

# Molecular Profil of Rice Land Races from Madagascar and Togo

## Abstract

Red pericarp on rice is one of the traits which interest the breeders and farmers in Madagascar. There is no genetic diversity study conducted on such rice varieties in Madagascar before. Understanding the genetic diversity in the available genotypes in the germplasm collection is very important for the crop improvement. The objectifs of this study are to assess the genetic diversity and relationship between rice accessions from Madagascar and those from AfricaRice genebank and to build a core collection of these accessions for conservation. 20 SSR markers were used to characterize 238 genotypes from FOFIFA and AfricaRice genebank and also famers preferred varieties. A total of 192 alleles were detected among the 238 genotypes. The number of alleles varied from 4 (RM21) to 20 (RM11 and RM164) with an average of 11.29 per locus. The mean PIC value per locus was 0.64 with maximum value of 0.81 recorded for SSR marker RM 001 and lowest value of 0.15 observed for SSR marker RM215. Heterozygosity varied from 0.01 (RM168 and RM 256) to 0.27 (RM205 and RM222) with the average of 0.12. The principal Coordinate plot showed two distinct clusters, African rice genotypes grouped in cluster I and Madagascar genotypes in cluster II. The cluster II is subdivided in three subgroups (A, B, C). The Analyse of Molecular VAriance revealed great variation among individuals (38%) and among population (47%) and low variation within individuals (15%). The core collection is reprinted by 61 genotypes which 17 are from Africa.

**Keywords:** Africarice; Agromorphological; Heterozygosity; Bioscience; Polymerase chain reaction; Genetic diversity

## Research Article

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## Introduction

Rice is one of the most cultivated cereals in the world. It is a staple food for over half of the population in the world, especially developing countries [1]. It represents 27% of energy intake of people in third world countries and 24% of their dietary protein (FAO 2004). It is belongs to grass family Poaceae ( $2n = 24$ ). 25 species of genus *Oryza* are known, but only two species are cultivated, *Oryza sativa* or Asian rice and *Oryza glaberrima* or African rice [2,3]. These two species have them own importance, the Asian rice is known as high yield potential but sensitive on some abiotics and biotics stresses, on the overhand the second is low yield potential but resistant to various challenges in Africa condition [4].

According to the previous study, Madagascar grown only Asian rice (*Oryza sativa*) [4,5] Agromorphological and isozyme characterization were used to determine the genetic diversity of Madagascar traditional variety by Ahmadi et al. [6]; he identified two subspecies indica and japonica and another group atypic specific to Madagascar [4]. Radaniela was found the same result by using SSR markers, done for only one region [5]. Asian rice is characterized by white caryopsis, few are with red caryopsis, however, *Oryza glaberrima* have mainly red caryopsis [7]. Madagascar has many red caryopsis rice varieties and mostly traditional variety. Among the countries of Sub Saharan Africa, Madagascar has already grown rice for a long time (Mather et

al., 2010). It is mainly grown in rainfed lowland conditions but upland was Introduced by FOFIFA and CIRAD since 1990 [5]. Rice cultures cover 41% of cultivated area and managed by 80% of the Malagasy which are mainly smallholder farmers. They use or not very few inputs, such as organic matter or chemical fertilizer, pesticides, traditional cultivars; their seeds come from the previous harvest or from seed exchange, they can't manage the irrigation and the yield become very low less than 2t/ha [8,9] (Pierre Bernard et al. 2007).

Rice is the staple food for Malagasy and the annual average consumption is 125 kg per person/year [10]. Rice production was 3.6 million tons (paddy rice) in 2013. This is not sufficient for the population that is constantly growing [8]. The use of improved variety can help to increase the yield. Last few year, FOFIFA has developed several improved lowland varieties trough crossing or varietal introduction, most of them have white caryopsis with high yielding but not accepted by the farmers. FOFIFA 172 and FOFIFA 173, upland varieties with red caryopsis were released by FOFIFA and they are appreciated and highly demanded. New improved red rice lowland varieties are also highly demanded but not available.

The farmer criteria to adopt new varieties are based on productivity, early duration, resistant to insect pests and disease, and with red caryopsis. The breeder must take all of these criteria in consideration if they want to succeed the breeding program.

Many consumers prefer colored rice nowadays because of the health benefits offered by this typical rice. It contains high nutritional value compared to white rice such as protein, amylose, vitamins and minerals according to the previous study [7]. The red rice is a source of anthocyanin which is well known as antioxidant, anti-inflammation, anti-cancer [11]; it may reduce the cholesterol rate [12] and prevent beriberi. It can be a source of iron and zinc for the pregnant to fight anemia. By these advantages, the red rice becomes popular and the demand in international and local markets is increased. The red rice produced by farmers are traditional cultivars with low productivity, long duration more than 180 days, susceptible to biotic and abiotic stresses (blast, RYMV, sheath rot, stem borer, weeds, drought, cold, iron toxicity and nutrient deficiency, etc...).

The development of new improved red varieties can help to improve the production and also assure the demand of this kind of rice. For this, screening the available genotypes from the local germplasm will help to identify new genes from diverse candidates. It will provide the best parental lines for rice breeding programs for improving new cultivars. Many traditional varieties are collected and conserved in FOFIFA germplasm. It contains red rice which are not yet evaluated and can be used for the varietal improvement. No study has been done on genetic diversity of red caryopsis rice in Madagascar. Molecular markers have been used by many authors for the molecular characterization to assess germplasm genetic diversity of different crops and rice [5,13-16]. It detects high levels of allelic variation, co-dominant and low cost [17].

In this study, the genetic diversity of the two cultivated rice with red caryopsis was evaluated using SSR markers to determine the genetic diversity and relationship of rice accessions from Madagascar and Africa Rice Center (AfricaRice) genebank, to build a core collection of these accessions for conservation. The results will help on the conservation strategy and of course easily utilization on breeding programs.

## Materials and Methods

### Plant materials

A total of 238 rice genotypes were used in this study. These genotypes were obtained from the FOFIFA Germplasm including *interspecific lines* (NERICA lowland and upland, new variety developed by AfricaRice), traditional varieties and farmers preferred varieties mainly *Oryza sativa*, and *Oryza glaberrima* from AfricaRice Genbank located in Cotonou.

### DNA extraction

The seeds were planted at the Bioscience eastern and central Africa Hub greenhouse. Fresh leaves of 15 to 20 days are collected for genomic DNA extraction. They were placed in 1.5mL eppendorf tube, labeled and putted immediately on ice to avoid the degradation. Before proceeding on grinding, young leaves in the eppendorf were putted in the liquid nitrogen and ground by Zymo grinder machine. DNA was extracted using ZYMO Research Kit (Seed and Plant D6020). The quantity of the genomic DNA was checked using the Nanodrop (Nanodrop One Thermo Scientific). After the nanodrop, concentrations were normalized at 15ng /ul

and 100µl each sample were putted on PCR plate 96 wells. The quality of the genomic DNA was checked on 1% agarose gel with 50 Voltage for 45 min, then, the bands in the agarose gels were visualized and photographed under GelDoc It<sup>2</sup> Imager Bioimage System).

