

# Diachronic (1979–2003) analysis of rice genetic diversity in Guinea did not reveal genetic erosion

Mamdou B. Barry · Jean Louis Pham ·  
Sedou Béavogui · Alain Ghesquière · Nour Ahmadi

Received: 24 May 2007 / Accepted: 3 September 2007 / Published online: 19 October 2007  
© Springer Science+Business Media B.V. 2007

**Abstract** Greater insight into the dynamics of genetic resources of crop plants is needed in order to pinpoint detrimental evolutionary patterns and draw up conservation priorities. Temporal evolution of rice genetic diversity was monitored in Maritime Guinea where subsistence-oriented agriculture prevails. Diachronic comparison was performed between samples collected in six villages during the 1979/1982-period and in 2003, based on the names and number of varieties inventoried and the polymorphism of microsatellite markers. The number of varieties appeared not to be comparable between the two dates, due to differences in the collection methods. The varietal composition had evolved very substantially between the two collection dates. Many long-duration varieties present in 1979/1982 had been

abandoned and several improved varieties had been introduced. The mean number of alleles per locus and per accession was significantly higher in accessions collected in 2003. Pairwise comparisons of the mean number of alleles per locus in 1979/1982–2003 homonymous accession pairs indicated higher intra-accession diversity for the 2003 collections. Genetic differentiation, measured with the  $F_{ST}$  values, was very high and significant for more than 80% of these pairs of accessions. The overall genetic differentiation between accessions from the two collection dates was also significant. Significant changes were also observed for allelic composition. However, alleles specific of each collection date had much lower frequency, compared to alleles common to the two collection dates. These results suggest that rice genetic diversity in Maritime Guinea has been maintained or even enhanced. Old collections of crop genetic resources are often not exhaustive enough to undertake perfect diachronic comparison. New methods to utilize this historical data for diversity monitoring are needed.

---

M. B. Barry · S. Béavogui  
Institut de Recherche Agronomique de Guinée, PB 1523,  
Conakry, Guinea

J. L. Pham  
UMR DGPC/IRD, Av. Agropolis, Montpellier Cedex 5  
34398, France

A. Ghesquière  
Laboratoire Génome et Développement, IRD, Av.  
Agropolis, Montpellier Cedex 5 34394, France

N. Ahmadi (✉)  
UPR Peuplements de riz, CIRAD, TA70/03, Av.  
Agropolis, Montpellier Cedex 5 34398, France  
e-mail: ahmadi@cirad.fr

**Keywords** Diversity · Erosion · Genetic resources · Guinea · *Oryza* · Rice

## Introduction

Biodiversity monitoring should be enhanced in view of the threatening constraints (Millenium Ecosystem

Assessment 2005). Greater insight into changes under way is essential in order to be able to pinpoint detrimental evolutionary patterns and draw up conservation priorities. Moreover, biodiversity monitoring is useful for assessing the impact of conservation policies.

Genetic resources of crop plants also represent a key biodiversity component. Diversity is eroded at three levels in agrosystems. As noted by Pernès (1984), “fewer the cultivated species, fewer varieties cultivated per species, and lower the diversity within each variety”. This general observation now needs to be objectively documented. On-farm conservation of crop plants, as a complement to standard *ex situ* conservation strategies, is now the focus of increasing attention. It is therefore essential to get a dynamic picture of the diversity actually existing on farms, so as to be able to accurately pinpoint areas where this conservation would be suitable and to design and target effective interventions.

Assessing changes in genetic diversity within agrosystems require diachronic comparison, between time zero and a subsequent time. Such comparisons can be based on different diversity measurements, e.g., species, varietal, agromorphological and molecular diversity. These three latter intraspecific diversity indicators have advantages and drawbacks. Varietal diversity is easy to measure but does not accurately portray the genetic diversity, especially due to inconsistencies in local variety names (unclear relationship between the genetic identity and the variety name). Agromorphological diversity provides an indication of the diversity that could be tapped for agricultural uses, but this is hard to measure in large samples collected under different agroecological conditions, and it is impossible to appraise the overall complexity (morphological traits, tolerance to biotic and abiotic stress, organoleptic qualities, etc.). Repeatable and environment interference-free data on diversity can be obtained with molecular markers, but markers currently available for most species are neutral and do not reflect the adaptive diversity, especially on a small geographical scale.

