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Chromosome Segment Substitution Lines: A Powerful Tool for the Introgression of Valuable Genes from *Oryza* Wild Species into Cultivated Rice (*O. sativa*)

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Abstract Wild species of rice (genus *Oryza*) contain many useful genes but a vast majority of these genes remain untapped to date because it is often difficult to transfer these genes into cultivated rice (*Oryza sativa* L.). Chromosome segment substitution lines (CSSLs) and backcross inbred lines (BILs) are powerful tools for identifying these naturally occurring, favorable alleles in unadapted germplasm. In this paper, we present an overview of the research involving CSSLs and BILs in the introgression of quantitative trait loci (QTLs) associated with the improved performance of rice including resistance to various biotic and abiotic stresses, and even high yield from wild relatives of rice and other unadapted germplasm into the genetic background of adapted rice cultivars. The CSSLs can be used to dissect quantitative traits into the component genetic factors and evaluate gene action as single factors (monogenic loci). CSSLs have the potential to uncover new alleles from the unadapted, non-productive wild rice accessions, develop genome-wide genetic stocks, and clone genes identified in QTL studies for functional genomics research. Recent development of high-density single-nucleotide polymorphism (SNP) arrays in rice and availability of custom-designed medium- and low-density SNP arrays will enhance the CSSL development process with smaller marker-defined segment introgressions from unadapted germplasm.

Keywords *Oryza* species · Backcross inbred lines · Chromosome segment substitution lines · Introgression lines · Genome-wide genetic stocks · *Oryza sativa*

Introduction

Rice (*Oryza sativa* L.) is a major cereal crop and a primary source of food for more than half of the world's human

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population (Khush 2005; www.csrees.usda.gov/newsroom/impact/2009/nri/02171_rice_gene.html). It is grown worldwide under a wide range of agro-climatic conditions. Rice productivity is affected by many abiotic and biotic stresses. Some of the major biotic stresses include diseases, such as bacterial leaf blight, blast, sheath blight, tungro virus, and insect pests, such as brown planthopper, green leafhopper, and stem borer. Similarly, abiotic stresses, such as drought, cold, salinity, acidity, iron toxicity, and submergence under water, severely affect rice production. Furthermore, changes in disease pathogen races and insect biotypes are continual threats to rice production. The genetic variability for some important traits including resistance to diseases, pests, and abiotic stresses are limited in the cultivated rice germplasm. During the course of domestication of cultivated rice from wild relatives, there was a significant reduction in genetic diversity (Brar and Khush 2003) as desirable agronomic traits were selected, leaving the number of alleles in cultivated rice reduced by 50–60% compared to wild rice (Sun et al. 2001). This emphasizes the importance of broadening the gene pool in rice by introgression of new genes from diverse sources.

In this paper, we review research illustrating the usefulness of chromosome segment substitution lines (CSSLs) and backcross inbred lines (BILs) for identifying new alleles of genes controlling agronomically important traits in rice wild relatives and their transfer into a cultivated rice genetic background to improve productivity, CSSL and BIL development strategies, and progress in developing CSSLs as genome-wide genetic stocks for gene cloning and functional genomics studies.

Potential of wild rice species

Wild species of rice have evolved in a wide range of environments over millions of years (Stebbins 1981). The *Oryza* wild species have either $2n=24$ or $2n=48$ chromosomes represented by the AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ, or HHKK genomes (Brar and Khush 1997; Jena and Khush 2000; Lu et al. 2010; Vaughan 1994). The geographical distribution of wild species with the AA genome is shown in Fig. 1. The AA genome is known to exist in Asia (*Oryza nivara* and *Oryza rufipogon*), Africa (African cultivated rice or *Oryza glaberrima*, *Oryza barthii*, and *Oryza longistaminata*), Australia (*Oryza meridionalis*), and Latin America (*Oryza glumaepatula*) (Vaughan et al. 2005).

Wild species of rice are a reservoir of many valuable genes which could be exploited for improving elite cultivars (Brar and Khush 1997). Almost all the wild rice species have been reported to contain some valuable traits (Table 1; Jena and Khush 2000). Although *O. glaberrima* is a cultivated species, mainly grown in Africa, it bears numerous interesting genes that can be used to improve *O. sativa*, including resistance to nematodes (both *Heterodera* and *Meloidogyne* genus) and insects (African gall midge; Brar and Khush 2006), viruses (rice yellow mottle virus, rice stripe necrosis virus; Ndjiondjop et al. 1999; Gutiérrez et al. 2010), and tolerance to drought stress (Ndjiondjop et al. 2010; Furuya et al. 1994). The first report of transfer of an agronomically important gene from a wild species to cultivated rice was the introgression of grassy stunt virus resistance from *O. nivara* (Khush et al. 1977). Other

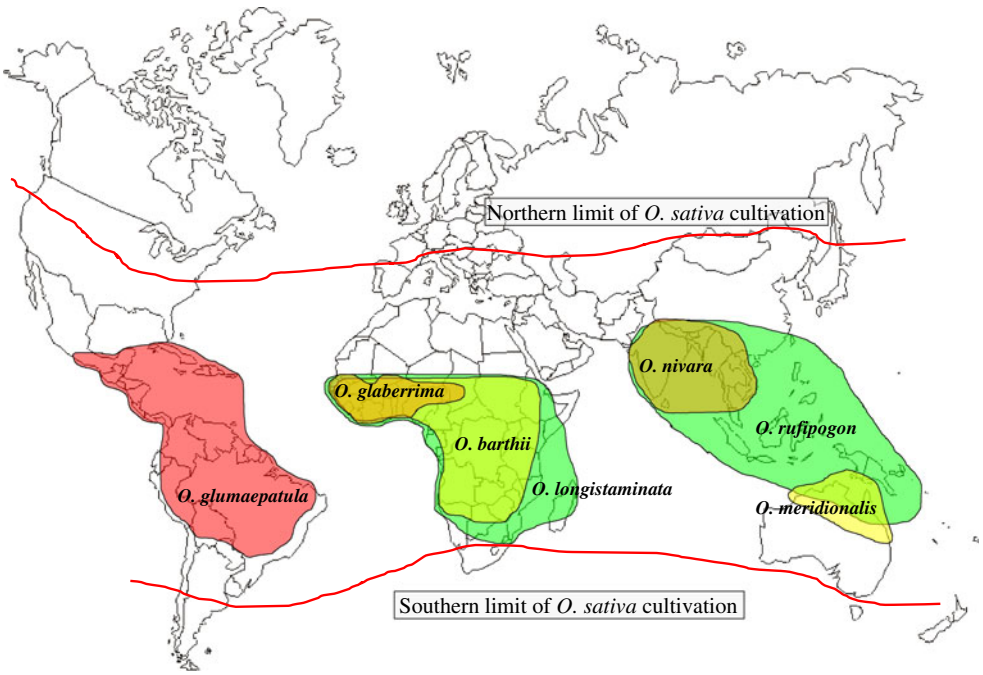


Fig. 1 Geographic distribution of AA genome wild *Oryza* species (Lu et al. 2010).

