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First steps towards a rational use of African rice, *Oryza glaberrima*, in rice breeding through a 'contig line' concept

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Summary

In the past, utilisation of African cultivated rice, *Oryza glaberrima*, in *O. sativa* breeding was hampered by high sterility in interspecific F_1 and in early progenies. Recent development of mapped molecular markers provides the opportunity to rationalise and to monitor introgressions between the two cultivated rice species. Development of such introgressed lines through interspecific hybridisation and backcrossing represents high potential to create new genetic and transgressive variation. We are initiating an original marker-aided backcross program with the aim of producing a set of 100 'contig lines', each one bearing an alien *O. glaberrima* chromosomal fragment of around 20 cM in the *O. sativa* genetic background. As a preliminary step, we are now developing a genetic linkage map based on STS and RAPD markers, using an interspecific backcross between *O. sativa* and *O. glaberrima*. BC_1 plants will then be selected to expedite the production of 'contig lines' exhibiting only the target fragment. The interspecific polymorphism is anticipated to be large enough to make this methodology feasible. Many advantages are expected from this approach, such as: (i) systematic assessment of the useful genetic resources displayed by *O. glaberrima*, (ii) improved and simplified detection of QTLs and (iii) comparative analysis of genomic organisation of *O. glaberrima* vs. *O. sativa*. Current work on evaluation of STS and RAPD polymorphism between parents and development of the BC_1 population is presented.

Abbreviations: AFLP^{TM} – amplified fragment length polymorphism; CAPS – cleaved amplified polymorphic sequence; CIRAD – Centre de Coopération Internationale en Recherche Agronomique pour le Développement (Centre for International Cooperation in Argonomic Research for Development); ORSTOM – L'Institut Francais de Recherche Scientifique pour le Développement en Coopération (French Institute for Scientific Research for Development in Cooperation); QTL – quantitative trait locus; RAPD – random amplified polymorphic DNA; RFLP – restriction fragment length polymorphism; RYMV – rice yellow mottle virus; SSCP – single-strand conformation profile; STS – sequence-tagged site; WARDA – West African Rice Development Association (Côte-d'Ivoire); PCR – Polymerase chain reaction

Introduction

Oryza glaberrima Steud. is one of the two cultivated rice species. It is endemic and only cultivated in tropical West Africa. *O. glaberrima* is increasingly being replaced by the Asian cultivated species, *O. sativa* L., because of its low yield potential (high shattering, lodging susceptibility). *O. glaberrima* is usually found in mixture with *O. sativa*, but under poor crop management or adverse ecological conditions, farmers still favour *O. glaberrima* (Baga rice in Guinea, floating rice in Niger Valley). *O. glaberrima* occupies a wide range of ecosystems in regard to water supply: from rainfed hill slopes to deep water conditions and coastal mangrove areas. Two major agroecotypes can be distinguished, a floating photosensitive type and an early erect type cultivated in upland conditions or in moderately inundated lowlands.

O. glaberrima was proposed to have been domesticated in West Africa (Central Niger Delta in Mali to Lake Chad and Guinea/Senegal) from the wild annual rice *O. breviligulata* A. Chev. & Roehr. (= *O. barthii* A. Chev.) long before the introduction of *O. sativa* in the 15^{th} or 16^{th} century (Portères, 1950). Isozyme polymorphism confirmed fairly well that *O. glaberrima* was a true cultivated species resulting from an independent domestication (Second, 1982). The genetic diversity of *O. glaberrima* is substantially lower compared to *O. sativa* and no classification into different subspecies such as *indica* and *japonica* in *O. sativa* can be recognised (Second, 1982). In the genus *Oryza*, the two cultivated rice species belong to the *sativa* group characterised by the A genome at diploid level (2n = 24). In spite of complete male sterility, meiosis and chromosome pairing in F_1 hybrids are regular (Nayar, 1973).

