Molecular Profiling of Interspecific Lowland Rice Progenies Resulting from Crosses between TOG5681 and TOG5674 (*Oryza glaberrima*) and IR64 (*Oryza sativa*)

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Abstract

Two Outstanding *Oryza glaberrima* ($2n = 24$, AA) varieties TOG5681 and TOG5674 were used as male donor parents with IR64, the high-yielding improved Asian rice variety used as recurrent female parent by the International Rice Research Institute (IRRI) to develop 18 BC_3F_1 interspecific lowland rice progenies. The proportion of parental genomic contribution and the extent of genetic differences among these lines were assessing using 36 microsatellites markers. The average genomic contribution of the donor TOG5681 and that of the recurrent IR64 within their 12 interspecific lines derived from IR64xTOG5681 cross were estimated to 13.2% and 79.8% respectively. Using 33 out of the 36 SSR markers, the average genome introgression rate of TOG5674 and that of IR64 within their 6 progenies were estimated to 8.7% and 85.5% respectively. In addition, heterozygosity and non-parental alleles were also identified. Clustering analysis technique using NTSYS classified the progenies into six groups and group five is closely related to IR 64.

Keywords: rice, *Oryza glaberrima*, interspecific, introgression, microsatellites, genetic diversity

1. Introduction

Plant genetic resources for food and agriculture are the basis of global food security. They provide livelihood to all the inhabitants of our planet (Dantsey-Barry et al., 2004). Among all the subsistence crops that contribute to human food, rice represents the 2nd grown cereal and the 3rd most important consumed and exported product in the world after wheat and maize (Hirsch, 2001). It contributes 27% of energy intake and 24% of food protein of the inhabitants from developing countries (FAO, 2001). Two species of rice are cultivated; *Oryza sativa L.* originated from Asia and *Oryza glaberrima Steud.* originated from Africa.

O. glaberrima is grown for over 3500 years in Africa (Porters, 1950), and has developed many traits due to its domestication history that make it adapted to the prevalent traditional systems of shifting cultivation. The cultivars of that species are known for several genes of resistance/tolerance to the local constraints including biotic (weeds, diseases, insects, etc.) and abiotic (drought, salinity, iron toxicity, etc.) stresses in West and Central Africa. Some of these genes have already been identified (Abo et al., 1998; Johnson et al., 1998; Ndjiondjop et al., 1999; Fofana & Rauber, 2000; Kawano et al., 2008; Thiémélé et al., 2010; Moukoumbi et al., 2011 etc.). Convinced about the rich potential of the genetic resources of *O. glaberrima Steud.*, Jones et al. (1997) and WARDA (1996) affirmed that this species represents a real gold mine and a rich reservoir of genes useful for improving the crop. However, *O. glaberrima* has a low-yielding potential due to some undesirable traits of which the most important are spontaneous shattering, lodging and poor panicles branching (WARDA, 1996; Futakuchi et al., 2008; Ndjiondjop et al., 2008; Futakuchi & Sié, 2009). Contrary to *O. glaberrima, O. sativa L.* has good yielding potential but poor

resistance/tolerance to several biotic and abiotic constraints prevailing in West and Central Africa. The development of interspecific varieties between *O. glaberrima* and the Asian species *O. sativa* offers an opportunity to exploit the useful traits in both species (Ghesquière et al., 1997). With that vision, the Africa Rice Center (AfricaRice) initiated a vast program to cross the two cultivated species with a view to exploiting the adaptive and organoleptic assets of *O. glaberrima* and combining them with the yield abilities of the Asian species *O. sativa*. The released interspecific varieties and the under development lines of the New Rice for Africa (NERICA) resulted from these efforts (Futakuchi & Sié, 2008). The development of the first interspecific lines (upland NERICAs) was described by Jones et al. (1997) and based on the same breeding concept, lowland NERICA (the WAS122, 161 and 191 series) have been developed (Futakuchi & Sié, 2009). The NERICA varieties give real hope for the improvement of rice productivity, profitability and the sustainability of rice production system in Sub-Saharan Africa (Ndjiondjop et al., 2008). According to the same author, breeders from AfricaRice and those of the National Agricultural Research Systems (NARS) have crossed the IR64 variety (*O. sativa indica)* used as recurrent parent and TOG5681 (*O. glaberrima*) variety used as donor parent for the development of the NERICAs (New Rice for Africa) that have good yield abilities and are adapted to lowland ecosystems. The molecular profiling of these interspecific has been studied by Ndjiondjop et al. (2008) using microsatellite markers which are co-dominant, polymorphic, multi-allelic on closely related individuals and uniformly distributed on the plants genome (Morgante & Olivieri, 1993; Sharma et al., 1995; Ndjiondjop et al., 2008). The positions and information about the sequences of these SSRs markers are available on (http://www.gramene.org).