### Microsatellite markers analysis

Twenty Simple Sequence Repeats (SSR) Markers were used during this study selected from the core set of rice genetic diversity markers and also based on the polymorphic reported for the *Oryza glaberrima* and *Oryza sativa* genetic study "Genetic diversity of the two cultivated rice species in Maritime Guinea Evidence for interspecific recombination Euphitica" by Barry (2007), "the Original features of rice genetic diversity Madagascar" by Radaniela [5] and "Analysis of Population Structure and Genetic Diversity in Rice Germplasm Using SSR Markers: An Initiative Towards Association Mapping of Agronomic Traits in *Oryza sativa*" by Nachimutu [24]. They are labeled with a dye colour: bleu (6 FAM), green (VIC), yellow (NED) and red (PET) (Table 1).

### Polymerase chain reaction (PCR)

The PCR reaction was conducted in a reaction volume of 10 µL containing 20 to 40 ng of genomic DNA, 5 µL of Master mix, 0.2 µL of MgCl<sub>2</sub> (25nM), 0.25 µL of forward and reverse SSR primer (3µM), and double distilled water. Multiplex of three or four markers depending on the dye colours and the annealing temperature were prepared. Amplifications were performed in the PCR machine. The thermal cycler was programmed as follows: initial denaturation at 94°C for 3 min followed by 30 cycles of 30s denaturation at 94°C, 1 min annealing depending on the marker used at 55/60°C, 1 min extension at 72°C, 20 min final extension at 72°C. PCR products were kept at 15°C until further use.

To verify the amplification, 4 µL of PCR products with 1µl loading dye were runned on electrophoresis in 1.5% agarose with 100V during 45 min. Bands in the agarose gels were detected and photographed under GelDoc It<sup>2</sup> Imager Bioimage System. If the DNA were amplified, 1 µL to 2 µL of PCR product were mixed with 9µl of HiDi Formamide with Liz (1000 µL HiDi with 15 µL Liz), denatured at 95°C for 3min, putted quickly on ice and sent for capillary electrophoresis.

### Alleles calling

Gene mapper V4.1 software was used to score the exact lengths of each allele after capillary electrophoresis. One peak was detected for homozygote or two peaks for heterozygote. Auto-binning was used to reduce the errors.

### Data analysis

For the statistical analysis to determine the molecular diversity such as heterozygosity, fixation index, Principal Coordinate Analysis, Nei Unbiased Genetic Distance and molecular variance (AMOVA) we used GenAlEx 6.501 software (Peakall and Smouse, 2006). Alleles frequency, number of alleles, heterozygosity, Polymorphism Information Content and fixation index were determined by using Power Marker V3.25 software. Phylogenetic tree was built using Unweighted Neighbor-Joining (UWNJ)

algorithm by DARwin V5 software. The population structure was determined using STRUCTURE 2.3.4 (Pritchard et al, 2000). The maximum number of populations for the simulation was set at

10 (K=10), the length of Burn in period was set at 50000 and the number of Markov chain Monte Carlo (MCMC) repetitions after analysis is equal to 100000.

**Table 1:** Details of SSR loci used.

	Marker	Chromosome N°	Annealing Temperature	Size	Repeat Motif	Dye Color
1	RM001	1	55	113	(GA) <sub>26</sub>	VIC
2	RM007	3	55	180	(GA) <sub>19</sub>	NED
3	RM011	7	55	140	(GA) <sub>17</sub>	PET
4	RM021	11	55	157	(GA) <sub>18</sub>	6-FAM
5	RM122	5	55	227	(GA) <sub>7</sub> (GA) <sub>2</sub> (GA) <sub>11</sub>	NED
6	RM164	5	55	246	(GT) <sub>16</sub> TT(GT) <sub>4</sub>	PET
7	RM168	3	55	116	T <sub>15</sub> (GT) <sub>14</sub>	6-FAM
8	RM205	9	55	122	(CT) <sub>25</sub>	VIC
9	RM214	7	55	112	(CT) <sub>14</sub>	6-FAM
10	RM222	10	55	213	(CT) <sub>18</sub>	NED
11	RM224	11	55	157	(AAG) <sub>8</sub> (AG) <sub>13</sub>	PET
12	RM229	11	55	116	(TC) <sub>11</sub> (CT) <sub>5</sub> C <sub>3</sub> (CT) <sub>5</sub>	6-FAM
13	RM25	8	60	146	(GA) <sub>18</sub>	VIC
14	RM256	8	60	127	(CT) <sub>21</sub>	NED
15	RM287	11	55	118	(GA) <sub>21</sub>	PET
16	RM316	9	55	192	(GT) <sub>8</sub> -(TG) <sub>9</sub> (TTTG) <sub>4</sub> (TG) <sub>4</sub>	6-FAM
17	RM320	7	55	167	(AT) <sub>11</sub> GTAT(GT) <sub>13</sub>	NED
18	RM324	2	55	175	(CAT) <sub>21</sub>	PET
19	RM332	11	55	183	(CTT) <sub>5</sub> -12-(CTT) <sub>14</sub>	6-FAM
20	RM536	11	60	243	(CT) <sub>16</sub>	VIC

## Results and Discussion

### Genetic diversity

Among twenty markers used during this study, the genotypes from Africa did not amplified by markers RM320 and RM324, all of the genotypes by marker RM536, we removed these three markers in the analysis.

### Comparison of genetic diversity parameters obtained at each locus by two origins

On this study, 174 alleles are observed on the genotypes from Madagascar varied from 4 (RM164) to 20 (RM021) with an average of 10.24, for the AfricaRice genotypes 117 alleles are scored varied from 3 (RM011) to 12 (RM332) with an average of 6.88. The alleles scored on Madagascar genotypes is higher than Africa genotypes. The genetic diversity of the Madagascar genotypes range from 0.28 (RM 256) to 0.91 (RM 021) with an average of 0.68 and the RM1, RM222, RM205, RM224 are also highly diverse with a value of 0.89, 0.85, 0.84 and 0.81. The average of the genetic diversity for the africa genotypes is 0.34 with a range of 0.07 to 0.70. The genetic diversity of the genotypes from Madagascar is higher than from AfricaRice.

The mean of heterozygosity and the Polymorphism Content Information of the genotypes from Madagascar is double of the one from Africa. The heterozygosity is almost low. The highest PIC value was found on the marker RM 021 of Madagascar genotypes (0.90) but in the Africa genotypes the PIC value is very low (0.08). We can assume that the PIC value for each marker between Madagascar and Africa genotypes were not similar, the PIC observed on Africa genotypes were lower than from Madagascar, except RM 168 wich are quite close (0.30 and 0.29) (Table 2).

### Genetic diversity parameters obtained at each locus

192 alleles are detected among the genotypes. The number of alleles per locus varied from 4 to 20 with an average of 11.29. The highest was detected for the loci RM21 and the lowest was found for the loci RM11 and RM164. This result is similar to the one reported by Barry during the genetic diversity analysis of the two cultivated rice species (*O. sativa* & *O. glaberrima*) in Maritime Guinea using 11 SSR Marker. The alleles number is higher than reported by Radanielina [5] from the diversity study of rice in the highlands of Madagascar using 14 SSR Marker which he detected 145 alleles, with an average 10.4 per locus.

On this study, more alleles were observed RM21 (20), RM 168

(18), RM 205 (17), RM1 (16), RM 332 (15) comparing to those detected in the previous study, it means that the materials that we used are more diverse.

The major alleles frequency ranged from 0.34 to 0.92 with an average of 0.46. Heterozygosity varied from 0.01 (RM168 and RM 256) to 0.27 (RM205 and RM222) with the average of 0.12, this low value is due to selfpollination of rice. This result is similar on observed in the previous study by Radaniela [5].

