number of clones in each library ranged between 36,864 to 147,456, totaling more than 1.36 million clones that are arrayed in 384-well plates and stored in -80°C freezers at the Arizona Genomics Institute's BAC/EST Resource Center (www.genome.arizona.edu). All libraries were rigorously characterized for the average insert size, organellar content and non-recombinant clones that ranged between 123 kb–161 Kb, 0.4%–4.1% and 0%–5% respectively. The randomness and representational heterogeneity of each library was tested by hybridization with gene specific probes—at least one per each chromosome, and the ability of the identified positive clones to form physical contigs (

Table 1. Wild species of *Oryza* used for genomic resource development in OMAP and their useful attributes

| Species | Genome type | Origin | Accession number | | Useful traits |
|-----------------------------|-------------|-------------|------------------|-------------|---|
| | | | NIG | IRRI (IRGC) | |
| <i>O. glaberrima</i> (CG14) | AA | Africa | NA | 96717 | Nematode resistance, Drought tolerance, Tolerance to soil acidity & Iron toxicity, Aluminium toxicity, Weed competitiveness. African gall midge, Resistance to RYMV |
| <i>O. rufipogon</i> | AA | Malaysia | W0120* | 105491 | Yield enhancing traits |
| <i>O. rufipogon</i> | AA | Vietnam | NA | 106424 | Rice Tungro Virus resistance, Tolerance to Aluminium toxicity, Tolerance to Phosphorous deficiency |
| <i>O. nivara</i> | AA | India | W0106* | 100897 | Grassy stunt virus resistance |
| <i>O. brarhii</i> | AA | Cameroun | W1588* | 105608 | Resistance to GLH and BB |
| <i>O. logistaminata</i> | AA | Africa | NA | 110404 | Resistance to BB |
| <i>O. meridionalis</i> | AA | Australia | W2112** | 105296 | Elongation ability; Drought tolerance |
| <i>O. glumaepatula</i> | AA | Cuba | GEN1233 | NA | Elongation ability; Source of CMS; Aluminum tolerance |
| <i>O. punctata</i> | BB | Africa | W1514* | 105690 | BB, BPH |
| <i>O. officinalis</i> | CC | Thailand | W0002* | 100896 | Resistance to BB, BPH, WBPH |
| <i>O. minuta</i> | BBCC | Philippines | W1342* | 101141 | Resistance to BB, blast, BPH, WBPH, Shb |
| <i>O. alta</i> | CCDD | S. America | W1182* | 105143 | Increased biomass and yield related traits |
| <i>O. australiensis</i> | EE | Australia | W0008* | 100882 | Resistance to BPH, BB, blast, tolerance to drought related traits |
| <i>O. brachyantha</i> | FF | Africa | W1401* | 101232 | Resistance to BB, leaf folder and YSB |
| <i>O. granulata</i> | GG | Thailand | W0067(B)* | 102118 | Resistance to BB, BPH, Shade tolerance, adaption to aerobic soils |
| <i>O. ridleyi</i> | HHJJ | Thailand | W0001* | 100821 | Resistance to BB, YSB |
| <i>O. coarctata</i> | KKLL | Bangladesh | NA | 104502 | Salt tolerance |

* Rank 1: The Rank1 core collection at NIG, Japan (<http://www.shigen.nig.ac.jp/rice/oryzabase/wild/coreCollection.jsp>)

** Rank 3: The Rank3 core collection NIG, Japan (<http://www.shigen.nig.ac.jp/rice/oryzabase/wild/coreCollection.jsp>)

BB = bacterial blight, BPH = brown planthopper, CMS = cytoplasmic male sterility, GLH = green leafhopper, RYMV = rice yellow mottle virus, Shb = sheath blight, WBPH = white-backed planthopper, YSB = yellow stem borer.

Trait information is from Brar and Khush (1997, 2003) and Brar (unpublished).

