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# The International *Oryza* Map Alignment Project: development of a genus-wide comparative genomics platform to help solve the 9 billion-people question

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The wild relatives of rice contain a virtually untapped reservoir of traits that can be used help drive the 21st century green revolution aimed at solving world food security issues by 2050. To better understand and exploit the 23 species of the *Oryza* genus the rice research community is developing foundational resources composed of: 1) reference genomes and transcriptomes for all 23 species; 2) advanced mapping populations for functional and breeding studies; and 3) *in situ* conservation sites for ecological, evolutionary and population genomics. To this end, 16 genome sequencing projects are currently underway, and all completed assemblies have been annotated; and several advanced mapping populations have been developed, and more will be generated, mapped, and phenotyped, to uncover useful alleles. As wild *Oryza* populations are threatened by human activity and climate change, we also discuss the urgent need for sustainable *in situ* conservation of the genus.

## Addresses

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## Introduction

In 2010, Science magazine published a special issue entitled ‘Feeding the Future’ that featured a series of articles concerning food security and the challenge of feeding 9 billion people [1], followed by an issue in the Economist magazine entitled ‘The 9-billion People Question’ (9BPQ) [2]. Both addressed a worldwide dilemma that is central to most if not all plant scientists,

that is, how can our society grow enough food to feed 2 billion additional human beings in less than 40 years? Rice (*Oryza sativa*) will play a key role in helping to solve the 9BPQ, as it presently provides 20% or more daily calories to half the world’s population, and will be the developing world’s most important food crop in 2050 [3]. Rice 2020 [4] is a key initiative aimed at solving the 9BPQ and calls for community mobilization to pool and coordinate all available resources with the common goal of creating new green super rice varieties, where ‘green’ means less input (e.g. water, fertilizer, pesticides, land), and ‘super’ means two- to threefold yields [5].

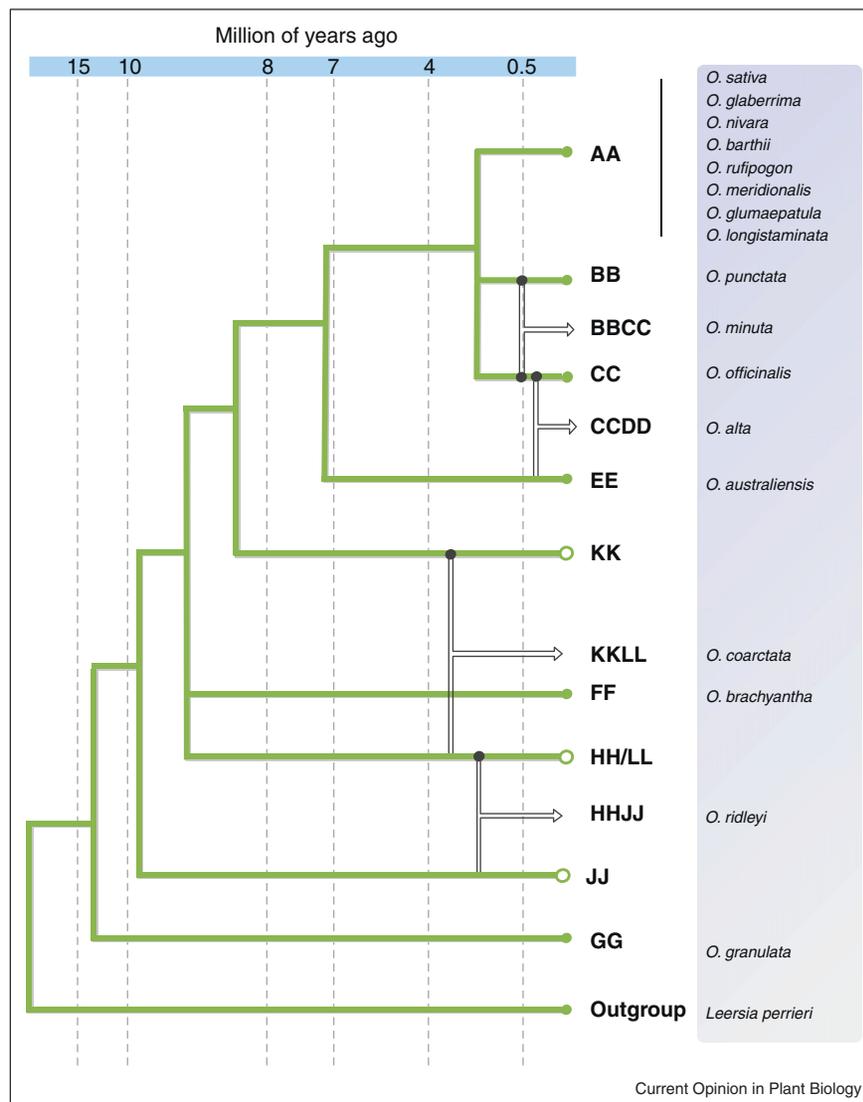
One of the most important resources that can be utilized to improve cultivated rice is the virtually untapped reservoir of genetic variation hidden within the wild relatives of *Oryza*. The genus *Oryza* spans approximately 15 MY of evolutionary history (Figure 1) [6] and is composed of 21 wild and 2 domesticated (*O. sativa*, *Oryza glaberrima*) species, 10 distinct genome types (AA, BB, CC, BBCC, CCDD, EE, FF, GG, KKLL, HHJJ), and a 3.6 genome size variation. Wild *Oryza* species have a broad habitat distribution, including Asia, Australia, Africa, South and Central America, and many novel biotic/abiotic resistances have been identified.

To lay the foundation for a complete genomic interrogation of the wild relatives of rice the *Oryza* Map Alignment (OMAP) and *Oryza* Genome Evolution (OGEP) Projects were funded to generate a 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 — fingerprinted, end-sequenced, and FPC assembled) representing 17 of the 23 recognized *Oryza* species, covering all eight AA genome species and one each of the other nine genome types (BB, CC, BBCC, CCDD, EE, FF, GG, KKLL, HHJJ) [6–8]; and a set of chromosome three short arm sequences from all eight 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 can be accessed through the <http://www.Gramene.org> and <http://www.genome.arizona> websites, respectively.

Analysis of these data sets revealed the following key points: first, LTR Retro-transposable element amplifications dramatically increased the size of both the *Oryza*

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Figure 1



Phylogenetic tree of the *Oryza* genus and outgroup *Leersia perrieri* (modified from [6]). Targeted OGE/I-OMAP species are indicated on each branch. Arrows indicate origins of allotetraploids. Dark circles indicate maternal parents. Open circles indicate unidentified diploid species. Divergence time is based on data from the literature [13,67,68,69].