Temporal comparisons also require data of the same type and those on the same scale for the different periods compared. It should otherwise be possible to infer the diversity at a given level on the basis of information from other indicators, e.g., infer molecular diversity from data on the ecogeographical

distribution of varietal groups with well-established genetic characteristics, or on the basis of similar situations with respect to agroecological and socio-economic characteristics.

These methodological difficulties highlight the fact that few studies have focused on measuring genetic erosion. Studies based on species or varietal diversity (Hammer et al. 1996; Ochoa 1975; Fowler and Mooney 1990; Peroni and Hanazaki 2002) have generally concluded that genetic erosion was under way. Brush (1999) pointed out that diversity gains and losses must be jointly assessed, rather than simply focusing on this latter factor. The conclusions of the few studies using molecular markers conducted to date have not been as clearcut with respect to the involvement of genetic erosion. Martos et al. (2005) used AFLP markers and concluded that wheat genetic diversity was preserved between 1945 and 2000 in Italy and Spain. Tin et al. (2001) also showed that the isoenzymatic diversity between *ex situ* and *in situ* conserved rice accessions cultivated in Vietnam was relatively stable.

For cultivated plants, genebank collections provide a unique opportunity to get a clear picture of the *in situ* diversity at the time of the collection, but the sampling must be properly carried out and the conservation conditions have to be efficient enough to avoid degradation of the initial material, which would blur the picture.

The aim of this study was to check whether these collections could be used for estimating changes in rice genetic diversity in Maritime Guinea over a period of around 20 years, between 1979–1982 (when the first collections were carried out) and 2003. We present the results of a temporal analysis of the diversity of rice varieties in six villages of Maritime Guinea, as determined through an inventory of cultivated varieties and a study of inter- and intra-varietal molecular diversity at microsatellite loci.

## Materials and methods

### Study periods and sites

The two reference dates for the temporal analysis were 1979/1982 and 2003. For the first date (hereafter called the 1979/1982 collections), two collections were carried out in 1979 (Bezançon and Koffi 1979)

and 1982 (De Kochko and Koffi 1982) by ORSTOM, IRAT and IDESSA, as part of a germplasm collection program supported by FAO. The second date corresponds to our collections in 2003.

The study sites included six villages in Maritime Guinea, i.e., Kifinda, Kaboguessy, Thia, Kibola, central Boffa and Meyenhouré. The first three sites were part of a sample of 15 villages that had been studied for an analysis of genetic diversity of rice in Maritime Guinea (Barry 2006). The latter three were randomly chosen, specifically for this study, amongst the villages surveyed for the 1979/1982 collections. Kifinda was the focus of a more in-depth study. Given its size, ethnical composition, production systems, management of rice varieties and seeds, Kifinda is a typical village of Maritime Guinea (Barry 2006). Thanks to our previous work in this village a good mutual confidence had been established between the village population and the research team which is very important for the acquisition of reliable information and seed samples.

#### Sample collection

Little information was available on the conditions of the 1979/1982 collections. However, we did discover that the collection team generally showed up in the villages without notice, spent around 1–2 h per village, conducted a quick inventory of rice varieties in the village and then collected one sample of each identified traditional rice variety. The aim was more to assess regional diversity in rice than to systematically collect all varieties in each village (Charrier, pers. comm.). We thus had access to a list of rice varieties that had been sampled in each village. The sampling conditions (bulk or panicle samples) were unknown. The passport data collected were limited to the village name and the variety name.

The 2003 collections were preceded by a community survey that was conducted to draw up an exhaustive list of varieties cultivated in the village in 2003. At the end of the survey session, for each inventoried variety, a 50 g sample was collected from a larger bulk sample supplied by the farmers. Varieties for which several farmers had supplied a sample were surveyed several times. Hereafter, we refer to these samples as accessions of the same variety. For each accession or variety the following

passport data were collected: village name, variety name, ecosystem (mangrove, rainfed lowland and rainfed upland) in which the variety was cultivated, and the name of the farmer who provided the seed sample.

