

10 Unlocking the *Oryza glaberrima* Treasure for Rice Breeding in Africa

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Domestication and Genetic Diversity of *Oryza glaberrima*

African cultivated rice (*Oryza glaberrima* Steud.) was domesticated independently of Asian cultivated rice (*Oryza sativa* L.) in Africa from the wild species *O. barthii* A.Chev. (syn. *O. breviligulata* A.Chev. & Roehr.) (Second, 1982), which diverged 0.6–0.7 million years ago from *O. sativa* (Zhu and Ge, 2005; Ammiraju *et al.*, 2008). While Asian rice has spread worldwide and become the most produced food crop in the world, African rice is grown only in tropical West Africa and there sporadically. *Oryza glaberrima* demonstrates a typical case of reduction of genetic diversity observed in crops compared to their wild progenitors as a result of the dual bottlenecks of domestication and breeding (Buckler *et al.*, 2001; Tenaillon *et al.*, 2004; Zeder *et al.*, 2006). Using isozyme, RFLP (restriction fragment length polymorphism), SSR (simple sequence repeat) and transposable element markers, previous studies have detected dramatic reduction of genetic diversity associated with the domestication of African rice and revealed substantially lower genetic diversity in *O. glaberrima* than

in *O. sativa* (Second, 1982; Wang *et al.*, 1992; Ishii *et al.*, 2001). The domestication of *O. glaberrima* occurred much later than that of *O. sativa*, but prior to the introduction of the latter into Africa (Sweeney and McCouch, 2007). The most recent analysis of diversity, comparing nucleotide variation of 14 independent nuclear loci between *O. glaberrima* and *O. barthii*, showed that *O. glaberrima* has lost 76% of the nucleotide diversity of its wild progenitor (Li *et al.*, 2011a), while *O. barthii* itself harbours slightly less diversity than *O. sativa* subsp. *indica*. The low genetic diversity of *O. glaberrima* compared to *O. sativa* is likely to have resulted from a reduction of diversity during the migration of the wild progenitor of African *Oryza* species into Africa plus a severe genetic bottleneck during its domestication from small initial populations of *O. barthii*. The ecological diversity seems to be so low that clustering analysis is unable to pinpoint the domestication places and dispersion of *O. glaberrima* (Li *et al.*, 2011a). Portères' (1970) hypothesis remains the most probable – that African rice was first domesticated in the inland delta of the upper Niger River and subsequently spread along Sahelian rivers and their tributaries to two

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secondary centres of diversity: one along the coast of The Gambia and Guinea-Bissau, and the other in the Guinea forest between Sierra Leone and western Côte d'Ivoire.

Although *O. glaberrima* was recognized as an interesting source of agronomically important traits for rice breeding by the French and international scientific community, its utilization was, for a long time, hampered by the strong reproductive isolation between the two cultivated rice species (Sano *et al.*, 1979) and by a lack of convenient tools and general strategy for rational introgression into *O. sativa*. Interspecific hybridization with formal breeding objectives was initiated in the 1990s by the Africa Rice Center (AfricaRice) and resulted in NERICA (New Rice for Africa) varieties adapted to upland and lowland growing conditions of Africa (Jones *et al.*, 1997; Saito *et al.*, 2010).

This chapter reviews recent advances of research on interspecific sterility in rice with emphasis on the reproductive barriers between the two cultivated rice species, and highlights how we can – with the advent of sequence information, genetic maps and markers – develop pre-breeding schemes suitable for gene and trait discovery, with the aim of easier transfer of *O. glaberrima* traits of agronomic interest into elite *O. sativa* cultivars.

Interspecific Sterility Between *O. sativa* and *O. glaberrima*

Two main types of reproductive barriers are observed in plants: pre-zygotic barriers that prevent the formation of hybrid zygotes and post-zygotic barriers (such as hybrid weakness, unviability and hybrid sterility) that hamper gene flow between species or subspecies, and which largely depend on divergence time between the species (Ouyang *et al.*, 2010). Hybrid sterility is the most common form of post-zygotic reproductive isolation. In rice, one of the best known examples is the hybrid sterility between the subspecies of *O. sativa* (*indica* and *japonica*), which show embryo-sac abortion and pollen sterility. A general gametic lethal model has been proposed in which two independent loci affect the gamete development and gametes carrying the recessive alleles at both loci are aborted during

the development while gametes of other genotypes were normal (Oka, 1957, 1974). These negative interactions can be also observed within a single locus as the consequence of independent evolution of its alleles causing sterility when two incompatible alleles are brought together in hybrids. The (recently cloned) sterility gene *S5* (Ikehashi and Araki, 1986; Chen *et al.*, 2008) is an example where neutral alleles are found in wide compatibility varieties (WCVs) and suppress the negative effects of *indica* and *japonica* alleles in hybrids. Nevertheless, the molecular basis of hybrid incompatibility is usually complex and often involves accumulative effects and interactions of genes at multiple loci. It is critical that we obtain a better understanding of the genetic bases and biological mechanisms of hybrid sterility, since it hinders the transfer of useful genes between the two cultivated rice species and is a major obstacle for utilization of the strong heterosis exhibited in *O. sativa* F₁ hybrids.