In the seventies, many samples of *O. glaberrima* were gathered during field collections by ORSTOM-CIRAD, WARDA and IITA with the help of National Institutions (Ng et al., 1983). More than 1000 accessions of *O. glaberrima* were collected and most of them are currently evaluated for genetic and agronomic characterisation. This permitted the identification of interesting agronomic traits and potential donors for resistance/tolerance to major stresses with appropriate plant type and grain quality properties. *O. glaberrima* was found to have different patterns of blast resistance (Silue & Notteghem, 1991), good to very high levels of resistance to Rice Yellow Mottle Virus (RYMV) (Attere & Fatokun, 1983; John et al., 1985), and insect resistances including resistance to *Diopsis* spp. (Alam, 1988; Sauphanor, 1985) and resistance to rice gall midge provoked by *Orseolia oryzivora*. Specific resistances to nematodes were also observed in *O. glaberrima* and *O. breviligulata* (Reversat & Destombes, 1995). *O. glaberrima* also possesses useful traits in terms of tolerance of abiotic stresses such as acidity, iron toxicity and drought (Sano et al., 1984). Moreover, its early, rapid and vigorous vegetative growth can contribute to a better control of weeds in rice fields (Jones et al., 1994).

Nevertheless, the utilisation of *O. glaberrima* in rice genetics and breeding was most often limited to obtaining interspecific hybrids and first backcross progenies. So far, no improved lines have been obtained by this way. Extensive analyses of reproductive barriers between the two species were made by several authors (Chu et al., 1969; Sano, 1985; Sano et al., 1979). Genetic models based on one-locus sporo-gametophytic interactions were proposed by these authors to account for the near complete F_1 male sterility and the rapid recovery of parental types in the hybrid derivatives. Consequently, conventional breeding programs do not seem to be appropriate for introduction of novelties or interesting traits coming from *O. glaberrima* into *O. sativa*. In order to by-pass sterility and recombination restriction, we propose here a new original action aiming at a systematic and rational utilisation of *O. glaberrima* in using an interspecific 'contig line' methodology.

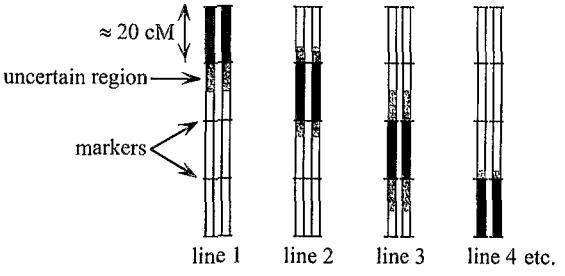

Figure 1. The concept of 'contig lines'. Each 'contig line' will have a genetic background coming from *O. sativa* (in white) and an introgressed 15 to 20 cM-long fragment coming from *O. glaberrima* (in black). This fragment will be delimited by two markers, with an uncertain region (in grey) of which the length will depend on the saturation of the map and on the number of backcrosses performed to obtain the line. The totality of the contiguous fragments will cover the whole genetic map. Only one chromosome is represented for clarity.

The utilisation of molecular markers is critical to the development of this approach. The association of mapped molecular markers (RFLPs, RAPDs) converted to STSs and of RAPDs is envisaged as a powerful combination of tools for that goal. We present here preliminary results relating the development of F_1 hybrids and backcross progenies and molecular polymorphism between the parents.

Materials and methods

General strategy

The main aim of this work is to obtain a set of lines with the same genetic background coming from *O. sativa*, and only one chromosomal fragment introgressed from *O. glaberrima*. Fragments belonging to the different lines will be arranged in a contiguous pattern in such a way that the totality of the fragments will represent all