This study addresses the reciprocal crossing of eighteen lines that were developed by International Rice Research Institute (IRRI). The lines comprised of twelve progenies from BC_3F_1 IR64 x TOG5681 and six progenies from BC_3F_1 IR64 x TOG5681 and two African rice varieties TOG5681 and TOG5674 were used as donor male parent. The objectives of this study were to compare the molecular profiling of these lines using microsatellite markers and to assess the effectiveness of the genomic contribution of the donor and recurrent parents as well as the genetic diversity existing among these interspecific lines. The results were compared with those obtained by Ndjiondjop et al. (2008) on NERICA lowland varieties which were derived from reciprocal crossing BC_3F_8 TOG5681 x IR64 where TOG5681 was used as donor female parent. Our study shown the efficiency of the donor parent *O. glaberrima* (TOG5681) that was introgressed and this depend on the approached used (nucleus or cytoplasm) in both studies.

2. Materials and Methods

2.1 Plant Materiel

The plant material used in this study consisted of 18 BC_3F_1 interspecific lines developed by IRRI. IR64 was used as recurrent female parent with TOG5674 and TOG5681 varieties of *Oryza glaberrima* used as donor male parent (Table 1 and 2). IR64 is an improved variety of irrigated rice having several desirable agronomic traits such as high yield potential, earliness and medium plant height suitable for irrigated rice cultivation conditions. On the contrary, TOG5681 is an *O. glaberrima* variety from Nigeria having a low yield potential which is mainly due to the spontaneous shattering of the grains and its sensitivity to lodging (John et al., 1997). The desirable features of TOG5681 are high tillering capacity, competitiveness with weeds, resistance to Rice Yellow Mottle Virus (RYMV) and to nematodes. In addition to these characteristics mentioned for TOG5681, TOG5674 is tolerant to important rice stresses. Among the 18 BC_3F_1 interspecific lines selected in this genetic diversity study, twelve (12) were selected from IR64 / TOG5681 cross and six (6) from the IR64 / TOG5674 cross. All the 18 lines are presented in bothTables 1 and 2.

2.2 Genotyping

The extraction of the DNA from the samples (18 interspecific lines and their 3 parents) was carried out using 300 mg of fresh leaves collected from 28 days-old plants. The leaves of each sample are then cut into pieces and grounded in a buffer solution (composition for 100 ml: 28 ml NaCl, 4 ml EDTA, 10 ml Tris, 3g MATAB, 0.5 g Sodium Bisulfite and adjusted to 100 ml with H_2O,) preheated at 65°C at the rate of 7 ml of the solution per sample. The resulting ground material was incubated at 74°C in an incubator for one hour. The freed proteins were precipitated by adding 7 ml of a mixture of chloroform / isoamyl alcohol (24:1, v: v). After centrifugation at 4000 rpm for 10 min, the supernatant containing the DNA is collected and precipitated with 5 ml of isopropanol at -20°C. After separation of the supernatant with the DNA pellet by centrifugation, the latter was washed with 7 ml of ethanol 70° then resuspended after drying in 400 μl of buffer TE 0.1% (mixture of 10 mM Tris-HCl and 1 mM EDTA at pH 8.0) for dissolution in a refrigerator. The quality was controlled by adjusting its quality so as to adjust its concentration for the amplification of microsatellite markers. Fifty four (54) SSR markers (Table 3) were selected based on their position on the genetic map (http://www.gramene.org) in order to first determine the

polymorphism among the three parents of the interspecific lines (TOG5681, TOG5674 and IR64). Out of these 36 primers were polymorphic and selected for the genotyping of the 18 interspecific lines. The amplification of the DNAs using PCR (Polymerase Chain Reaction) was followed by the separation of the products by electrophoresis. The results were coded based on the parental alleles (**A** = genome of the donor parent TOG5681, **B** = genome of the recurrent parent IR64, **C** = genome of the donor parent TOG5674, H = heterozygosity, **M** = missing allele and **U** = non parental allele.) and the statistical analysis was performed on the scoring data.

