Theor Appl Genet (2000) 100:593–601

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A first interspecific *Oryza sativa*×*Oryza glaberrima* microsatellite-based genetic linkage map

Received: 1 June 1991 / Accepted: 22 June 1999

Abstract *Oryza glaberrima* is an endemic African cultivated rice species. To provide a tool for evaluation and utilisation of the potential of *O. glaberrima* in rice breeding, we developed an interspecific *O. glaberrima*×*Oryza sativa* genetic linkage map. It was based on PCR markers, essentially microsatellites and STSs. Segregation of markers was examined in a backcross (*O. sativa*/*O. glaberrima*//*O. sativa*) population. Several traits were measured on the BC_1 plants, and major genes and QTLs were mapped for these traits. Several of these genes correspond well to previously identified loci. The overall map length was comparable to those observed in *indica*×*japonica* crosses, indicating that recombination between the two species occurs without limitation. However, three chromosomes show discrepancies with the *indica*×*japonica* maps. The colinearity with intraspecific maps was very good, confirming previous cytological observations. A strong segregation-distortion hot spot was observed on chromosome 6 near the *waxy* gene, indicating the presence of s_{10}, a sporo-gametophytic sterility gene previously identified by Sano (1990). The main interests of such a PCR-based map for African rice breeding are discussed, including gene and QTL localisation, marker-assisted selection, and the development of interspecific introgression lines.

Key words *O. glaberrima* · *O. sativa* · Rice · Genetic maps · Microsatellites · Sequence-tagged sites · Interspecific hybridisation · Gene/QTL mapping · Segregation distortion

Communicated by H.F. Linskens

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Introduction

Oryza glaberrima Steud. is the African cultivated rice species. It is of economical importance only in West Africa. Its domestication from *Oryza breviligulata* A. Chev. & Roehr was independent to that of *Oryza sativa* (Portères 1950; Second 1982). *O. glaberrima* is separated from *O. sativa* by a strong reproductive barrier, including pollen sterility as well as female sterility (Pham and Bougerol 1993; Sano 1985, 1990; Sano et al. 1979).

Although *O. glaberrima* accessions present unfavourable traits, such as high shattering or lodging susceptibility, it is of great interest to study segregation and introgression events between *O. sativa*×*O. glaberrima*. First, *O. glaberrima* accessions often show superior values for several agronomic traits under poor management conditions as well as for resistance to biotic (viruses, nematodes, insects) or abiotic (acidity, iron toxicity, drought) stresses (Attere and Fatokun 1983; Sano et al. 1984; Ghesquière et al. 1997; Reversat and Destombes 1998). Second, for quantitative traits where *O. glaberrima* is equal or inferior to *O. sativa*, we can expect transgressions in interspecific progeny, meaning that favourable alleles may come from *O. glaberrima*. Hence, the identification of favourable alleles from *O. glaberrima* at the genes or QTLs (quantitative trait loci) responsible for these traits would be of great value for breeding new *O. sativa* cultivars. Finally, another benefit of such studies would be the assessment of the genetic basis of the sterility system observed between the two species.

Several saturated genetic linkage maps based on molecular markers have been developed for rice, using *indica*×*japonica* or *O. sativa*×*O. longistaminata* crosses (Causse et al. 1994; McCouch et al. 1988; Harushima et al. 1998). However, no extended linkage study has so far been reported between the two cultivated rice species; only linkage studies on small portions of the genome have been carried using morphological markers (Sano 1990). A molecular linkage map based on *O. sativa*×*O. glaberrima* progeny would represent a very useful tool for several studies: (1) localising genes or QTLs of interest from *O.*

glaberrima, providing the basis for marker-assisted selection (MAS), (2) localising sterility loci between molecular markers in order to easily screen large progenies for fertility, (3) systematically surveying the genetic recombination between the two species over all the genome, and (4) monitoring the development of introgressed genetic material with the help of molecular markers.

To be useful for all these aspects, a genetic map has to follow several criteria: technical criteria such as rapidity, simplicity, robustness, transferability and cost of the marker technology and methodological criteria such as efficient and rapid checking of advanced-generation plants for some specific chromosomal regions. To fit these requests, we initiated the development of a genetic map based on microsatellite and sequence-tagged site (STS) markers, which have recently been published for rice (Ghareyazie et al. 1993; Wu and Tanksley 1993; Inoue et al. 1994; Akagi et al. 1996; Panaud et al. 1996; Chen et al. 1997). These markers present several advantages: they are simple to generate, they are codominant and they are specific for loci on known linkage groups. Moreover, microsatellites are extremely polymorphic, permitting one, for example, to compare different rice maps in order to maximise the percentage of common markers between maps.

Here we report on the development of an interspecific (*O. sativa*×*O. glaberrima*) microsatellite-based rice genetic map and its comparison to previously published intraspecific microsatellite maps.

Materials and methods

Population development and trait evaluation

We developed a backcross population from the cross IR64/TOG5681//IR64. IR64 (*O. sativa*, *indica* subspecies) is the most widespread irrigated variety in the world and was obtained from IRRI (the Philippines). TOG5681 is a photosensitive *O. glaberrima* cultivar found to be highly resistant to Rice yellow mottle virus (RYMV) Attere and Fatokun 1983; Ndjiondjop et al. 1999) and to several nematode species (*Meloidogyne spp.*, *Heterodera sacchari*) (Reversat and Destombes 1998). A total of 144 BC_1 individuals were obtained. This population will hereafter be referred to as the BC_1 population. Although the main aim of this study was to develop an interspecific map, several traits were measured in the population in order to search for genes or QTLs for these traits. The entire population was measured for height, tillering, flowering time in decreasing day length, awning, and the coloration of stem base and stigma (see Table 2 for details on measurements). A subset of the population was also measured for the number of primary and secondary branches on the panicles.