Approximately 50 loci controlling hybrid fertility have been identified in the 'A genome' *Oryza* species, mainly from studies of *indica*–*japonica* crosses. Post-zygotic sterility is the most frequent and includes loci causing female-gamete abortion and others inducing pollen sterility. There is increasing documentation of interspecific sterility and the identification of sterility genes in *O. sativa*–*O. glaberrima* hybridization. Near complete pollen sterility is generally observed in F₁ hybrids of the two species. Pollen sterility is accompanied by a reduction in female fertility (Bouharmont *et al.*, 1985). Nevertheless, obtaining backcross progenies is feasible and allows us to derive advanced-backcross lines and near-isogenic lines suitable for identifying and mapping sterility genes (Garavito *et al.*, 2010). More than 12 pollen-sterility loci have been described, some of which are associated with embryo-sac sterility (see Table 10.1 for review). Transmission ratio distortion (TRD) of parental alleles at sterility loci is commonly observed in interspecific progenies and may be the result of many different post-zygotic effects. Considering, for example, a locus with two *a* and *A* alleles coming from *O. sativa* and *O. glaberrima*, respectively, a backcross between a hybrid plant with *Aa* genotype and an *O. sativa* plant with *aa* genotype is expected to give a 1:1 segregation between *aa* and *Aa* genotypes in the following generation. The TRD refers to the departure from this ratio and its origin is

Table 10.1. A non-exhaustive list of loci involved in reproductive barriers between the cultivated rice species.

Locus	Chromosome	Effects on sterility ^a	Reference(s)
<i>S1</i>	6	P, ES	Sano (1990); Koide <i>et al.</i> (2008c); Garavito <i>et al.</i> (2010)
<i>S3</i>	11	P, ES	Sano (1983, 1986)
<i>S4</i>	6	P, ES	Sano (1986)
<i>qSS-6a</i>	6	P	Li <i>et al.</i> (2011c)
<i>S18</i>	10	P	Doi <i>et al.</i> (1998a, 2003b)
<i>S19/qSS-3</i>	3	P, ES	Taguchi <i>et al.</i> (1999); Doi <i>et al.</i> (2003b); Li <i>et al.</i> (2011c)
<i>S20/qSS-7a</i>	7	P	Doi <i>et al.</i> (1999, 2003b); Li <i>et al.</i> (2008)
<i>S21</i>	7	P	Doi <i>et al.</i> (2003b)
<i>S29(t)^b/qSS-2</i>	2	P	Hu <i>et al.</i> (2006); Li <i>et al.</i> (2008); Zhu <i>et al.</i> (2005)
<i>S33(t)^b</i>	1	P, ES	Ren <i>et al.</i> (2005); Jing <i>et al.</i> (2007)
<i>S34(t)^b</i>	3	P	Zhang <i>et al.</i> (2005); Jing <i>et al.</i> (2007)
<i>S36(t)</i>	2	P	Li <i>et al.</i> (2011b)
<i>qSS-1</i>	1	P	Li <i>et al.</i> (2008)
<i>qSS-7b</i>	7	P	Li <i>et al.</i> (2008, 2011c)

^aES: embryo sac sterility; P: pollen sterility.

^bLocus name given in sterility studies of *O. sativa* subsp. *indica* × subsp. *japonica* crosses, but with different chromosome positions.

inferred according to the cross analysed. When an interspecific F_1 plant is used as the female parent, the TRD can be attributed to the female TRD (fTRD), since interspecific F_1 hybrids are almost completely male sterile. Reciprocally, when an advanced interspecific isoline is used as the male parent for crossing with an *O. sativa* parental line, we measure male TRD (mTRD).

A genetic map has been constructed from 125 individuals of an (*O. sativa* × *O. glaberrima*) × *O. sativa* (IR64/TOG5681/IR64) backcross population, using 140 SSR anchor markers derived from a Universal Core Genetic Map (UCGM; Orjuela *et al.*, 2010) to monitor loci involved in hybrid sterility through the detection of fTRD (Garavito *et al.*, 2010). A majority of sterility loci between the two species and previously identified by Li *et al.* (2008, 2011b,c) and Doi *et al.* (1998b) could be co-localized with fTRD regions mapped by Garavito *et al.* (2010) and confirmed the link between fTRD and pollen and spikelet sterility (Plate 2). In one case (S_{33}), sterility genes seemed specific for pollen sterility as no accompanying fTRD was found. Variations in direction and intensity of fTRD were also observed and may suggest the existence of different alleles involved in the sterility barriers. Occurrence of TRD in genomic regions where no sterility loci have been recorded could be explained by new sterility loci

still to be discovered or other mechanisms not related to gamete elimination, such as pre-zygotic sterility favouring, for example, pollen germination on hybrid stigmas or post-zygotic effects leading to hybrid weakness or endosperm abortion which are also reported in interspecific hybridization (Matsubara *et al.*, 2003; Koide *et al.*, 2008a,b). Epistatic interactions are suspected to be acting between regions harbouring TRD and sterility locus, similar to barriers between *indica* and *japonica* (Li *et al.*, 2008). The strongest fTRD ($P < 10^{-5}$) was found at the S_1 locus and is characterized by an extreme distortion in favour of the *O. glaberrima* allele whatever the orientation of crosses or genetic background of the parents. Thus, the S_1 locus is presumed to be the major determinant of female sterility, while pollen sterility is the result of S_1 plus additive and epistatic effects of other sterility loci dispersed on the different chromosomes (Garavito *et al.*, 2010).