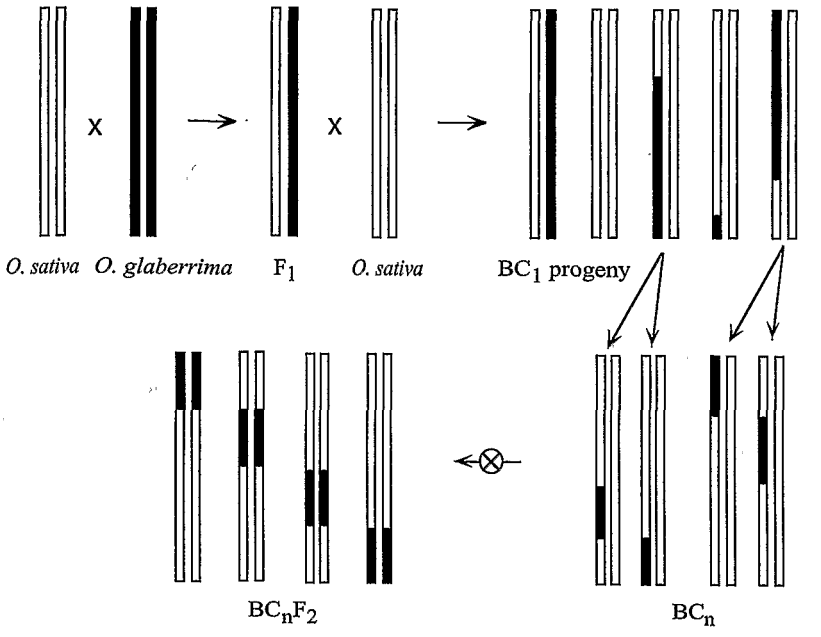

Figure 2. The initial cross is made with the *O. sativa* parent as female. The backcross uses the *O. sativa* parent as male. BC_1 plants will be chosen among the progeny, according to their allelic configuration. These plants will be backcrossed with *O. sativa* parent, and about eight BC_2 plants per BC_1 individual will be analysed for at least four markers flanking the target fragments carried by the BC_1 plants. The process will be repeated until complete elimination of linkage drag. Finally, plants will be self-pollinated to allow fixation of the alien fragments.

the genome of *O. glaberrima* (Figure 1). We will thus call the resulting material 'contig lines'. Introgression of selected chromosomal fragments will be monitored by molecular markers. Since the rice genetic linkage map length is about 1600 cM (Kurata et al., 1994), our goal is to build a set of around a hundred 'contig lines', each one carrying an alien *O. glaberrima* chromosomal fragment of about 15–20 cM.

The first step consists of the development of a backcross population between *O. sativa* and *O. glaberrima*. We thus made crosses between six *O. glaberrima* varieties (CG 14, CG 20, SG 329, IG 10, TOG 5673 and TOG 5681) and four *O. sativa* lines (WAB 181-18, WAB 56-14, WAB 56-104 and IR 64). CG 14, CG 20, SG 329, and IG 10 *O. glaberrima* accessions are traditional cultivars coming from Senegal and Côte-d'Ivoire selected by WARDA for their good plant type after several years of evaluation. TOG 5673 and TOG 5681 represented a special interest because of their resistance of RYMV. The three WAB cultivars (*japonica* subspecies) are promising lines selected by WARDA for upland conditions with good resistance/tolerance to drought and blast. IR 64 (*indica* subspecies) is the most widespread irrigated variety in the world and was obtained from IRRI (the Philippines). The crosses were made using *O. sativa* as the female parent, in order to limit unfavourable nucleocytoplasmic interactions.

The second step is the construction of a new genetic linkage map based on the interspecific backcross progeny. Ideally, the markers should be regularly distributed on the linkage groups and sufficiently numerous to identify 15–20 cM-long chromosome fragments with a reasonable precision.

Then, BC_1 plants will be selected on the basis of their marker pattern to expedite the production of 'contig lines' receiving only the target fragment. Each fragment will be delimited by two markers. A subset of BC_1 plants will be selected for BC_2 in order to keep all the potential *O. glaberrima* fragments. Each BC_1 candidate should have the selected fragments and the minimum of undesirable *O. glaberrima* genetic background in order to reduce the heterogeneity in advanced generations. At each further backcross generation, plants will be analysed by markers to check for the presence of *O. glaberrima* fragments. Only the plants having the desirable fragments will be sown for the next backcross. At the same time, the material will be characterised and evaluated with a special emphasis

Table 1. Numbers of F_1 hybrids obtained for sixteen *O. sativa* · *O. glaberrima* combinations

	CG 14	CG 20	IG 10	SG 329	TOG 5673	TOG 5681
WAB 181-18	2	5	7	–	36	9
WAB 56-104	–	9	6	3	2	2
WAB 56-14	18	30	33	–	3	–
IR 64	–	–	–	–	8	4

–: undone cross.

on fertility restoration. The process will be repeated until having only one fragment per line and complete elimination of linkage drag. Finally, plants will be self-pollinated for allele fixing and to control their fertility (Figure 2).