Table 1 Taxonomic classification of *Oryza* species with the chromosome number and genomic composition based on Lu et al. (2010), and potentially useful traits

Species	2*n*	Genome	Potentially useful traits[a]	Reference(s)[b]
Section Oryza				
Series Sativae				
O. sativa	24	AA	Cultigen	
O. nivara	24	AA	Resistance to grassy stunt virus, blast, sheath blight, stemborer and whorl maggot; drought avoidance, CMS, hybrid breakdown locus, pollen sterility locus	2, 3, 4, 14, 16, 21, 28, 30, 39
O. rufipogon	24	AA	Resistance to BB, stem rot, tungro virus, blast, stem borer and WBPH; elongation ability, tolerance to aluminum and soil acidity, source of CMS, improved yield, salinity tolerance, fertility restoration ability	2, 3, 4, 5, 7, 11, 16, 18, 21, 22, 23, 24, 27, 32
O. glaberrima	24	AA	Cultigen. Resistance to yellow mottle virus, rice stripe necrosis virus and nematodes; tolerance to aluminum, soil acidity and iron toxicity; drought avoidance, source of CMS	3, 12, 13, 15, 21, 34
O. barthii	24	AA	Resistance to BB, BLS, blast, brown spot, sheath blight, and GLH; drought avoidance, source of CMS	2, 3, 8, 9, 10, 29
O. glumaepatula	24	AA	Elongation ability, source of CMS	2, 3, 21
O. longistaminata	24	AA	Resistance to BB, blast, BPH, nematodes and yellow stemborer; drought avoidance, pollen/spikelet fertility	2, 3, 6, 21, 37
O. meridionalis	24	AA	Elongation ability, drought avoidance, sheath blight	2, 30
Series Latifoliae				
O. punctata	24 48	BB BBCC	Resistance to BPH, zigzag leafhopper	17
O. malampuzhaensis	48	BBCC	–	
O. minuta	48	BBCC	Resistance to sheath blight, blast, BB, BPH and GLH; grain size, awn length, heading date, panicle number, spikelet number	1, 17, 19, 25, 26, 31
O. schweinfurthiana	48	BBCC	–	
O. eichingeri	24	CC	Resistance to yellow mottle virus, BPH, WBPH and GLH	17
O. officinalis	24	CC	Resistance to thrips, BPH, GLH and WBPH	17
O. rhizomatis	24	CC	Drought avoidance, rhizomatous	17
O. alta	48	CCDD	Resistance to striped stemborer, high biomass production	17
O. grandiglumis	48	CCDD	High biomass production	17
O. latifolia	48	CCDD	Resistance to BPH, WBPH, GLH	17
Series Australienses				
O. australiensis	24	EE	Drought avoidance, resistance to blast, BPH	17, 35
Section Brachyantha				
Series Brachyanthae				
O. brachyantha	24	FF	Resistance to yellow stemborer, leaf-folder, whorl maggot and BB; tolerance to laterite soil	17, 33
Section Padia				
Series Meyerianae				
O. granulata	24	GG	Shade tolerance, adaptation to aerobic soil	17
O. meyeriana	24	GG	Shade tolerance, adaptation to aerobic soil	17
O. neocaledonica	24	GG	–	
Series Ridleyanae				
O. longiglumis	48	HHJJ	Resistance to blast, BB	17
O. ridleyi	48	HHJJ	Resistance to stemborer, whorl maggot, blast, BB	17
Series Schlechteriana				
O. schlerchteri	48	HHKK	–	

The potentially useful traits identified are listed by species with a reference to the report

[a] *BPH* brown planthopper, *GLH* green leafhopper, *WBPH* white-backed planthopper, *BB* bacterial blight; *BLS* bacterial leaf streak, *CMS* cytoplasmic male sterility

[b] *1* Amante-Bordeos et al. 1992, *2* Brar and Khush 1997, *3* Brar and Khush 2003, *4* Chaudhary and Khush 1990, *5* Chen et al. 2008, *6* Chen et al. 2009, *7* Chen et al. 2010, *8* Chu 1970, *9* Chu and Oka 1970, *10* Devadath 1983, *11* Fu et al. 2010, *12* Furuya et al. 1994, *13* Gutiérrez et al. 2010, *14* Heinrichs et al. 1985, *15* Heuer and Miezan 2003, *16* Hoan et al. 1997, *17* Jena and Khush 2000, *18* Chen et al. 2006, *19* Jin et al. 2004, *20* Jungtsung et al. 1986, *21* Khush and Brar 2002, *22* Kobayashi et al. 1993, *23* Kobayashi et al. 1994, *24* Li et al. 2002, *25* Linh et al. 2006, *26* Liu et al. 2002, *27* McCouch et al. 2007, *28* Miura et al. 2008, *29* Nayar 1968, *30* Prasad and Eizenga 2008, *31* Rahman et al. 2007, *32* Ram et al. 2005, *33* Ram et al. 2010a, b, *34* Sakamoto et al. 1990, *35* Suh et al. 2009, *36* Tseng and Oster 1994, *37* Vales 1985, *38* Velusamy et al. 1995, *39* Win et al. 2009

noteworthy reports include the transfer of cytoplasmic male sterility (CMS) from *O. sativa* f. *spontanea* (Lin and Yuan 1980) and the *Xa-21* gene for bacterial blight resistance from *O. longistaminata* (Khush et al. 1990). In these cases, the donor *Oryza* species were of the same genome class (AA) as cultivated rice (*O. sativa*). More recently, successful introgression of traits from non-AA genome species include the transfer of blast resistance from *Oryza minuta* (CCDD) and *Oryza australiensis* (EE) into *O. sativa* (Brar and Khush 2002; Fu et al. 2008; Jena and Khush 2000). Also, there are several more recent successful gene transfers from wild AA genome *Oryza* species into cultivated rice (Table 2).

Wild species contain novel yield-enhancing genes

In addition to being a largely untapped reservoir of genes for resistance to various biotic or abiotic stresses, wild species also contain genes that can enhance productivity and yield in the background of adapted cultivars. In general, wild species have smaller fruit (seeds), produce fewer seeds that often shatter, and other undesirable traits compared to cultivars, and thus appear to be poor as donors for enhancing yield. However, an early study in oats (*Avena* spp.) showed the transgressive segregants had about 20% yield increase over the recurrent parent (Lawrence and Frey 1975), and another study revealed that the yield of cultivated oats, *A. sativa*, was increased by 4–7% through crossing with wild species, *Avena sterilis* (Frey et al. 1984). In tomato (*Lycopersicon esculentum*), lines derived from crosses with the wild species, *Lycopersicon chmielewskii*, which has small green fruit, led to progenies with large red fruit and increased fruit weight (Rick 1974). Using advanced backcross lines or introgression lines, novel alleles from the wild progenitor of cultivated rice, *O. rufipogon* (IRGC 105491) were simultaneously identified, mapped, and introgressed into the genetic background of several adapted cultivars (McCouch et al. 2007). Alleles associated with the yield-enhancing traits from this *O. rufipogon* accession were incorporated into the genetic background of the well-known *indica* cultivar IR64 (Cheema et al. 2008; Septiningsih et al. 2003), the US *tropical japonica* cultivar Jefferson (Thomson et al. 2003), the *indica* V20B maintainer line of a widely used CMS line 'V20A' (Xiao et al. 1998), and the Korean *temperate japonica* cultivar Hwaeongbyeo (Xie et al. 2006). Alleles associated with the yield-related traits, such as spikelet number, grain weight, and panicle length also were identified in this *O. rufipogon* accession using a backcross inbred line population derived from a cross with Zhenshan 97B, an *indica* cultivar (Yu, personal communication). Similarly, alleles associated with the improved traits related to yield from two other *O. rufipogon* accessions were introgressed into the Chinese cultivars TeQing (Tan et al. 2007) and Guichao 2 (Tian et al. 2006). Yield trials of selected Jefferson/*O. rufipogon* near-isogenic lines (NILs) revealed yield enhancing QTLs when compared to the donor parent (Kimball et al. 2009). These studies suggest that additional novel alleles associated with high productivity and yield may be found in other accessions of *O. rufipogon*, as well as other wild *Oryza* species.