Molecular analyses

The DNAs of parents, the F_1 hybrid, and 77 BC_1 individuals were isolated from lyophilised leaves using the CTAB method (Murray and Thompson 1980).

To generate simple sequence length polymorphism (SSLP), we used the microsatellite primer sequences published by Akagi et al. (1996); Chen et al. (1997); Panaud et al. (1996) and Wu and Tanksley (1993). PCR conditions were similar to those of Chen et al. (1997) except that 0.4 µCi of [α-^{33}P] dATP was incorporated during the PCR reaction. PCR products were run on 6% polyacrylamide gels and the gels exposed to X-ray films (Kodak Biomax) for 7 h.

Primer aliquots or sequences for STS markers were kindly provided by J. Bennett from IRRI (Ghareyazie et al. 1993). These sequences correspond to the RG (genomic) and RZ (cDNA) probes already mapped on the interspecific *O. sativa*×*O. longistaminata* saturated map (Causse et al. 1994). Other STS primer sequences were used as published in Inoue et al. (1994). This set of 63 STSs correspond to restriction fragment length polymorphism (RFLP) markers mapped on the *indica*×*japonica* saturated map (Kurata et al. 1994; Harushima et al. 1998). PCR and migration conditions were as described in Ghesquière et al. (1997). Polymorphism was revealed either by migration of amplification products or after enzyme restriction.

A few amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD) markers were also added to the map. For AFLP, the standard protocol was followed (Vos et al. 1995). For RAPD, we used the protocol described in Ghesquière et al. (1997).

Map construction

The map was estimated by multipoint analysis (Lander and Green 1987), using MapMaker v. 3.0 (Lander et al. 1987). Conversion of recombination fractions into centimorgans (cM) was obtained using Kosambi's mapping function (Kosambi 1944). This map function is the one most commonly used for rice genetic mapping, and using it systematically permits a direct comparison between different rice maps.

A comparison with the two main RFLP-based *indica*×*japonica* microsatellite maps (Akagi et al. 1996; Chen et al. 1997, hereafter referred to as RIL_1 and DH_1 populations, respectively) was established on the basis of common microsatellite loci. STS markers could also be used to establish the comparison, as they correspond either to RFLP markers mapped on the microsatellite maps, or to markers on the saturated RFLP genetic maps (Xiao et al. 1992; Causse et al. 1994; Harushima et al. 1998) which have numerous markers in common with the microsatellite maps.

Marker segregations were checked for deviations from an expected 1:1 ratio. As segregation distortion may affect the correct estimation of recombination fractions (Lorieux et al. 1995), we estimated recombination fractions between markers showing deviations from Mendelian expectations using appropriate models. Computations were done using MapDisto, a program developed for such situations (http://www.mpl.ird.fr/~lorieux).

According to their segregation mode, the genes and QTLs responsible for the measured traits were mapped either by conventional Mendelian mapping using MapMaker 3.0 or by interval mapping using MapMaker/QTL 1.1(Lander and Botstein 1989). Significant interval mapping tests were confirmed by Kruskall and Wallis tests using MapQTL 2.4 (van Ooijen 1992). The correspondence with previously located genes (Kinoshita 1995) was also examined.

Results

Marker efficiency

As expected, microsatellite markers showed a high polymorphism. Among 159 non-redundant primer couples, 94 (60%) gave SSLPs and 65 couples (40%) either gave faint or bad amplifications or else monomorphic patterns. On the other hand, STS markers gave a low polymorphism, even after restriction by 4-base cutters. Indeed, among 171 available primer couples, 150 (88%) gave faint or bad amplifications or else monomorphic patterns. The remaining 21 (12%) couples gave polymorphic patterns.

Table 1 Microsatellite markers showing total cosegregation in the present work and their correspondance with previous studies

Pairs[a]	Chromosome	Distance[b]	Reference[c]
OSR19–OSR25	6	0	A
OSR28–OSR29	9	0	A
OSR9A–G357	2	0	A
RM211–RM233A– RM236	2	<3	C
RM20a–RM4a	12	<3	P
OSR26a–RM48	2		
RM12–RM17	12		
RM163–RM164	5	0	W
RM232–RM251	3	2.8	C
RM168–RM49	3	22	C
RM42a–RM223	8	4.4	C

[a] Cosegregating microsatellites in our map
[b] Interval size in original mapping work (in cM)
[c] Original mapping work which showed cosegregation or linkage between microsatellites. A: Akagi et al. 1996; C: Chen et al. 1997; P: Panaud et al. 1996; W: Wu and Tanksley 1993

Genome coverage

The map comprised 129 markers (94 SSLPs, 21 STSs, 11 AFLPs and 3 RAPDs), representing 112 discrete marker loci. The total map length was of 1923 cM. The mean interval length is thus about 17 cM.

The genome coverage was about the same as that of the two main microsatellite maps. However, it is somewhat limited, especially for chromosomes 4 and 10, due to the number of available markers (Fukui and Iijima 1991; Chen et al. 1997). Also, the end of the short arm of chromosome 7 could not be covered due to the lack of polymorphism with available markers.

Some STS markers were helpful to fill gaps between microsatellite loci (i.e. G144 on chromosome 3, RG143 on chr. 4, G342 on chr. 6, G278 on chr. 8, G103 on chr. 9).

Redundancy and new loci

Several microsatellites showed a total cosegregation in the BC_1 population. In several cases, these cosegregations were either also found, or else corresponded to tightly linked markers, in previous studies. In other cases, cosegregation was found between markers originally mapped in differents studies (Table 1).