Table 2. The *Oryza* BAC libraries, their characteristics, and associated genomics tools

| Species | Genome type | Accession number | | Genome Size | BAC resources | | | | Whole genome BES resources | Physical maps |
|-------------------------|-------------|------------------|--------------|-------------|---------------|---------------------|------------------|-----------------|----------------------------|---------------|
| | | NIG | IRRI (IRGC) | | Library name | Average Insert size | Number of clones | Genome coverage | | |
| <i>O. glaberrima</i> | AA | NA | 96717 (CG14) | 354 | OG_BBa | 130 | 55296 | 20 | √ | √ |
| <i>O. glaberrima</i> | AA | NA | 103544 | 354 | OG_Fba | 110 | 36864 | 11 | NA | NA |
| <i>O. rufipogon</i> | AA | W0120 | 105491 | 439 | OR_CBa | 133 | 64512 | 20 | √ | √ |
| <i>O. rufipogon</i> | AA | NA | 106424 | 450 | OR_Ba | 134 | 92160 | 27 | NA | NA |
| <i>O. nivara</i> | AA | W0106 | 100897 | 448 | OR_BBa | 161 | 55296 | 20 | √ | √ |
| <i>O. brarhii</i> | AA | W1588 | 105608 | 411 | OB_ABa | 136 | 36864 | 12 | √ | √ |
| <i>O. logistaminata</i> | AA | NA | 110404 | 352* | OL_Aba | 126 | 36864 | 13 | In progress | In progress |
| <i>O. meridionalis</i> | AA | W2112 | 105296 | 435* | OM_ABa | 147 | 36864 | 12 | In progress | In progress |
| <i>O. glumaepatula</i> | AA | GEN1233 | NA | 464* | OG_GBa | 139 | 36864 | 11 | In progress | In progress |
| <i>O. punctata</i> | BB | W1514 | 105690 | 423 | OP_Ba | 141 | 51840 | 17 | √ | √ |
| <i>O. officinalis</i> | CC | W0002 | 100896 | 653 | OO_Ba | 140 | 92160 | 20 | √ | √ |
| <i>O. minuta</i> | BBCC | W1342 | 101141 | 1124 | OM_Ba | 124 | 129024 | 14 | √ | √ |
| <i>O. alta</i> | CCDD | W1182 | 105143 | 1124 | OM_Ba | 124 | 129024 | 14 | √ | √ |
| <i>O. australiensis</i> | EE | W0008 | 100882 | 960 | OA_CBa | 152 | 73728 | 12 | √ | √ |
| <i>O. brachyantha</i> | FF | W1401 | 101232 | 338 | OB_Ba | 130 | 36864 | 14 | √ | √ |
| <i>O. granulata</i> | GG | W0067(B) | 102118 | 862 | OG_ABa | 133 | 73728 | 11 | √ | √ |
| <i>O. ridleyi</i> | HHJJ | W0001 | 100821 | 1283 | OR_ABa | 126 | 129024 | 13 | √ | √ |
| <i>O. coarctata</i> | KKLL | NA | 104502 | 771 | OC_Ba | 122 | 147456 | 23 | √ | √ |

NA = not available.

The library data is from Ammiraju *et al.* (2006), Song *et al.* (unpublished); Associated genomics tools for each BAC library (i.e. the physical maps and BES) are described in Kim *et al.* (2008) and www.omap.org.

Genome size data is from Ammiraju *et al.*, Kim *et al.* (2008); * Wing *et al.* (unpublished data).



Fig. 2. Genetic diversity of *Oryza*. Representative species for each *Oryza* genome type were shown in the picture.

genome.arizona.edu/BAC_special_projects/). Each library represents approximately 10 fold or higher redundancy as determined empirically and experimentally (Table 2). At this depth, the probability of finding any random genomic sequence of *Oryza* species is over >99%, therefore providing a critical resource for rapidly isolating any target gene(s) and regulatory elements from these wild species. Convenient public access to all *Oryza* BAC libraries, individual clones and macroarray filters are provided through the Arizona Genomic Institute's BAC/EST Resource Center (www.orders.genome.arizona.edu).