The relative information per marker is represented by the PIC value, in the study, the average value is 0.64. The maximum value of 0.81 was recorded for SSR marker RM 001 and the lowest was observed for SSR marker RM215 (0.15). Population specific F-statistic indices showed the values of inbreeding index (Fis) for loci ranged between 0.444 and 0.969 with a mean of 0.749, indicating that high levels of genetic differentiation were present in the populations (Table 3).

**Table 2:** Comparison of genetic diversity parameters obtained at each locus by two origins.

Marker	Madagascar				Africa			
	Allele No	Gene Div	Het	PIC	Allele No	Gene Div	Het	PIC
RM001	16	0.89	0.175	0.88	8	0.54	0.08	0.51
RM007	10	0.74	0.083	0.72	7	0.17	0.03	0.17
RM011	5	0.55	0.092	0.5	3	0.16	0.01	0.15
RM021	20	0.91	0.167	0.9	5	0.08	0.02	0.08
RM122	9	0.64	0.125	0.61	8	0.21	0.03	0.2
RM164	4	0.49	0.25	0.38	5	0.57	0.24	0.52
RM168	7	0.31	0.017	0.3	7	0.3	0	0.29
RM205	16	0.84	0.4	0.82	11	0.46	0.12	0.44
RM214	13	0.66	0.108	0.63	7	0.21	0.05	0.2
RM222	13	0.85	0.208	0.84	7	0.65	0.31	0.6
RM224	10	0.81	0.158	0.79	9	0.26	0.03	0.26
RM229	10	0.65	0.133	0.62	6	0.38	0.08	0.36
RM25	10	0.72	0.083	0.68	7	0.43	0.15	0.4
RM256	6	0.28	0.017	0.27	4	0.07	0.01	0.07
RM287	8	0.77	0.192	0.74	6	0.43	0.02	0.4
RM316	7	0.61	0.15	0.57	5	0.16	0.03	0.16
RM332	10	0.77	0.225	0.74	12	0.7	0.27	0.67
Total	174				117			
Mean	10.23529	0.68	0.152	0.65	6.88	0.34	0.09	0.32

**Table 3:** Estimated genetic diversity parameters obtained at each locus across 238 genotypes.

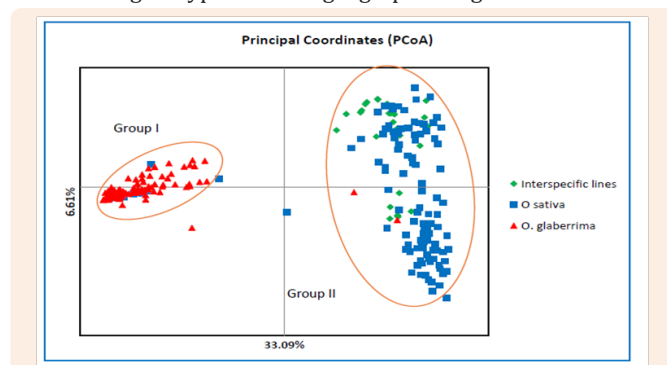
Marker	MAF	A No	Het	PIC	Fis	Fit	Fst
RM001	0.38	16	0.15	0.81	0.782	0.833	0.234
RM007	0.45	12	0.05	0.69	0.868	0.921	0.399
RM011	0.51	4	0.05	0.5	0.776	0.848	0.322
RM021	0.52	20	0.09	0.69	0.764	0.819	0.232
RM122	0.45	11	0.08	0.65	0.777	0.876	0.443
RM164	0.34	4	0.25	0.63	0.527	0.605	0.165
RM168	0.44	8	0.01	0.55	0.969	0.984	0.493
RM205	0.37	17	0.27	0.79	0.444	0.605	0.29
RM214	0.44	18	0.08	0.66	0.777	0.86	0.372
RM222	0.34	12	0.27	0.76	0.767	0.809	0.181
RM224	0.43	12	0.1	0.73	0.757	0.844	0.357

RM229	0.38	12	0.11	0.7	0.734	0.834	0.377
RM25	0.37	10	0.12	0.74	0.855	0.888	0.227
RM256	0.92	5	0.01	0.15	0.8	0.827	0.138
RM287	0.41	9	0.12	0.73	0.807	0.869	0.32
RM316	0.76	7	0.09	0.39	0.635	0.679	0.121
RM332	0.34	15	0.26	0.77	0.69	0.74	0.163
Total		192					
Mean	0.46	11.29	0.12	0.64	0.749	0.814	0.285

MAF: Major Allele Frequency; A No: Allele No; Het: Heterozygosity; PIC: Polymorphism Information Content; Fis: Inbreeding Index

### Principal coordinate

Principal Coordinates plot showing the genetic difference among Madagascar genotypes (*O sativa*) (group II) and African rice (group I) (Figure 1). Majority of genotypes from AfricaRice clustered in group I; few of them clustered in group II in addition with interspecific lines (NERICA). There is a distinct relationship between the genotypes and the geographic origine.



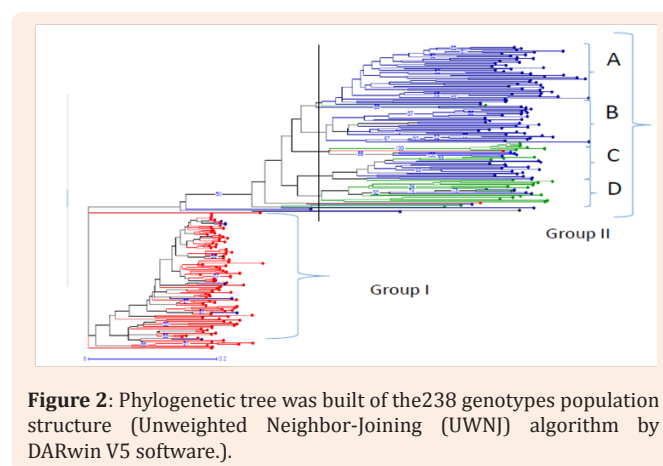
**Figure 1:** Principal Coordinates plot showing the genetic difference among Madagascar genotypes (*O sativa*) (group II) and African rice (group I).

### Patterns of genetic differentiation

The unrooted neighbor-joining tree put the 238 genotypes into two clusters I and II (Figure 2). Cluster I consisted the genotypes from Africa and cluster II consisted the genotypes from Madagascar with few from Africa. Its shown that the *Oryza glaberrima* grouped in cluster I is completely different of Red caryopsis rice from Madagascar grouped in cluster II in term of genetic composition. African rice genotypes are less diverse than the genotypes from Madagascar. Few genotypes from Africa cluster with Madagascar population closed to Nerica genotypes, they must be interspecific genotypes.

Madagascar rice germplasm consist mainly four subgroup which are indica and japonica and the third subgroup is atypic subspecies, it confirm the result obtained by the previous study [4,5] and the fourth subgroup is composed by the interspecific lines. The introduction of the interspecific lines in Madagascar changed the population structure observed by the previous study. Through this result, the clustering revealed that the genotypes

called on the same name « rojomena » don't have the same genetic characteristics. We observed some *O glaberrima* genotypes cluster with the group II, it may be because of the mistake during the labelling or during the planting in the greenhouse. Unrooted neighbour-joining tree based on 238 genotypes and 17 SSR loci.



**Figure 2:** Phylogenetic tree was built of the 238 genotypes population structure (Unweighted Neighbor-Joining (UWNJ) algorithm by DARwin V5 software.).