*australiensis* [EE] and the *Oryza granulata* [GG] genomes by as much as 400 and 200 Mb, respectively [9,10]; second, the AA genomes of *Oryza nivara*, *Oryza 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 [11]; and third, analysis of the *Adh1* region (~100–200 kb) across the entire *Oryza* phylogeny (diploid and polyploid) showed significant perturbations of synteny including dynamic evolution of gene families, transposable element mediated gene movement, mutations and large scale physical rearrangements [12,13].

The overriding conclusion from these studies, and from many others (e.g. [14]), 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 within the genus to help solve the 9BPQ.

To address this resource/knowledge gap the International *Oryza* Map Alignment Project (I-OMAP) was initiated in 2007 and has held six grand challenge meetings (Japan 07, Korea 08, Philippines 09, Brazil 10, Taiwan 11, Thailand 12) in conjunction with the annual International Symposia on Rice Functional Genomics (ISRFG). The three

Table 1

Sequencing status of 16 *Oryza* genomes and *Leersia perrieri* (outgroup species)

Species [genome type]	Genome size	Lead seq. group	Seq. method	Status
(1) <i>O. sativa</i> ssp. <i>indica</i> [AA]	~400 Mb	BGI	WGS	2002 (Draft)
(2) <i>O. sativa</i> ssp. <i>japonica</i> [AA]	~400 Mb	IRGSP, Sasaki T.	CBC/PM	2004 (RefSeq)
(3) <i>O. glaberrima</i> [AA]	~354 Mb	AGI, Wing R.A.	BP	2010 (RefSeq)
(4) <i>O. barthii</i> [AA]	~411 Mb	AGI, Wing R.A.	WGS/PM	2012 (RefSeq)
(5) <i>O. brachyantha</i> [FF]	~260 Mb	CAS, Chen M.	WGS/PM	2011 (RefSeq)
(6) <i>O. longistaminata</i> [AA]	~352 Mb	CAS, Wang W.	WGS	2011 (Draft)
(7) <i>O. nivara</i> [AA]	~448 Mb	AS, Hsing Y.	BP/WGS/PM	Assembly IP
(8) <i>O. rufipogon</i> [AA]	~445 Mb	NIG, Kurata N./NCGR, Han B.	WGS	2013 (Draft)
(9) <i>O. glumaepatula</i> [AA]	~464 Mb	UFP, Oliveira A.C.	WGS/PM	Assembly IP
(10) <i>O. punctata</i> [BB]	~423 Mb	AGI, Wing R.A.	BP/WGS/PM	2012 (RefSeq)
(11) <i>O. meridionalis</i> [AA]	~435 Mb	SCU, Henry R.J./UP, Panaud O.	WGS/PM	2013 (Draft)
(12) <i>O. australiensis</i> [EE]	~960 Mb	UP, Panaud O.	WGS/PM	Sequencing IP
(13) <i>O. officinalis</i> [CC]	~653 Mb	NIG, Kurata N.	WGS/PM	Sequencing IP
(14) <i>O. eichingeri</i> [CC]	~650 Mb	NIG, Kurata N.	WGS	Sequencing IP
(15) <i>O. rhizomatis</i> [CC]	~650 Mb	NIG, Kurata N.	WGS	Sequencing IP
(16) <i>O. granulata</i> [GG]	~862 Mb	CAS, Gao L.	WGS/PM	Sequencing IP
(17) <i>L. perrieri</i> [outgroup]	~323 Mb	AGI, Wing R.A.	WGS/PM	2012 (Draft)

Key sequencing method: WGS, Whole Genome Shotgun; CBC, Clone-by-Clone; BP, BAC Pool; PM, Physical Map Integration. Group: BGI, Beijing Genomics Institute China; IRGSP, International Rice Genome Sequencing Project; AGI, Arizona Genomics Institute, USA; CAS, Chinese Academy of Sciences, China; AS, Academia Sinica, Taiwan; NIG, National Institute of Genetics, Japan; NCGR, National Center for Gene Research, China; UFP, Universidade Federal de Pelotas, Brazil; SCU, Southern Cross University Australia; UP, Université de Perpignan France.

primary focus areas of I-OMAP are to: first, generate RefSeqs & Transcriptome data sets for all eight AA genome species, and representative species of the nine other genome types; second, generate, map, and phenotype advanced ABC, CSSL, RIL populations for the AA genome species for functional and breeding studies; and third, identify collections of naturally occurring populations of the wild *Oryza* species for diversity and evolutionary analyses, and for conservation. Here all three I-OMAP focus areas will be discussed.

### Sequencing the collective *Oryza* genome

As stated, a major goal of focus area 1 is to generate reference quality sequences from representatives of all 23 *Oryza* species. Table 1 lists the current status of each I-OMAP genome project to date. Sixteen of the 23 genome sequencing projects are in progress or have been completed, and include all of the diploid *Oryza* species. Draft sequences of two subspecies of *O. sativa* were published a decade ago [15,16], followed by the release of IRGSP 'gold standard' RefSeq of *O. sativa* ssp. *japonica* (cv. Nipponbare) in 2005 [17]. Significant progress has been achieved over the past two years with completion of the *O. glaberrima* [AA], *Oryza barthii* [AA], *Oryza longistaminata* [AA], *O. punctata* [BB], and *O. brachyantha* [FF] genomes (all unpublished but in GenBank for early access). Assembly is currently in progress for the *O. nivara* [AA], *Oryza glumaepatula* [AA] and *O. meridionalis* [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 significant advantage over other next generation genome sequencing projects (e.g. *Drosophila* 12 genomes) [18] in

that physical maps are available for all AA genome species as well as representatives of all other nine genome types. Such resources facilitate the assembly of more complete genome sequences versus ones that rely solely on next generation short-read sequence data and assembly algorithms, the so called 'gene space assemblies'.

Once the assemblies are linked to their respective physical maps and edited for mis-assemblies and errors, they will then be annotated using a common annotation platform—for example, MAKER [19]. To date, all available assemblies have been annotated, including both *O. sativa* subspecies *japonica* and *indica*. It is important that all the *Oryza* genome be annotated using a common platform for ease of downstream comparative structural and evolutionary analyses.

In the future, I-OMAP plans to sequence all the polyploid *Oryza* species but has yet to secure funding. The BBCC genome of *O. minuta* will likely be the first one attempted as RefSeqs for both the CC and the BB genomes will soon be available, which can help to guide the assembly process of the BBCC genome. In addition a robust physical map for the *O. minuta* genome is available and thus a combination of WGS and BAC pool sequencing can be used to tame this wild genome.