Focus on the S_1 Locus

The S_1 locus was first mapped on chromosome 6 thanks to its strong linkage with waxy (*Wx*) gene, which can be identified by pollen staining of F_1 hybrids (Sano, 1983, 1990). Sano's group

has also suggested that the S_1 locus has at least two components, one controlling mTRD mapped in a 45 kb interval of the Nipponbare chromosome 6 sequence, and a second controlling the female sterility component (Koide *et al.*, 2008c). On the assumption that the degree of fTRD would be inversely proportional to the genetic distance between the sterility factor and the markers subjected to fTRD, several backcross populations between Nipponbare, IR64, Curinga (*O. sativa*) and CG14, MG12 (*O. glaberrima*, alias IRGC103544) were fine-mapped using SSR (Garavito *et al.*, 2010). Taken together, these maps enabled determination of a sharp peak of the fTRD value, corresponding to a maximum common fTRD, and leading to high-resolution mapping of S_1 in a region of a 27.8 kb on chromosome 6.

To compare the chromosomal regions between the two species, we used the large genomic library of the *O. glaberrima* cv. CG14 prepared in bacterial artificial chromosomes (BAC) for the *Oryza* Mapping Alignment project (OMAP; Kim *et al.*, 2008). The BAC clone OG-BBa0049I08 containing a 164,664-bp genomic sequence of *O. glaberrima* was selected as it carried the markers with the highest fTRD. Additional convenient markers were derived from the *O. glaberrima* sequence and were used to analyse the recombination events around the peak of fTRD in the rare BC₁ plants homozygous for the *O. sativa* allele of S_1 (S_1^s), since each of these plants originated from a viable S_1^s female gamete transmitted by the F₁ hybrids and the other S_1^s from the recurrent *O. sativa* parent. No recombination was observed in a chromosomal segment of at least 7.3 cM around S_1^s (886 kb in the Nipponbare genome) which was shared by all the S_1^s/S_1^s plants. Thus, results suggested the necessity of inheriting other *O. sativa* factors in the vicinity of S_1 to ensure the viability of the S_1^s gametes. The existence of an additional factor on each side of S_1 (hereafter denoted as S_1A and S_1B) was proposed to infer a sterility system based on three components (S_1 , S_1A , S_1B) playing a role in female sterility of F₁ hybrids and the associated fTRD, through epistatic interactions. The frequency of S_1^s elimination and the length of the genomic block surrounding the S_1 locus that does not recombine differ between the subspecies – *indica* or *japonica* – used in interspecific combinations, but also according to the presence of another factor (S_1C) located approximately 50 cM from S_1 (Garavito *et al.*, 2010).

A General Model to Explain the Reproductive Barrier Between *O. glaberrima* and *O. sativa*

A general model to explain the reproductive barrier between the two species has been developed based on the assumption that the post-zygotic barriers in rice are due to the accumulation of incompatibilities in epistatic interactions between genes. It is based on the very general Bateson–Dobzhansky–Muller (BDM) model (Bateson, 1909). This model says that a specific epistasis among S_1A , S_1 and S_1B is the cause of the female hybrid sterility between the two species and explains the elimination of the S_1^s gamete. Considering that $S_1A^s-S_1^s-S_1B^s$ and $S_1A^g-S_1^g-S_1B^g$ are the original haplotypes observed in *O. sativa* and *O. glaberrima*, respectively, the epistatic interaction among the three *O. glaberrima* factors will cause the abortion of daughter cells carrying S_1^s during the two first meiotic divisions and will cause an fTRD. When recombination occurs in the mother cell and eliminates S_1B^g , S_1A^g or both, the epistatic incompatible interaction acting against S_1^s would cease, allowing the development of the corresponding S_1^s daughter cells and eliminating the fTRD. Finally, at the end of the megagametogenesis, only the S_1^s megaspores carrying the non-recombinant haplotype $S_1A^s-S_1^s-S_1B^s$ could survive and would lead to a functional embryo sac (Fig. 10.1). Cytological observations support this genetic model and the resulting reduction of seed set, since different types of female-gamete abortion occur during female gametogenesis of the F₁ hybrids: some are characterized by a complete absence of embryo sacs and are probably due to an early abortion just after meiosis, and others are characterized by embryo sacs with fewer than seven cells (Bouharmont *et al.*, 1985; Koide *et al.*, 2008c).