### Population genetic structure

The peak of Evanno Delta K shown K=2, it classify the two major groups corresponding to two rice species, *Oryza glaberrima* from Togo and *Oryza sativa* from Madagascar (Figure 3). We have two gene pools. Interspecific lines cluster with *O. sativa*, it is because the backcrossing with *O. sativa* during the breeding process. Our Principal Coordinates and Unrooted neighbour-joining tree confirme the result.

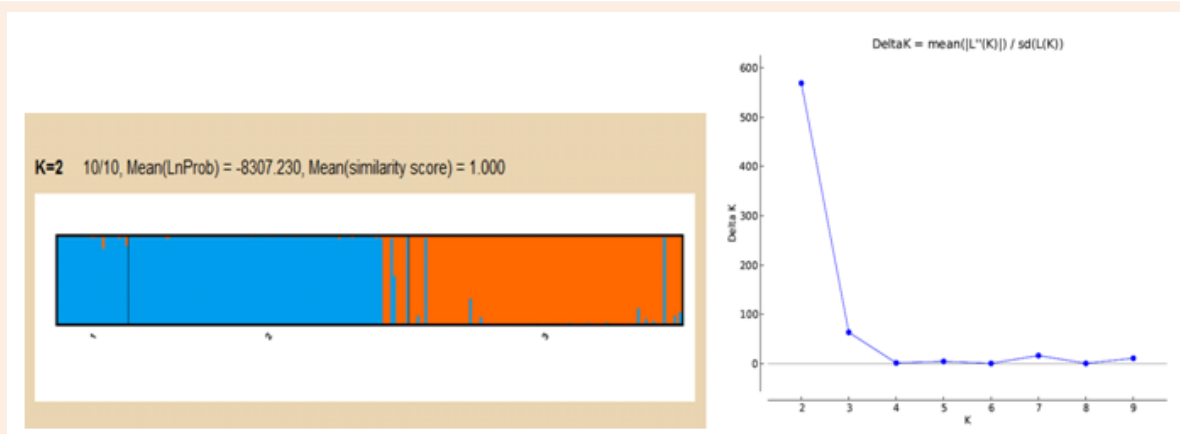
### Genetic diversity pattern

The SSR markers used in this study were able to structure Madagascar and Togo accessions. The allelic richness of the different populations according to the data provides significant variation among the populations. The expected heterozygosity ( $H_e$ ) was higher than that observed ( $H_o$ ) for all of the populations. *O. sativa* and interspecific lines showed higher observed heterozygosity compare to *O. glaberrima* with 0.66 and 0.62 followed by *O. glaberrima* with 0.24. (Table 4). Fixation index for all populations which ranged between 0.73 observed in *O. glaberrima* and 0.79 in *O. sativa* (Table 4).

**Table 4:** Population estimates on number of different alleles, Heterozygosity and Fixation Index.

POP	N	Na	Ho	He	uHe	F	Np
<i>Interspecific lines</i>	26.05 ± 0.37	5.11 ± 0.47	0.12 ± 0.026	0.62 ± 0.03	0.63 ± 0.04	0.79 ± 0.04	0.647
<i>O sativa</i>	104 ± 1.17	9.88 ± 1.03	0.16 ± 0.02	0.66 ± 0.04	0.67 ± 0.05	0.77 ± 0.03	3.765
<i>O. glaberrima</i>	101.52 ± 0.84	4.94 ± 0.49	0.08 ± 0.02	0.24 ± 0.04	0.24 ± 0.05	0.73 ± 0.04	0.706

N: Sample Size; Na: No. of Different Alleles; Ho: Observed Heterozygosity He: Expected Heterozygosity; uHe: Unbiased Expected Heterozygosity; F: Fixation Index; SE: Standard Errors in Parenthesis



**Figure 3:** Population structure of 238 genotypes and Evanno curve.  
Population: 1: *Interspecific lines*. 2: *O sativa*. 3: *O glaberrima*

### Private alleles

*Interspecific* has 11 private alleles, *O. sativa* had 64, and *O. glaberrima* had 12 private alleles. Higher number of private alleles was found on *O. sativa* genotypes, however, the few private alleles found on *O. glaberrima* must be source of resistant or tolerance on biotics and abiotics stresses which can be used for *O. sativa* genotypes improvement in Madagascar.

### Genetic variance analysis

The hierarchical distribution of molecular variance by AMOVA and pair-wise analysis revealed that the most of the variation is among individuals 47% followed by among population 38%. 15% of variation was associated within individuals (Table 5). The high variation among individual may be because of the two different species which is very far in term of genetic (two different species) and also the geographic distance. The variation is highly significant. This diversity among population and among individuals can be used and considered when we want to proceed on the crossing for the variety improvement in Madagascar.

### Core collection

The objectives of core collection is to reduce the redundancy, reduce the number of accession. The core collection is limited set of accession which represent the genetic variability of the entire collection [18]. During this study, we used DARwin V5 software to determine the core collection. The data from alleles scoring were used. We obtained a number of 61 genotypes in the core collection, 17 of these are Africa genotypes. The result

shown that the number of alleles in the initial collection and core collection are the same (192), we didn't lose the alleles from the initial collection. The PIC of the core collection (0.64) is less than the initial collection (0.72) but the difference is not significant (Table 6).

**Table 5:** Summary AMOVA Table.

Source	df	SS	MS	Est. Var.	%
Among Pops	2	776.714	388.357	2.693	38%
Among Indiv	235	1775.723	7.556	3.268	47%
Within Indiv	238	243	1.021	1.021	15%
Total	475	2795.437		6.982	100%

F-Statistics	Value	P(rand >= data)
Fst	0.386	0.001
Fis	0.762	0.001
Fit	0.854	0.001

### Principal coordinates of collection

The principal coordinates plot and Unrooted neighbour-joining tree the shown the same result as the entire collection, it confirms that our core collection is representative of the core set. Unrooted neighbour-joining tree based on core collection and 17 SSR loci.

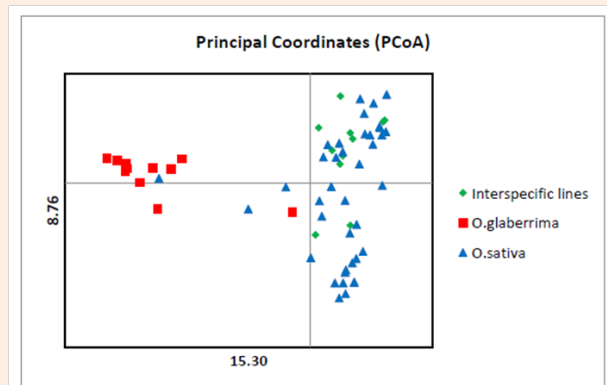
**Table 6:** Comparison of genetic diversity parameters obtained at each locus of Initial collection and Core collection.