Challenges of transferring genes from unadapted germplasm or wild species

Most of the world's rice is produced from inbred varieties which have been developed almost exclusively from crosses between the accessions of the same subpopulation or between related subpopulations (*tropical japonica*×*temperate japonica*, *indica*×*aus*; Lu et al. 2005; Ni et al. 2002; Ali et al. 2011; in review). This is because it is difficult to obtain a random array of fertile recombinants from crosses between the *indica*×*japonica* varietal groups (in other words *O. sativa* ssp. *indica*×*O. sativa* ssp. *japonica*), largely due to the prevalence of subpopulation incompatibilities (Harushima et al. 2002; Oka 1988; Sano 1993; Win et al. 2009). The long-term consequence of restricting crossing and population development to within subpopulations is a limited pool of genetic variation available to rice breeders for identifying new and useful combinations of genes. Over time, this has compounded the problems associated with genetic bottlenecks that were primarily created during the domestication process and led to the cryptic form of genetic erosion that dramatically slows the rate of genetic gain (McCouch et al. 2007).

Inter-specific breeding offers a way of expanding the gene pool of cultivated rice and enlarging the range of genetic variation available for plant improvement (McCouch et al. 2007). Wide hybridization has been used for many years to introduce qualitative characters from wild species into elite breeding materials, such as disease and insect resistance, and male sterility (Brar and Khush 1997; Dalmacio et al. 1995). However, several incompatibility barriers, such as low crossing success, increased sterility, and limited recombination between the chromosomes of wild and cultivated species seriously hampered the transfer of useful genes (Brar and Khush 1986; Fu et al. 2008; Miura et al. 2008). Recent advances in tissue culture and genomics helped the production of wide hybrids between distantly related species. A major challenge is the difficulty in identifying genes from wild or unadapted materials that are likely to enhance the performance of elite cultivars without disrupting favorable gene complexes or allele

Table 2 List of the rice introgression lines populations (CSSLs/BILs/NILs) reported or under development from crosses between an African rice (*O. glaberrima*) or a wild *Oryza* species as the donor parent and an *O. sativa* cultivar as the recurrent parent

Donor parent	Recurrent parent (*O. sativa*)	Potentially useful traits/genes	References
O. nivara (IRGC105444)	*Japonica* (cv. Koshihikari)	Hybrid breakdown locus [*-hbd 1(t)*]	Miura et al. 2008
O. nivara (IRGC105444)	*Japonica* (cv. Taichung 65)	Pollen sterility gene (*S27-niv*^{s})	Win et al. 2010
O. nivara (IRGC105715)	*Japonica* (cv. Taichung 65)	Green rice leafhopper resistance	Fujita et al. 2004
O. rufipogon (DXCWR)	*Indica* (cv. Guichao 2)	Yield-related traits, drought tolerance	Tian et al. 2006; Zhang et al. 2006a, b.
O. rufipogon (IRGC105491)	*Indica* (cv. IR64)	Yield and yield components	Cheema et al. 2008; Septiningsih et al. 2003.
O. rufipogon (YJCWR)	*Indica* (cv. TeQing)	Yield and yield components	Tan et al. 2007
O. rufipogon (Dwr)	*Indica* (cv. Xieqingzao B)	WBPH and BPH resistance	Chen et al. 2010
O. rufipogon (IRGC105491)	*Indica* (cv. Zhenshan 97B, maintainer line)	Yield-related traits	Yu, personnel communication
O. rufipogon (YJCW)	*Indica* (cv. 93–11, restorer line)	Yield-related traits	Fu et al. 2010.
O. rufipogon (IRGC105491)	*Japonica* (cv. Hwaseongbyeo)	Grain weight	Xie et al. 2006
O. rufipogon (W1944)	*Japonica* (cv. Hwayeongbyeo)	Heading date, panicle number, shattering, grain characters	Lee et al. 2003
O. rufipogon (W1962)	*Japonica* (cv. Taichung 65)	Green rice leafhopper resistance	Fujita et al. 2003
O. glaberrima (Tog5675)	*Indica* (cv. IR64)	BPH resistance (*Bph1*)	Ram et al. 2010b
O. glaberrima (Tog5681)	*Indica* (cv. IR64)	Rice yellow mottle virus resistance	Ghesquiere et al. 1997
O. glaberrima	*Japonica* (cv. Koshihikari)	Glabrous gene	Angeles-Shim et al. 2009
O. glaberrima (IRGC103544)	*Indica* (cv. Milyang 23)	Yield and yield components	Kang et al. 2008
O. glaberrima (IRGC104038)	*Japonica* (cv. Taichung 65)	Pollen fertility, days to heading	Doi et al. 1997
O. glaberrima (CG14/IRGC96717)	*Japonica* (WAB56-104)	Drought resistance, early vigor	Ndjiondjop et al. 2010
O. glaberrima (MG12/IRGC103544)	*Tropical japonica* (cv. Caiapo)	Rice stripe necrosis virus resistance	Gutiérrez et al. 2010
O. glumaepatula (RS-16)	*Indica* (BG90-2)	Grain yield, cooking quality	Rangel et al. 2008
O. glumaepatula (IRGC105668)	*Japonica* (cv. Taichung 65)	Heading date, seed shattering	Sanchez et al. 2002; Sanchez et al. 2001
O. longistaminata	*Indica* (cv. RD23)	Pollen/spikelet fertility, plant height	Chen et al. 2009
O. meridionalis (Ng, W1625)	*Japonica* (cv. Taichung 65)	Seed shattering, awn presence	Sanchez et al. 2002; Kurakazu et al. 2001; Matsushita et al. 2003.
O. minuta (IRGC101141)	*Indica* (IR31917-45-3-2)	BPH resistance	Ram et al. 2010b
O. minuta (IRGC101141)	*Japonica* (cv. Hwaseongbyeo)	Awn length, heading date, grain traits	Linh et al. 2006; Jin et al. 2004
O. grandiglumis (IRGC101154)	*Japonica* (cv. Hwaseongbyeo)	Plant height, heading date, grain size, spikelets/panicle	Ahn et al. 2002a
O. brachyantha (IRGC101232)	*Indica* (cv. IR56)	Bacterial blight	Ram et al. 2010a
CSSLs under development			
O. nivara (IRGC100898, IRGC104705)	*Tropical japonica* (cv. Bengal)	To be evaluated for sheath blight and leaf blast resistance	Eizenga, personal communication
O. meridionalis (IRGC105306)	*Tropical japonica* (cv. Lemont)	To be evaluated for sheath blight resistance	Eizenga, personal communication
O. rufipogon (IRGC105491), *O. barthii* (IRGC10193), *O. glumaepatula* (GEN1233), *O. meridionalis* (OR44/W2112)	*Tropical japonica* (cv. Curinga)	To be evaluated for yield related traits, grain traits, tolerance to biotic/abiotic stresses	Lorieux, personal communication
O. nivara (IRGC106148), *O. rufipogon* (IRGC105567), *O. rufipogon* (W1944)	*Indica* (cv. IR64), *Tropical japonica* (cv. Cybonnet)	To be evaluated for yield related traits, grain traits, plant traits, tolerance to biotic/abiotic stresses	Tung et al. 2010
O. nivara (IRGC100897), *O. rufipogon* (IRGC105491, IRGC106424, Dongxiang), *O. barthii* (IRGC101937),	*Indica* (cv. Zhenshan 97B and cv. 93–11)	To be evaluated for yield related traits, grain traits, tolerance to biotic/abiotic stresses	Yu, personal communication