The S_1 Locus as an Entry Point to Analyse Domestication and Evolution in Rice

The genomic sequence of the 50.38 kb interval presumed to contain the female component of the S_1 locus was carefully annotated to identify putative candidate gene for S_1 . Seven genes and three pseudogenes were predicted and were

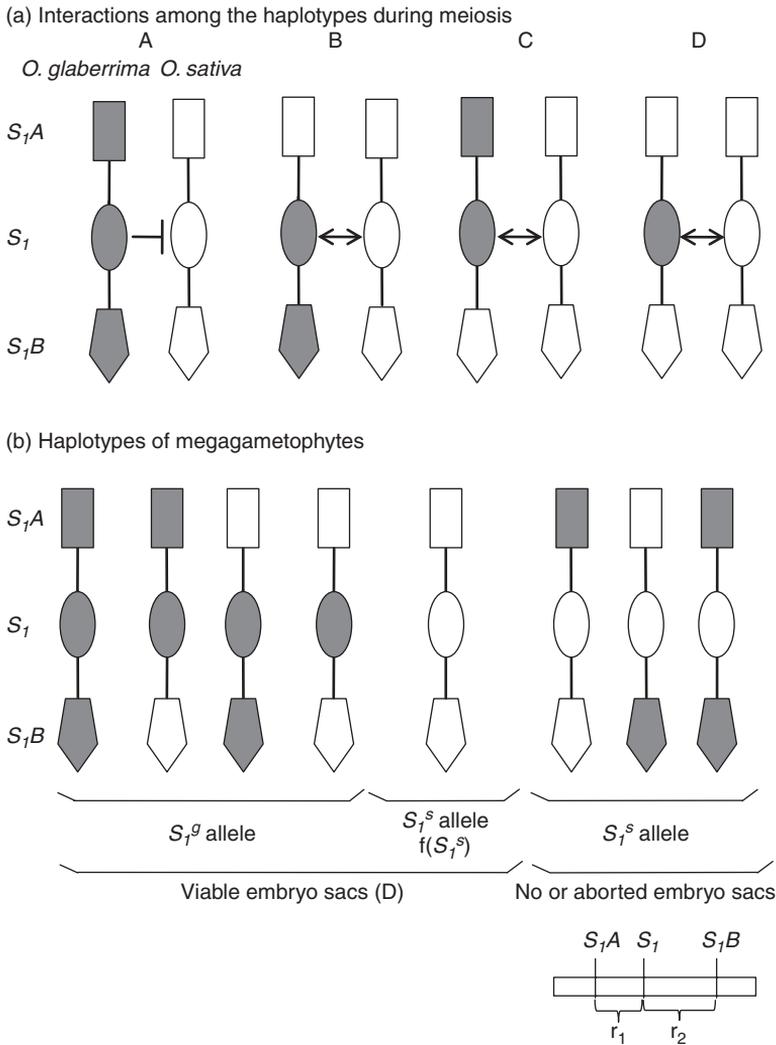


Fig. 10.1. Genetic model based on a Bateson–Dobzhansky–Muller (BDM) model of interspecific incompatibility (Bateson, 1909) and consequences on the elimination of S_7^s female gametes. (a) During the meiotic divisions of the mother cell, the epistatic interactions of the *O. glaberrima* S_7A^g - S_7^g - S_7B^g haplotype cause the abortion (-) of the daughter cells carrying S_7^s and will originate a female Transmission Distortion Ratio (fTRD). If a recombination eliminates S_7B^g (B), S_7A^g (C) or both (D), the epistatic incompatible interaction against S_7^s would cease (double arrow) allowing the development of S_7^s gametes and eliminating the fTRD. (b) Among the S_7^s gametes, only the small fraction corresponding to the non-recombinant *O. sativa* S_7A^s - S_7^s - S_7B^s haplotype ($f(S_7^s)$) can survive and develop in a normal embryo sac, since it is derived from a recombination event in the daughter cell during the meiotic process which suppresses the epistasis against S_7^s . All the other S_7^s allelic configurations will result in the abortion of megaspores or abnormal embryo sacs. Configurations giving viable embryo sacs (D) estimate the reduction of the seed set observed in F_1 hybrids. Therefore, the fTRD is directly dependent on the recombination fraction between S_7A - S_7 (r_1) and S_7 - S_7B (r_2).

considered as candidates. Among the predicted genes, two deserved special attention as their putative functions, differential presence between *O. glaberrima* and *O. sativa*, and greater divergence

between the two species, could justify relationships with reproductive effects (Garavito *et al.*, 2010). The first gene (49108-10) is carried by a Pack-Mutator-like transposable element (Pack-MULE)