For Kifinda, in addition to the 2003 inventory, a list of varieties cultivated in 1979 (first collection date) was also drawn up during a community survey session that was focused specifically on this topic. The year of the change in political regime in Guinea (1984) was set as the reference year.

The wild relatives of *O. sativa* L. were also present in the villages (*O. longistaminata* A. Chev. et Roehr. in the lowland ecosystem and *O. breviligulata* A. Chev. et Roehr. in the upland ecosystem) but were not surveyed.

#### Plant material

For each village except Kifinda, varieties assessed by molecular analysis were only the one present in the village at the two collection dates (Table 2). For Kifinda, all varieties collected in 1979/1982 and in 2003 were analyzed (Table 3). The 1979/1982 collection samples were taken from the original bulk samples (not regenerated plants) maintained in a cold room at IRD, Montpellier (France). The 2003 samples were from our own collections, which are preserved at CIRAD, Montpellier (France).

For the 2003 collections, each variety and accession was represented by 27 individuals. For the 1979/1982 collections, the number ranged from 9 to 21 depending on seed availability. In Kifinda, two varieties out of ten from the 1979/1982 collections could not be studied due to a lack of available seed.

#### Genotyping

Total DNA was extracted using the MATAB method described by Risterucci et al. (2000). DNA amplification was performed by polymerase chain reaction (PCR) in 384-well plates using a Mastercycler (Eppendorf) or DYAD (MJ Research) thermocycler. Multiplex migration (two primer pairs) of PCR products was performed on acrylamide gel (7 or 8%) using a LI-COR IR<sup>2</sup> automatic sequencer (genotyping platform of Génopole Montpellier

Languedoc Roussillon, hosted by CIRAD's IRU Polymorphisms of Interest in Agriculture).

The genotype of each individual was determined at 10 microsatellite loci that were selected for their high polymorphism (Luce et al. 2001; Simon Mande pers. com.), i.e., RM1, RM7, RM11, RM21, RM122, RM164, RM168, RM224, RM229 and RM332.

### Data analysis

The diversity of each accession was analyzed on the basis of the following three statistical parameters: number of alleles per locus (Na) heterozygosity rate (Ho) and polymorphism information content (PIC), using the Power Marker version 3.20 software package. An analysis of variance was performed using SAS software to compare the intra-accession genetic diversity of homonymous accessions collected in the same village at the two collection dates. The number of genotyped individuals for the 1979/1982 accessions was variable and lower than that of the 2003 accessions. Hence, to avoid bias due to this difference in sample size, the number of individuals between homonymous accessions was equated by randomly

choosing the same number of individuals in the 2003 accessions as there were in the 1979/1982 accessions. Genetic differentiation between accessions pairs was evaluated by the  $F_{ST}$  factor (Wright 1931) calculated using ARLEQUIN version 2.000 software.

In the Kifinda village, the total diversity of the 1979/1982 and 2003 collections was compared on the basis of the same three statistical parameters (Na, Ho and PIC) using the Power Marker version 3.20 software package (Liu and Muse 2001–2004). The mean intra-varietal diversity in the 1979/1982 and 2003 collections was compared on the basis of Na, Ho and the expected heterozygosity (Hs) for each variety using FSTAT software (Goudet 1995), which corrects sample size differences by random re-sampling.

### Results

Number of varieties per village and common varieties at two collection dates

The total number of varieties inventoried and collected in each village in 2003 was, in average,

**Table 1** Main characteristics of the six study villages in 2003 and number of rice varieties inventoried during the 1979/1982 and 2003 collection surveys