### Unfolding the genetic architecture of the wild relatives of rice with advanced mapping populations

The wild relatives of rice constitute an important reservoir of valuable genes but the association of these species with several weedy traits and incompatibility barriers has limited the transfer of useful genes into

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cultivated species. The major consideration in alien gene transfer is to selectively transfer agronomically and commercially important genes from wild species, while at the same time avoiding linkage drag, using a combination of strategies involving conventional and molecular plant breeding and tissue culture [20]. Most of the important traits present in the wild relatives of rice are cryptic and need to be extracted in the cultivated species background. A substantial amount

of ‘pre-breeding’ research can provide a useful platform to identify and transfer favorable alleles from wild and unadapted sources into elite cultivars [21].

Conventional mapping populations including  $F_2$ , backcross, recombinant inbred lines (RILs), and doubled haploids (DH) have been used for mapping both major genes and quantitative trait loci (QTL). Although such populations are relatively simple to generate, they have

##### Box 1 Advanced mapping populations

**Backcross Inbred Lines (BILs).** BILs are derived by crossing a wild species with a cultivated one, followed by backcrossing with the cultivated parent two or more times and selecting for a target trait in each generation. For the purposes of genetic analysis, the advantages of using BILs include: first, high genetic and morphological similarity between lines that enables more precise estimates of quantitative traits; second, the opportunity to study QTL-by-environment interactions more accurately; and third, relatively rapid and straightforward utilization of BILs for commercial plant breeding [37].

**Near Isogenic Lines (NILs).** An alternative type of immortal experimental population commonly used in breeding is the development of sets of introgression lines or NILs. Unlike BILs, NILs differ at a limited number of loci. Small-effect QTL can be detected more precisely with NILs than with RIL populations, although the local resolution may be lower. In general, population size is more important than the number of replicates to increase the mapping power of RILs, whereas for NILs, many replicates are absolutely required [38].

**Advanced Intercrossed Lines (AILs).** AILs are produced by randomly, but sequentially, intercrossing a population that initially originated from a cross between two inbred lines or some variant, thereof. AILs provide an increased probability of recombination between any two loci. Consequently, the genetic length of the entire genome is stretched, and QTL are identified with higher precision as compared with conventional RIL populations [39]. AILs derived from crosses between known inbred lines may be a useful resource for fine genetic mapping as well; however, it takes additional time to generate a new set of inbreds after intermating.

**Chromosome Segment Substitution Lines (CSSLs).** A CSSL is an introgression line that contains a small portion of a given chromosome introgressed from another line, and a set of CSSLs encompass an entire chromosome. CSSLs are developed both from intraspecific and interspecific crosses with a series of backcrosses and the identification of individual lines with molecular markers. Genome wide CSSLs can be used as powerful QTL mapping populations for the elucidation of the molecular basis of interesting traits especially derived from wild species [23]. A major limitation of using CSSLs is that unidentified introgression of small untargeted chromosomal segments, which are not tagged by markers, sometimes generate experimental noise that makes it more difficult to detect QTL effects in particular chromosomal regions. Secondly, it may be difficult to detect phenotypic differences generated by a combination of two or more donor alleles in different chromosomal regions. In this case, the mapping resolution of respective QTL can be improved by fine mapping to develop NILs using the CSSLs/BILs as base materials [22]. Graphical genotyping software programs, such as GGT [40] or CSSL Finder (<http://mapdisto.free.fr/CSSLFinder>), are very useful for the development and usage of CSSLs.

**High-Throughput Genotyped CSSLs.** In this case, every CSSL is genotyped by whole-genome re-sequencing, and thus combines the advantages of an ultrahigh-quality physical map with high mapping accuracy. This approach has great potential value for gene discovery and genetic mapping but has not been reported for interspecific crosses of rice. High-throughput genotyped CSSLs may provide powerful tools for future whole genome large-scale gene discovery in rice and offers foundations to enable the development of superior rice varieties [41].

**Multiparent Advanced Generation Inter-Cross (MAGIC) populations.** Under this scheme, as many diverse founder lines as an investigator wishes to deploy are used to create a mapping population. If  $n$  founder lines are chosen, they need to be intercrossed for  $n/2$  generations until all founders are combined in equal proportion. Once the intercrossing is completed, RILs may be derived from these after selfing [42,43]. The major limitation of the use of MAGIC populations is that with increased founder size, intercrossing cycles also proportionately increase. Another limitation is that MAGIC populations are likely to show extensive segregation for phenological traits, like maturity and plant height. Segregation for such traits may influence the overall performance of complex traits like yield or drought tolerance, and hence may yield false QTL.

**Association Panels.** Association panels are open mapping populations that utilize samples of individuals from germplasm collections or natural populations. Compared with conventional and linkage disequilibrium (LD) mapping, the use of nonrandom associations of loci in haplotypes is an effective tool to connect structural genomics and phenomics in plants. Association mapping exploits historical recombination events that have occurred during establishment of the sample population [44]. A number of publications have appeared on association mapping in rice. The most comprehensive study was published by Huang *et al.* [45] who used 950 diverse lines representing the worldwide collection of rice germplasm to map flowering time and grain yield traits. Major limitations of using association panels are population stratification, and an unequal distribution of alleles within a population which results in non-functional, spurious associations.

**Nested Association Mapping (NAM).** NAM populations are developed by pooling equal numbers of progenies from a large number of crosses involving one common parent. NAM, as currently implemented for maize, is an extremely powerful strategy for dissecting the genetic basis of quantitative traits in a species with low LD. The controlled crosses reduce the confounding effects of population structure, while the large numbers of progeny derived from such crosses allow for family mapping with substantial statistical power [46]. The major limitations are the large number of crosses required to generate NAM populations, and QTL interactions with genetic backgrounds cannot be examined as one parent is common in all component subpopulations.

A comparison of all the advanced mapping populations discussed above with respect to practical utility as mapping resources and their suitability toward mapping different genetic components is presented in Table 2.