Marker	Allele No		PIC	
	Intitial Collection	Core Collection	Intitial Collection	Core Collection
RM001	16	16	0.89	0.81
RM007	12	12	0.81	0.69
RM011	4	4	0.51	0.5
RM021	20	20	0.84	0.69
RM122	11	11	0.77	0.65
RM164	4	4	0.51	0.63
RM168	8	8	0.54	0.55
RM205	17	17	0.86	0.79
RM214	18	18	0.81	0.66
RM222	12	12	0.82	0.76
RM224	12	12	0.85	0.73
RM229	12	12	0.78	0.7
RM25	10	10	0.8	0.74
RM256	5	5	0.22	0.15
RM287	9	9	0.77	0.73
RM316	7	7	0.59	0.39
RM332	15	15	0.81	0.77
	192	192		
Mean	11.29	11.29	0.72	0.64

## Conclusion

In conclusion, the molecular markers used during this study to determine genetic diversity in the germplasm were highly informative especially RM001, RM205, RM332 and RM 222. We can say that red caryopsis rice in Madagascar is completely different from African rice, there is no genetic relationship. Madagascar genotypes grouped in one main cluster and three sub clusters thereby demonstrating that Madagascar germplasm contain a great reservoir of genetic diversity with high level of allelic richness, on the overhand African rice from Togo are genetically narrow but it contain also rare alleles, it is very usefull and important for the rice variety improvement. The varieties called on the same name (Rojomena) are genetically different. We can use the SSR Markers RM320 and RM324 to identify *O sativa* with *O glaberrima*. The introduction of NERICA varieties changed the population structure in Madagascar. The result of this work and the core collection will be very usefull for the plant breeders for choosing the genotypes in the futur breeding program and

improve molecular information of FOFIFA germplasm [19-26] (Table 7 & Table 8).

**Figure 4:** Principal Component Analysis of core collection.

**Table 7:** Liste of the genotypes used during the study.

	Collection Number	Sample Name	Species	Origine	POP
1		NL 47	<i>Interspecific lines</i>	Senegal	Middle East
2		F 173	<i>O sativa</i>	Antsirabe	Central Highland
3		ROJOFOTSY	<i>O sativa</i>	Fofifa	Central Highland
4		Tangongo	<i>O sativa</i>	Ambohibary Sambaina	Central Highland
5		Ramavo	<i>O sativa</i>	Ambohibary Sambaina	Central Highland
6		Riz rouge Sambaina	<i>O sativa</i>	Ambohibary Sambaina	Central Highland
7		Rojomena CR	<i>O sativa</i>	Fofifa	Central Highland
8		Vary gasy Fara	<i>O sativa</i>	Mahitsy	Central Highland
9		Riz noir	<i>O sativa</i>	Fofifa	Central Highland
10		Rojomena MJK	<i>O sativa</i>	Manjakandriana	Central Highland
11		Rojomena CV	<i>O sativa</i>	Fofifa	Central Highland
12		NL 19	<i>Interspecific lines</i>	Senegal	Middle East
13		Rojomena Sambaina	<i>O sativa</i>	Ambohibary Sambaina	Central Highland
14		Manjamena	<i>O sativa</i>	Fofifa	Central_Highland
15		NL 3	<i>Interspecific lines</i>	Senegal	Middle East
16		NL 38	<i>Interspecific lines</i>	Senegal	Middle East
17		N 15	<i>Interspecific lines</i>	Senegal	Middle West
18		Betsilaizina	<i>O sativa</i>	Ambohibary Sambaina	Central Highland
19		Telorirana	<i>O sativa</i>	Ambohibary Sambaina	Central Highland
20		F 171	<i>O sativa</i>	Antsirabe	Central Highland
21		N 14	<i>Interspecific lines</i>	Senegal	Middle West
22		Chomrong Dhan	<i>O sativa</i>	Nepal	Central Highland
23		NL 11	<i>Interspecific lines</i>	Senegal	Middle_East
24		N 4	<i>Interspecific lines</i>	Benin	Middle West
25		F 172	<i>O sativa</i>	Antsirabe	Central Highland
26		N 16	<i>Interspecific lines</i>	Bouaké	Middle West
27	SMNJ 187	Lohambitro Masompera	<i>O sativa</i>	Mananjary	East
28	SMNJ 192	Tsimahory	<i>O sativa</i>	Mananjary	East
29	SMNJ 171	Vary kitrana	<i>O sativa</i>	Mananjary	East
30	7031	Rojomena	<i>O sativa</i>	Fofifa	Central Highland
31	SMNJ 156	Fotsiavarina mena	<i>O sativa</i>	Mananjary	East
32	SNV 416	Menamongo	<i>O sativa</i>	Nosy varika	East
33	SFB 032	Oviben'ampotaka	<i>O sativa</i>	Fenoarivobe	Middle West
34	NL 10	NL 10	<i>Interspecific lines</i>	Senegal	Middle East
35	NL 7	NL 7	<i>Interspecific lines</i>	Senegal	Middle East
36	SSB 097	Mamoriaka mena	<i>O sativa</i>	Sambava	North
37	SNV 427	Manarivo	<i>O sativa</i>	Nosy varika	East



38	SMNJ 177	Ranilona mena	<i>O sativa</i>	Mananjary	East
39	SNV 419	Menalava	<i>O sativa</i>	Nosy varika	East
40	SMNJ 161	Keliasangotry	<i>O sativa</i>	Mananjary	East
41	SNV 410	Vary kalina	<i>O sativa</i>	Nosy varika	East
42	SNV 401	Tomorongo	<i>O sativa</i>	Nosy varika	East
43	SNV 407	Tsimanavaka mena	<i>O sativa</i>	Nosy varika	East
44	SMNJ 163	Vatomandry tenany	<i>O sativa</i>	Mananjary	East
45	716	Botry	<i>O sativa</i>	Andramasina	Central Highland
46	1036	Tsiasialahy	<i>O sativa</i>	Antsirabe	Central Highland
47	SMNJ 194	Tsimanolana	<i>O sativa</i>	Mananjary	East
48	SMNJ 164	Vary gony manantsomotra	<i>O sativa</i>	Mananjary	East
49	SFP 460	Lavambozona	<i>O sativa</i>	Foul point	East
50	SMNJ 153	Vary hosy tenany	<i>O sativa</i>	Mananjary	East
51	SMNR 541	Komonjamena	<i>O sativa</i>	Mananara	East
52	SABA 351	Langaka mena	<i>O sativa</i>	Nosibe an'Ala	East
53	SSI 480	Samiaraka mena	<i>O sativa</i>	Ivoloina	East
54	SSI 467	Betahavana mena	<i>O sativa</i>	Ivoloina	East
55	SMNJ 188	Mazantoka mena	<i>O sativa</i>	Mananjary	East
56	SMNJ 186	Berija	<i>O sativa</i>	Mananjary	East
57	SNV 420	Ramazava	<i>O sativa</i>	Nosy varika	East
58	SNV 429	Tsy avela	<i>O sativa</i>	Nosy varika	East
59	SMNJ 165	Boriziny mena	<i>O sativa</i>	Mananjary	East
60	SUT 447	Kitrana vary mena	<i>O sativa</i>	Vavavantenina	East
61	1199	Lovasoavina	<i>O sativa</i>	Fianarantsoa	Central Highland
62	654	Rojomena	<i>O sativa</i>	Manandona	Central Highland
63	SMNR 545	Tsimila ro	<i>O sativa</i>	Mananara	East
64	SMNJ 182	Garagea	<i>O sativa</i>	Mananjary	East
65	SFB 024	Ambanjabe	<i>O sativa</i>	Fenoarivobe	Middle West
66	SKJV 109	Vatomandry maroaandrano mena	<i>O sativa</i>	Kianjavato	East
67	SFE 444	Vary gony mena	<i>O sativa</i>	Fenoarivo Est	East
68	SABA 337	Vary longotra	<i>O sativa</i>	Nosibe an'Ala	East
69	SMNJ 178	Ramaditra na Vihimboahangy	<i>O sativa</i>	Mananjary	East
70	6912	Vary manga	<i>O sativa</i>	Antsirabe	Central Highland
71	6914	Vary mangarirana	<i>O sativa</i>	Antsirabe	Central Highland
72	6916	Vary mena	<i>O sativa</i>	Antsirabe	Central Highland
73	6917	Vary omby	<i>O sativa</i>	Antsirabe	Central Highland
74	6816	Rojomena	<i>O sativa</i>	Antsirabe	Central Highland
75	6803	rojomena mangavava	<i>O sativa</i>	Antsirabe	Central Highland
76		Japone mena	<i>O sativa</i>	Mahitsy paysans	Central Highland