Table 2 (continued)

Donor parent	Recurrent parent (*O. sativa*)	Potentially useful traits/genes	References
O. glumaepatula (IRGC100969), *O. meridionalis* (IRGC104092)			
O. glaberrima (IRGC96717)	*Indica* (cv. Zhenshan 97B)	Drought tolerance, grain quality	Yu, personal communication
O. glaberrima, O. barthii	*Tropical japonica* (cv. LaGrue), *Temperate japonica* (cv. M-202)	To be evaluated for yield related traits, grain traits, tolerance to biotic/ abiotic stresses	Eizenga and Sanchez, personal communication.

BPH brown planthopper, *WBPH* white-backed planthopper

combinations. A substantial "pre-breeding" research can provide a platform to identify and transfer favorable alleles from wild and unadapted sources into elite rice cultivars (McCouch et al. 2007). Often, the genetic potential of an individual is obscured by the presence of a few deleterious alleles that confound the breeders' ability to accurately determine its breeding value. Today, with the advent of quality sequence information, genetic maps and molecular markers, it is possible to identify regions of the genome associated with specific components of a phenotype and determine which parent contributes the favorable allele(s) at a particular locus. This information is very helpful for selecting which genes or components of quantitative trait variation to introduce from a wild or exotic gene pool into an elite cultivar.

CSSL development strategies

In the crossing program to develop rice CSSLs, the recurrent parent, which is usually an adapted cultivar or germplasm accession, is used as the female, while the donor parent, usually a wild species or unadapted germplasm accession, is used as the male in order to avoid cytoplasmic sterility in subsequent generations. A typical backcross scheme to develop CSSLs is shown in Fig. 2. A set of polymorphic markers (either SSRs or SNPs) is used in the selection of backcross lines during the backcrossing process. For backcrossing, either the F_1 hybrid plant(s) or the recurrent parent can be used as female, depending on the fertility of the F_1s. Next, a set of 50–60 randomly chosen BC_1F_1 individuals is backcrossed again to generate BC_2F_1s. A set of 10–12 individuals from each of the BC_2F_1 families (50–60 BC_1founder lines) is selected for the first-round of genotyping with DNA markers. About 150–200 selected BC_2F_1 individuals (see Fig. 3 showing introgressed segments) are used for the next round of backcrossing to generate BC_3F_1s. About 120–150 BC_3F_1 individuals are selected for selfing after genotyping a second time from 400 to 500 BC_3F_1 individuals, to develop homozygous lines or for the next round of backcrossing to generate BC_4F_1 plants. During each generation of selection, one must identify individuals that carry the introgression(s) of interest along each chromosome. Graphical genotyping software programs, such as GGT (van Berloo 2008) or CSSL Finder (http://mapdisto.free.fr/CSSLFinder), are very useful tools to select desired backcross progenies based on their genotypic content. The third or last round of selection is made after genotyping of BC_3F_2 or BC_4F_2 individuals. At this stage, each individual generally contains only one marker defined-target segment and all the selected individuals together cover the whole donor genome in an overlapping manner as shown in Fig. 4. In order to obtain adequate seed, 10 plants are selfed from each of the selected BC_4F_2 families carrying the desired traits and target introgressed segments. These introgression lines are suitable for phenotypic evaluation of the "trait(s) of interest", and once they are fixed, may be evaluated in multiple environments.

A useful tool for developing rice CSSLs is the rice universal core genetic map (Orjuela et al. 2010). This map can easily be used to select polymorphic SSR markers between the parents in a specific cross or crosses, and provide a platform for making comparisons across a wide range of maps derived from crosses involving parents belonging to diverse groups. Markers on the universal core map of rice provide a set of uniformly distributed polymorphisms that readily distinguish cultivars and wild accessions, especially within the AA genome.

Use of 384-SNP assays substantially increases the efficiency of genotyping the backcross lines (Tung et al. 2010). Using multiple 384-SNP assays, a large number of SNP markers can be employed for genotyping which will allow more precise identification of the genome-wide introgression lines. The SNP-linked genes/QTLs on the introgressed segments can also be mapped and cloned with relative ease, due to the small size of the substituted chromosome segments in the CSSLs, and the known physical locations of SNPs (Zhu et al. 2009). Even larger numbers of polymorphic SNP markers with uniform