The markers OSR17 and RM12, whose map positions were unknown, were mapped in the present study on chromosomes 2 and 12, respectively. Moreover, several microsatellite primer couples generated new loci: RM260 was monomorphic for the locus on chromosome 11 in the DH_1 map, but generated a marker on chromosome 12 that we called RM260b in the BC_1 map. Likewise, RM42 and RM81 generated new loci on chromosome 5 (RM42b and RM81c), and RM3 generated two loci on chromosomes 3 and 9 (RM3c and RM3b, respectively).

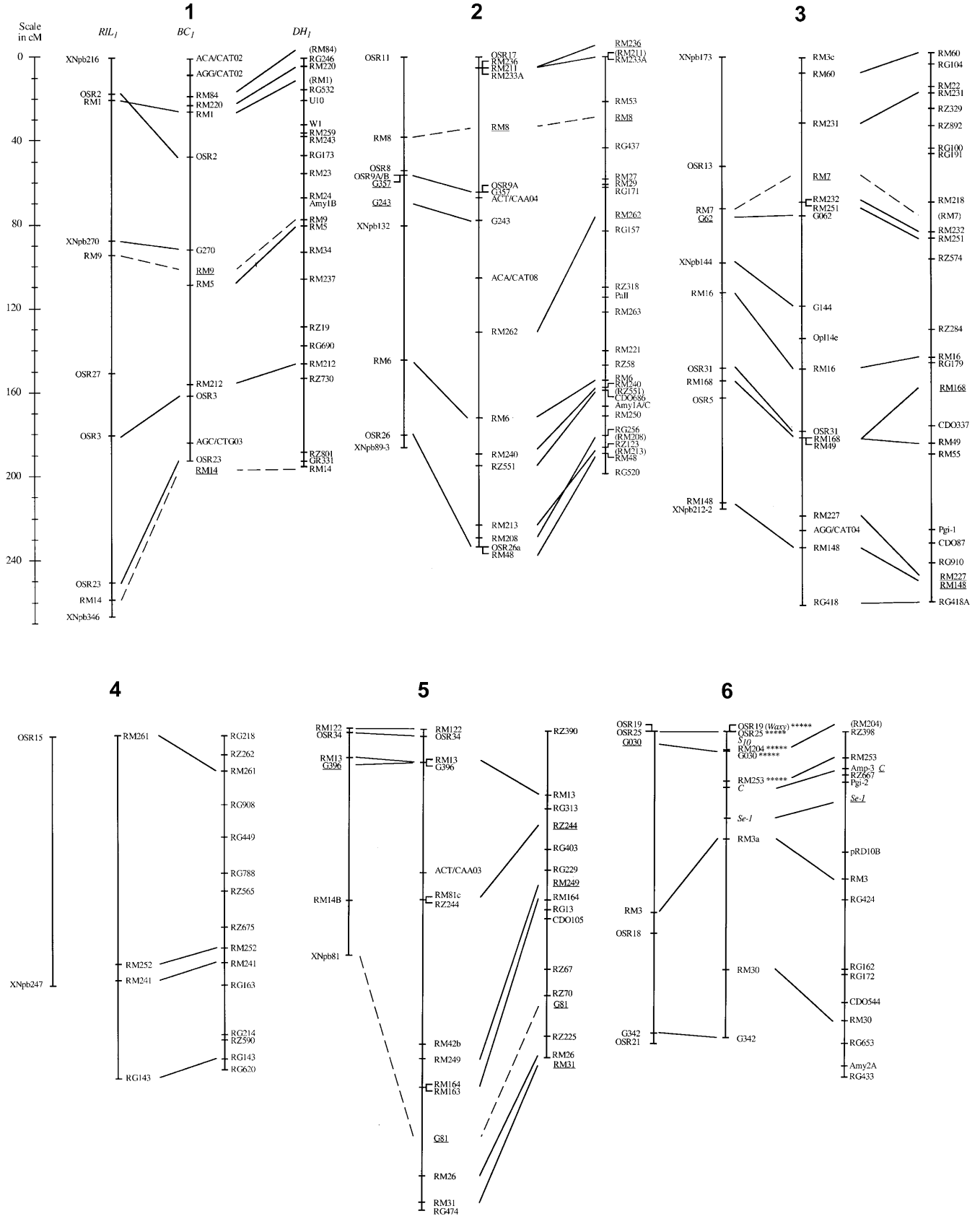
Genome colinearity and recombination

A very good colinearity was observed between the BC_1 map and the intraspecific DH_1 and RIL_1 maps (Fig. 1). A small number of very localised inversions of marker orders were observed, which were not statistically significant. For instance, on chromosome 2, the microsatellites RM208 and RM 213 were inverted between the (IR64/Azucena) map and this work, but these markers were not placed with good confidence on the (IR64/Azucena) map (LOD <2).

To compare the linkage-group sizes, we have considered the maximum common interval size (MCIS) between two markers shared by both maps. This represents a better parameter than the total linkage group size (TLGS) for a comparison of map sizes, because it eliminates errors due to the lack of genome coverage at chromosome ends. The respective lengths (TLGS and MCIS) of the 12 chromosomes in the BC_1 and DH_1 maps are reported in Table 3. It appears that the majority of linkage groups had globally similar sizes. Moreover, the two genomes had similar total sizes (1923 and 1935 cM). This could be related to the fact that the genetic distance between *O. glaberrima* and *O. sativa* is about the same as the distance between the 'ancestral' *indica* and *japonica* subspecies of *O. sativa* (Second 1985). However, considering the MCIS, chromosomes 6 and 11 showed a notably lower mean recombination rate, and chromosome 5 showed a larger mean recombination rate in the interspecific hybrid. Moreover, recombination differences between the BC_1 and DH_1 maps could vary along the same chromosome (e.g. chromosome 3). Similar observations have already been made, for instance, in the *O. sativa*×*O. longistaminata* BC_1 population (Causse et al. 1994).