77	1233	Tsitahavana	<i>O sativa</i>	Befandriana Nord	North
78	716-A	Botry	<i>O sativa</i>	Andramasina/ Ambatolampy	Central Highland
79	1214	Rakaraka	<i>O sativa</i>	Befandriana Nord	North
80	720	Bemavo	<i>O sativa</i>	Antanifotsy/ Ambatolampy	Central Highland
81	721	Benapanga	<i>O sativa</i>	Antanifotsy/ Ambatolampy	Central Highland
82	694	Mangakely	<i>O sativa</i>	Soavina/Betafo	Central Highland
83	479	Mahia	<i>O sativa</i>	Mandritsara	North
84	726	Rojomena	<i>O sativa</i>	Antanifotsy/ Ambatolampy	Central Highland
85	654	Rojomena	<i>O sativa</i>	Manandona Antsirabe	Central Highland
86	686	Rojomena	<i>O sativa</i>	Mahaiza Betafo	Central Highland
87	431	Roka	<i>O sativa</i>	Bealanana	North
88	676	Mangakely	<i>O sativa</i>	Ambohibary Sambaina	Central Highland
89	669	Tokambany	<i>O sativa</i>	Ambohibary Sambaina	Central Highland
90	674	Rojomena	<i>O sativa</i>	Ambohibary Sambaina	Central Highland
91	670	Botry	<i>O sativa</i>	Ambohibary Sambaina	Central Highland
92	876	Makalioka noir 80 n°	<i>O sativa</i>	Alaotra	Middle East
93	833	Hybride (34 x 9 (15.416))	<i>O sativa</i>	Alaotra	Middle East
94	482	Roka	<i>O sativa</i>	Maevatanana	North
95	469	Tsipala	<i>O sativa</i>	Madirovalo	North
96	6811	Rojomena	<i>O sativa</i>	Antsirabe	Central Highland
97	6722	Latsika	<i>O sativa</i>	Antsirabe	Central Highland
98		Latsika bozaka (n°20)	<i>O sativa</i>	Faratsiho	Central Highland
99		Latsika (n°18)	<i>O sativa</i>	Faratsiho	Central Highland
100	673	Rojomena	<i>O sativa</i>	Ambohibary	Central Highland
101	691	Mangakely	<i>O sativa</i>	Mahaiza Betafo	Central Highland
102	1232	Be	<i>O sativa</i>	Befandriana Nord	North
103	1231	Tsipala	<i>O sativa</i>	Befandriana Nord	North
104		Dista	<i>O sativa</i>	Alaotra	Middle East
105		MK 34	<i>O sativa</i>	Alaotra	Middle East
106	D19-Red	ARD51-ABE19-R	<i>Interspecific lines</i>	Antsirabe	Central Highland
107	D19-white	ARD51-ABE19-W	<i>Interspecific lines</i>	Antsirabe	Central Highland
108	D27	ARD51-ABE27	<i>Interspecific lines</i>	Antsirabe	Central Highland
109	D36	ARD51-ABE36	<i>Interspecific lines</i>	Antsirabe	Central Highland
110	D44	ARD51-ABE44	<i>Interspecific lines</i>	Antsirabe	Central Highland
111	E3	ARD52-ABE3	<i>Interspecific lines</i>	Antsirabe	Central Highland
112	E8	ARD52-ABE8	<i>Interspecific lines</i>	Antsirabe	Central Highland
113	E9	ARD52-ABE9	<i>Interspecific lines</i>	Antsirabe	Central Highland

114	E14	ARD52-ABE14	<i>Interspecific lines</i>	Antsirabe	Central Highland
115	F7	ARD53-ABE7	<i>Interspecific lines</i>	Antsirabe	Central Highland
116	G12	ARD54-ABE12	<i>Interspecific lines</i>	Antsirabe	Central Highland
117	G20	ARD54-ABE20	<i>Interspecific lines</i>	Antsirabe	Central Highland
118	H14	ARD55-ABE14	<i>Interspecific lines</i>	Antsirabe	Central Highland
119	H17	ARD55-ABE17	<i>Interspecific lines</i>	Antsirabe	Central Highland
120		Nerica 1	<i>Interspecific lines</i>	Bouaké	Middle West
121	TG08-001	Xlétiévé	<i>O. glaberrima</i>	Todomé	Togo
122	TG08-003	Akpesse	<i>O. sativa</i>	Todomé	Togo
123	TG08-005	Xlétiétoé	<i>O. glaberrima</i>	Todomé	Togo
124	TG08-006	Xlétiétoé	<i>O. glaberrima</i>	Todomé	Togo
125	TG08-007	Agbolébè	<i>O. sativa</i>	Todomé	Togo
126	TG08-008	Xlétiétoé	<i>O. glaberrima</i>	Todomé	Togo
127	TG08-011	Danyimoli	<i>O. glaberrima</i>	Todomé	Togo
128	TG08-012	Ewento hi	<i>O. glaberrima</i>	Todomé	Togo
129	TG08-013	Xlétiévé	<i>O. glaberrima</i>	Todomé	Togo
130	TG08-015	Xlétiévé	<i>O. glaberrima</i>	Todomé	Togo
131	TG08-016	Xlétiétoé	<i>O. glaberrima</i>	Todomé	Togo
132	TG08-017	Xlétiétoé	<i>O. glaberrima</i>	Todomé	Togo
133	TG08-020	Venutohe	<i>O. glaberrima</i>	Atigba	Togo
134	TG08-021	Ewemoli	<i>O. glaberrima</i>	Atigba	Togo
135	TG08-022	Danyimoli	<i>O. glaberrima</i>	Atigba	Togo
136	TG08-023	Ewemoli	<i>O. glaberrima</i>	Atigba	Togo
137	TG08-027	Danyimoli	<i>O. glaberrima</i>	Atigba	Togo
138	TG08-028	Danyimoli	<i>O. glaberrima</i>	Atigba	Togo
139	TG08-030	Venutoyibo	<i>O. sativa</i>	Atigba	Togo
140	TG08-031	Danyimoli	<i>O. glaberrima</i>	Atigba	Togo
141	TG08-032	Danyimoli	<i>O. glaberrima</i>	Atigba	Togo
142	TG08-033	Danyimoli	<i>O. glaberrima</i>	Atigba	Togo
143	TG08-035	Ewento yibo	<i>O. glaberrima</i>	Atigba	Togo
144	TG08-037	Danyimoli	<i>O. glaberrima</i>	Atigba	Togo
145	TG08-040	Danyimoli	<i>O. glaberrima</i>	Atigba	Togo
146	TG08-041	Xlétiétoé	<i>O. glaberrima</i>	Danyi Kétémé	Togo
147	TG08-042	Danyimoli	<i>O. glaberrima</i>	Danyi Kétémé	Togo
148	TG08-043	Xlétiétoé	<i>O. glaberrima</i>	Danyi Kétémé	Togo
149	TG08-044	Danyimoli	<i>O. glaberrima</i>	Danyi Kétémé	Togo
150	TG08-045	Danyimoli	<i>O. glaberrima</i>	Danyi Kétémé	Togo
151	TG08-047	Xlétiéné	<i>O. sativa</i>	Danyi Kétémé	Togo
152	TG08-051	Ewinto moli	<i>O. glaberrima</i>	Danyi Kétémé	Togo