BAC resources for all remaining species of the genus *Oryza* or even beyond *Oryza* are currently being developed at AGI. For example, efforts are already underway to develop *Leersia perreri* and *Zizania paulstris* BAC libraries that could serve as important evolutionary outgroups for *Oryza* comparative genomic studies.

Applications of *Oryza* BAC resources

The *Oryza* BAC library resource described here represents a first of its kind, the most comprehensive genus-wide tool to study genome evolution and leverage novel genetic diversity that span only a 15 MY divergence window. Because of the key attributes of the *Oryza* species described above, the BAC resources described here will have an unprecedented impact on many facets of rice and plant biology in general. These resources will broadly apply and benefit a large and diverse group of end-users, ranging from biochemists to plant breeders to ecologist to taxonomists and geneticists to genome biologists. *Oryza* BAC resources have attracted

considerable attention since their availability from 2006, and have provided essential research tools for many groups around the world, including the distribution of 204 library screening filters, over 300,000 clones, and several research alliances under the umbrella of International OMAP (I-OMAP), a consortium of basic and applied rice researchers. The value of the *Oryza* BAC resource set was greatly enhanced by the development of physical maps and BAC end sequence (BES) resources for each species. Nearly 10 genome equivalent BAC clones from 13 *Oryza* BAC libraries, including four allopolyploids were fingerprinted, end sequenced, assembled into physical maps and aligned to IRGSP Refseq (Goicoechea 2009, Hass-Jacobus *et al.* 2006, Kim *et al.* 2007, 2008, Rice Chromosome 3 Sequencing Consortium 2005, www.omap.org). Efforts are underway to produce three additional comparative physical maps (Table 2) from *O. longistaminata*, *O. glumaepatula* and *O. meridionalis*. These genus-wide and genome-scale comparative physical maps offer a rapid translational tool for exchange of genomic knowledge across *Oryza* through cross-species synteny, and for efficient exploitation of the sequenced rice genome. In addition, these physical maps will serve as critical templates for targeted (i.e. individual chromosomes, arm, regions, BACs) or whole genome sequencing projects, complementing and correcting sequence assemblies, and providing access to complex repetitive regions that are avoided in *de novo* shotgun genome sequencing projects.

A number of genome browsers have been developed for the rice genome including Gramene (<http://www.gramene.org/cmap/>; http://www.gramene.org/Oryza_sativa/

Info/Index), Michigan State University (MSU) Rice Annotation Project (<http://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice/>), and Rice Annotation Project (RAP) (<http://rapdb.dna.affrc.go.jp/>) all of which have integrated OMAP BES datasets. The Gramene and SyMAP (Soderlund *et al.* 2006), in particular have integrated the comparative map viewers for the OMAP physical maps. These browsers are populated with an extensive list of known QTLs, genes, variations, and phenotypes of the reference species. Therefore having an additional layer of *Oryza* BESs and physical maps can reduce efforts toward the isolation of orthologous genomic segments of interest from wild relatives to the mere picking of clones. In addition, the *Oryza* BES data set has allowed for the identification of large numbers of genetic variations, such as Simple Sequence Repeats (SSRs) and Single Nucleotide Variations (SNVs), that can readily serve as functional and molecular breeding tools (Kim *et al.* 2008).

A large number of long range *Oryza* sequence data sets developed from these BAC resources are already publicly available, and include genus-wide orthologous sequences for the *Adh1*, *MOC1*, *Hd1* (Ammiraju *et al.* 2008, 2010, Lu *et al.* 2009, Sanyal *et al.* 2010), individual loci from specific lineage such as DREB (Zhang *et al.* 2007), *Pi2/9* regions (Dai *et al.* 2010) and two large BAC contigs from chromosome 4 (Feng *et al.* 2009), and entire short arm sequences of chromosome 3 for six species (www.gramene.org).

Complex patterns of genome ev

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