Segregation distortion

Segregation distortion is frequently observed in rice mapping studies (Causse et al. 1994; Kurata et al. 1994; Lorieux et al. 1996; Xu et al. 1997; McCouch et al. 1988). In our study, strong segregation distortion was observed on chromosome 6 close to the microsatellite markers OSR19 and OSR25, both contained in the *waxy* gene (Fig. 1). This is due to the presence of a sporo-


1
2
3
4
5
6
Scale in cM
RIL1
BC1
DH1
0
40
80
120
160
200
240
XNpb216
OSR2
RM1
XNpb270
RM9
OSR27
OSR3
OSR23
RM14
XNpb346
ACA/CAT02
AGG/CAT02
RM84
RM220
RM1
OSR2
G270
RM9
RM5
RM212
OSR3
AGC/CTG03
OSR23
RM14
(RM84)
RG246
RM220
(RM1)
RG532
U10
W1
RM259
RM243
RG173
RM23
RM24
Amy1B
RM9
RM5
RM34
RM237
RZ19
RG690
RM212
RZ730
RZ801
GR331
RM14
OSR11
RM8
OSR8
OSR9A/B
G357
G243
XNpb132
RM6
OSR26
XNpb89-3
OSR17
RM236
RM211
RM233A
RM8
OSR9A
G357
ACT/CAA04
G243
ACA/CAT08
RM262
RM6
RM240
RZ551
RM213
RM208
OSR26a
RM48
RM236
(RM211)
RM233A
RM53
RM8
RG437
RM27
RM20
RG171
RM262
RG157
RZ318
PstI
RM263
RM221
RZ58
RM6
RM240
(RZ551)
CDO686
Amy1A/C
RM250
RG256
(RM208)
RZ123
(RM213)
RM48
RG520
XNpb173
OSR13
RM7
G62
XNpb144
RM16
OSR31
RM168
OSR5
RM148
XNpb212-2
RM3c
RM60
RM231
RM7
RM232
RM251
G062
G144
Opl14e
RM16
OSR31
RM168
RM49
RM227
AGG/CAT04
RM148
RG418
RM60
RG104
RM22
RM231
RZ329
RZ892
RG100
RG191
RM218
(RM7)
RM232
RM251
RZ574
RZ284
RM16
RG179
RM168
CDO337
RM49
RM55
Pgi-1
CDO87
RG910
RM227
RM148
RG418A
OSR15
XNpb247
RM261
RM252
RM241
RG143
RG218
RZ262
RM261
RG908
RG449
RG788
RZ565
RZ675
RM252
RM241
RG163
RG214
RZ590
RG143
RG620
RM122
OSR34
RM13
G396
RM14B
XNpb81
RM122
OSR34
RM13
G396
ACT/CAA03
RM81c
RZ244
RM42b
RM249
RM164
RM163
G81
RM26
RM31
RG474
RZ390
RM13
RG313
RZ244
RG403
RG229
RM249
RM164
RG13
CDO105
RZ67
RZ70
G81
RZ225
RM26
RM31
OSR19
OSR25
G030
RM3
OSR18
G342
OSR21
OSR19 (Waxy) *****
OSR25 *****
Sh
RM204 *****
G030 *****
RM253 *****
C
Se-1
RM3a
RM30
G342
(RM204)
RZ398
RM253
Amp-3
C
RZ667
Pgi-2
Se-1
pRD10B
RM3
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RG162
RG172
CDO544
RM30
RG653
Amy2A
RG433