Table 1. Genomic composition of the 12 interspecific lines resulting from the ***IR64 / TOG5681*** cross

Code	Lines	Pedigree	Genomic composition				
			TOG5681 A (%)	IR64 B (%)	H (%)	M (%)	U (%)
int1	IR75871-2-13-B-WAB1	IR 64/TOG 5681/3*IR64	18.5	81.4	0.1	0.0	0.0
int 2	IR75871-2-43-19-WAB1	IR 64/TOG 5681/3*IR64	5.3	85.2	6.6	0.0	2.9
int 3	IR75871-2-43-26-WAB1	IR 64/TOG 5681/3*IR64	26.5	63.6	0.0	3.0	6.8
int 4	IR75871-3-17-28-WAB1	IR 64/TOG 5681/3*IR64	14.5	84.1	1.4	0.0	0.0
int 5	IR75871-4-29-13-WAB1	IR 64/TOG 5681/3*IR64	11.8	81.7	0.0	6.6	0.0
int 6	IR75871-4-29-B-WAB1	IR 64/TOG 5681/3*IR64	9.2	87.7	0.0	0.0	3.0
int 7	IR75871-4-B-4-WAB1	IR 64/TOG 5681/3*IR64	3.1	95.3	1.6	0.0	0.0
int 8	IR75871-4-B-B-WAB1	IR 64/TOG 5681/3*IR64	2.3	89.5	0.0	8.2	0.0
int 9	IR75871-5-2-1-WAB1	IR 64/TOG 5681/3*IR64	19.4	54.7	6.4	7.8	11.7
int 10	IR75871-7-1-6-WAB1	IR 64/TOG 5681/3*IR64	10.8	80.2	5.1	3.8	0.0
int 11	IR75871-8-14-21-WAB1	IR 64/TOG 5681/3*IR64	30.5	65.2	0.0	0.0	4.4
int 12	IR75871-9-8-5-WAB1	IR 64/TOG 5681/3*IR64	6.1	88.8	0.0	5.0	0.0
Minimum			2.3	54.7	0.0	0.0	0.0
Maximun			30.5	95.3	6.6	8.2	11.7
average			13.2	79.8	1.8	2.9	2.4

A = % of the genome of the donor parent (*TOG5681*), **B** = % of the genome of the recurrent parent (*IR64*), **H** = % heterozygosity, **M** = missing % and **U** = % non parental allele.

Table 2. Genomic composition of the 06 interspecific lines resulting from the ***IR64 / TOG5674*** cross

Code	Lines	Pedigree	Genomic composition				
			TOG5674 C (%)	IR64 B (%)	H (%)	M (%)	U (%)
nt13	IR75866-1-B-8-WAB1	IR 64/TOG 5674/3*IR64	11.7	88.3	0	0	0
int14	IR75866-2-7-1-WAB1	IR 64/TOG 5674/3*IR64	4.9	87.8	0.6	6.7	0
int15	IR75866-2-18-23-WAB1	IR 64/TOG 5674/3*IR64	2.3	86.4	9.4	0	0
int16	IR75866-9-21-4-WAB1	IR 64/TOG 5674/3*IR64	3.4	85.8	2.1	8.8	0
int17	IR75866-17-B-12-WAB1	IR 64/TOG 5674/3*IR64	20.7	77.8	0	1.5	0
int18	IR75866-18-30-19-WAB1	IR 64/TOG 5674/3*IR64	8.9	87.1	0	0	4
Minimum			2.3	77.8	0	0	0
Maximun			20.7	88.3	9.4	8.8	4
average			8.7	85.5	2.0	2.8	0.66

B = % of the genome of the recurrent parent (IR64), **C** = % of the genome of the donor parent (TOG5674), **H** = % heterozygosity, **M** = missing % and **U** = % non parental allele.