and is present only in the *O. glaberrima* S_1 sequence. This gene showed similarities with an APETALA (AP2) transcription factor and mutations in AP2 in *Arabidopsis* are known to stop mega-sporogenesis after the first meiotic division (Byzova *et al.*, 1999). Pack-MULEs are known to have an important role in rice genome evolution, as they can capture and relocate gene fragments to other genomic contexts (Jiang *et al.*, 2004) leading to modulation of both MULEs and paralogous gene expression (Hanada *et al.*, 2009). If the complete paralogous AP2 gene had a function similar to that of the *Arabidopsis* genes, this pack-MULE could affect its expression in the heterozygotes, in a dose-dependent fashion, causing abortion of female gametes, and would appear very close to hybrid dysgenesis mechanism (Michalak, 2009). The second candidate gene of interest (*49108-11*) belongs to the super-family of F-Box proteins. Members of this family constitute protein complexes known as SCF (Skp1–Cullin–F-Box) involved in the control of a wide range of processes (Xu *et al.*, 2009). Several F-box genes and their associated proteins have been related with the progression of the cell cycle, especially during sporogenesis and gametogenesis (Wang and Yang, 2006; Pesin and Orr-Weaver, 2008; Gusti *et al.*, 2009). A strong functional similarity can be seen with the complex composed by an F-box and a SUMO E3 ligase-like protein that controls the inter-subspecific hybrid sterility mediated by the *Sa* locus in *O. sativa* (Long *et al.*, 2008).

The structural analysis was extended to the 813 kb genomic sequence of *O. glaberrima* covering the S_1A and S_1 regions and most of the S_1B region, which has been compared to its orthologous regions in *O. sativa* (Guyot *et al.*, 2011). A strong structural conservation was observed all along the S_1 locus between the two cultivated species. This was in accordance with the relatively recent divergence (approximately 0.7 million years) of Asian and African rice lineages by geographical isolation (Ma and Bennetzen, 2004; Ammiraju *et al.*, 2008) and it reinforced the consistency of candidate genes provided by F-Box genes as they represented the most important structural genetic variation between the two cultivated rice species. Thus, effects of duplicated F-Box genes strengthened by a local inversion, which increases the epistatic cohesion of the factors, may contribute greatly to the repro-

ductive isolation between the two species (Guyot *et al.*, 2011). Further experiments are being carried out at Institut de recherche pour le développement (IRD) to confirm candidate genes and to better understand the mechanism controlling the post-zygotic barrier at the S_1 locus.

Identification of the main locus involved in the reproductive barrier between the cultivated rice species reflects the divergence accumulated between their respective wild progenitors. The colocalization of sterility locus between *O. sativa* and *O. glaberrima* with the ones observed between *indica* and *japonica* accessions or between *O. rufipogon* and *O. sativa* can provide useful guidelines for in-depth analysis of the evolution of critical loci responsible for BDM reproductive barriers, which may have fixed different alleles of incompatibilities between the species (Plate 2). S_{10} and S_7 , for example, or S_{21} – S_{23} on chromosome 2 and S_{22} – $S_{29(i)}$ – S_{32} – $qSS-2$ on chromosome 7 are the most striking colocalizations of sterility genes between different A genome species. Accumulation of effects of additional loci before and during the domestication of rices may explain why the reproductive barrier is so pronounced between the two cultivated species. Identification of sterility genes may also help in refining the analysis of the potential gene flow between the cultivated species, since they coexist in West Africa. Hence, specific transposable elements identified in the *O. glaberrima* and *O. sativa* S_1 sequences were used to determine Retrotransposon-based insertion polymorphism (R-BIP) markers, which proved to be very useful for the analysis of the integrity of the S_1 locus in a representative collection of wild and cultivated African rices. Results confirmed fairly well that no recombination occurred in the entire S_1 region even in very rare off-types or accessions supposed to have been introgressed elsewhere in the genome (our unpublished results).

Development of Chromosome Segment Substitution Lines (CSSLs) and Near-isogenic Lines (NILs) to Exploit the Genetic Diversity of *O. glaberrima*

Interspecific hybridization offers an attractive way of enlarging the genetic diversity for crop improvement, but it is hampered by reproductive

barriers, increased sterility and reduced recombination between the cultivated and the related species. The development of markers and genetic mapping provides powerful tools to more systematically introgress a genome of a distant species into a cultivated one, and to characterize phenotypic variation through the development of specific populations. Among such populations, complete sets of chromosome segment substitution lines (CSSLs), which are based on the representation of small chromosome fragments coming from a donor species in another recipient species, are particularly suitable in the case of interspecific hybridization between *O. sativa* and *O. glaberrima* (Ghesquière *et al.*, 1997). The general scheme for developing a CSSL population is based on successive backcrosses and selection of individuals bearing only one or a small number of targeted chromosomal segments of the *O. glaberrima* donor in the *O. sativa* background, using well-distributed molecular markers (Plate 3). Usually a first set of 50–60 randomly chosen BC₁F₁ individuals is large enough to cover the complete genome of the donor parent. The BC₁F₁ plants are backcrossed again to generate the BC₂ families. Then 10–15 individuals from each BC₂F₁ family are completely genotyped and a total of about 150–200 BC₂F₁ plants showing a targeted segment are selected and used for the next backcrossing generation to produce BC₃F₁s. Again, 120–150 BC₃F₁ individuals are selected from 400–500 BC₃F₁ individuals after a second round of genotyping. These individuals are selfed to develop BC₃F₂s and then homozygous lines for the target segment generated through single-seed descent (SSD), or they can be backcrossed again to generate BC₄F₁ plants if additional isogenization is needed. To optimize the coverage of the entire genome of the donor in a minimum set of lines, graphical genotyping software such as GGT (van Berloo, 2008) and CSSL FINDER (<http://mapdisto.free.fr/CSSLFinder>) have been developed. A universal core genetic map is also available to select SSR markers anchored in the Nipponbare physical map and polymorphic across a large range of cultivars and species with the A genome (Orjuela *et al.*, 2010).