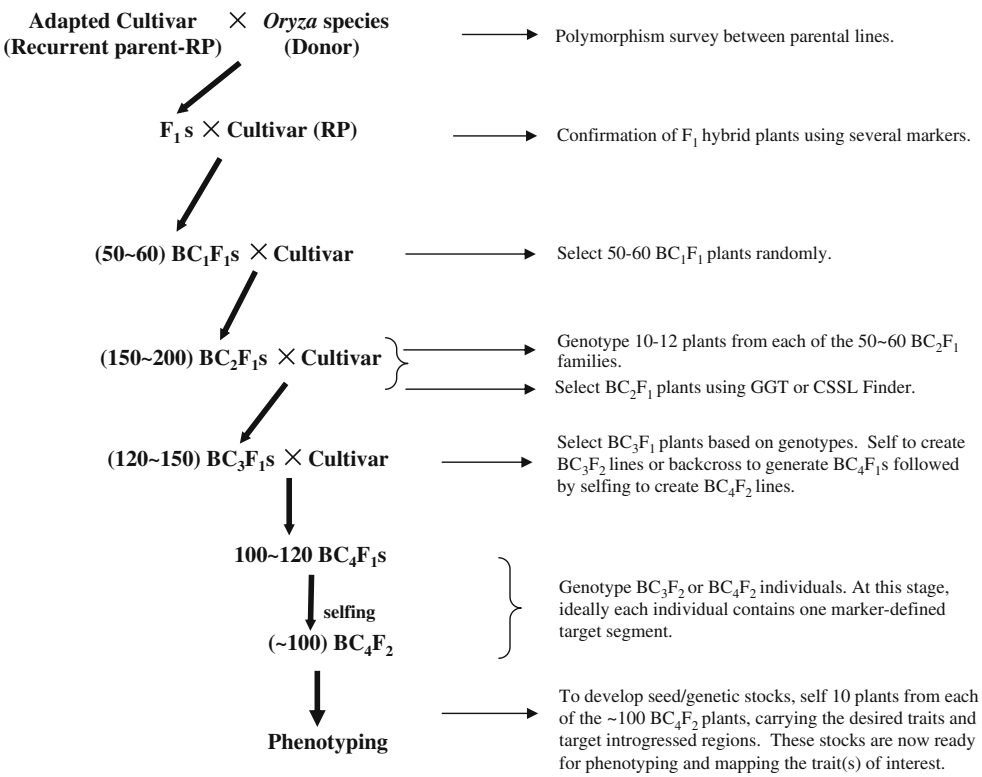


Fig. 2 A backcross scheme for developing chromosome segment substitution lines (CSSL) utilizing molecular markers for selecting the progeny each generation.

distribution over the 12 rice chromosomes can be chosen from the newly constructed high density 44K-SNP array or 1 million SNP array (a future endeavor) after genotyping the parental lines (Tung et al. 2010). Using saturated maps of SNPs would help in identifying the double recombination events that cannot be revealed by lower density maps. Identification of these double recombination events is critical as they can result in false interpretation of the results.

Indica–japonica inter-subspecific CSSLs

In addition to being an effective tool for introgressing valuable genes from wild species to cultivated rice and subsequently transferring genes to commercial cultivars, CSSLs have been used to introgress genes from one subspecies to another in rice, especially between the varietal groups, *indica* into *japonica* or *japonica* into *indica*. Because of the incompatibility barriers encountered,

Fig. 3 Graphical genotypes of 20 BC_2F_1s showing introgressed segments from *O. rufipogon* in the background of IR64 based on 384-SNP assays. The heterozygous segments are purple and the homozygous IR64 segments are light blue while yellow represents missing segments. Genotypes were created in GGT (van Berloo 2008). The consensus genotype identifies the homozygous IR64 background across all lines.

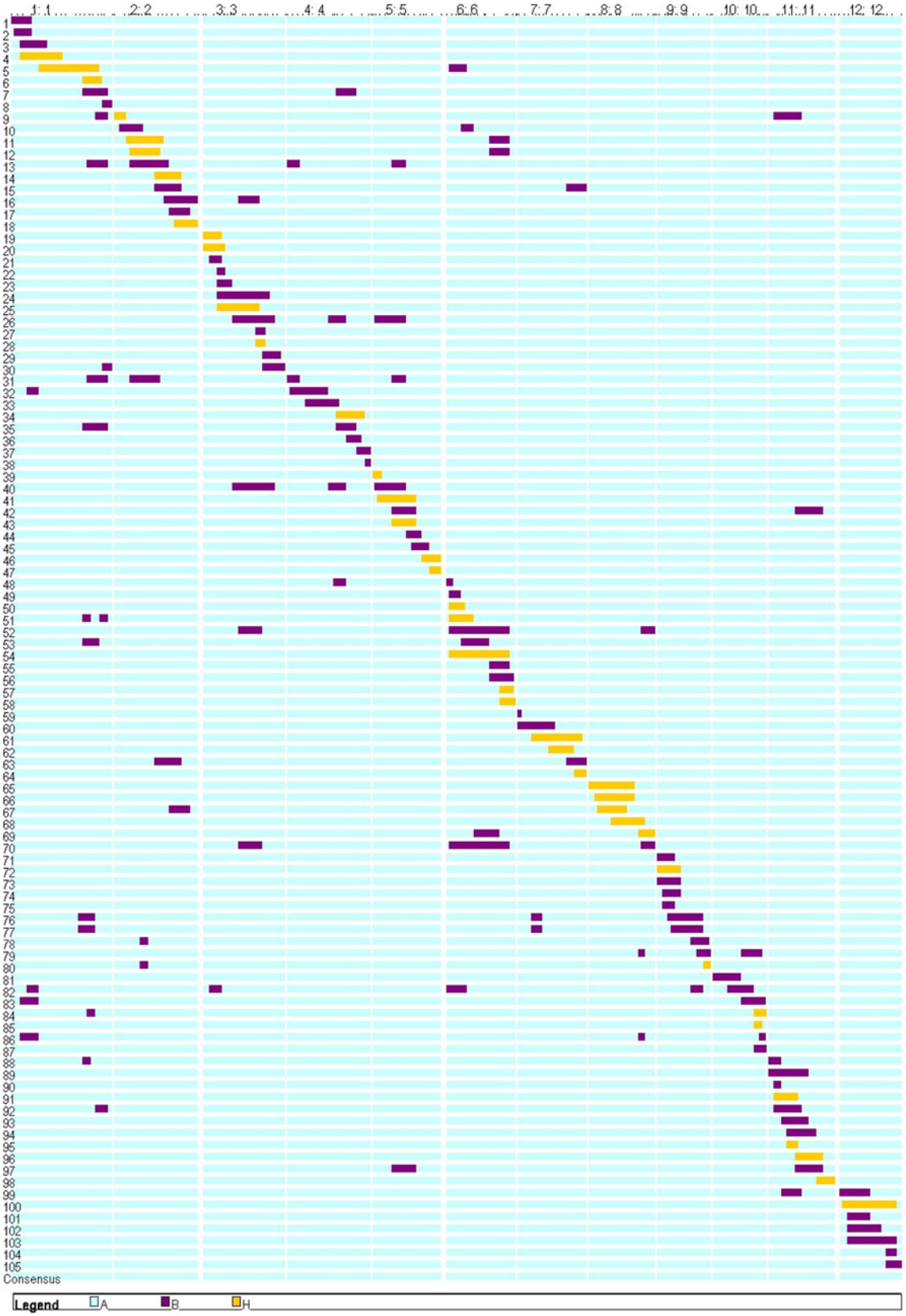
some researchers have chosen to develop CSSLs to introgress chromosome segments containing genes of interest from either *indica* or *japonica* cultivars into the desired genetic background (Harushima et al. 2002; Oka 1988; Sano 1993) even though limited success was achieved because of high sterility or hybrid breakdown (Miura et al. 2008; Win et al. 2009). Reported successful intra-specific CSSLs include 'IR24', an *indica* donor parent, introgressed into 'Asominori', a *japonica* recurrent parent, in which alleles for some important quantitative traits were mapped including eating quality (Wan et al. 2004), grain dimension and chalkiness (Wan et al. 2005; Wang et al. 2007), grain length (Wan et al. 2006), and the sterility gene, *S31* (Zhao et al. 2007). Similarly, CSSLs with donor segments from Kasalath, an *Indica* landrace from India, in the genetic background of the *Japonica* cultivar, Koshihikari, have been used to introgress and map QTLs affecting heading date (Ebitani et al. 2005), cadmium concentration in brown rice (Ishikawa et al. 2005), increased panicle number per plant, increased grain number per panicle, and increased root mass (Maduoka et al. 2008), among others. Other examples of CSSLs containing donor segments from an *indica* cultivar in a *japonica* genetic background include: Koshihikari (*japonica* recurrent parent) × Nona Bokra (*indica* donor parent) developed to map QTLs for salt tolerance and heading date (Takai et al. 2007), and Sasanishiki (*japonica*, recurrent parent) × Habataki (high yielding *indica* donor) which was used to dissect and map QTLs for panicle architecture (Ando et al. 2008).