Table 3. List of the 36 polymorphous microsatellite markers and their position on each of the 12 chromosomes

Markers	Chromosomes	Map distance (cM)	Markers	Chromosomes	Map distance (cM)
RM220	1	28.4	RM 429	7	96.9
RM 449	1	15.31	RM 82	7	27.33
RM486	1	153.5	RM433	8	116.0
RM475	2	92.5	RM404	8	69.0
RM208	2	188.4	RM 316	9	1.08
RM007	3	64.00	RM 464	9	6.58
RM471	4	53.8	RM 434	9	15.66
RM564	4	73.1	RM 242	9	18.81
RM470	4	115.5	RM 215	9	21.19
RM348	4	135.4	RM 205	9	22.72
RM13	5	31.4	RM 474	10	1.8
RM164	5	80.1	RM 239	10	9.36
RM26	5	122.7	RM 258	10	17.76
RM204	6	25.1	RM 228	10	21.98
RM508	6	0.0	RM167	11	37.5
RM 436	7	2.55	RM224	11	120.1
RM 234	7	22.35	RM17	12	109.1
RM 455	7	26.81	RM20	12	3.2

2.3 *Data Analysis*

Three types of analysis were carried out. The first type is the computation and interpretation of the percentage of polymorphic markers that enabled to reveal the genomic proportions of the parents. The second type is related to the determination of the proportion of the parental genome of the donors and the recurrent using GGT software package, 2007 edition (http://www.dpw.wau.nl/pv/pub/ggt; Van Berloo, 1999). For that type of analysis, the genetic distance (in cM) between the markers has been used as the basis to estimate the genomic contribution of the parents per chromosome and per line. The proportion of heterozygosity and extra-alleles (non-parental allele) through the three backcross generations were also determined. The averages of the proportions were calculated using EXCEL software whereas the graphic representation of the chromosomes were completed using the CSSL FINDER software.

Regarding the third type of analysis, a distance matrix (tree-diagram) between the various characterized lines was generated and at the same time, groups were formed following the UPGMA (Unweighted Pair Group Method with Arithmetic mean) by using the NTSYS-pc program, version 2.02 Exeter Software (Rohlf, 1998).

3. Result

3.1 *Polymorphism of the Markers*

A total of 54 microsatellite markers distributed over all the 12 chromosomes of rice genome has been selected in order to assess the introgression rate of the parental genes and to study the genetic diversity of the 18 interspecific lines. Out of these, 36 markers (i.e. 66.66%) showed polymorphism between TOG5681 and IR64 parents, while 33 polymorphic markers out of 43 (i.e. 76.74%) were identified between TOG5674 and IR64. These polymorphic markers were used to genotype 12 and 06 descendants of the respective crosses.

The statistic analysis of the scoring data relating to the various proportions of the polymorphic markers identified within the interspecific genomes showed that 61.11% and 100% of the 36 polymorphic markers were enabled to reveal the genome of the donor parent (TOG5681) and that of the recurrent parent (IR64) within the twelve BC_3F_1 interspecific lines respectively. In these lines, the number of polymorphic markers identified varies from one (1) on chromosome three (3) to six (6) on chromosome nine (9) with an average of 3 markers per chromosome. On the

other hand, in the 06 BC_3F_1 interspecific lines from IR64 x TOG5674 cross, 30.30% and 96.96% of the polymorphic markers enabled to reveal the genome of the donor (TOG5674) and that of the recurrent (IR64) respectively. The number of polymorphic markers varies from one (01) on the chromosome 2, 3 and 11 to six (06) on the chromosome 9 with an average of three polymorphic markers per chromosome.

Moreover, 16.66% and 12.12% of the polymorphic markers, enabled to reveal the heterozygous alleles in the two groups of 06 and 12 interspecific lines respectively. Table 3 presents the list of the 36 polymorphic markers and the position of each of them on the chromosomes (source: www.gramene.org). However, among these markers, only RM475, RM348 and RM224 were not used on the six (06) BC_3F_1 interspecific lines of the IR64 / TOG5674 cross. This brings the number of the used polymorphic markers to 33 on these lines.