The CSSL approach has become popular for identifying and introgressing genes from one varietal group to another in rice, namely from *indica* to *japonica* and vice versa. Special effort has been made to use recipient varieties with

available genomic sequences, such as Nipponbare (a temperate *japonica* variety), Zhenshen 97B (a hybrid rice maintainer line) and 93-11 (a restorer line sequenced by the Beijing Genomics Institute). The latter two lines are also used in an ambitious project to develop 14 CSSL libraries comprising seven A genome wild species accessions and included in the OMAP (Wing *et al.*, 2007). Using CSSL populations developed from 93-11 and Zhenshen 97B with Nipponbare as donor, quantitative trait loci (QTLs) have been identified for enhancing the cultivability of *indica* rice (Zhao *et al.*, 2009), number of panicles and grain yield under low nitrogen and phosphorus conditions (Wang *et al.*, 2009), and grain length and width (Zhu *et al.*, 2009). CSSLs from Kasalath (an *Aus* landrace from India) were developed in the *japonica* genetic background of Koshihikari to map QTLs affecting heading date (Ebitani *et al.*, 2005), cadmium concentration in brown rice (Ishikawa *et al.*, 2005), increased number of panicles per plant, increased number of grains per panicle and increased root mass (Maduoka *et al.*, 2008). For African rice species, several CSSL initiatives have been developed or are in progress (Table 10.2; Ali *et al.*, 2010). The most advanced libraries concern two CSSL populations using the *O. glaberrima* accession MG12 in the background of Caiapo (a tropical *japonica*) and the multi-purpose *O. glaberrima* accession TOG5681 in IR64 (*indica*) (Gutiérrez *et al.*, 2010).

Identification, Mapping and Transfer of Valuable Genes from *O. glaberrima*

Systematic introgression is time- and labour-intensive, so it needs to be conceived as an investment for the future and focused on important recipient *O. sativa* varieties or lines and on multi-purpose *O. glaberrima* donors. As different lines of a CSSL population show strong morphological similarities, they represent an ideal tool to identify QTLs from remote or unadapted donor germplasm with the opportunity to reveal small effects that are usually masked by major QTLs in direct F₂ or recombinant inbred line (RIL) primary populations. In addition, genotype × environment interactions can be precisely monitored through replicated trials over years, across different

Table 10.2. Summary of chromosome segment substitution lines (CSSLs), backcross inbred lines (BILs) and near-isogenic lines (NILs) developed or in development, using *O. glaberrima* or *O. barthii* as donors and *O. sativa* as recurrent parent.

Donor accession	Recurrent parent (<i>O. sativa</i>)	Potential traits targeted ^a	Reference(s)
<i>O. glaberrima</i> TOG 5675	<i>indica</i> IR64	BPH resistance (<i>Bph1</i>)	Ram <i>et al.</i> (2010)
<i>O. glaberrima</i> TOG 5681	<i>indica</i> IR64	RYMV resistance (<i>rymv1-2</i> ; <i>rymv1-3</i>)	Albar <i>et al.</i> (2006)
<i>O. glaberrima</i> TOG 5681	<i>indica</i> IR64	Nematode resistance (<i>Meloidogyne</i> spp.)	S. Bellafiore (personal communication); Bimpong <i>et al.</i> (2010)
<i>O. glaberrima</i> IRGC 104589	<i>indica</i> IR64	RYMV resistance (<i>Rymv2</i>)	Thiémélé <i>et al.</i> (2010)
<i>O. glaberrima</i> IRGC 96726/TOG 5307	<i>indica</i> IR64	RYMV resistance (<i>Rymv3</i>)	L. Albar (personal communication)
<i>O. glaberrima</i> IRGC 103544/MG 12	Tropical <i>japonica</i> Caiapo	<i>Rice stripe necrosis virus</i> resistance	Gutiérrez <i>et al.</i> (2010)
<i>O. glaberrima</i> IRGC 103544/MG 12	<i>indica</i> Mylang 23	Yield and yield components	Kang <i>et al.</i> (2008)
<i>O. glaberrima</i> IRGC 104038	<i>japonica</i> Taichung 65	Pollen fertility and heading date	Doi <i>et al.</i> (1997) (2003a)
<i>O. glaberrima</i> IRGC 96717/CG 14	Tropical <i>japonica</i> WAB 56-104	Drought tolerance and weed competitiveness	Ndjondjop <i>et al.</i> (2010)
<i>O. glaberrima</i>	Temperate <i>japonica</i> cv. Koshihikari	Glabrous gene	Angeles-Shim <i>et al.</i> (2009)
CSSLs under construction			
<i>O. barthii</i> IRGC 10193	Tropical <i>japonica</i> Curinga	To be evaluated for yield-related traits, grain traits, tolerance to biotic and abiotic stresses	M. Lorieux (cited in Ali <i>et al.</i> , 2010)
<i>O. barthii</i> IRGC 101937	<i>indica</i> Zhenshen 97B and 93-11	To be evaluated for yield-related traits, grain traits, tolerance to biotic and abiotic stresses	S.B. Yu (cited in Ali <i>et al.</i> , 2010)
<i>O. glaberrima</i> IRGC 96717/CG 14	<i>indica</i> Zhenshen 97B	Drought tolerance and grain quality	S.B. Yu (cited in Ali <i>et al.</i> , 2010)
<i>O. glaberrima</i> and <i>O. barthii</i>	Tropical <i>japonica</i> LaGrue and temperate <i>japonica</i> M-202	To be evaluated for yield-related traits, grain traits, tolerance to biotic and abiotic stresses	P. J. Sanchez and G. C. Eizenga (cited in Ali <i>et al.</i> , 2010)

