

Molecular characterization of a sub-collection of African rice (*Oryza glaberrima*)

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Introduction

WARDA's germplasm collection consisting in *O. glaberrima* is about 1,200 accessions. WARDA has played a significant role in supporting genetic enhancement activities in Africa. A tangible example is the development of NERICA rice from glaberrima accessions collected more than 20 years ago. *O. glaberrima* constitutes a great reservoir of useful genes for the improvement of elite lines. It has developed adaptive/protective mechanisms for resisting major biotic and abiotic stresses such as drought, iron toxicity, weed, nematodes, African gall midge... (Jones *et al.*, 1997; Linares, 2002).

To fully exploit the natural genetic variation of this glaberrima collection we need to assess the genetic diversity of the material. Very few genetic diversity studies have been carried out to date on African rice (Semon *et al.*, 2005; Barry *et al.*, 2007). *O. glaberrima* shows less genetic diversity than his Asian counterpart *O. sativa*. However, it displays a large range of variation for many morphological and agronomic traits important for breeders (Jones *et al.*, 1997).

Methodology

Ninety two accessions (Table 1) were sown in pots after a pregermination in Petri dishes. Leaves from 1 to 4 plants coming from the same panicle were bulk harvested and their DNA extracted according to Risterucci *et al.* (2000). PCR amplifications were performed using 30 labeled markers well dispersed on the rice genetic map (Table 2). The ABI3100 sequencer was used to identify the alleles (Fig. 1). Analysis was done by using GeneMapper version 4.0, ABAS (Ghosh *et al.* 1997) and DARwin 5.0 software.

Table 2: SSR markers used and diversity detected

Marker	Chr.	Dye	Freq.	N.A.	Gene Diversity	PIC
RM1	1	PET	0.84	6	0.29	0.28
RM312	1	PET	0.51	10	0.67	0.63
RM237	1	PET	0.69	9	0.51	0.49
RM431	1	VIC	0.43	6	0.65	0.58
RM154	2	6 FAM	0.78	10	0.38	0.37
RM109	2	PET	0.37	10	0.73	0.69
RM7	3	VIC	0.86	6	0.25	0.25
RM338	3	6 FAM	0.88	3	0.22	0.2
RM261	4	NED	0.44	6	0.67	0.62
RM124	4	VIC	0.89	4	0.21	0.19
RM507	5	6 FAM	0.86	3	0.26	0.24
RM249	5	NED	0.26	19	0.86	0.85
RM3	6	NED	0.58	7	0.62	0.58
RM125	7	PET	0.49	7	0.59	0.5
RM11	7	NED	0.49	8	0.63	0.56
RM455	7	NED	0.58	9	0.6	0.56
RM234	7	6 FAM	0.23	13	0.86	0.85
RM408	8	NED	0.85	6	0.27	0.25
RM152	8	NED	0.82	10	0.33	0.32
RM223	8	NED	0.3	10	0.78	0.75
RM433	8	6 FAM	0.67	9	0.51	0.47
RM447	8	PET	0.46	7	0.61	0.53
RM316	9	6 FAM	0.54	9	0.65	0.61
RM219	9	VIC	0.2	17	0.91	0.9
RM171	10	VIC	0.51	8	0.59	0.52
RM333	10	6 FAM	0.25	19	0.88	0.87
RM536	11	VIC	0.79	7	0.36	0.35
RM287	11	PET	0.66	8	0.52	0.49
RM19	12	6 FAM	0.83	6	0.3	0.29
RM277	12	PET	0.79	4	0.36	0.33
Mean			0.595	8.53	0.54	0.5
Min			0.2	3	0.21	0.19
Max			0.89	19	0.91	0.9

Chr: chromosome; Freq: major allele frequency; NA: number of alleles; PIC: polymorphism information content

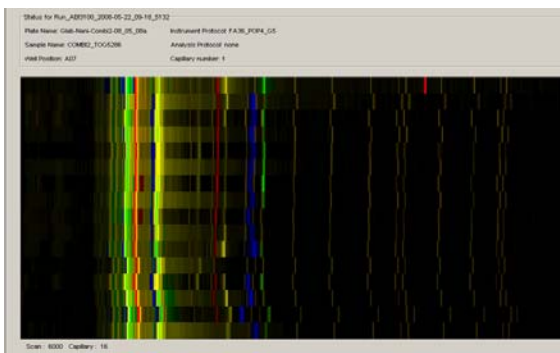


Fig.1: ABI3100 screen capture showing the sequencing of a multiplex (RM312, RM408, RM234 and RM7) with 4 different dyes. The size standard is the Gene Scan-Liz labeled in orange

Table 1: Accessions, species and ecologies

Species	Accessions	Ecologies
Wild	2	
Barthii (IRGC101937)	1	
Longistaminata (IER-Mali)	1	
O. glaberrima	84	
Upland	36	
Lowland (rainfed/irrigated)	11	
Forest swamp (deep/shallow)	34	
Floating	3	
O. sativa	6	
japonica	3	
sativa	3	

Results & Discussion

A total of 256 alleles were detected using the 30 markers. On average, 8.5 alleles/locus were detected ranging from 3 to 19 alleles/locus (Table 2). Allele size ranged from 78 bp to 347 bp. The mean PIC value of 0.5 obtained was slightly higher than those obtained by Semon *et al.* (2005) and Barry *et al.* (2007) who respectively found PIC values of 0.34 and 0.45 in their studies.

Genetic distance based analysis showed 3 main genetic groups. One consisted in a mixture of sativas, glaberrimas and the wild longistaminata accession. The second is constituted exclusively of glaberrimas. The third one represented glaberrimas plus the wild barthii accession (Fig. 2). The existence of glaberrimas closely related to sativas was also reported by Semon *et al.* (2005) and Barry *et al.* (2007). This confirmed the existence of intermediates or admixtures between the two cultivated species.

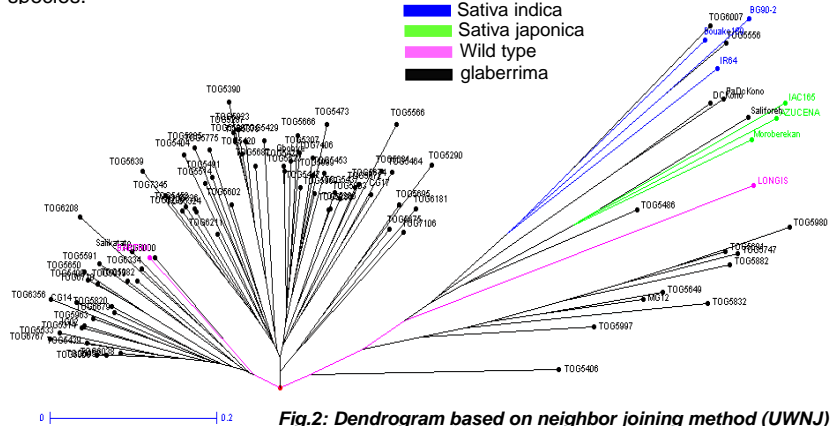


Fig.2: Dendrogram based on neighbor joining method (UWNJ)

Conclusion & Perspectives

The set of microsatellites used here allowed the detection of a high genetic diversity in African rice. These results will be confronted to the agromorphological data to see the correlations between genetic groups and specific ecosystems and morpho-types. For future work these markers could be used for the genotyping of new collected accessions in order to identify new genetic groups not identified so far. This will help to cover the entire distribution area of *O. glaberrima*, to enrich the database with new information and to broaden the genetic diversity in the genebank collection in order to constitute a core collection of African rice

References

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