153	TG08-052	Evinto hi	<i>O. glaberrima</i>	Danyi Kétémé	Togo
154	TG08-054	Ewento hi	<i>O. glaberrima</i>	Danyi Kétémé	Togo
155	TG08-057	Evinto hi	<i>O. glaberrima</i>	Danyi Kétémé	Togo
156	TG08-060	Xlétiétoé	<i>O. glaberrima</i>	Kpeve	Togo
157	TG08-063	Xlétiétoé	<i>O. glaberrima</i>	Kpeve	Togo
158	TG08-064	Xlétiétoé	<i>O. glaberrima</i>	Kpeve	Togo
159	TG08-068	Xlétiétoé	<i>O. glaberrima</i>	Kpeve	Togo
160	TG08-072	Xlétiétoé	<i>O. glaberrima</i>	Mempeassem	Togo
161	TG08-073	Xlétiévé	<i>O. glaberrima</i>	Mempeassem	Togo
162	TG08-074	NERICA6	<i>Interspecific lines</i>	Mempeassem	Togo
163	TG08-075	Evinto hi	<i>O. glaberrima</i>	Mempeassem	Togo
164	TG08-076	Ewento yibo	<i>O. glaberrima</i>	Mempeassem	Togo
165	TG08-079	Xlétiévé	<i>O. glaberrima</i>	Mempeassem	Togo
166	TG08-082	Xlétiétoé	<i>O. glaberrima</i>	Mempeassem	Togo
167	TG08-084	Danyimoli	<i>O. glaberrima</i>	Mempeassem	Togo
168	TG08-085	Ewento hi	<i>O. glaberrima</i>	Mempeassem	Togo
169	TG08-086	Xlétiétoé	<i>O. glaberrima</i>	Mempeassem	Togo
170	TG08-087	Danyimoli	<i>O. glaberrima</i>	Mempeassem	Togo
171	TG08-088	Xlétiévé	<i>O. glaberrima</i>	Mempeassem	Togo
172	TG08-089	Xlétiétoé	<i>O. glaberrima</i>	Mempeassem	Togo
173	TG08-092	Xlétiétoé	<i>O. glaberrima</i>	Mempeassem	Togo
174	TG08-097	Xlétiétoé	<i>O. glaberrima</i>	Amouta	Togo
175	TG08-098	Wento hi	<i>O. sativa</i>	Amouta	Togo
176	TG08-099	Xlétiétoé	<i>O. glaberrima</i>	Amouta	Togo
177	TG08-100	Xlétiétoé	<i>O. glaberrima</i>	Amouta	Togo
178	TG08-102	Wento hi	<i>O. sativa</i>	Amouta	Togo
179	TG08-103	Danyimoli	<i>O. glaberrima</i>	Amouta	Togo
180	TG08-104	Danyimoli	<i>O. glaberrima</i>	Amouta	Togo
181	TG08-107	Xlétiétoé	<i>O. glaberrima</i>	Amouta	Togo
182	TG08-110	Xlétiétoé	<i>O. glaberrima</i>	Amouta	Togo
183	TG08-111	Xlétiévé	<i>O. glaberrima</i>	Danyi Kpéto	Togo
184	TG08-112	Xlétiévé	<i>O. glaberrima</i>	Danyi Kpéto	Togo
185	TG08-113	Ewento yibo	<i>O. glaberrima</i>	Danyi Kpéto	Togo
186	TG08-114	Ewento hi	<i>O. glaberrima</i>	Danyi Kpéto	Togo
187	TG08-115	Xlétiévé	<i>O. glaberrima</i>	Danyi Kpéto	Togo
188	TG08-116	Ewento yibo	<i>O. glaberrima</i>	Danyi Kpéto	Togo
189	TG08-117	Ewento yibo	<i>O. glaberrima</i>	Danyi Kpéto	Togo
190	TG08-119	Xlétiétoé	<i>O. glaberrima</i>	Danyi Kpéto	Togo
191	TG08-121	Ewento yibo	<i>O. glaberrima</i>	Danyi Kpéto	Togo

192	TG08-123	Ewento hi	<i>O. glaberrima</i>	Danyi Kpéto	Togo
193	TG08-124	Ewento yibo	<i>O. glaberrima</i>	Danyi Kpéto	Togo
194	TG08-127	Xlétiétoé	<i>O. glaberrima</i>	Danyi Dafo	Togo
195	TG08-128	Xlétiétoé	<i>O. glaberrima</i>	Danyi Dafo	Togo
196	TG08-134	Xlétiétoé	<i>O. glaberrima</i>	Danyi Dafo	Togo
197	TG08-136	Kaki moli	<i>O. sativa</i>	Danyi Dafo	Togo
198	TG08-138	Xlétiétoé	<i>O. glaberrima</i>	Danyi Dafo	Togo
199	TG08-141	Ewento hi	<i>O. glaberrima</i>	Danyi Dafo	Togo
200	TG08-142	Dapango	<i>O. sativa</i>	Danyi Dafo	Togo
201	TG08-147	Xlétiétoé	<i>O. glaberrima</i>	Danyi Wetrofé	Togo
202	TG08-152	Xlétiétoé	<i>O. glaberrima</i>	Danyi Wetrofé	Togo
203	TG08-153	Xlétiétoé	<i>O. glaberrima</i>	Danyi Wetrofé	Togo
204	TG08-154	Ewento hi	<i>O. glaberrima</i>	Danyi Wetrofé	Togo
205	TG08-155	Ewento hi	<i>O. glaberrima</i>	Danyi Wetrofé	Togo
206	TG08-158	Xlétiétoé	<i>O. glaberrima</i>	Danyi Wetrofé	Togo
207	TG08-162	Xlétiévé	<i>O. glaberrima</i>	Danyi Wetrofé	Togo
208	TG08-166	Ewento hi	<i>O. glaberrima</i>	Danyi Wetrofé	Togo
209	TG08-168	Xlétiétoé	<i>O. glaberrima</i>	Afiadenyigba	Togo
210	TG08-173	Ewenoutohe	<i>O. sativa</i>	Afiadenyigba	Togo
211	TG08-181	Xlétiétoé	<i>O. glaberrima</i>	Afiadenyigba	Togo
212	TG08-182	Ewenoutohe	<i>O. sativa</i>	Afiadenyigba	Togo
213	TG08-184	Wento hi	<i>O. sativa</i>	Bavé-Edzéré	Togo
214	TG08-188	Xlétiétoé	<i>O. glaberrima</i>	Bavé-Edzéré	Togo
215	TG08-189	Xlétiétoé	<i>O. glaberrima</i>	Bavé-Edzéré	Togo
216	TG08-191	Danyimoli	<i>O. glaberrima</i>	Bavé-Edzéré	Togo
217	TG08-200	Xlétiétoé	<i>O. glaberrima</i>	Bavé-Edzéré	Togo
218	TG08-205	Ewento hi	<i>O. glaberrima</i>	Djidjolé	Togo
219	TG08-210	Kpakpalikpè	<i>O. glaberrima</i>	Djidjolé	Togo
220	TG08-211	Ewento hi	<i>O. glaberrima</i>	Djidjolé	Togo
221	TG08-220	Ewento yibo	<i>O. glaberrima</i>	Danyi Héhéatro	Togo
222	TG08-221	Ewento hi	<i>O. glaberrima</i>	Danyi Héhéatro	Togo
223	TG08-222	Ewento yibo	<i>O. glaberrima</i>	Danyi Héhéatro	Togo
224	TG08-226	Xlétiétoé	<i>O. glaberrima</i>	Danyi Héhéatro	Togo
225	TG08-227	Xlétiétoé	<i>O. glaberrima</i>	Danyi Héhéatro	Togo
226	TG08-231	Kpakpalikpè	<i>O. glaberrima</i>	Danyi Elavanyo	Togo
227	TG08-232	Ewento hi	<i>O. glaberrima</i>	Danyi Elavanyo	Togo
228	TG08-237	Kpakpalikpè	<i>O. glaberrima</i>	Danyi Elavanyo	Togo
229	TG08-246	Ewento yibo	<i>O. glaberrima</i>	Danyi Elavanyo	Togo
230	TG08-247	Xlétiétoé	<i>O. glaberrima</i>	Danyi Elavanyo	Togo