At any step of the backcrossing process, the genetic material can be fixed by selfing with selection for interesting traits and introduced into breeding programs. The phenotypic effect of the most interesting *O. glaberrima* fragments in the final material will be confirmed by linkage analysis on F_2 populations derived from F_1s between 'contig lines' and recurrent parent.

Molecular markers

The number of analyses necessary to check at each backcross generation for the presence of the complete set of target fragments requires the use of PCR technology. STS and RAPD markers have been tested for polymorphism between the parents. STSs are PCR markers obtained by amplification of DNA between two primers corresponding to the two bounds of a genomic RFLP probe. They combine the advantage of RAPD markers (they are based on PCR) and those of RFLP markers (they are locus-specific and codominant). A first set of 63 STSs mapped on an intraspecific saturated map (Kurata et al., 1994) was published by Inoue et al. (1994) and was used in this study to score the polymorphism between parents. A second set made of 250 STSs located on an interspecific saturated map (Causse et al., 1994) is in preparation at IRRI (Ghareyazie et al., 1993). It should permit the complete coverage of the rice genome with STSs.

The polymorphism was anticipated to be large enough to make the program feasible. The first reason rests in the evolution of the wild progenitor of *O. glaberrima*, which diverged independently from corresponding wild ancestors of *O. sativa* 2 or 3 million years ago (Second, 1982). The other reason relies on the strong reproductive barriers, which hampered natural introgressions between the two cultivated species after the introduction of *O. sativa* in Africa. As a comparison, Causse et al. (1994) found a very high polymorphism (54% on average per restriction enzyme) between *O. sativa* and the perennial African wild rice species, *O. longistaminata*. Moreover, isozyme polymorphism between *O. sativa* and *O. glaberrima* is quite high (Second, 1982). Due to the method, the STS/CAPS polymorphism is expected to be relatively low compared to other marker types (here, we call CAPS markers the bands generated by restriction of STS amplification products). Saturation of the STS core map will be obtained by RAPDs.

The parental DNAs were isolated from lyophilised leaves using the CTAB method (Murray & Thompson, 1980).

For STS markers, PCR reactions were carried out in 25 or 50 μl, with the following reagent concentrations: primer (forward and reverse) 0.4 μM, Taq polymerase (Promega) 0.02 U/μl, buffer mix (Promega) 1X, dNTP 200 μM. Conditions were 94 °C – 5 min; 35 × (94 °C – 1 min; 55 to 70 according to the primers – 2 min; 72 °C – 2 min); 72 °C – 3 min. Polymorphism was revealed either directly or after restriction with restriction enzymes (CAPS). To this aim, eight four-bases cutters were used: Hae III, Rsa I, Hpa II, Cfo I, Alu I, Hsp92 II, Taq I and Sau 3A. Migrations of PCR/restriction products were carried out on 2–3% agarose gels or on 8% polyacrylamide gels for fragments below to 350 bp. Gels were stained with ethidium bromide.

For RAPD markers, PCR amplifications were carried out in 25 μl, with the following reagent concentrations: primer 0.4 μM, Taq polymerase (Appligene) 0.02 U/μl, buffer mix (Appligene) 1X, dNTP 150 μM. Conditions were 95 °C – 5 min; 45 × (95 °C – 1 min; 35 °C – 1 min; 72 °C – 2 min); 72 °C – 6 min. Migrations of PCR products were carried out on 1.5% agarose gels and stained with ethidium bromide.

Table 2. Polymorphism observed between IR 64 and TOG 5681, for 23 STS markers located on chromosomes 2, 3, 4, 8 and 12

STS name	Chromosome	Bp	Polymorphism of amplicon size	Restriction occurrence	Restriction polymorphism
G 39	2	136	no	no	no
G 45	2	900	no	yes	no
G 57	2	500	no	yes	no
G 132	2	1300	yes (sb)	–	–
G 243	2	200	yes	–	–
G 275	2	271	no	yes	no
G 357	2	137	yes	–	–
G 365	2	350	yes (sb, ni)	yes	no
G 55	3	144	no	–	–
G 62	3	276	no	no	no
G 144	3	376	yes (p/a)	–	–
G 164	3	580	yes (s/b)	–	–
G 177	4	297	no	no	no
G 271	4	1100	no	no	no
G 282	4	237	no	no	no
G 56	8	1000	yes (sb, ni)	yes	yes
G 104	8	780	no	yes	yes
G 187	8	1400	no	yes	no
G 278	8	1000	no	yes	yes
G 124	12	109	no	–	–
G 148	12	177	no	–	–
G 193	12	256	no	–	–
G 402	12	272	no	–	–

Bp : number of base pairs of the amplicon obtained with Nipponbare variety (Inoue et al., 1994). –: untested combination. sb: supplementary band. ni: non-informative polymorphism in backcross. p/a: presence/absence polymorphism.