3.2 Allelic Contribution of the Parents to the Genomes of the Descendants

Knowledge about the proportions and contribution of the parent genome within the interspecific lines by the way of the molecular markers provide useful information on the selection and development of the varieties. In the context of this study, the introgression rate of the genome of the donor parent TOG5681 (*O. glaberrima*) in those of the twelve (12) BC_3F_1 interspecific lines derived from IR64 x TOG5681 cross varies from 2.3 to 30.5% with an average introgression proportion estimated at 13.2%. Regarding the contribution of the alleles of TOG5681, Figure 1 shows that no allele of that parent is on chromosome 1 of the 12 lines. Furthermore, chromosomes 7, 8 and 11 of those lines are poorly covered by its alleles contrary to chromosomes 6 and 10 which are well-stocked with them. On the other hand, the contribution of the donor parent TOG5674 (*O. glaberrima*) in the 6 interspecific lines derived from IR64 xTOG5674 cross varies from 2.3 to 20.7% with an average proportion of the introgressed alleles estimated at 8.7%. The examination of figure 2 shows that the genome of the 6 interspecific lines is devoid of the alleles of the donor parent TOG5674 on chromosomes 1, 2, 5, 7, and 11. Moreover, chromosome 3, 4, and 9, as far as they are concerned, are poorly covered whereas chromosome 6 was the most well-stocked. In summary, at the level of those two types of crosses, chromosome 6 has the highest introgression proportion of the alleles of the donor parent *Oryza glaberrima* contrary to chromosome 1 which does not possess any one.
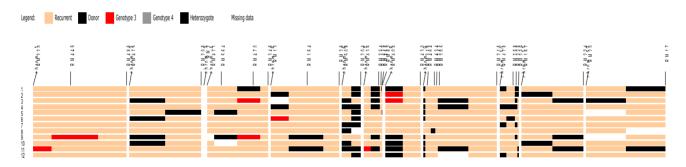

Figure 1. Graphical representation of the 12 chromosomes in the twelve (12) BC3F1 IR64 / TOG5681 interspecific lines showing the genomic introgressions inherited from each of the parents
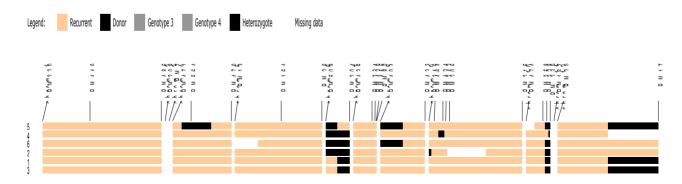

Figure 2. Graphical representation of the 12 chromosomes in the six (06) BC3F1 IR64 / TOG5674 interspecific lines showing the genomic introgression inherited from each of the parents.1= int 13; 2= int 14; 3= int 15; 4= int 16; 5= int 17; 6= int 18