Village	Eco-system	Accessibility	Number of inhabitants	Landuse intensity	Main crops	Extension services	Species	Number of varieties inventoried		Evolution in the number of varieties inventoried (%)	
								1979/1982	2003	Village	Ecosystem
Kaboguessy	LL	Very good	350	Low	MR, RLLR, F, C, P, SP	Present	Os	10	16	+54.5	+84.4
							Og	1	1		
Kibola	LL	Good	1,400	Low	MR, C, SP	Present	Os	10	18	+72.7	
							Og	1	1		
Kifinda	LL	Good	1,600	Low	MR, RLLR, C, SP	Present	Os	9	23	+130.0	
							Og	1	0		
Boffa	UL	Good	1,200	High	ULR, F, P	Present	Os	11	15	+30.8	+6.5
							Og	2	2		
Meyenhouré	UL	Bad	450	High	ULR, F, P	Absent	Os	3	5	+28.6	
							Og	4	4		
Thia	UL	Bad	750	High	ULR, MR, F, P, C	Absent	Os	6	4	-36.4	
							Og	5	3		
Mean							Os	8.17	13.50	+65.3	
							Og	2.33	1.83		-21.4
Total								63.00	92.00	+46.0	

LL, Lowland ecosystem; UL, Upland ecosystem; Os, *Oryza sativa*; Og, *Oryza glaberrima*; MR, Mangrove rice; RLLR, Rainfed lowland rice; F, Fonio, C, Cassava; P, Peanut; SP, Sweet potatoes

46% higher than that inventoried in 1979/1982 (Table 1). Note, however, that the 1979/1982 data were estimated on the basis of the number of varieties collected. The average value also span a broad range of situations depending on the village (range –36 to +130%), on the rice species (–21% for the number of *O. glaberrima* Steud. varieties and +65% for *O. sativa* varieties) and on the ecosystem, i.e., 6% in upland ecosystems and 84% in lowland ecosystems.

We compared names of varieties collected in each village in 1979/1982 and 2003 to identify common or homonymous varieties for the two collection dates. At least one common variety was identified in each village. Only 12 common varieties were noted for the two collection dates (Table 2).

In Kifinda, 23 cultivated varieties were inventoried in 2003 (Table 3) and 20 of them were collected. The inventory of varieties cultivated in the village in 1979, based on the retrospective survey in 2003, recorded 19 names. Only 10 of them had been collected in the survey of Bezançon and Koffi (1979). This suggests that only about 50% of the varieties present in the village had been inventoried and sampled during the 1979 collection. The varietal composition had evolved very substantially between the two collection dates. Only two common varieties were noted for the two collection dates. The only *O. glaberrima* variety *Bagas Malé* and many long-duration *O. sativa* varieties present in 1979/1982 had been abandoned. Several improved *O. sativa* varieties had been introduced.

## Genetic diversity in the six villages

For the six villages, a total of 12 varieties were present at the two collection dates. For six of these varieties, two or three copies (accessions) were collected in 2003 in two or three different farms of the same village (Table 3). This gave 21 pairs of homonymous accessions, which enabled us to perform 21 pairwise comparisons between the 1979/1982 and 2003 collections.

The mean number of alleles per locus and per accession ( $N_a$ ) was 1.62 for the 10 accessions from the 1979/1982 collection and 2.78 for the 21 accessions from 2003, and this difference was highly significant ( $P < 0.0001$ ). The same trend was noted for PIC, i.e., 0.255 and 0.134, respectively. Genetic differentiation between accessions from the two collections dated was low but significant ( $F_{ST} = 0.047$ ;  $P < 0.0001$ ).

Pairwise comparisons of the mean number of alleles per locus in the 21 homonymous accession pairs indicated higher intra-accession diversity for the 2003 collections. In over 80% of the pairwise comparisons, mean  $N_a$  and PIC values for the 2003 accessions were significantly higher than those of their 1979/1982 homonyms. Only one 1979/1982 accession had a higher mean  $N_a$  than its 2003 homonym.

More than 90% of the  $F_{ST}$  values per 1979/1982–2003 homonymous accession pair were significantly different from zero, and more than 80% of the

**Table 2** List of varieties common to the 1979/1982 and 2003 collections and number of accessions per variety collected in 2003

Villages	Varieties	Species	Varieties collected 1979/1982	Varieties and accessions 2003 <sup>a</sup>
Kaboguessy	Baga-Malé	Og	1	3
	Alaya (Kab)	Os	1	1
	Phom	Os	1	1
	Mafoudia	Os	1	1
Kibola	Alaya (Kib)	Os	1	2
Meyenkhourè	Malé-Forè (Mey)	Og	1	1
	Ségué-Malè	Og	1	1
Kifinda	Kinsampena	Os	1	3
	Katoko	Os	1	2
Thia	Malè-Forè (Thia)	Og	1	3
Boffa Centre	Mamoussou	Os	1	1
	Kaoulaka-Gbeli	Os	1	2
Total			12	21