Results

Crosses

From the twenty-four possible *O. sativa* × *O. glaberrima* combinations, sixteen were performed in the glasshouse at ORSTOM, Montpellier. We obtained 177 F_1 hybrids (Table 1). For all combinations, the F_1 plants were tall, very vigorous, highly tillered, with large and awned spikelets. However, no grain was found in the panicles of the 177 hybrids under natural self pollination. Pollen staining with Alexander coloration (Alexander, 1969) showed that pollen grains were completely empty and confirmed that complete male sterility is a general rule in *O. sativa*× *O. glaberrima* F_1 hybrids whatever the combination of parental varieties (Pham & Bougerol, 1989; Sano, 1985; Sano et al., 1979).

Two combinations, (IR 64 × TOG 5673) and (IR 64 × TOG 5681) were preferred for backcrossing in order (1) to attempt to transfer RYMV resistance into *O. sativa* and (2) to determine if a wider adaptation of *O. sativa* to various growing conditions (lowland to upland) may be obtained in resulting lines.

Large backcross seed sets (50 and 150 seeds respectively) were obtained from those two backcrosses.

STS/CAPS polymorphism

Twenty-three STSs located on chromosomes 2, 3, 4, 8 and 12 (Inoue et al., 1994) were tested (Table 2). All pairs of primers amplified the DNAs of the three parents, IR 64, TOG 5673 and TOG 5681, with the sole exception of marker G144, which did not amplify IR 64 (repeated three times). Two other DNAs were also always amplified by all primers: Azucena, a *japonica*

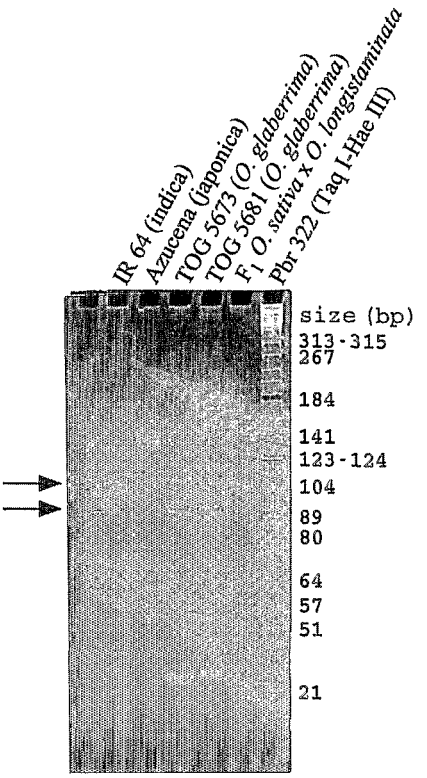

Figure 3. Detection of polymorphism of amplicon size between *O. sativa* (ssp. *indica* and *japonica*) and *O. glaberrima* with STS marker G357 (Polyacrylamide gel).

variety and an F_1 hybrid between *O. sativa* and *O. longistaminata*.

Three STSs (13%) showed a difference in the amplicon sizes detectable by migration on agarose or polyacrylamide gel (Figure 3). Four other markers (18%) showed a polymorphism coming from supplementary bands. These bands came from repeatable but unexpected amplification. They will have to be used with caution for mapping due to their unknown *a priori* map location. Two of them (9%) gave informative polymorphism in a backcross, i.e., an additional band coming from *O. glaberrima*. The two others were not informative, i.e., the additional band came from *O. sativa*.

Among the sixteen STSs which did not show a difference in amplicon sizes, thirteen were searched for variation in restriction pattern (CAPS). Seven (54%) showed a restriction of the amplicon, and three (23%) revealed polymorphism (Table 2). Note that polymorphism after restriction may be ambiguous for STSs generating supplementary bands. As expected, bands with high molecular weight were more frequently cut than smaller ones.