As far as recurrent parent *O. sativa* (IR64) is concerned, its introgression percentage varies from 54.7 to 95.3% for the twelve (12) BC_3F_1 interspecific lines with an average introgression rate of the genome estimated at 79.8%. The highest introgression proportions of the alleles of that parent are found on chromosome 1 with a coverage rate of 94.46%. In the same lines, the proportions of the heterozygous alleles vary from 0 to 6.6% with an average of 1.8%. In total, 4 chromosomes (2, 7, 10 and 11) containing those heterozygous alleles were identified. The highest proportions of heterozygous were especially identified on the chromosomes 7, 10 and 11 with a coverage rate of 16.7%. The proportions of non-parental alleles inventoried throughout the genomes of the descendants vary from 0 to 11.7% with an average of 2.4%. These non-parental alleles are found on chromosomes 1, 4, 5, 7 and 8 of the 12 interspecific lines (Table 1). On the other hand, in the second group of the six (06) interspecific lines (Table 2), the introgression percentage of the genome of the recurrent parent IR64 varies from 77.8 to 88.3% with an average introgression estimated at 85.5%. The highest introgression rate of the alleles of that recurrent parent is found on chromosomes 1 and 7 with a coverage rate of 100%. The heterozygous alleles' proportions vary from 0 to 9.4% with an average of 2%. These heterozygous alleles were located on chromosomes 6, 10, 11 and 12, with very high heterozygosity rates observed on chromosomes 10 and 11 which are covered at 33.3%. The non-parental alleles' rate varies from 0 to 4% with an average of 0.66% was observed only on the chromosome 5 of one single subject IR75866-18-30-19-WAB1 (int18) among the 6 lines.
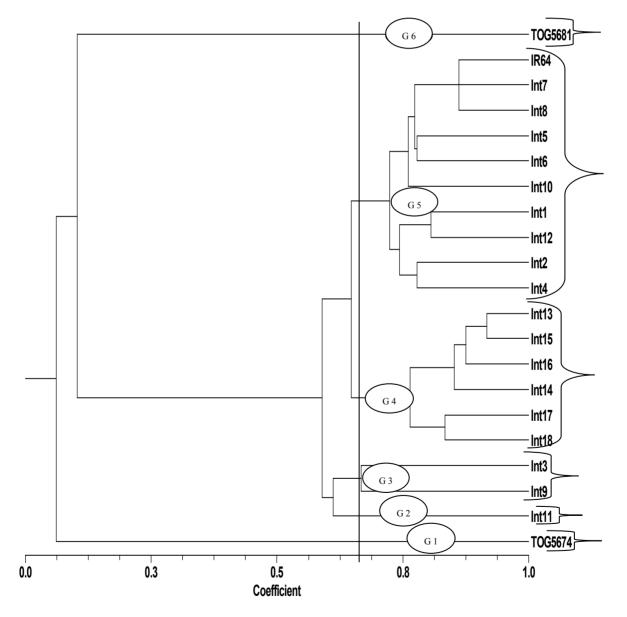

Figure 3. Molecular dendrogram of the 18 interspecific lines and their 03 parents

3.3 Genetic Similarity and Relation between the Interspecific Lines

The analysis of the genetic similarities between the studied interspecific lines through the hierarchical clustering enables to classify the homogenous lines based on the SSR markers used with a view to select the good ones. The review of the tree diagram shows the distribution of the 18 interspecific lines into six (6) different groups with a similarity coefficient estimated at 70% (Figure 3). The twelve (12) BC_3F_1 interspecific lines of the IR64 x TOG5681 cross were distributed into 3 groups: G2, G3 and G5 which is made up of 9 interspecific lines, is the most important one. It has a genetic similarity estimated at 76.5% with the recurrent parent IR64 (*O.sativa*). On the other hand, group.4 is only made up of the six (6) BC_3F_1 interspecific lines derived from IR64 x TOG5674 cross with at least 83% of similarity between the genome of these descends. Moreover, the two parents of the African species *O. glaberrima,* namely TOG5674 and TOG5681 belonging to group.1 (G1) and group.6 (G6) respectively, present a respective average similarity of 7.5% and 12% with all the 18 studied interspecific lines and their parent *sativa* IR64. In those interspecific lines, the lowest genetic similarity (63%) was noted in IR75871-8-14-21-WAB1 (int11) whereas the highest similarity (93.5%) was observed in IR75866-1-B-8-WAB1 (Int13) and IR75866-2-18-23-WAB1 (Int15). They are followed by IR75871-4-B-4-WAB1 (Int7) and IR75871-4-B-B-WAB1 (Int8**)** which have the same genetic similarity with the recurrent parent IR64.