^aBPH, brown planthopper; RYMV, *Rice yellow mottle virus*.

environments and growing conditions. A good example of direct utilization of CSSLs was the identification of a major resistance gene to *Rice stripe necrosis virus* (RSNV) and yield-component QTLs in the (MG12 × Caiapo) CSSL population (Gutiérrez *et al.*, 2010; Fig. 10.2). CSSL lines usually contain more than their single targeted segment, but additional backcrossing can be performed to focus on a specific chromosome segment or on a confirmed line with special

interest to derive true NILs ready for fine mapping and positional cloning. For instance, using the (IR64 × Tog5681) CSSL population, a NIL has been developed for the *Rice yellow mottle virus* (RYMV) resistance gene *rymv1-3*/IR64 (Albar *et al.*, 2006). Similarly, three confirmed IR64 NILs resistant to nematode *Meloidogyne incognita* have been derived from an (IR64 × Tog5681) CSSL population to map and to ultimately clone the resistance genes to *Meloidogyne*

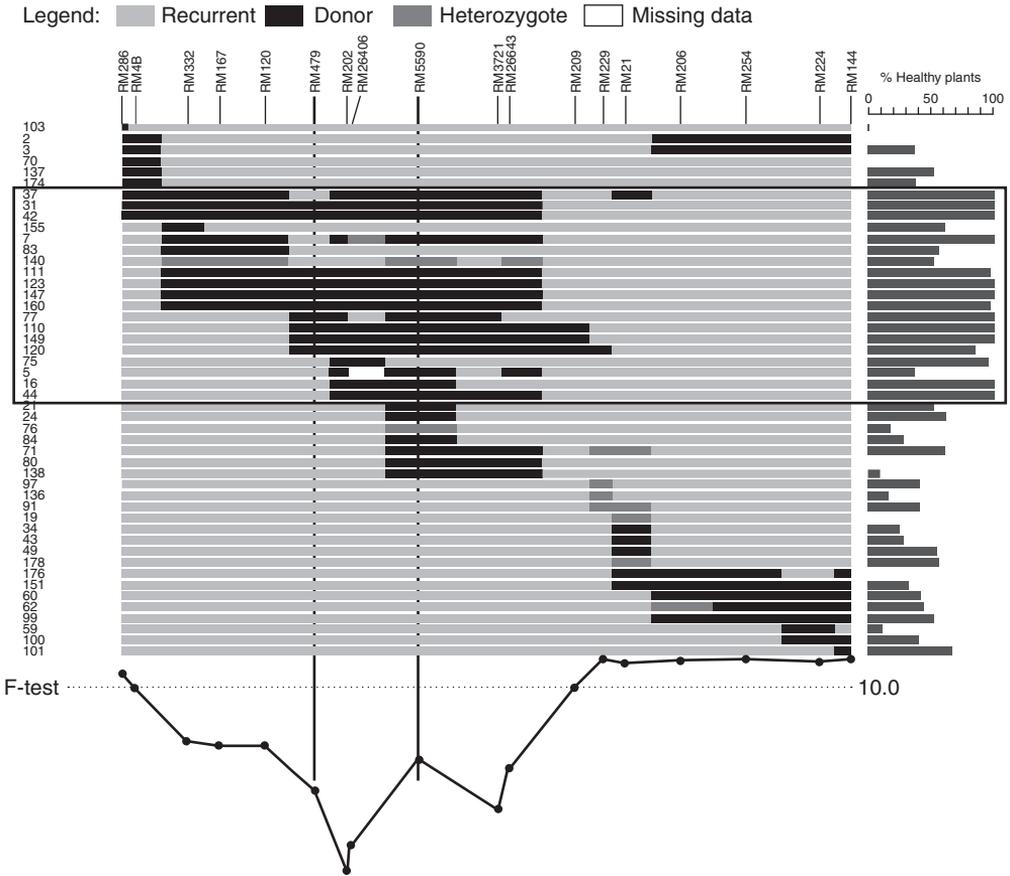


Fig. 10.2. Identification of a major QTL for resistance to *Rice stripe necrosis virus* (RSNV) in *O. glaberrima* MG12. A major resistance QTL is located on chromosome 11 between SSR markers RM479 and RM5590 ($F = 70.63$, $P \sim 0.0$, significance threshold = 10.0). On the right, solid bars indicate the percentage of healthy plants for each line. The resistant lines (more than 85% healthy plants) are located within the black frame. The most probable location of the resistance QTL is given by the intersection of the black frame and the positions of the markers RM479 and RM5590, which define a common introgressed region between the resistant lines.