Table 2

## Comparison of advanced mapping populations for QTL mapping and their suitability for measuring various genetic components

Properties	RILs/DH	BILs	NILs	AILs	CSSL	MAGIC	Assoc.	NAM
Founder parents	2	2	2	2	2	≥8	≥100	≥10
Crossing requirement	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Time to establish	Moderate	Long	Long	Long	Long	Long	Low	Long
Population size	~200	~100	~100	>200	>200	~1000	~100	>1000
Suitability for fine mapping	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Amount of genotyping required	Low	Low	Low	High	High	High	High	High
Amount of phenotyping required	Low	High	Low	High	Low	High	High	High
Statistical complexity	Low	Low	Low	High	Low	High	High	High
Use of germplasm variation	Low	Low	Low	Low	High	High	High	High
Suitability for mapping QTL in interspecific crosses	No	Yes	Yes	No	Yes	No	Yes	No
Practical utility	Low	High	High	High	High	High	High	High
Suitability for measuring genetic components								
i. Additive	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
ii. Dominance	No	No	No	Yes	No	No	No	No
iii. Additive × additive	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
iv. Additive × dominance	No	No	No	Yes	No	No	No	No

Modified from [42,43].

one or more significant limitations. F<sub>2</sub> and backcross mapping populations are the simplest to generate but are not efficient for mapping QTL as they cannot be replicated over multiple environments, and single plant data is not reliable. On the other hand, RIL and DH populations are immortal, and can be replicated over locations and years. However, both undergo limited recombination events, and mapping analyses can be limited because of the masking effects of major QTL and epistatic interactions of multiple QTL. Advanced

mapping populations (AMPs) like backcross inbred lines (BILs), advanced intercrossed lines (AILs), near isogenic lines (NILs), chromosome segment substitution lines (CSSL), high throughput genotypes CSSLs, multiparent advanced generation inter-cross (MAGIC), association panels, and nested association mapping (NAM) populations potentially address the limitations of conventional mapping populations, and are likely to bring paradigm shifts toward QTL analysis in plants. A brief description of these populations is presented in Box 1.

Table 3

Advanced mapping populations reported between 2007 and 2012 and traits introgressed/mapped between *O. glaberrima* or a wild *Oryza* species as the donor parent, and an *O. sativa* cultivar as the recurrent parent

Population	Donor parent (accession)	Recurrent parent (cultivar)	Traits introgressed/mapped	Ref.
IL	<i>O. glaberrima</i> (Tog5681)	<i>Indica</i> (IR64)	Drought tolerance	[47]
CSSL	<i>O. glaberrima</i> (IRGC103544)	<i>Tropical japonica</i> (Caiapo)	Rice stripe necrosis virus resistance	[48]
BIL	<i>O. glaberrima</i> (Tog5675)	<i>Indica</i> (IR64)	BPH resistance (Bph1)	[49]
BIL	<i>O. glaberrima</i> (IRGC96717)	<i>Japonica</i> (WAB56-104)	Drought resistance, early vigor	[50]
CSSL	<i>O. glaberrima</i>	<i>Japonica</i> (Koshihikari)	Glabrous gene	[51]
BIL	<i>O. glaberrima</i> (IRGC103544)	<i>Indica</i> (Milyang 23)	Yield and yield components	[52]
CSSL	<i>O. glaberrima</i>	<i>Japonica</i> (Wuyujing-7)	Spreading panicle	[53]
IL/BIL	<i>O. nivara</i> (IRGC105444)	<i>Japonica</i> (Taichung 65)	Pollen sterility gene (S27-nivs)	[54]
BIL	<i>O. nivara</i> (IRGC105444)	<i>Japonica</i> (Koshihikari)	Hybrid breakdown locus [-hbd 1(t)]	[55]
BIL	<i>O. rufipogon</i> (W630)	<i>Japonica</i> (Nipponbare)	Drought tolerance	[56]
BIL	<i>O. rufipogon</i> (IRGC105491)	<i>Tropical japonica</i> (Jefferson)	Early flowering	[57]
BIL/CSSL	<i>O. rufipogon</i> (Dwr)	<i>Indica</i> (Xieqingzao B)	WBPH and BPH resistance	[58]
BIL	<i>O. rufipogon</i> (YJCW)	<i>Indica</i> (93-11, restorer line)	Yield-related traits	[59]
CSSL	<i>O. rufipogon</i>	<i>Indica</i> (Teqing)	Small grain panicle and dwarfness	[60]
BIL	<i>O. rufipogon</i> (IRGC105491)	<i>Indica</i> (IR64)	Yield and yield components	[61]
NIL	<i>O. rufipogon</i> (IRGC105491)	<i>Japonica</i> (Hwaseongbyeol)	Yield components	[62]
BIL	<i>O. rufipogon</i> (YJCWR)	<i>Indica</i> (TeQing)	Yield and yield components	[63]
BIL/NIL	<i>O. longistaminata</i>	<i>Indica</i> (RD23)	Pollen/spikelet fertility, plant height	[64]
BIL	<i>O. glumaepatula</i> (RS-16)	<i>Indica</i> (BG90-2)	Grain yield, cooking quality	[65]
BIL	<i>O. minuta</i> (IRGC101141)	<i>Indica</i> (IR31917-45-3-2)	BPH resistance	[49]
BIL	<i>O. brachyantha</i> (IRGC101232)	<i>Indica</i> (IR56)	Bacterial blight	[66]

Modified from [23].

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AMPs developed with the wild relatives of rice help not only to minimize genetic noise and reveal the true expression of individual loci, but also permit the ability to map and clone these cryptic alleles. While in case of interspecific crosses, especially with wild and unadapted germplasm, conventional mapping populations again lose their worth for these additional reasons: first, hybrid sterility often hampers their development and second, progeny derived from wide crosses often exhibit too much phenotypic noise and wide variation in heading date, particularly as a result of strong transgressive segregation. Therefore, it may be difficult to precisely evaluate phenotypic traits among segregants [22]. For transfer and mapping of QTL from wild and unadapted germplasm, BILs, NILs, AILs, CSSL are the most powerful populations that can be used. A number of such populations have already been developed for rice and are summarized in Table 3. Developing AMPs can require more than six years, even with the adoption of accelerated generations [22]. But once developed, they have the potential to uncover new alleles from the unadapted, non-productive wild rice accessions, develop genome-wide genetic stocks and clone identified genes/QTLs for functional genomic research [23].

CSSLs and ILs using different wild rice accessions have or are being developed around the world (e.g. USA, China, India, Japan, the Philippines, South America, and Europe) (for details see [23]). At Punjab Agricultural University in India, BILs have been developed in the background of two *indica* cvs. PR114 and Pusa44 by crossing these with 72 accessions selected from African cultivated rice *O. glaberrima* and five AA genome wild species (*O. barthii*, *O. nivara*, *O. rufipogon*, *O. longistaminata* and *O. glumaepatula*), followed by two successive backcrosses and selfing. These BILs are currently being evaluated over a five-year period for variation in yield and yield component traits (K Singh, personal communication). Alleles derived from the wild species in the cultivated recurrent parent background have already been shown to contribute to significant increases in yield component traits including spikelets/panicle and grain weight as shown in Figure 2.

With the advent of next generation sequencing, combined with genetic maps and molecular markers, it is now possible to rapidly map and identify regions of the genome associated with specific components of a phenotype and to determine which parental line contributes the favorable allele(s) at a particular locus. These are helpful tools only with precise phenotyping which is critical to the success of gene/QTL mapping experiments. Unfortunately, the importance of refining and developing new methods for precise phenotypic measurements has so far been neglected in the genomics era. Overcoming the phenotyping gap represents the next greatest challenge for scientists involved in the rice genomics research.