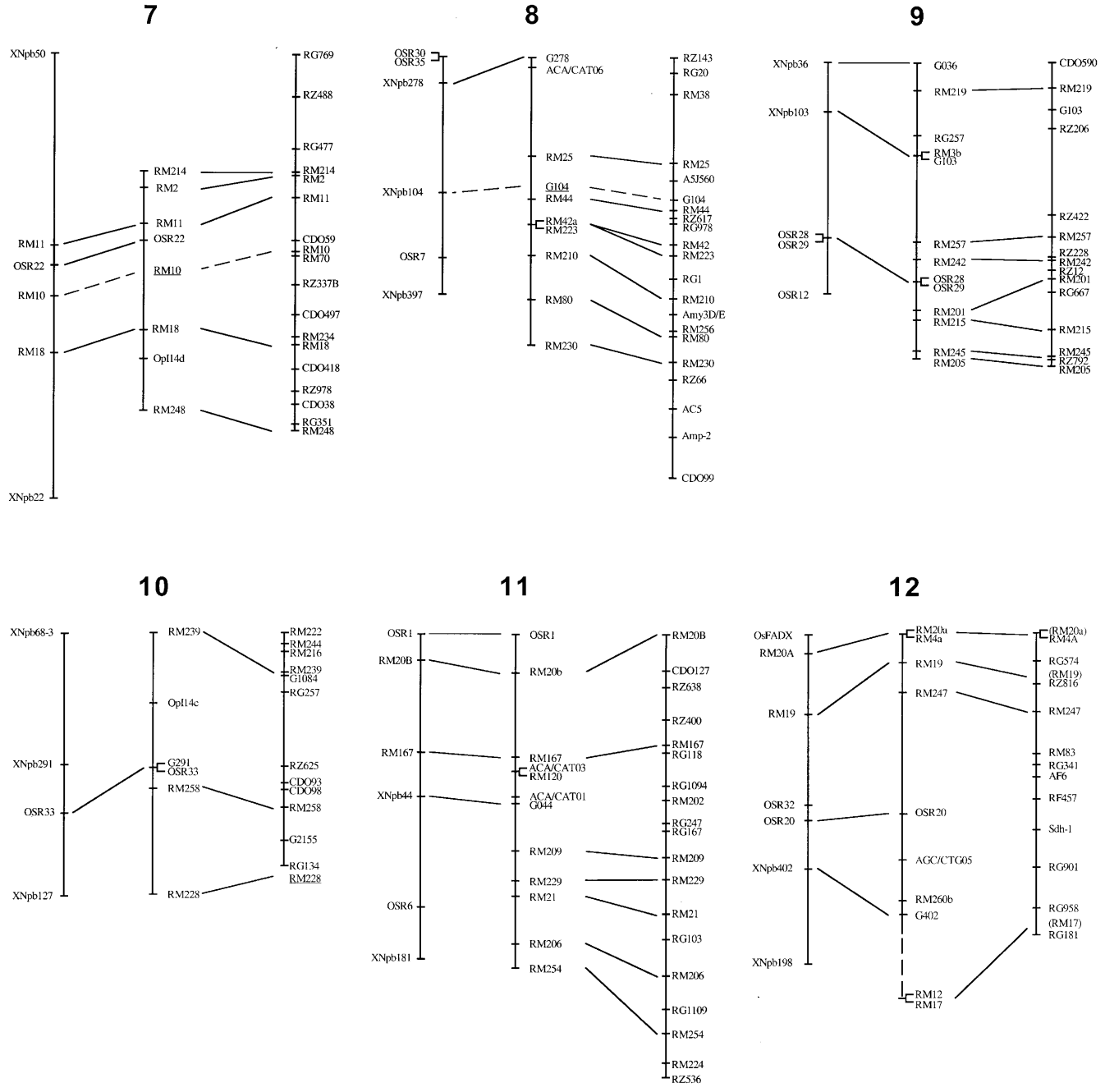

Fig. 1 PCR-based genetic map developed from a backcross population (*O. sativa*/*O. glaberrima*//*O. sativa*) and its comparison with two other rice genetic maps. Linkage groups are oriented as in Chen et al. (1997). *Left* linkage groups: RFLP-based microsatellite map developed on a RIL_1 population by Akagi et al. (1996). *Center* linkage groups: microsatellite-based map developed on a BC_1 population (present study). *Right* linkage groups: RFLP-based microsatellite map developed on a DH_1 population by Chen et al. 1997. Markers *underlined* are placed by inference from another mapping population. Markers in *parenthesis* on the DH_1 map are in their most-probable position but with a LOD <2. Common markers between maps are joined by a *plain line* or by a *dashed line* when marker position is inferred in the BC_1 map from positions in RIL_1 and DH_1 maps. The stars ('*****') following markers on chromosome 6 indicate a $P<0.00001$ for χ^2 testing the deviation from Mendelian segregation. No segregation distortion was observed for other chromosomes

gametophytic sterility factor, s_{10}, which was already found to be tightly linked to *waxy* (Sano 1990). The main effect of this factor is that, in heterozygous genotypes, male gametes are systematically eliminated, and female gametes carrying the *O. glaberrima* allele for s_{10} are selected against compared to gametes carrying the *O. sativa* allele. In our BC_1 population, this resulted in a very large proportion (95.4%) of heterozygous plants at markers close to s_{10}. We inferred the approximate position of s_{10} from marker segregation ratios (Fig. 1). This location corresponds well with that found by Sano (1990). It has been shown that segregation distortion caused by a single locus, or by a locus cluster acting mainly as a single locus, has theoretically no impact on

Table 2 Summary of major gene or QTL positions found by interval-mapping analysis in BC_1 population and their possible correspondance with previously mapped genes

Trait[a]	Chromosome	Marker or interval	LOD score	R^2 [b]	Pop. size[c]	Gene nature	Corresp. major gene[d]
HTR	1	OSR23	13.28	52.6	64	Major gene	*sd1*
HTR	3	RM148/RM418	2.32	20.4	101	QTL	
FT	6	RM253/RM3	20.49	66.4	143	Major gene	*Se-1*
R I	6	RM3/RM30	5.61	72.1	49	Major gene	Unknown
R I	8	RM210/RM80	2.10	24.2	48	QTL	
AWN	4	RM241/RM252	5.46	24.0	98	Major gene	*Awn-1*
AWN	8	RM080	5.51	26.8	70	Major gene	*Awn-4*
COLste	6	RM253/RM3	15.59	40.8	141	Major gene	*C*
COLsti	6	RM253/RM3	34.93	69.4	139	Major gene	*C*

[a] Trait: HTR: mean hight of the three tallest panicules; R I: mean number of primary branching on three panicules; AWN: awning of spikelets (three classes); FT: flowering time in decreasing day length (which was mainly governed by a photosensitivity gene as plants were cultivated at the end of summer, i.e. in long days followed by short days); COLste: stem base coloration (two classes); COLsti: stigmate coloration (three classes); COLap: apiculus coloration (two classes)
[b] R^2: percentage of variation of the trait explained by the QTL or major gene
[c] Pop. size: population size for which both marker and trait data were available
[d] Corresp. major gene: possible correspondance with known genes localised in other studies (Kinoshita 1995)

Table 3 Comparison of map lengths between interspecific (IR64/Tog5681//IR64) and intraspecific (IR64/Azucena) rice genetic maps. The majority of chromosomes have similar sizes, especially when considering the MCIS[c]. Chromosomes 6 and 11 show a lower mean recombination rate in the interspecific hybrid, and chromosome 5 shows a lower mean recombination rate in the intraspecific hybrid