Table 3. Polymorphism observed between IR 64 and TOG 5681, for 15 RAPD markers (primers from Operon). Thirteen primers (87%) gave at least one polymorphic band coming from *O. glaberrima*. Polymorphic bands coming from *O. sativa* were not counted, as they provide no information in the backcross

RAPD primer	Number of polymorphic bands
A9	2
A12	2
J1	3
J11	2
J12	2
J15	1
K17	3
M9	1
M13	3
M16	1
N8	0
X4	2
Y2	0
Y5	4
Z7	0
Mean	1.73

RAPD polymorphism

Fifteen primers (from Operon) were tested for polymorphism between IR 64 and TOG 5681. Very high polymorphism was observed, as thirteen primers (87%) produced at least one band present in *O. glaberrima* and absent in *O. sativa*. The mean number of polymorphic bands per primer was 1.73. Results are summarised in Table 3.

Discussion

Obtaining F_1 hybrids and backcrosses was successful. It is the first time that sufficient progeny (n = 150) to develop a reliable genetic map was obtained from a single backcross involving the two cultivated species of rice. We may expect that recombination events in our *O. sativa* × *O. glaberrima* F_1s were not too abnormal. As an informative example, the overall *O. sativa* × *O. longistaminata* map length (1491 cM) (Causse et al., 1994) is reduced by only 5% compared to that

of the *indica* × *japonica* map (1575 cM) (Kurata et al., 1994), indicating that interspecific recombinations in rice can occur almost normally. Further, the genetic distance between *O. glaberrima* and *O. sativa* is smaller than between *O. longistaminata* and *O. sativa* (Second, 1985).

The overall STS/CAPS non-redundant polymorphism was about 30%, only taking into account informative bands. This result may be compared to STS/CAPS polymorphism between *indica* and *japonica* subspecies found at IRRI, which is about 21% after restriction of amplification products with 9 restriction enzymes (Mendoza et al., 1995). As expected, this shows that *O. glaberrima*/*O. sativa* STS/CAPS polymorphism is higher than intraspecific polymorphism. Moreover, as some STS/enzyme pairs have not been tested yet, we can expect greater variation. In some cases, we expect to overcome lack of polymorphism by using the SSCP technique (Orita et al., 1989). This technique has been used with success for the detection of polymorphism for STSs in rice (Fukuoka et al., 1994).

For RAPD markers, a very high level of polymorphism was observed. As a comparison, the polymorphism observed between *indica* and *japonica* was about 8% in the interspecific saturated map using a double-RAPD technique (Kurata et al., 1994). We may thus expect a very rapid saturation of the interspecific map with this type of marker. In backcross progenies, RAPD markers are as informative as codominant markers such as STSs and RFLPs, for estimating recombination fractions and ordering loci since all genotypes may be classified. Moreover, RAPD markers may map on the marker-rare regions (Kurata et al., 1994). However, the map location of RAPDs is unknown *a priori* and they tend to cluster in rice (Kurata et al., 1994). Consequently, they have to be used as a complement to locus-specific markers such as STSs.

RFLPs or AFLPs are very useful markers. RFLPs are locus-specific, and AFLPs can very quickly generate many polymorphic markers. However, they are not included in our program, essentially for cost reasons. We will only need two markers per line in advanced generations. Thus, RFLPs would not be profitable since Southern blots would be hybridised only one or two times. Consequently, this technique will be used only if no polymorphism is found with STSs on a long chromosome segment. AFLPs are profitable only when at least 4 or 5 bands are usable per gel lane. In our case, we would generally have to generate two lanes per individual with only one usable band per lane. Moreover, as with RAPDs, their location on other rice genetic maps is unknown.