### Conservation of the wild relatives of rice

Whereas progress in genomics and breeding techniques will certainly enable the successful transfer of complex traits present in the wild relatives of rice, including such divergent species such as *Porteresia* or *Hygroryza*, into cultivated rice, the long-term availability of these wild genetic resources is not assured [24]. Risks for populations of the wild relatives of rice, and plant biodiversity overall, include threats related to human activities and climate change [25\*\*]. The expansion of urban and agriculture areas leads to habitat destruction and fragmentation. For example, in India (Andhra Pradesh), a population of *O. officinalis* ssp. *malampuzhaensis* is endemic to the Nallamalais of Eastern Ghats, and needs urgent collection and conservation as its narrow distribution makes it more vulnerable to the disruption of its habitat [26]. Populations of *O. rufipogon* have been threatened by overgrazing and its consequences on water flow in Queensland, Australia [27], and by the construction of buildings in the Central Plains of Thailand [28]. Rising sea levels may be a problem for *Oryza* populations in the wetlands of Northern-Australia [27]. Populations of *O. meridionalis* in the same area are threatened by the infestation of an exotic grass (Para Grass) that inhibits germination [29], showing that invasive species are also a potential risk. When populations are fragmented, their genetic diversity is reduced, and they may not be able to adapt to rapid environmental changes. Sustainable conservation of the wild relatives of rice is thus an urgent problem that needs to be addressed before described and undescribed populations that could provide desirable traits of interest are lost forever.

The characterization and collection of species and populations are the first step for conservation and are well documented in many regions of the world where the wild relatives of rice are found [30,31]. However, extensive identification and collection are still needed in some areas, for example, Australia [27] and Venezuela [32]. To implement conservation strategies with an optimum result, it is urgent to understand the genetic diversity and structure among populations. A strong correlation between genetic and geographic distances, as well as high enzymatic polymorphism, was found among *O. glumaepatula* accessions in South-America [33]. This kind of information is necessary to evaluate the need to protect specific populations, and increased international efforts should be organized to systematically assess genetic diversity and screen germplasm for useful genes [24]. A critical step is the assessment of existing risks and conservation status of species and populations. Unfortunately, the IUCN Red List, which applies the most common criteria to assess threats to wild taxa, currently covers a low percentage of crop wild relatives (CWR). Only three *Oryza* species are listed (*O. neocaledonica*, *O. officinalis*, *O. rufipogon*), where *O. neocaledonica* is considered endangered. Moreover there is a need to develop new criteria suitable to estimate genetic diversity loss within a species [25\*\*].

Figure 2



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**(a)** Introgression for spikelet number/panicle in BILs derived from *O. longistaminata* acc. IRGC104301 (left) and *O. glumaepatula* acc. IRGC104387 (right) in the background of *indica* cv. PR114 (middle). **(b)** Introgression for grain weight in BILs derived from *O. rufipogon* acc. IRGC104301 (left) and *O. glumaepatula* acc. IRGC104387 (right) in the background of *indica* cv. PR114 (middle).

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Once such information is available, it will be possible to implement a systematic conservation plan for the wild relatives of rice. *Ex situ* conservation is already well developed, with extensive seed collections in GenBanks, the two largest being the International Rice Research Institute in the Philippines (4370 wild species and hybrids accessions at IRRI, <http://irri.org>), and Oryzabase in Japan (1703, <http://www.shigen.nig.ac.jp>). The downside of *ex situ* conservation is genetic erosion resulting from the lack of exposure to environmental variability and selection pressures, as well as genetic drift associated with germplasm regeneration [34\*,35]. Comparison of *ex situ* and *in situ* conservation efficiency of *O. rufipogon* populations in Dongxiang, China, showed that *ex situ* failed to maintain genetic diversity, thereby reducing allelic polymorphism by 34%, and genetic heterozygosity by 16% in 13 years [34\*]. On the contrary, *in situ* conservation of germplasm, along with its habitat, is a dynamic form of protection. It allows populations to adapt to environmental stress, leading to the creation of genetic novelties useful for future research and breeding activities. It is necessary to complement *ex situ* with *in situ* conservation to protect genetic diversity effectively, as well as ecosystem's biodiversity. However full practical implementation of *in situ* for the wild relatives of rice remains limited [36]. On the nine populations of *O. rufipogon* in Dongxiang studied in 1978, only three remained in 1995 [34\*]. A brick fence was constructed to protect two of these populations threatened by human activity. The challenges to implement and manage new protected sites on the long term remain the cost and the need for collaboration between national governments with international agriculture research programs. A short-term solution would be to improve the management of populations of the wild relative of rice found within existing protected areas.

On the positive side, the value of CWRs and the importance of their conservation have been of increased interest and are now considered a high priority for numerous national and international initiatives, like the FAO of the United Nation's project to establish a network for the *in situ* conservation of CWRs [25\*\*]. As nations are interdependent with regards to the global issues of food security and the loss of biodiversity, these problems should be addressed by all parties interested in agriculture and conservation.

### Opinion

As stated in the introduction, the improvement of rice in terms of increased yield, reduced environmental impact, and enhanced nutrition are important goals and key factors in helping to solve the 9 billion people question by 2050. In reality, we only have about 25 years to solve this pressing issue if we are to supply breeders with designed germplasm that will need to be adapted to different and changing growing conditions across the planet.

The ultimate goal of the International *Oryza* Map Alignment Project is to create a basic and translational research platform that can provide immediate access to virtually any region of the collective *Oryza* genome both genomically (i.e. access to high quality reference genome sequences linked to population resequencing data, and clone resources), and functionally (i.e. access to phenotyped advanced interspecific mapping populations and *in situ* conserved natural populations). Such a platform would facilitate the rapid identification of genes, molecular markers and germplasm from an evolutionary perspective that could be used to efficiently transfer a number of potentially important traits into cultivated rice.

A strong, collaborative and active synthesis of the three I-OMAP focus areas will be required if we are to accelerate the understanding and incorporation of useful wild alleles into cultivated rice and jump over the domestication bottleneck hurdle that has slowed conventional rice breeding for years. Ideally, advanced mapping populations should be developed and genotyped for a minimum of 10 diverse accessions of each *Oryza* species crossed with several elite varieties derived from the major and emerging rice growing regions around the world. Priority should be given to wild accessions possessing known abiotic and biotic stress resistances/tolerances (e.g. salt tolerance). Once created the AMPs should be extensively phenotyped under both field and greenhouse conditions in facilities like those designed at Huazhong Agricultural University (e.g. rice high-throughput automatic phenotyping center). Once a specific phenotype is linked to a set of markers the genomic region of the wild *Oryza* species can be pinpointed, extensively characterized, and targeted for introgression into elite cultivars and molecular cloning.