4. Discussions

The study of the genetic diversity conducted with the molecular markers enables us to get useful information in order to assess the genomic contribution of the parents within the intra and interspecific lines (Bernardo et al., 1997, 2000; Heckenberger et al., 2005a). The microsatellite markers used in this study are representative and have a good distribution along the 12 rice chromosomes. These SSR markers used are considered to be very appropriate in molecular genetics studies because they are co-dominant with multiple alleles and very polymorphic even among very closely linked subjects (Morgante et al., 1993; Sharma et al., 1995 and Ndjiondjop et al., 2008). The efficiency of these markers and their superiority over the RFLP markers in the assessment of the parental contribution has been reported by Bernardo et al. (1997 & 2000). In this study, the main targeted objective is to get the introgression of the parental genome within the interspecific lines, i.e. small fragments of *Oryza glaberrima* in the genetic pool of *Oryza sativa* in the form of isogenic lines which are more favorable for the assessment of genetic diversity. The findings of this study showed that the average proportion of the genome of *Oryza glaberrima* is estimated at 13.2% (with EC= ±9.5%) for the twelve (12) BC_3F_1 lines of the IR64 x TOG5681 cross while it was evaluated at 8.7% (with EC= ±6.87%) for the six (06) BC_3F_1 lines of the IR64 x TOG5674 cross. Furthermore, the highest proportion of the alleles inherited from the donor male parents *Oryza glaberrima* (TOG5681 and TOG5674) is found on chromosome 6 which is the most well-stocked contrary to chromosome 1 which does not have any one but only inherited alleles from the parent *sativa* IR64 for the 36 polymorphic markers used. However, the study confirmed results obtained by Ndjiondjop et al. (2008) on the interspecific lowland varieties (NERICA-L) developed by AfricaRice. TOG5681 was used as donor female Parent and IR64 was used as recurrent male Parentt. The results shown an average introgression rate of 8.5% (EC = ±2.49%) of the genome of the donor parent *Oryza glaberrima* calculated for 17 BC_3F_8 varieties. These estimated proportions are higher than the theoretical proportions expected from the third backcross which were estimated at 6.25% for BC_3 lines (Zahour, 1992).

Such deviation between the estimated parental contribution and the expected one can be explained in two ways according to Ndjiondjop et al. (2008).

- ✓ First, the intensive selection during the self-fertilizations in both BC_2 and BC_3 generations could have been done for the parent *O. glaberrima* (donor parent) for a number of characters concerned by many genes; and

- ✓ Second, the selection and genetic drift during the endogamy could have caused some differences between the real genomic proportions and the expected proportions that a line inherits from its parents (St. Martin, 1982; Lorenzen et al., 1995; Visscher, 1996; Bernardo et al., 1997; Bernardo et al., 2000; Heckenberger et al., 2005b; Frisch et Melchinger, 2006).

Moreover, compare to the two aforementioned reasons, when we take as basic concept the theoretical laws of genetics on the proportions inherited at each backcross generation while developing lines, the difference between the introgression rates of the parental genomes of those two types of reciprocal cross can also be explained by the following:

- Firstly, the number of microsatellite markers used in this study (36) was lower than the number of polymorphic markers (60) used by Ndjiondjop et al. (2008) i.e. only 40% (24 out of 60) of common and identical markers for both studies;

- Secondly, the maternal effect due to the expression of cytoplasmic DNA (Zahour, 1992) of TOG5681, the African rice variety used as donor female parent (by AfricaRice) and the varying number of self-fertilization generations for the two types of crosses: BC_3F_1 for this study and BC_3F_8 for the study conducted by Ndjiondjop et al. (2008).

- Furthermore, the comparison of the average introgression rate of the TOG5681 genome estimated at 13.2% (with EC= 9.5%) in this study for the BC_3F_1 interspecific lines derived from IR64 x TOG5681 cross versus those obtained by Ndjiondjop et al. (2008) estimated at 8.5% (with EC = 2.49%) for the 17 sister lines of BC_3F_8 from reciprocal cross TOG5681 x IR64 shows clearly different results at the level of the two crosses. Moreover, the results are higher than the theoretical proportion expected from the 3rd backcross generation estimated at 6.25%. This would be more interesting if the useful genes of the *Oryza glaberrima* were introgressed as it's a donor parent with poor agronomic performance and only used to introduce a particular trait to the *O. sativa* parent.