Another interesting product of this research will be the assessment of reproductive barriers which can act as a strong localised restriction of genetic recombination and limitation in transfer of interesting traits due to M-V linkage (Sano et al., 1980). Considering the genetic model from Sano et al. (1979), gamete eliminator(s) will be promoted during the backcross process and can favour introgression of alien fragments despite the morphological recovery to parental types. Then, according to the origin of alleles at S (sterility) loci in F_1s, introgression will either be favoured or be more difficult to achieve. Segregation distortion of markers can give a first approximation of location of these genes. For instance, the segregation of isozyme marker Est-2 on chromosome 6 was found strongly distorted in favour of the *O. glaberrima* allele in a BC_1 progeny (Pham & Bougerol, 1989). This confirmed previous observations of distortions for *wx* gene which is located at the end of chromosome 6 (Sano, 1985). Subsequently, intensive mapping of corresponding alien fragments by test crosses with the recurrent parent could lead to precise localisation of S (sterility) genes.

The above strategy can help in predicting the feasibility of transferring a specific trait from *O. glaberrima* in the case of genetic linkage with reproductive barriers. Some *O. glaberrima* varieties such as TOG 5681 show a high level of resistance to RYMV and were demonstrated by serological (Elisa) tests to be totally immune to this virus (IITA, 1986). However, transfer by backcrossing was unsuccessful. If such a trait is linked to a sterility gene, S_a, coming from *O. glaberrima*, in theory it should be eliminated during backcrossing. Then, large backcross progeny can be helpful to identify recombinant individuals to be backcrossed again. The blind approach of 'contig lines' is also suitable in the case of recessive traits since it can make sure that target genes will not be lost before fertility restoration necessary for the analysis of selfed progenies. Another example of interesting traits to be transferred is resistance to nematodes, *Meloidogyne* spp. and *Heterodera sacchari* (Diomandé, 1984; Reversat & Destombes, 1995). Reversat & Destomes (1995) tested forty-two *O. sativa* varieties for *Heterodera sacchari* root penetration, and found no resistance. In contrast, nineteen of thirty *O. glaberrima* varieties were found resistant and no development of juvenile nematodes was observed. For both RYMV and nematodes, it is suggested that a coevolution between

host and pathogens permitted *O. glaberrima* to develop specific resistance presumed absent in *O. sativa*.

Two pathways of future development can be envisaged for the strategy that we present. The first one consists of starting to genotype of few BC_1 individuals (25–30), and then to develop additional 'contig lines' containing smaller fragments than in BC_1 lines by exploiting recombination events in successive backcrosses. Note that the development of a genetic map is not absolutely necessary if sufficient STS markers with known map locations are available. Also, few crosses are needed in this approach. The other possibility is to type more BC_1 progeny (100–150) and to construct a reliable genetic map. Thus, more lines could be directly chosen with better cutting of the fragments and a higher percentage of recurrent parent genetic background. Moreover, this option allows a search for QTLs using BC_1F_2s. The information about these QTLs could therefore be used to derive 'contig line' sets for specific traits, allowing for identification of epistatic effects. To this aim, the STS map would be used, complemented by the use of RAPD markers where necessary. STS locus-specific markers are crucial since we aim to compare QTLs coming from *O. glaberrima* to QTLs found in *O. sativa* crosses. However, the number of controlled crosses necessary to follow this strategy will constitute a limitation. A compromise between the two approaches is probably a more realistic solution.

This 'contig line' approach will provide a systematic assessment of useful genetic diversity coming from *O. glaberrima*. This process has the potential to mimic the introgressions between *indica* and *japonica* subspecies which led to the large genetic diversity observed in *O. sativa* species (Second, 1982). One of its main advantages is to allow a direct comparison of controlled introgressed lines with the recurrent parent, allowing the identification of alien sources of genetic variation for any interesting trait. It is thus an improved and simplified way of QTL detecting. Furthermore, as the genetic linkage map becomes more and more saturated, the characterisation of alien fragments will be refined. By backcrossing 'contig lines' to the recurrent parent, it will be possible to split the original fragments into smaller ones and to increase the precision of QTL localisation.

The development of a complete set of 'contig lines' introgressed with *O. glaberrima* will provide genetic material amenable to be distributed to any interested institution in rice breeding. Such genetic material will also be very useful for multilocational evaluations. The resulting lines will be multiplied in the field at WARDA. Molecular markers linked to useful traits coming from *O. glaberrima* will be at hand. Tagging of these genes may be expected in many instances to be valid for *O. sativa* and will give reliable information on the allelic diversity for useful traits in wild and cultivated rices.

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