Given our accelerated time frame to solve the 9BPQ, the process described above should be transparent and fully integrated with the above Rice 2020 and Green Super Rice initiatives overall.

### Conclusion

OMAP, OGE & I-OMAP consortia have generated a vast array of *Oryza* genomic tools and data that can now be used to help solve the 9BPQ. It is anticipated that a full array of 16 reference quality *Oryza* genome sequences will be available by the summer of 2013 at the latest. Such a data set will facilitate rapid gene discovery and provide the evolutionary insights needed to feed the future.

### References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C: **Food security: the challenge of feeding 9 billion people**. *Science* 2010, **327**:812-818.

2. Parker J: *The 9 Billion-People Question*. The Economist; 2011.: (February 24th Issue).
  3. Timmer CP, Block S, Dawe D: **Long-run dynamics of rice consumption, 1960–2050**. In *Rice in the Global Economy: Strategic Research and Policy Issues for Food Security*. Edited by Pandey S, Byerlee D, Dawe D, Dobermann A, Mohanty S, Rozelle S, Hardy B. Los Banos: International Rice Research Institute; 2010: 39-47.
  4. Zhang Q, Li J, Xue Y, Han B, Deng XW: **Rice 2020: a call for an international coordinated effort in rice functional genomics**. *Mol Plant* 2008, **1**:1715-1719.
  5. Zhang Q: **Strategies for developing Green Super Rice**. *Proc Natl Acad Sci U S A* 2007, **104**:16402-16409.
  6. Ammiraju JSS, Song X, Luo M, Sisneros N, Angelova A, Kudrna D, Kim HR, Yu Y, Goicoechea JL, Lorieux M *et al.*: **The *Oryza* BAC resource: a genus-wide and genome scale tool for exploring rice genome evolution and leveraging useful genetic diversity from wild relatives**. *Breed Sci* 2010, **60**:536-543.
  7. Ammiraju JS, Luo M, Goicoechea JL, Wang W, Kudrna D, Mueller C, Talag J, Kim H, Sisneros NB, Blackmon B *et al.*: **The *Oryza* bacterial artificial chromosome library resource: construction and analysis of 12 deep-coverage large-insert BAC libraries that represent the 10 genome types of the genus *Oryza***. *Genome Res* 2006, **16**:140-147.
  8. Kim H, Hurwitz B, Yu Y, Collura K, Gill N, SanMiguel P, Mullikin JC, Maher C, Nelson W, Wissotski M *et al.*: **Construction, alignment and analysis of twelve framework physical maps that represent the ten genome types of the genus *Oryza***. *Genome Biol* 2008, **9**:R45.
  9. Ammiraju JS, Zuccolo A, Yu Y, Song X, Piegu B, Chevalier F, Walling JG, Ma J, Talag J, Brar DS *et al.*: **Evolutionary dynamics of an ancient retrotransposon family provides insights into evolution of genome size in the genus *Oryza***. *Plant J* 2007, **52**:342-351.
  10. Piegu B, Guyot R, Picault N, Roulin A, Sanyal A, Kim H, Collura K, Brar DS, Jackson S, Wing RA *et al.*: **Doubling genome size without polyploidization: dynamics of retrotransposon-driven genomic expansions in *Oryza australiensis*, a wild relative of rice**. *Genome Res* 2006, **16**:1262-1269.
  11. Hurwitz BL, Kudrna D, Yu Y, Sebastian A, Zuccolo A, Jackson SA, Ware D, Wing RA, Stein L: **Rice structural variation: a comparative analysis of structural variation between rice and three of its closest relatives in the genus *Oryza***. *Plant J* 2010, **63**:990-1003.
  12. Ammiraju JS, Fan C, Yu Y, Song X, Cranston KA, Pontaroli AC, Lu F, Sanyal A, Jiang N, Rambo T *et al.*: **Spatio-temporal patterns of genome evolution in allotetraploid species of the genus *Oryza***. *Plant J* 2010, **63**:430-442.
  13. Ammiraju JS, Lu F, Sanyal A, Yu Y, Song X, Jiang N, Pontaroli AC, Rambo T, Currie J, Collura K *et al.*: **Dynamic evolution of *Oryza* genomes is revealed by comparative genomic analysis of a genus-wide vertical data set**. *Plant Cell* 2008, **20**:3191-3209.
  14. Huang X, Kurata N, Wei X, Wang ZX, Wang A, Zhao Q, Zhao Y, Liu K, Lu H, Li W *et al.*: **A map of rice genome variation reveals the origin of cultivated rice**. *Nature* 2012, **490**:497-501.
  15. Goff SA, Ricke D, Lan T-H, Presting G, Wang R, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H *et al.*: **A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*)**. *Science* 2002, **296**:92-100.
  16. Yu J, Hu S, Wang J, Wong GK-S, Li S, Liu B, Deng Y, Dai L, Zhou Y, Zhang X *et al.*: **A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*)**. *Science* 2002, **296**:79-92.
  17. **The International Rice Genome Sequencing Project: the map-based sequence of the rice genome**. *Nature* 2005, **436**:793-800.