Chromosome	Marker number	Discrete point number[a]	TLGS[b]			MCIS[c]			
			BC_1[d]	DH_1[e]	BC_1/DH_1	Interval	BC_1	DH_1	BC_1/DH_1
1	12	12	191.3	194.3	0.98	RM220-RM212	132.6	141.3	0.94
2	17	13	233.1	198.4	1.17	RM233A-RM48	228.2	189	1.21
3	16	14	259.7	259	1.00	RM60-RG418	252.7	259	0.98
4	4	4	117.1	158.2	0.74	RM261-RG143	117.1	126.9	0.92
5	14	11	226.6	155	1.46	RM13-RM26	195.9	124.8	1.57
6	8	7	145.6	163.5	0.89	RM253-RM30	89.5	125.6	0.71
7	7	7	97.1	146	0.67	RM214-RM248	97.1	100.6	0.97
8	9	8	111.7	163	0.69	RM25-RM230	73.8	77.5	0.95
9	13	11	115.1	117.9	0.98	RM219-RM205	104.3	108.4	0.96
10	6	5	101.7	90.6	1.12	RM239-RM258	60.4	52.1	1.16
11	13	12	129.4	172.3	0.75	RM20B–RM254	114.5	155	0.74
12	10	8	194.8	116.9	1.67	RM20A-RM247	35.4	29.8	1.19
Total (cM)			1923.2	1935.1			1501.5	1490.0	

[a] Discrete point number: number of discrete loci defined by non-cosegregating markers on a linkage group
[b] TLGS: total linkage group size in centimorgans
[c] MCIS: interval on a linkage group defined by the two extreme common markers between the two maps (in centimorgans)
[d] BC_1: (IR64/Tog5681//IR64) backcross population
[e] DH_1: (IR64/Azucena) doubled-aploid population

the estimation of recombination fractions of neighbour intervals (Lorieux et al. 1995). This has been numerically confirmed for chromosome 6 in comparing the classical estimate to other estimates taking into account different allelic selection patterns using the MapDisto program. For the other chromosomes, segregations followed Mendelian expectations. The classical estimate implemented in Mapmaker v. 3.0 was thus kept for all chromosomes.

Trait analysis

The combined analysis of phenotypic and molecular data permitted us to identify major genes and QTLs responsible for three quantitative traits (height, flowering time, number of primary branches) and for three qualitative traits (awning, coloration of the stem base and coloration of the stigma) (see Table 2 for details). When possible, we tried to establish correspondences with major genes which have already been mapped [see Kinoshita (1995) for a report of genes located in rice].

For all three quantitative traits, interval-mapping analysis revealed the presence of a major gene. The ma-

jor gene responsible for 53% of the variance of height and located on chromosome 1 is almost certainly *sd-1*, a semi-dwarf allele present in IR64. The QTL for height on chromosome 3 could correspond to that identified at the same location using the DH_1 population (Albar et al. 1998). For flowering time, in terms of decreasing day length, the major gene on chromosome 6 corresponds to the photosensitivity gene *se-1*.

The two genes identified for awning could correspond to *An-1* and *An-4* that have been located on chromosomes 4 and 8, respectively. Coloration of the stem base and the stigma are probably governed by the same gene, as they map to the same location, which is that of the previously identified gene *C* (chromogen for anthocyanin).

No QTL was found for tillering or the number of secondary branches on the panicles, probably due to the low power of detection by reason of the limited population-size available.

Discussion

According to our objectives, mainly the development of 'contig' lines, the coverage was satisfactory for the major part of the genome, except for chromosome 4 and the short-arm end of chromosome 7. Some gaps remain unfilled and will be filled when new microsatellite markers become available. Use of SSCP (single strand conformation polymorphism) could also be helpful to this aim. Fukuoka et al. (1994) showed that 39 of 70 (55.7%) STS markers showed polymorphism by this technique between two *indica* and *japonica* varieties, so we can expect that SSCP polymorphism between *O. sativa* and *O. glaberrima* will be at least 50%. RAPD and AFLP markers can not be used extensively in an introgression backcross program, since their map location is unknown *a priori* and as they tend to cluster in rice (Kurata et al. 1994). Moreover, AFLPs are not suitable for following genome fragments in a backcross programme, as we would generally have to generate at least four lanes per individual with only one interesting band per lane. Microsatellite markers thus represent the best choice, as they are simple to generate, highly polymorphic, locus-specific and codominant. However, their development is expensive and time-consuming and obtaining a saturated microsatellite map represents a major effort.

Our results show a very good colinearity of *O. sativa* and *O. glaberrima* species with only small non-significant marker inversions. This means that no major rearrangements like inversions or translocations have occurred between the two species. Moreover, except for local differences, the BC_1 interspecific map is about the same size as the DH_1 (*indica*×*japonica*) map. It is interesting to compare these results on recombination and colinearity with available cytogenetic studies: Morinaga and Kuriyama (1957) showed a normal behaviour of the meiotic chromosomes of *O. sativa*×*O. glaberrima* hybrids. A recent study showed a very strong resemblance between the chromosomes of both species, with only minor cytogenetic differences (Ohmido and Fukui 1995). This genetic mapping study thus confirms that the interspecific hybrids can recombine normally along the whole genome. The region in the vicinity of the s_{10} gene on chromosome 6 showed segregation distortion, which to some extent may reflect the rapid recovery of parental types and the MV linkage in the segregating population as proposed by Sano et al. (1980).

This would be very different for distant *indica*×*japonica* crosses, where segregation distortion can involve many chromosomal regions (Causse et al. 1994; Kurata et al. 1994; Lorieux et al. 1996; McCouch et al. 1988; Xu et al. 1997) and can be explained by different sets of gametophytic sterility genes (Oka 1974). In our BC_1 population, it is also probable that several sterility genes are acting on pollen viability but were masked by the total elimination of male gametes due to s_{10}.