With similar objectives as described above, CSSLs have also been developed in the background of an *indica* cultivar with donor segments from *japonica* varieties. These populations include HJX74 (*indica*, recurrent parent) × four *japonica* donors (Suyunuo, IRAT261, Lemont, and JAPAR9) developed to map QTLs for different traits including days to heading and grain length (Xi et al. 2006); Zhenshen 97B (*indica* recurrent parent) × Nipponbare (*japonica* donor) to map QTL alleles enhancing the culturability of *indica* rice (Zhao et al. 2009); and '93–11' (*indica* recurrent parent) × Nipponbare (*Japonica* donor) to detect QTLs for panicle number per plant and grain yield under low nitrogen and phosphorus conditions (Wang et al. 2009) and seed shattering, grain length, and grain width (Zhu et al. 2009).

Backcross inbred lines, an alternative tool to CSSLs for gene introgression

Like CSSLs, populations of BILs can be used to introgress useful genes from wild species into the genetic background of cultivated rice. Wehrhahn and Allard (1965) demonstrated the effects of individual QTLs in wheat (*Tritcum aestivum*) that can be measured by using backcross inbred lines. BILs are characterized by a low proportion of donor parent in each line and they are well suited for mapping inter-specific variation. Since each line carries only a small fraction of the wild species genome, most of the fertility problems can be eliminated and yield-associated traits can be measured (Eshed and Zamir 1995). BIL development, like CSSL development, involves crossing the wild species accession containing genes of interest to a cultivated parent. The F_1 hybrid is backcrossed with the cultivated parent and the BC_1F_1 plants (50–75 founder lines) produced are backcrossed with the cultivated parent to generate BC_2F_1 plants. The BC_2F_1 plants representing all the founder lines are generally used for developing BILs. A single BC_2F_1 seed selected from each of the approximately 200 lines composing the population is selfed to produce BC_2F_2 plants. If sterility is a major problem, these lines may need to be backcrossed an additional generation, to the BC_3F_1 before being selfed. Following the single-seed descent method, the $BC_{2:3}F_2$ plants are advanced to $BC_{2:3}F_6$ to create BILs. At this stage, these BILs are nearly homozygous and may be genotyped with molecular markers. Based on genotype, populations of approximately 100 BILs containing one or more introgressed wild segments may be selected to represent the genome of the wild species donor. If any regions of the genome are not represented in the population, additional backcrosses can be made to recover the missing wild segment(s) in the cultivated parent background. A set of ~100 BILs comprise a library of BILs, similar in nature to the aforementioned CSSLs, but each line often has several homozygous donor segments randomly distributed in the genetic background of the cultivated (recurrent) parent, as compared to the CSSLs, where each line generally carries a single, defined introgression segment from the divergent (wild) donor genome. The BIL libraries are not developed with the concept of whole donor genome coverage unlike CSSL libraries which are basically designed to recover the whole donor genome. Ideally, CSSLs are a collection of NILs because each CSSL carries a single donor segment in the near-isogenic background of the recurrent parent genotype (Yamamoto and Yano 2008). But in reality, in CSSL populations generated after three or four rounds of backcrossing, some individual lines still may contain more than the one targeted segment (Fig. 4). In this case, through additional backcrossing of these individuals, lines with purely a single segment introgression could be generated in order to obtain a final set of single introgressions, thus these could also be referred to as NILs.

For the purposes of genetic analysis, the advantages of using BIL or CSSL populations include (1) the presence of a high genetic and morphological similarity between lines that enables more precise estimates of quantitative traits


1: 1
2: 2
3: 3
4: 4
5: 5
6: 6
7: 7
8: 8
9: 9
10: 10
11: 11
12: 12
Consensus
Legend
A
B
H

◀ **Fig. 4** The graphical genotypes for a set of 105 CSSLs with donor segments from *O. rufipogon* (IRGC105491) in the background of Zhenshan 97B, an *indica* hybrid rice maintainer line. The CSSLs are arranged vertically in order of their chromosome substitution segments, with purple and yellow indicating homozygous and heterozygous wild alleles, respectively. Genotypes were created in GGT (van Berloo 2008). The consensus genotype identifies the homozygous Zhenshen 97B background across all CSSLs.

and (2) the opportunity to study QTL×environment interactions more accurately because lines are homozygous, making it possible to evaluate phenotypic variation collected from multiple replications across years and environments (Jeuken and Lindhaut 2004). A practical advantage of BILs or CSSLs for commercial breeding purposes is the low percentage of the wild species parent genome, which allows the transfer of an interesting trait into a commercial cultivar to be relatively straightforward and rapid. As with CSSLs, the development of a complete set of BILs is labor- and time-intensive, and the marker evaluation is relatively expensive. For genetic studies involving epistasis, individual BILs or CSSLs may be crossed to identify interacting loci.

There are a number of reports of using BILs to introgress QTLs from the wild rice species into cultivated rice, some of which are: alleles from *O. rufipogon* associated with the increased number of grains (Tian et al. 2006) and improved drought tolerance (Zhang et al. 2006a, b) in the background of the *indica* cultivar Guichao 2, alleles associated with increased yield and its components in the background of the *indica* cultivar IR64 (Cheema et al. 2008), alleles associated with early flowering (Maas et al., 2010) in the background of the *tropical japonica* cultivar Jefferson, alleles from *O. glumaepatula* increased yield in the background of *indica* cultivar BG90-2 (Rangel et al. 2008), and alleles from *O. glaberrima* improved grain size in the background of *indica* male sterile line V20A (Li et al. 2004; Table 2). There are also reports of introgression between the subspecies, *indica* and *japonica*, such as alleles from the *indica* donor parent 'Kasalath' increased tiller number and salt tolerance (Takehisa et al. 2004) and low temperature germination ability (Miura et al. 2001) in the background of the *temperate japonica* cultivar 'Nipponbare'.

CSSL libraries in rice currently under development

Currently, over two dozen CSSL or BIL libraries in rice are being developed at different research institutions around the world (Table 2). As a collaboration between the University of Arizona, United States Department of Agriculture-Agricultural Research Service (USDA-ARS), and the University of Arkansas, it is anticipated that four BIL libraries will be developed using both *O. glaberrima* and *O. barthii* donors and 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 (Table 2; Eizenga and Sanchez, personal communication). At USDA-ARS, Stuttgart, AR, under the RiceCAP program, three BILs libraries are being developed using *O. nivara* and *O. meridionalis* as donors and two US *tropical japonica* cultivars, Bengal and Lemont as recipient parents with a view to map sheath blight and blast resistance genes (Table 2; Eizenga, personal communication).