Os, *Oryza sativa*; Og, *Oryza glaberrima*

<sup>a</sup> In 2003, for some varieties several accessions belonging to different farmers were collected in the same village

**Table 3** Rice varieties present in Kifinda village in 1979/1982 and 2003

N	Rice varieties present in 1979/1982		Rice varieties present in 2003	
	Inventoried	Collected	Inventoried	Collected
1	Baga-Malé (Og)	Baga-Malé (Og)	B30D2 (I)	B30D2 (I)
2	Fodè Linsény	–	BA8A (I)	BA8A (I)
3	Guinèkobi	–	Balanta	Balanta
4	Kaback	Kaback	Barka-Madina	Barka-Madina
5	<b>Katako</b>	<b>Katako</b>	Bjogoya	Bjogoya
6	<b>Kinsampéna</b>	<b>Kinsampéna</b>	Kaoulaka	Kaoulaka
7	Kobaya	Kobaya	<b>Katako</b>	<b>Katako</b>
8	Mafoudia	–	Khobè-Gbeli	Khobè-Gbeli
9	Mambambé	–	<b>Kinsampéna</b>	<b>Kinsampéna</b>
10	Mamousso	–	Kissosso	Kissosso
11	Manaré	Manaré	Kkobè	Kkobè
12	Mandéka	–	Koba	Koba
13	Missi Missi	Missi Missi	Lakhatanyi	Mandeka
14	Parkomen	–	Mabinty	Manènè
15	Tankoro Fickè	–	Mandeka	–
16	Tankoro Forè	–	Manènè	–
17	Wereya	Wereya	Matini	–
18	Yaka	Yaka	Rok5 (I)	Rok5 (I)
19	Yölon Yölon	Yölon Yölon	Tamba-Yeguety	Tamba-Yeguety
20			Tankoro	Tankoro
21			War73 (I)	War73 (I)
22			War77 (I)	War77 (I)
23			Yampony	Yampony
Total	19	8	23	20

Og, *O. glaberrima* varieties; I, improved varieties; Names in bold: varieties common to 1979/1982 and 2003 collections

**Table 4** Distribution of  $F_{ST}$  of homonymous pairs of accessions

Collection periods	Number of accession pairs compared	Number of $F_{ST}$ in different genetic differentiation classes <sup>a</sup>				Number of significant $F_{ST}$	Mean $F_{ST}$
		<0.05	0.05–0.15	0.16–0.25	>0.25		
1979/1982 and 2003	21	0	3	1	17	17	0.494
2003 and 2003	12	2	2	2	6	6	0.206

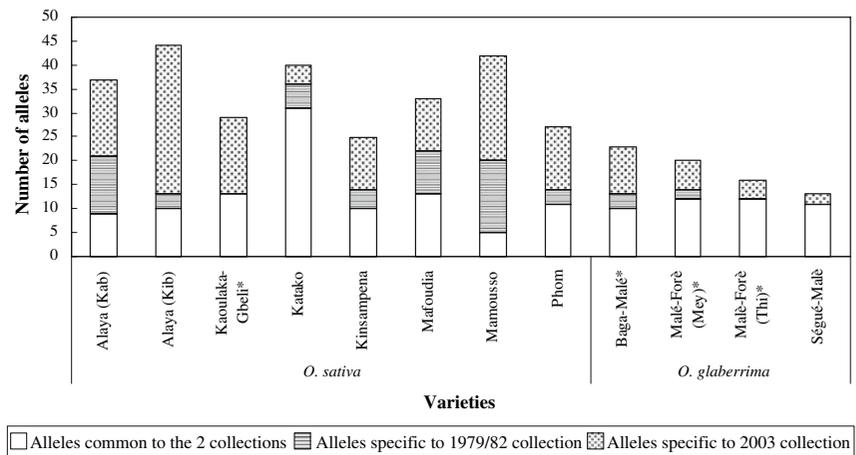
<sup>a</sup> Wright (1978) proposed the following classification of  $F_{ST}$  values:  $0 < F_{ST} < 0.05$ : low genetic differentiation between the two populations;  $0.05 < F_{ST} < 0.15$  moderate;  $0.15 < F_{ST} < 0.25$  high;  $F_{ST} > 0.25$ , very high