  18. ***Drosophila* 12 Genomes consortium: evolution of genes and genomes on the *Drosophila* phylogeny**. *Nature* 2007, **450**:203-218.
  19. Holt C, Yandell M: **MAKER2: an annotation pipeline and genome-database management tool for second-generation genome projects**. *BMC Bioinform* 2011, **12**:491.
  20. Brar DS, Kuldeep S: ***Oryza***. In *Wild Crop Relatives: Genomic and Breeding Resources: Cereals*. Edited by Kole C. Berlin/Heidelberg: Springer-Verlag Publications; 2011:321-365.
  21. McCouch SR, Sweeney M, Li J, Jiang H, Thomson M, Septiningsih E, Edwards J, Moncada P, Xiao J, Garris A *et al.*: **Through the genetic bottleneck: *O. rufipogon* as a source of trait-enhancing alleles for *O. sativa***. *Euphytica* 2007, **154**:317-339.
  22. Fukuoka S, Nonoue Y, Yano M: **Germplasm enhancement by developing advanced plant materials from diverse rice accessions**. *Breed Sci* 2010, **60**:509-517.
  23. Ali ML, Sanchez PL, Si-bin Y, Lorieux M, Eizenga GC: **Chromosome segment substitution lines: a powerful tool for the introgression of valuable genes from *Oryza* wild species into cultivated rice (*O. sativa*)**. *Rice* 2010, **3**:218-234.
  24. Ford-Lloyd BV, Schmidt M, Armstrong SJ, Barazani O, Engels J, Hadas R, Hammer K, Kell SP, Kang D, Khoshbakht K *et al.*: **Crop wild relatives-undervalued, underutilized and under threat?** *Bioscience* 2011, **61**:559-565.
  25. Maxted N, Kell S, Toledo A, Dulloo E, Heywood V, Hodgkin T, Hunter D, Guarino L, Jarvis A, Ford-Lloyd B: **A global approach to crop wild relative conservation: securing the gene pool for food and agriculture**. *Kew Bull* 2010, **65**:561-576.
- This paper proposes a step-by-step guide to identify and estimate the need to conserve crop wild relatives and implement systematic and effective conservation. They give multiple examples of studies on CWR distributions, as well as a map of the locations of high priority for CWR *ex situ* and *in situ* conservation.
26. Elangovan M, Kiran Babu P, Tonapi VA, Subba Rao LV, Sivaraj N: **Cultivated grasses and their wild relatives in Andhra Pradesh and their conservation concerns**. *Indian J Plant Genet Resour* 2012, **25**:166-173.
  27. Henry RJ, Rice N, Waters DLE, Kasem S, Ishikawa R, Hao Y, Dillon S, Crayn D, Wing R, Vaughan D: **Australian *Oryza*: utility and conservation**. *Rice* 2010, **3**:235-241.
  28. Nonomura K, Morishima H, Miyabayashi T, Yamaki S, Eiguchi M, Kubo T, Kurata N: **The wild *Oryza* collection in National BioResource Project (NBRP) of Japan: history, biodiversity and utility**. *Breed Sci* 2010, **60**:502-508.
  29. Williams PR, Collins EM, Grice AC, Nicholas DM, Perry JJ: **The role of fire in germinating Wild Rice (*Oryza meridionalis*), an annual grass of northern Australian wetlands threatened by exotic grass invasion**. *Ecol Manage Restor* 2011, **12**:74-76.
  30. Vaughan DA: (*IRRI*): *The Wild Relatives of Rice. A Genetic Resources Handbook*. IRRI press; 1994.
  31. Vaughan DA, Morishima H, Kadowaki K: **Diversity in the *Oryza* genus**. *Curr Opin Plant Biol* 2003, **6**:139-146.
  32. Berlingeri C, Crespo MB: **Inventory of related wild species of priority crops in Venezuela**. *Genet Resour Crop Evol* 2012, **59**:655-681.
  33. Veasey EA, De Andrade Bressan E, Zucchi MI, Vencovsky R, Cardim DC, Meireles da Silva R: **Genetic diversity of American wild rice species**. *Sci Agric* 2011, **68**:440-446.
  34. Xie J, Agrama HA, Kong D, Zhuang J, Hu B, Wan Y, Yan W: **Genetic diversity associated with conservation of endangered Dongxiang wild rice (*Oryza rufipogon*)**. *Genet Resour Crop Evol* 2010, **57**:597-609.
- This paper shows the importance of *in situ* conservation in maintaining the genetic diversity of populations of *O. rufipogon* from Dongxiang county, China, by comparing the structure of *ex situ* and *in situ* populations. As *O. rufipogon* accessions from Dongxiang are very closely related to the domesticated rice *japonica*, they are valuable germplasms that need to be protected. An *in situ* conservation plan was implemented to protect their habitat from human activity.
35. Sun J, Cao G, Ma J, Chen Y, Han L: **Comparative genetic structure within single-origin pairs of rice (*Oryza sativa* L.) landraces, from *in situ* and *ex situ* conservation programs in Yunnan of China using microsatellite markers**. *Genet Resour Crop Evol* 2012 <http://dx.doi.org/10.1007/s10722-011-9786-2>.
  36. Lu B: **The challenge of *in situ* conservation of crop wild relatives in the biotechnology era – a case study of wild rice**