The studies conducted by Cadanlène (1999) quoted by WARDA (1999) on 83 varieties of the *O. glaberrima* enabled to identify 155 alleles with 30 microsatellite markers and this gave an average of 5.16 alleles per locus. These results showed the genetic diversity within the *O. glaberrima* species as compared to the number of alleles and their efficacy as compared to the microsatellite markers. Furthermore, the efforts deployed to transfer the useful genes of *Oryza sativa* into the cytoplasm of Oryza *glaberrima* achieved limited success due to the sterility barriers between the two species. Sterility is said to have various causes including meiotic irregularities (Heur & Miezan, 2003), the low germination and proportion of viable pollens, the early elimination of female gametes and zygote from the female side (Kitampura, 1962) and finally the cytoplasm and its interaction effects. In order to overcome those barriers, some genetic models based on the interactions in a sporogametophyte locus have been suggested after comprehensive analyses on the reproductive barriers between the two species (Sano, 1986). It's a model of pollen killer and gamete eliminator. A series of sterility loci in the *Oryza glaberrima* species such as S1, gamete eliminator and S3, S18, S19, S20, and both S21 and S29 (t) pollen killers respectively located on chromosomes 6, 11, 10, 3, 7 and 2 has therefore been identified. Moreover, Ishii et al. (2001), Jonhnson et al. (1998) and Ikehashi et al. (1998) have also shown that repeated backcrosses enable to restore fertility as long as some embryo sacs of F_1 are fertile. The restoration of that fertility varies from 30 to 65% Jones et al. (1997), 90% Heuer and Miezan (2003) or even 98% Bourgol (1989) after two backcrosses. This method enables not only to restore the fertility by transferring genes but also to introgress desirable genes into a variety that doesn't possess them.

The ascending hierarchical classification (CAH) conducted on the 18 interspecific lines and their 03 parents permitted to identify the varieties with a higher level of similarity. Similar results were obtained by Aliyu and Fawole in 2000. The analysis of the tree diagram revealed that lines IR75871-4-B-4-WAB1 (Int7) and IR75871-4-B-B-WAB1 (Int8) have strong similarity with the parent s*ativa* IR64. The lowest introgression proportions of the parent *glaberrima* TOG5681 was estimated at 3.1 and 2.3% respectively. On the other hand, line IR75871-9-8-5-WAB1 (Int12) happens to be the single line that virtually respects the theoretical proportions expected from the 3rd generation of the backcross (6.1% of *O. glaberrima* genome and 88% for *O. sativa*). Furthermore, for parent TOG5674, lines IR75866-2-7-1-WAB1 (Int14), IR75866-2-18-23-WAB1 (Int15) and IR75866-9-21-4-WAB1 (Int16) have the lowest donor genome introgression rates.

Regarding the non-parental allele, the average proportions are estimated at 2.4% and 0.66% for the twelve (12) and six (06) interspecific lines derived from the two crosses respectively (Table 1 and 2). Smith et al. (1997) quoted by Ndjiondjop et al. (2008) have given many possible reasons on the existence of the non-parental allele. Firstly, the residual heterozygosity can be present in the parental lines. One issue with molecular markers analysis between the lines and their parents is that the DNA samples are taken during plant cultivation or in seeds stocks. In the best case, DNA samples were supposed to be taken from the plant that has been used to make the original cross from which the lines have been developed. Residual heterozigosity can therefore make the genotypes of the markers different between the seed stocks and the current plant used for the cross. Secondly, contamination by the foreign pollen can occur during the development of the lines. The third scenario is that the seed stocks of the lines could have changed genetically at any time: through spontaneous mutations at the level of the loci of the SSRs, or a physical mixture with the seeds of the lines, of other parents or as a result of unknown crosses during the generation. However, the relatively low rates of non-parental alleles with the increasing number of backcross generations in this study suggest that probably there has been contamination by foreign pollen at the first self-fertilization generation. The highest frequencies of non-parental alleles are observed on chromosomes 4 and 8 of the 12 lines and on chromosome 5 of the six (6) BC_3F_1 lines.

4. Conclusion

This study presents the comparative study of the molecular profiles of interspecific lowland rice developed by IRRI. The results obtained from the BC_3F_1 cross between the improved variety IR64 (*Oryza sativa* L.) and *Oryza*

glaberrima varieties TOG5681 and TOG5674 has shown average introgression rates of the donor male parents (*Oryza glaberrima*). It was observed higher than the theoretical proportion expected from the 3rd backcross generation with an efficiency of the introgressed genes of the donor TOG5681 compared to the expected one. However, to show the useful traits of *O. glaberrima* inherited by these interspecifics lines, agro-morphological characterization with screening against biotic and abiotic stresses may be carried out to highlight the gene of interest of *O. glaberrima* that were introgressed into the progenies. In conclusion, the best lines could also be selected from these materials through participatory varietal selection to satisfying farmers' needs.

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