At Huazhong Agricultural University in China, backcrossing programs are underway to develop 14 CSSL/IL libraries using seven AA genome wild *Oryza* species accessions that have BAC-end sequence as part of the *Oryza* Map Alignment project (Wing et al. 2007) as donors and two *indica* cultivars as recurrent parents, Zhenshan 97B, a hybrid rice maintainer line, and 93–11, a restorer line sequenced by the Beijing Genomics Institute (Table 2; Yu, personal communication). The donor accessions belong to the AA-genome wild species, *O. barthii*, *O. glumaepatula*, *O. meridionalis*, *O. nivara*, and *O. rufipogon.* The objective of generating these introgression lines is to develop a resource platform for identification of QTLs and gene discovery in these wild species which have the potential to improve the performance of cultivated rice. Under this program, a set of 105 CSSLs with donor segments from *O. rufipogon* (IRGC105491) in the background of Zhenshan 97B has been developed (Fig. 4). A companion mapping population (BC_2F_5) derived from the same cross combination was also developed and evaluated in the field. More than 50% of the alleles derived from the wild parent contributed to the increase of yield-related traits, such as spikelet number per panicle, grain weight, and panicle length, confirming earlier reports using the same donor in five different recurrent parent backgrounds (McCouch et al. 2007). In addition, a set of ILs (BC_3F_4) carrying donor segments from *O. glaberrima* in the genetic background of Zhenshen 97B are being developed with the objective of improving drought tolerance and grain quality.

The International Center for Tropical Agriculture and Institut de Recherche pour le Développement (CIAT/IRD) 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-CNPAF (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_2.pdf; Lorieux, personnel

communication). Development of introgresion lines from the *O. sativa*×*O. glumaepatula* interspecific cross (Rangel et al. 2008) was also undertaken in the GCP initiative.

Six CSSL libraries are being constructed through collaboration between Cornell University, USDA-ARS Stuttgart, Arkansas and the University of Arkansas (Tung et al. 2010) using three diverse *O. rufipogon/O. nivara* accessions as donors and two *O. sativa* recurrent parents, IR64, an *indica* developed by the International Rice Research Institute (IRRI) in the Philippines, and Cybonnet, a long-grain *tropical japonica* cultivar adapted to the southern USA (Table 2).

As these introgression lines are developed, the CSSL libraries will be evaluated under field and/or greenhouse conditions for different agronomic and quality parameters. This was recently illustrated by Gutiérrez et al. (2010) where 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. Selected introgression lines will be used as breeding lines to transfer various traits of interest into the new cultivars, for cloning genes, and for functional genomics research.

Potential of CSSLs for allele discovery

The development of CSSLs in *O. sativa* represents a powerful approach for broadening the genetic variation in the cultivated rice gene pool and better utilizing the genetic potential of wild rice germplasm resources. CSSLs contain marker-defined chromosome segment(s) from agriculturally unadapted donor plants in the background of an adapted cultivar. A population of CSSLs ideally covers the entire donor genome and these lines have been referred to as introgression lines (Eshed and Zamir 1995), contig lines (Ghesquiere et al. 1997), NILs (Tanksley and McCouch 1997) and are collectively referred to as an exotic library (Zamir 2001). CSSL development involves a series of backcrosses to the recurrent (recipient) parent until the recurrent parent's genome is fully recovered and provides a method of broadening the genetic variation of plant materials for plant breeding (Tanksley and McCouch 1997; Zamir 2001).

CSSLs are also effective tools for the detection of genes and QTLs controlling various traits from wild or unadapted germplasm. As differences between CSSLs and their parental lines must be due to the genes and QTLs located in the introgressed regions (Aida et al. 1998; Eshed and Zamir 1994), CSSLs are considered excellent starting materials for dissecting complex traits into a set of monogenic loci (Zhu et al. 2009). Through additional backcrossing and selfing of CSSLs, NILs can be generated for fine mapping genes, as well as, for precise estimation of the effect of each gene after testing in multiple environments.

In tomato, CSSLs covering the entire genome were used to identify alleles from a wild species that were associated with the increased total soluble solids and green fruit weight (Eshed and Zamir 1995). Similarly, Knorff et al. (2004) reported using introgression lines to identify an allele originating from an exotic barley accession that was associated with early flowering in cultivated barley (*Hordeum vulgare*). At Kyushu University in Japan, a series of CSSLs in rice were developed using accessions of *O. glaberrima* and several wild rice species (*O. glumaepatula*, *O. meridionalis*, *O. nivara*, *O. rufipogon*) as donor parents in the background of *O. sativa* cv. Taichung 65 (Doi et al. 1997; Kurakazu et al. 2001; Sobrizal and Yoshimura 2002; Matsushita et al. 2003) with the objective of uncovering new, naturally occurring quantitative trait variation. Using these introgression lines, alleles associated with a number of desirable traits were identified, such as awn character (Matsushita et al. 2003), seed shattering (Sanchez et al. 2001), blast resistance (Jeung et al. 2003), green leafhopper resistance (Fujita et al. 2004), bacterial blight resistance (Ram et al. 2010a), and brown planthopper resistance (Deen et al. 2010). At CIAT, using introgression lines of *O. glaberrima* cv. MG12 (IRGC103544) in the background of the *O. sativa tropical japonica* cultivar Caiapo, a rice stripe necrosis virus resistance locus was identified (Table 2; Gutiérrez et al. 2010).

Backcross introgression lines are often the only type of segregating population that can be derived between cultivated and wild species. This is because a strong sterility barrier often separates the species and does not allow one to derive classical segregating populations such as F_2, BC, DH (doubled haploid), and RILs (recombinant inbred lines) from the interspecific F_1 hybrids. Although sterility prevents developing populations through selfing, the backcrossing used to develop CSSLs and other kinds of ILs enables the recovery of fertility.

Most agronomic traits of interest to breeders are genetically complex, governed by multiple genes or QTLs that interact with each other, as well as the environment. In the genetic analysis of such quantitative traits, CSSLs are a powerful tool as they can be used to systematically detect QTLs with small additive effects that are masked by QTLs with larger effects in primary mapping populations, such as F_2 populations or RILs (Ebitani et al. 2005; Keurentjes et al. 2007). Because QTL analysis is based on a statistical calculation, it is difficult to identify the precise location of an individual QTL, even using a large population and saturating the QTL region with many markers. On the contrary, NILs, which carry only one target QTL in a

unique genetic background, facilitate the dissection, cloning, and comprehensive analysis of target QTLs. CSSLs, which represent a genome-wide collection of NILs, are very useful as precursors for the fine mapping of QTLs.