## 10 Genome studies and molecular genetics

- species. In *Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives*. Edited by Maxted N, Dulloo ME, Ford-Lloyd BV, Iriondo JM, Frese L. CABI Press; 2012:211-216.
37. Jeuken MJW, Lindhaut P: **The development of lettuce backcross inbred lines (BILs) for exploitation of the *Lactuca salina* (wild lettuce) germplasm.** *Theor Appl Genet* 2004, **109**:394-401.
  38. Keurentjes JJB, Bentsink L, Alonso-Blanco C, Hanhart CJ, De Vries HB, Effgen S, Vreugdenhil D, Koornneef M: **Development of a near-isogenic line population of *Arabidopsis thaliana* and comparison of mapping power with a recombinant inbred line population.** *Genetics* 2007, **175**:891-905.
  39. Balint-Kurti PJ, Wissner R, Zwonitzer JC: **Use of an advanced intercross line population for precise mapping of quantitative trait loci for gray leaf spot resistance in maize.** *Crop Sci* 2008, **48**:1696-1704.
  40. van Berloo R: **GGT 2.0: versatile software for visualization and analysis of genetic data.** *J Hered* 2008, **99**:232-236.
  41. Xu J, Zhao Q, Du P, Xu C, Wang B, Feng Q, Liu Q, Tang S, Gu M, Han B, Liang G: **Developing high throughput genotyped chromosome segment substitution lines based on population whole-genome re-sequencing in rice (*Oryza sativa* L.).** *BMC Genomics* 2010, **11**:656.
  42. Cavanagh C, Morell M, Mackay I, Powell W: **From mutations to MAGIC: resources for gene discovery, validation and delivery in crop plants.** *Curr Opin Plant Biol* 2008, **11**:215-221.
  43. Rakshit S, Rakshit A, Patil JV: **Multiparent intercross populations in analysis of quantitative traits.** *J Genet* 2012, **91**:111-117.
  44. Flint-Garcia SA: **Structure of linkage disequilibrium in plants.** *Annu Rev Plant Biol* 2003, **54**:357-374.
  45. Huang X, Zhao Y, Wei X, Li C, Wang A, Zhao Q, Li W, Guo Y, Deng L, Zhu C *et al.*: **Genome-wide association study of flowering time and grain yield traits in worldwide collection of rice germplasm.** *Nat Genet* 2012, **44**:32-39.
  46. Yu J, Holland JB, McMullen MD, Buckler ES: **Genetic design and statistical power of nested association mapping in maize.** *Genetics* 2008, **178**:539-551.
  47. Boccoa R, Lorieux M, Seck PA, Futakuchi K, Manneh B, Baimey H, Ndjiondjop MN: **Agro-morphological characterization of a population of introgression lines derived from crosses between IR64 (*O. sativa* ssp. *indica*) and TOG5681 (*O. glaberrima*) for drought tolerance.** *Plant Sci*, in press.
  48. Gutiérrez AG, Carabalí SJ, Giraldo OX, Martínez CP, Correa F, Prado G, Tohne J, Lorieux M: **Identification of a rice stripe necrosis virus resistance locus and yield component QTLs using *Oryza sativa* × *O. glaberrima* introgression lines.** *BMC Plant Biol* 2010, **10**:6.
  49. Ram T, Deen R, Gautam SK, Ramesh K, Rao YK, Brar DS: **Identification of new genes for brown planthopper resistance in rice introgressed from *O. glaberrima* and *O. minuta*.** *Rice Genet Newsl* 2010, **25**:67.
  50. Ndjiondjop MN, Manneh B, Cissoko M, Drame NK, Kakai RG, Boccoa R, Baimey H, Wopereis M: **Drought resistance in an interspecific backcross population of rice (*Oryza* spp.) derived from the cross WAB56-104 (*O. sativa*) × CG14 (*O. glaberrima*).** *Plant Sci* 2010, **179**:364-373.
  51. Angeles-Shim RB, Asano K, Takashi T, Kitano H, Ashikari M: **Mapping of the glabrous gene in rice using CSSLs derived from the cross *Oryza sativa* ssp. *japonica* cv. *Koshihikari* × *O. glaberrima*.** *Proc 6th Int Rice Genet Symp [CD-ROM]; Manila, Philippines, 16-19 November 2009*: 2009.
  52. Kang JW, Suh JP, Kim DM, Oh CS, Oh JM, Ahn SN: **QTL Mapping of agronomic traits in an advanced backcross population from a cross between *Oryza sativa* L. cv. Milyang 23 and *O. glaberrima*.** *Kor J Breed Sci* 2008, **40**: 243-249.
  53. Luo JJ, Hao W, Jin J, Gao JP, Lin HX: **Fine mapping of *Spr3*, a locus for spreading panicle from African cultivated rice (*Oryza glaberrima* steud.).** *Mol Plant* 2008, **1**:830-838.
  54. Win KT, Yamagata Y, Miyazaki Y, Doi K, Yasui H, Yoshimura A: **Independent evolution of a new allele of a F1 pollen sterility gene *S27* encoding mitochondrial ribosomal protein *L27* in *Oryza nivara*.** *Theor Appl Genet* 2011, **122**:385-394.
  55. Miura K, Yamamoto E, Morinaka Y, Takashi T, Kitano H, Matsuoka M, Ashikari M: **The hybrid breakdown 1(t) locus induces interspecific hybrid breakdown between rice *Oryza sativa* cv. *Koshihikari* and its wild relative *O. nivara*.** *Breed Sci* 2008, **58**:99-105.
  56. Thanh PT, Phan PDT, Mori N, Ishikawa R, Ishii T: **Development of backcross recombinant inbred lines between *Oryza sativa* Nipponbare and *O. rufipogon* and QTL detection on drought tolerance.** *Breed Sci* 2011, **61**:76-79.
  57. Maas LF, McClung A, McCouch SR: **Dissection of a QTL reveals an adaptive, interacting gene complex associated with transgressive variation for flowering time in rice.** *Theor Appl Genet* 2010, **120**:895-908.
  58. Chen J, Huang D-R, Wang L, Liu GJ, Zhuang JY: **Identification of quantitative trait loci for resistance to whitebacked planthopper, *Sogatella furcifera*, from an interspecific cross *Oryza sativa* × *O. rufipogon*.** *Breed Sci* 2010, **60**:153-159.
  59. Fu Q, Zhang P, Tan L, Zhu Z, Ma D, Fu Y, Zhan X, Cai H, Sun C: **Analysis of QTLs for yield-related traits in Yuanjiang common wild rice (*Oryza rufipogon* Griff.).** *J Genet Genomics* 2010, **37**:147-157.
  60. Shan JX, Zhu MZ, Shi M, Gao JP, Lin HX: **Fine mapping and candidate gene analysis of *spd6*, responsible for small panicle and dwarfness in wild rice (*Oryza rufipogon* Griff.).** *Theor Appl Genet* 2009, **119**:827-836.
  61. Cheema KK, Bains NS, Mangat GS, Das A, Brar DS, Khush GS, Singh K: **Introgression of quantitative trait loci for improved productivity from *Oryza rufipogon* into *O. sativa*.** *Euphytica* 2008, **160**:401-409.
  62. Xie X, Jin F, Song MH, Suh JP, Hwang HG, Kim YG, McCouch SR, Ahn SN: **Fine mapping of yield-enhancing QTL cluster associated with transgressive variation in an *Oryza sativa* × *Oryza rufipogon* cross.** *Theor Appl Genet* 2008, **116**:613-622.
  63. Tan L, Liu F, Xue W, Wang G, Ye S, Zhu Z, Fu Y, Wang X, Sun C: **Development of *Oryza rufipogon* and *O. sativa* introgression lines and assessment for yield-related quantitative trait loci.** *J Integr Plant Biol* 2007, **49**:871-884.
  64. Chen Z, Hu F, Xu P, Li J, Deng X, Zhou J, Li F, Chen S, Tao D: **QTL analysis for hybrid sterility and plant height in interspecific populations derived from a wild rice relative, *O. longistaminata*.** *Breed Sci* 2009, **59**:441-445.
  65. Rangel PN, Brondani RPV, Rangel PHN, Brondani C: **Agronomic and molecular characterization of introgression lines from the interspecific cross *Oryza sativa* (BG90-2) × *Oryza glumaepatula* (RS-16).** *Genet Mol Res* 2008, **7**:184-195.
  66. Ram T, Laha GS, Gautam SK, Deen R, Madhav MS, Brar DS, Viraktamath BC: **Identification of new gene introgressed from *Oryza brachyantha* with broad-spectrum resistance to bacterial blight of rice in India.** *Rice Genet Newsl* 2010, **25**:57.
  67. Guo Y, Ge S: **Molecular phylogeny of *Oryzaceae* (Poaceae) based on DNA sequences from chloroplast, mitochondrial, and nuclear genomes.** *Am J Bot* 2005, **92**:1548-1558.
  68. Wang X, Tang H, Bowers JE, Paterson AH: **Comparative inference of illegitimate recombination between rice and sorghum duplicated genes produced by polyploidization.** *Genome Res* 2009, **19**:1026-1032.
  69. Tang L, Zou X, Achoundong G, Potgieter C, Second G, Zhang D, Ge S: **Phylogeny and biogeography of the rice tribe (*Oryzaceae*): evidence from combined analysis of 20 chloroplast fragments.** *Mol Phylogenet Evol* 2010, **54**:266-277.