Nevertheless, a marker-assisted introgression strategy between the two cultivated rice species should succeed and the selection of the rare semi-fertile BC_1 individuals which are homozygous for the *O. sativa* allele of s_{10} is a possible starting point. In this way, the elimination of remaining gametophytic or gametic sterility genes could be achieved by a conventional process. Another possible way to overcome hybrid sterility would be the development of doubled haploid lines by anther culture from *O. sativa*×*O. glaberrima* F_1s or BC_1s. If anther culture permits the bypass of sporo-gametophytic interaction, a higher percentage of homozygous semi-fertile DH lines could be obtained than with conventional backcrossing. The rice breeding program at the West Africa Rice Development Association (WARDA) succeeded in producing fertile DH lines from various interspecific combinations between *O. glaberrima* and both *japonica* and *indica* varieties. This suggests that interspecific sterility barriers may be efficiently overcome in this way (Jones et al. 1997).

An *O. sativa*×*O. glaberrima* linkage map may have several interesting applications. First, it permits the localisation of several genes or QTLs from *O. glaberrima*. Indeed, we were able to map several loci responsible for different traits in the BC_1 progeny. Genetic mapping of resistance genes is currently being carried out for Rice Yellow Mottle Virus and for the nematode *Heterodera sacchari* on the basis of the present linkage map.

Second, although this species was not bred as intensively as *O. sativa*, it can carry numerous agronomically important alleles. Even if *O. glaberrima* alleles are, under optimised management conditions, often of lower value than *O. sativa* alleles when taken separately, transgressions may be produced in interspecific progeny. Such transgressions have been identified in *O. sativa*×*O. rufipogon* progeny (Xiao et al. 1998). For 12 agronomically important traits, the *O. rufipogon* accession was phenotypically inferior to *O. sativa*. However, transgressive segregants that outperformed the original elite hybrid variety were observed for all traits examined. Similar results were obtained in the present study.

For instance, we observed BC_1 plants with tillering at 45 days which was almost two-times higher than that of the *O. sativa* parent, IR64. This means that we may expect to identify QTLs acting in a complementary way and thus use them in a marker-assisted selection program.

Another main interest of a PCR-based *O. sativa*×*O. glaberrima* map is its utility for monitoring the development of a set of introgression lines (called 'contig lines'; Ghesquière et al. 1997) containing only targeted fragments of *O. glaberrima* in an *O. sativa* genetic background. This genetic material represents a valuable genetic resource with a known genetic content, and will permit the rapid identification of major factors responsible for segregating traits between the two cultivated rice species. A BC_3 progeny is currently being developed, and a molecular survey of BC_2 plants shows that the complete genome is represented by a small number of genotypes. The genomic regions identified using 'contig' lines will then have to be compared with those identified by QTL analysis in segregating interspecific populations.

Finally, knowledge of the *O. sativa ssp. indica*×*O. glaberrima* polymorphism and of the map position of a set of PCR-based markers will permit the rapid characterisation of introgressed material obtained by a conventional backcross strategy (i.e. without the aid of molecular markers). Such material would increase the precision and the power of analyses using the material of 'contig' lines.

An additional interest of the present study is the integration in a single map of microsatellite and STS markers coming from different studies. This permits one to define a framework map with 'anchor' PCR markers regularly dispersed throughout the genome. Such a map would be useful to carry out rapid segregation studies on multiple crosses.

The present study has generated the first PCR-based *O. sativa* × *O. glaberrima* genetic linkage map, which will constitute a valuable tool for the utilisation of *O. glaberrima* in the breeding of rice varieties adapted to the African environment.

Acknowledgements This research was supported by IRD and the Africa/Asia joint Research on Interspecific Hybridization between the African and Asian rice species (*O. glaberrima* and *O. sativa*) through WARDA. Many thanks to J. Bennett for having kindly provided us for STS primers aliquots and sequences.

References

Akagi H, Yokoseki Y, Inagaki A, Fujimura T (1996) Microsatellite DNA markers for rice chromosomes. Theor Appl Genet 93:1071–1077

Albar L, Lorieux M, Ahmadi N, Rimbault I, Pinel A, Sy AA, Fargette D, Ghesquière A (1998) Genetic basis and mapping of the resistance to rice yellow mottle virus. I: QTLs identification and relationship between resistance and plant morphology. Theor Appl Genet 97:1145–1154

Attere A, Fatokun C (1983) Reaction of *Oryza glaberrima* accessions to rice yellow mottle virus. Plant Dis 67:420–421

Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu K, Xiao J, Yu Z, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley SD (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138:1251–1274

Chen X, Temnykh S, Xu Y, Cho YG, McCouch SR (1997) Develoment of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). Theor Appl Genet 95:553–567

Fukui K, Iijima K (1991) Somatic chromosome map of rice by imaging methods. Theor Appl Genet 81:589–596

Fukuoka S, Inoue T, Miyao A, Monna L, Zhong H, Sasaki T, Minobe Y (1994) Mapping of sequence-tagged sites in rice by single-strand conformation polymorphism. DNA Res 1:271–277

Ghareyazie B, Huang N, Second G, Bennett J, Khush GS (1993) Comparison between PCR-based RFLP and Southern-based RFLP as DNA markers for germplasm classification in rice (*Oryza sativa* L.). Rice Genet Newslett 10:129–132

Ghesquière A, Séquier J, Second G, Lorieux M (1997) First steps towards a rational use of African rice, *Oryza glaberrima*, in rice breeding through a 'contig line' concept. Euphytica 96:31–39

Harushima Y, Yano M, Shomura A, Sato M, Shimano T, Kuboki Y, Yamamoto T, Lin S, Antonio B, Parco A, Kajiya H, Huang N, Yamamoto K, Nagamura Y, Kurata N, Khush G, Sasaki T (1998) A high-density rice genetic linkage map with 2275 markers using a single F_2 population. Genetics 148:479–494