CSSLs as genetic stocks for functional genomics research

The most common types of genetic stocks for functional genomics studies include genome-wide insertion and deletion mutants which have been developed in rice by several groups (Guiderdoni et al. 2003; Leung et al. 2003; Upadhyaya et al. 2003). The major limitation of using mutants for functional genomics research is the absence of detectable (visible) phenotypes for most mutants even when the disrupted gene(s) in a mutant line is known. Also, it remains an unanswered question as to whether the mutant stocks with single gene mutations are suitable for functional genomics studies of complex phenotypes, which are controlled by many genes and influenced by the environment (Li et al. 2005). Most rice traits, such as grain yield, resistance to abiotic stresses, and quality parameters are quantitative in nature. Understanding the genetic basis of complex phenotypes in rice is a major challenge. Over the past two decades, researchers worldwide have dissected quantitative trait variation in rice and other crops by QTL mapping and thousands of QTLs influencing the quantitative traits have been identified (Ni et al. 2009). There are many QTLs affecting specific quantitative traits and they are widely distributed in the rice genome but a few QTLs are detectable in a single mapping population and environment (Li et al. 2005). With the completion of the rice genome sequence, QTL cloning, while still time consuming and labor intensive, has become increasingly routine in rice where genes underlying QTL are isolated based on CSSLs/NILs and characterized on a regular basis (Shomura et al. 2008; Wei et al. 2010; Zhang et al. 2006a, b).

Although artificially induced variation, such as the mutants mentioned earlier, have been used historically for genetic studies in plant species, the development of new, cost-effective genomics, and re-sequencing technologies have made it possible to directly investigate the functional significance of natural variation in plants (Gilad et al. 2009; Morozova and Marra 2008; Mitchell-Olds and Schmitt 2006). In combination with these new genomic technologies, the availability of genome-wide libraries of introgression lines (i.e., CSSLs) represent particularly useful resources for assessing the phenotypic consequences associated with diverse donor alleles in specific genetic backgrounds, and subsequently for map-based cloning of genes (Ahn et al. 2002a, b; Doi et al. 2002; Koornneef et al. 2004; Sobrizal et al. 1996). Because the CSSLs could be used to dissect complex quantitative traits into a set of monogenic loci and to assign phenotypic values to different alleles at the locus of interest (Xi et al. 2006), they offer biologists access to essential tools that can be used to determine the genetic and functional basis of trait(s) down to the base pair level.

Application of CSSLs or introgression lines to breeding schemes

When CSSL libraries are generated in the genetic background of an adapted, elite cultivar, it provides plant breeders with an opportunity to select superior introgression lines possessing particular traits of interest. The best performing introgression lines may be recommended as new cultivars or used as parents in a breeding program to transfer the introgressed segments with superior performing genes into new cultivars under development. At IRRI, genes for resistance to brown planthopper, bacterial blight, blast, grassy stunt virus and tungro virus, and genes for tolerance to acid soil and cytoplasmic male sterility have been introgressed from different AA genome *Oryza* species, as well as, from distantly related genomes of wild species into elite breeding lines (Brar and Khush 2002; Multani et al. 2003). In some instances, selected lines carrying the introgressed genes have been released as commercial cultivars. Also, in some cases favorable alleles for multiple traits were detected in the same chromosome regions, thus CSSLs with these chromosome segments are potentially useful breeding materials for simultaneously improving multiple traits.

After the discovery of interesting traits in introgression lines (ILs), desirable QTLs can be combined through crossing of IL-QTLs to pyramid into a common genetic background with the aid of molecular markers (Ashikari and Matsuoka 2006; Xi et al. 2006). The validity of this approach has been demonstrated by pyramiding two QTLs involved in plant height and grain number (Ashikari et al. 2005). QTL pyramiding into the Koshihikari (*temperate japonica* cultivar) genetic background showed increased grain production (+23%) and reduced plant height (−20%) when compared to Koshihikari without the introgression from Kasalath, the *indica* donor parent. This illustrates that breeders can produce new cultivars with interesting traits by combining introgression lines carrying the desired QTLs using marker assisted selection.

Summary and conclusion

There is no doubt that wild *Oryza* species are reservoirs of genes that confer many valuable qualitative and quantitative traits which, if transferred to the cultivated rice, would

broaden the gene pool and improve productivity. There are thousands of wild species accessions collected from a range of geographic, climatic, and ecological conditions. To date, this wealth of germplasm is largely untapped owing to the difficulty of identifying agronomically important genes and transferring these genes into cultivated rice without any linkage drag or deleterious traits. CSSLs and BILs have proven to be very useful tools for identifying and transferring desirable genes from wild relatives into the genetic background of adapted cultivars. These populations are excellent materials for dissecting complex quantitative traits into the component variation as a set of monogenic loci, thus providing a platform for discovery of new QTL alleles. Individual CSSLs with one or more introgressed QTLs in the background of elite rice cultivars could be used as parents to transfer genes of interest into new commercial cultivars or could be used directly as commercial cultivars. Many introgressed genes or QTL alleles in the genetic background of an adapted rice cultivar show improved performance over the initial recurrent parent. Introgression lines with genes (QTLs) of interest can be readily used to pyramid these genes and create transgressive variants, thus improving multiple traits in a single breeding line.

Individual introgression lines having desirable trait variation can be used for fine mapping of QTLs with the ultimate aim of cloning the gene(s) and characterizing its function. Although about two dozen of the CSSL/BIL libraries have been developed in rice, representing several different *O. sativa* backgrounds and wild donors, the range of natural variation represented by the wild gene pool remains largely unexplored. A collaborative effort to evaluate these resources in different environments would allow the rice community to gain valuable insights into the breeding value of alleles from diverse wild species and to understand how diverse wild introgressions affect phenotypic performance across different elite genetic backgrounds. As pre-breeding material, the CSSLs represent a valuable resource for broadening the genetic base of high yielding rice cultivars around the world, and provide new sources of resistance to biotic or abiotic stresses. It is interesting to note that introgressions from wild species often carry 'gain of function' alleles in cultivated backgrounds. Thus, RNA*i* technology could be effectively used to knockout candidate genes underlying QTLs in selected lines to validate their function.

Most of the donors and recurrent parents involved in current CSSL and BIL development efforts have been re-sequenced using second-generation sequencing technology (McCouch SR, Cornell University, personnel communication and Wing RA, University of Arizona, personnel communication) and/or genotyped using the 44,100 SNP array described by Tung et al. (2010). Therefore, a large number of well-distributed polymorphic SNP markers can be readily selected for genotyping assays and used to develop smaller, 384-SNP assays that can be economically used to genotype the backcross progenies, allowing rapid selection of lines to advance during the backcrossing process. High-throughput SNP assays will enhance the efficiency of the CSSL development process, and allow for the selection of introgression lines with smaller marker-defined segments for genome-wide coverage in the CSSL library. SNP markers across the introgressed donor regions will facilitate rapid fine mapping and cloning of genes underlying target QTLs, aided by the availability of physical maps of 17 *Oryza* species (representing the 10 genome types) developed by the *Oryza* Map Alignment Project (Wing et al. 2007; Kim et al. 2008; http://www.omap.org). In an era where sequencing technology has essentially solved the problem of high throughput genotyping, the value of genetic resources, such as CSSLs is underscored because they link natural variation with breeding potential, promote more effective use of the wild gene pool link and simultaneously help geneticists explore the fundamental relationship between sequence variation and phenotypic diversity. Thus, the public availability of introgression libraries enhances the value of the genetic resources available in our in situ and ex situ germplasm collections and leads to more efficient development of improved rice varieties for agricultural producers and consumers around the world.

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