231	TG08-248	Ekoudzi	<i>O. glaberrima</i>	Danyi Elavanyo	Togo
232	TG08-253	Métamawu	<i>O. sativa</i>	Danyi Elavanyo	Togo
233	TG08-257	Ewento yibo	<i>O. glaberrima</i>	Danyi Elavanyo	Togo
234	TG08-262	Xlétiévé	<i>O. glaberrima</i>	Dzogbegan	Togo
235	TG08-263	Evenuto hi	<i>O. glaberrima</i>	Dzogbegan	Togo
236	TG08-272	Danyimoli	<i>O. glaberrima</i>	Dzogbegan	Togo
237	TG08-055	Akpesse	<i>O. sativa</i>	Danyi Kétémé	Togo
238	TG08-056	Xlétiévé	<i>O. glaberrima</i>	Danyi Kétémé	Togo

Table 8: Core collection liste.

S.no	Collection Number	Sample Name	Population
1	670	Botry	Central Highland
2		F 171	Central Highland
3		F 173	Central Highland
4		Japone mena	Central Highland
5		Mangakely	Central Highland
6		Manjamena	Central Highland
7		Riz noir	Central Highland
8		ROJOFOTSY	Central Highland
9	6816	Rojomena	Central Highland
10	7031	Rojomena	Central Highland
11	MJK	Rojomena	Central Highland
12		Tangongo	Central Highland
13	6914	Vary mangarirana	Central Highland
14	SMNJ 186	Berija	East
15	SMNJ 165	Boriziny mena	East
16		Dista	East
17	SMNJ 156	Fotsiavarina mena	East
18	SMNJ 182	Garagea	East
19	SABA 351	Langaka mena	East
20	SMNJ 187	Lohambitro Masompera	East
21	SMNJ 188	Mazantoka mena	East
22	SNV 419	Menalava	East
23	SNV 416	Menamongo	East
24		MK 34	East
25	SMNJ 178	Ramaditra Vihimboahangy	East
26	SNV 420	Ramazava	East

27	SNV 401	Tomorongo	East
28	SMNJ 192	Tsimahory	East
29	SMNR 545	Tsimilaro	East
30		ARD51 ABE19	Fofifa
31		ARD52 ABE14	Fofifa
32		ARD52 ABE3	Fofifa
33		ARD53 ABE7	Fofifa
34		ARD54 ABE12	Fofifa
35		ARD54 ABE20	Fofifa
36	833	Hybride(34x9(15.416))	Middle East
37		NL 19	Middle East
38		NL 7	Middle East
39		N 16	Middle West
40	SFB 032	Oviben ampotaka	Middle West
41	1232	Be	North
42	SSB 097	Mamoriaka mena	North
43	431	Roka	North
44	TG08-055	Akpesse	Togo
45	TG08-022	Danyimoli	Togo
46	TG08-040	Danyimoli	Togo
47	TG08-042	Danyimoli	Togo
48	TG08-191	Danyimoli	Togo
49	TG08-142	Dapango	Togo
50	TG08-221	Ewento hi	Togo
51	TG08-121	Ewento yibo	Togo
52	TG08-117	Ewento yibo	Togo
53	TG08-136	Kaki moli	Togo
54		NERICA 1	Middle West
55	TG08-074	NERICA6	Togo
56	TG08-030	Venutoyibo	Togo
57	TG08-047	Xlétiéné	Togo
58	TG08-068	Xlétiétoé	Togo
59	TG08-110	Xlétiétoé	Togo
60	TG08-013	Xlétiévé	Togo
61	TG08-088	Xlétiévé	Togo

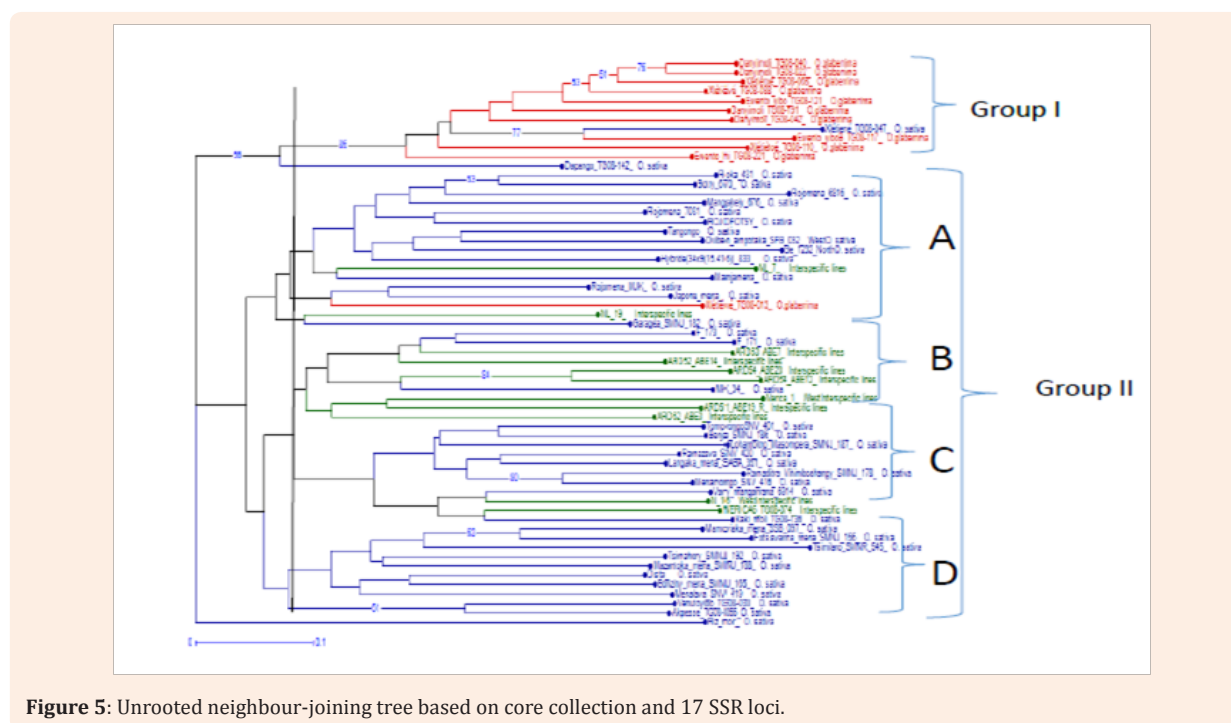


Figure 5: Unrooted neighbour-joining tree based on core collection and 17 SSR loci.

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## Conflict of Interest

The authors declared that they have no conflict of interest.

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