Inoue T, Zhong HS, Miyao A, Ashikawa I, Monna L, Fukuoka S, Miyadera N, Nagamura Y, Kurata N, Sasaki T, Minobe Y (1994) Sequence-tagged sites (STSs) as standard landmarkers in the rice genome. Theor Appl Genet 89:728–734

Jones M, Dingkhun M, Aluko GK, Semon M (1997) Using backcrossing and doubled haploid breeding to generate weed-competitive rices from *O. sativa* L.×*O. glaberrima* Steud. genepools. In: Proc Wkshp Africa/Asia Joint Res on Interspecific Hybridization between the African and Asian rice species (*O. glaberrima* and *O. sativa*), WARDA, M'bé, Bouaké, Côte-d'Ivoire, December 16–18, 1996, pp 61–70

Kinoshita T (1995) Report of Committee on gene symbolization, nomenclature and linkage groups. Rice Genet Newslett 12:9–153

Kosambi DD (1944) The estimation of map distance from recombination values. Ann Eug 12:172–175

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio BA, Shomura A, Shimizu T, Lin S-Y, Inoue T, Fukuda A, Shimano T, Kuboki Y, Toyama T, Miyamoto Y, Kirihara T, Hayasaka K, Miyao A, Monna L, Zhong HS, Tamura Y, Wang Z-X, Momma T, Umehara Y, Yano M, Sasaki T, Minobe Y (1994) A 300-kilobase interval genetic map of rice including 883 expressed sequences. Nature Genet 8:365–372

Lander ES, Botstein D (1989) Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Lander ES, Green P (1987) Construction of multilocus genetic linkage maps in humans. Proc Natl Acad Sci USA 84:2363–2367

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lorieux M, Goffinet B, Perrier X, González de León D, Lanaud C (1995) Maximum-likelihood models for mapping genetic markers showing segregation distortion. 1. Backcross populations. Theor Appl Genet 90:73–80

Lorieux M, Petrov M, Huang N, Guiderdoni E, Ghesquière A (1996) Aroma in rice: genetic analysis of a quantitative trait. Theor Appl Genet 93:1145–1151

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

Morinaga T, Kuriyama H (1957) Cytogenetical studies on *Oryza sativa* L. IX. The F_1 hybrid of *O. sativa* L. and *O. glaberrima* Steud Jpn J Breed 12:153–165

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325

Ndjiondjop M-N, Abar A, Fargette D, Fauquet C, Ghesquière A (1999) The genetic basis of high resistance to rice yellow mottle virus (RYMV) in cultivars of two cultivated rice species. Plant Dis (in press)

Ohmido N, Fukui K (1995) Cytological studies of African cultivated rice, Oryza glaberrima. Theor Appl Genet 91:212–217

Oka H (1974) Analysis of genes controlling F_1 sterility in rice by the use of isogenic lines. Genetics 77:521–534

Ooijen JW van (1992) Accuracy of mapping quantitative trait loci in autogamous species. Theor Appl Genet 84:803–811

Panaud O, Chen X, McCouch SR (1996) Development of microsatellite markers and characterization of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). Mol Gen Genet 252:597

Pham J, Bougerol B (1993) Abnormal segregations in crosses between two cultivated rice species. Heredity 70:466–471

Portères R (1950) Vieilles agricultures de l'Afrique intertropicale. Centres d'origine et de diversification variétale primaire et berceaux de l'Agriculture antérieure au XVIe siècle. Agron Trop 5:489–507

Reversat G, Destombes D (1998) Screening for resistance to *Heterodera sacchari* in the two cultivated rice species, *Oryza sativa* and *O. glaberrima*. Fund and Appl Nematol 21:307–317

Sano Y (1985) Sterility barriers between *Oryza sativa* and *O. glaberrima*. In: Rice genetics, Proc Int Genet Symp, IRRI, Los Baños, the Philippines, 27–31 May 1985, pp 109–118

Sano Y (1990) The genic nature of gamete eliminator in rice. Genetics 125:161–191

Sano Y, Chu Y, Oka H (1979) Genetic studies of speciation in cultivated rice. 1. Genic analysis for the F_1 sterility between *Oryza sativa* and *O. glaberrima* Steud. Jpn J Genet 54:121–132

Sano Y, Chu Y, Oka H (1980) Genetic studies of speciation in cultivated rice. 2. Character variations in backcross derivatives between *Oryza sativa* and *O. glaberrima* : M-V linkage and key characters. Jpn J Genet 55:19–39

Sano Y, Sano R, Morishima H (1984) Neighbour effects between two occurring rice species, *Oryza sativa* and *O. glaberrima*. J Appl Ecol 21:245–254

Second G (1982) Origin of the genic diversity of cultivated rice (*Oryza spp.*): study of the polymorphism scored at 40 isozyme loci. Jpn J Genet 57:25–57

Second G (1985) Evolutionary relationships in the Sativa group of *Oryza* based on isozyme data. Génét Sél Evol 17:89–114

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Fritjers A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP : a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Wu K, Tanksley S (1993) Abundance, polymorphism and genetic mapping of microsatellites in rice. Mol Gen Genet 241:225–235

Xiao J, Fulton T, McCouch S, Tanksley S, Kishimoto N, Ohsawa R, Ukai Y, Saito A (1992) Progress in integration of the molecular maps of rice. Rice Genet Newslett 9:124–128

Xiao J, Li J, Grandillo S, Ahn S, Yuan L, Tanksley S, McCouch S (1998) Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon*. Genetics 150:899–909

Xu Y, Zhu L, Xiao J, Huang N, McCouch S (1997) Chromosomal regions associated with segregation distortion of molecular markers in F_2, backcross, doubled haploid, and recombinant inbred populations in rice (Oryza sativa L.). Mol Gen Genet 253:535–545