spp. found in *O. glaberrima* (our unpublished results). CSSLs are also a starting point for dissecting complex traits, identifying lines with individual desirable QTLs, which can be re-assembled efficiently in a common genetic background through a recurrent marker selection process. Pyramiding of QTLs for plant height and number of grains (Ashikari *et al.*, 2005) to efficiently increase those traits in the Koshihikari *japonica* cultivar demonstrated how introgressed lines could be efficiently connected to breeding objectives and varietal release (Ashikari and Matsuko, 2006).

Conclusion: Application for New Breeding Schemes Adapted for *O. glaberrima*

Since the early 2000s, two major breakthroughs have been made for better gene discovery in rice genetic resources: user-friendly mapped markers such as SSRs, and suitable populations for trait analysis. In addition, single-nucleotide polymorphism (SNP) markers derived from rice-sequencing projects offer a quasi-infinite number of markers. Specifically designed 384-SNP sets will increase genotyping efficiency of any segregating progeny

for gene and QTL identification. Also, a 44,000 array is already available and a 700,000 SNP array is under development (Tung *et al.*, 2010; see also McCouch *et al.*, Chapter 9, this volume). Using the *O. glaberrima* CG14 genomic sequence, new sets of versatile SNPs well distributed on the rice genome and showing intra- and interspecific polymorphism are being developed by IRD for the study and use of *O. sativa* × *O. glaberrima* introgressions. Outsourcing of genotyping via dedicated platforms such as the Molecular Breeding Platform of the Generation Challenge Programme (GCP) to private companies will greatly alleviate activities in plant breeding, allowing breeders and geneticists to focus on phenotypic evaluation of the traits of interest. The first generation of upland NERICA varieties developed with *O. glaberrima* CG14 have been scored for their *O. glaberrima* and *O. sativa* contents (Semagn *et al.*, 2006). According to the S_1 locus genetic model described above, the presence of S_1^g allele in some of them is expected to restore the F_1 interspecific reproductive barrier if S_1^g NERICA varieties are used in crosses with *O. sativa* lines. The S_1 locus is not the only locus responsible for the reproductive barrier, but the S_1^s allele is an important prerequisite for favouring fertility restoration

in backcross generations. The female transmission of the S_1^s allele in *O. sativa* backcross progenies may vary from 1.5% to 10% according to recipient parents and combinations (Garavito *et al.*, 2010; our unpublished results). We developed the concept of interspecific bridge lines (iBridges) based on marker-assisted selection of homozygous S_1^s/S_1^s individuals, which resulted in an increase of the proportion of fertile individuals in the first backcross generation (Table 10.3). Combined with CSSL or backcross inbred lines (BIL) strategies as described above, this process allows accelerated fertility restoration and more efficient development of fertile introgressed lines for phenotypic evaluation and gene discovery. Producing lines that are able to give fertile progeny when crossed with more diverse *O. sativa* elite lines is expected to greatly facilitate the incorporation of *O. glaberrima* germplasm in conventional rice breeding schemes. This concept is being applied through international collaborations (AfricaRice, International Center for Tropical Agriculture, IRD and national agricultural research systems) within the framework of the Global Rice Science Partnership (GRISP) to enlarge the use of other important *O. glaberrima* donors and to diversify the target recurrent *O. sativa* varieties.

Table 10.3. First steps of the development of iBridges through marker-assisted selection for S_1^s allele and subsequent fertility restoration in the *O. sativa/O. glaberrima/O. sativa* backcross progenies. Off-types refer to some natural accessions showing evidence of introgression events based on SSR and SNP marker data.

Parent	No. comb ^a	No. BC ₁ seed set	No. BC ₁ screened by MAS ^b	No. S_1^s (RM190)	S_1^s allele transmission in F_1	Range	Fertile individuals (S_1^s/S_1^s genotype, %)	Fertile individuals (S_1^s/S_1^g genotype, %)
<i>O. sativa</i> line								
IR64	12	1536	831	45	0.055	0–19	54	2
Curinga	11	1633	797	33	0.041	0–16	45	5
WAB165	11	1989	669	33	0.049	0–10	16	3
Total	34	5158	2297	112	0.049	0–11	40	3
Off-types	10	1503	878	233	0.265	16–46	72	23

^acomb, combinations; ^bMAS, marker-assisted selection.

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