accession pairs had very high genetic differentiation ( $F_{ST} > 0.25$ ). By comparison, the  $F_{ST}$  values were much lower between homonymous accession pairs collected in 2003, and only 50% of them were significantly different from 0 (Table 4). Only four of the 21 accession pairs compared, including three

*O. glaberrima* and one *O. sativa* accession pairs, showed non-significant genetic differentiation.

We compared the allelic composition of 12 pairs of 1979/1982 and 2003 homonymous varieties, while randomly selecting one accession of the 2003 collection. On average, 47% of the alleles inventoried

**Fig. 1** Comparison of the allelic composition of homonymous varieties collected in 1979/1982 and 2003. Names followed by an \* indicate non-significant  $F_{ST}$  between the two collection dates



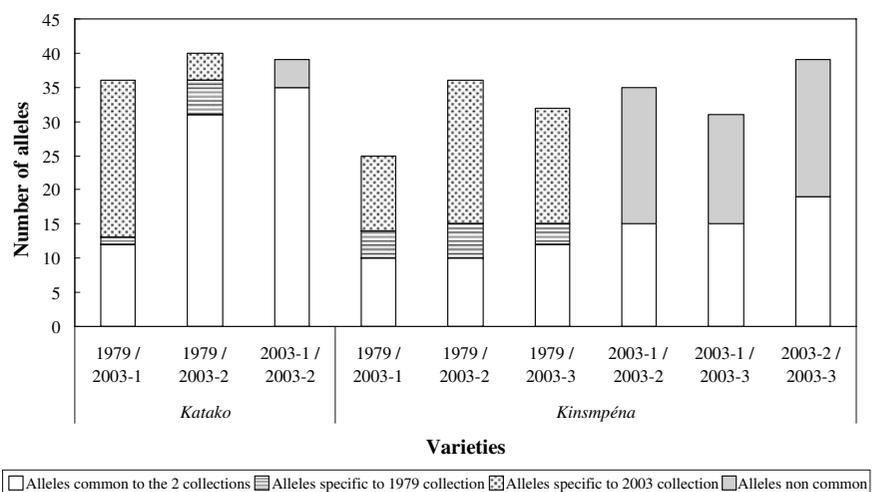
at the 10 loci studied were common to both collection periods, 14% were specific to 1979/1982 and 39% were specific to 2003. The proportions varied according to varieties, and the percentage of alleles specific to accessions collected in 1979/1982 was especially low for *O. glaberrima* varieties, i.e., only 0–13% (Fig. 1). Note that for these varieties (Baga-Malé, Malé-Forè-Mey and Malè-Forè-Thi) also the  $F_{ST}$  values between accessions of the 1979/1982 and 2003 collections were not significant. Since alleles specific to 1979/1982 represented a diversity loss and those specific to 2003 represented a diversity gain between the two collection periods, the evolutionary balance was positive for all variety pairs except one, i.e., cv. *Katako* in the Kifinda village. However, the

allelic frequency analysis indicated that all of the acquired and lost alleles had a frequency of at most 20% (Fig. 2), whereas more than 80% of the alleles common to 1979/1982 and 2003 had a high frequency of over 20%.

Genetic diversity in the village of Kifinda

The mean number of alleles per locus was six for the eight varieties of the 1979 collection as compared to 11 for the 20 varieties of the 2003 collection. The PIC values were identical (0.656 and 0.671, respectively). This trend also applied to  $H_o$  (1.3 and 1.5%). The mean number of alleles per locus and per variety was

**Fig. 2** Comparison of the allelic composition of homonymous accessions of two varieties collected in 1979 and 2003 in Kifinda village. Numbers after the year 2003 identify different accessions of the variety collected in 2003



1.7 for the eight varieties of the 1979 collection and 2.5 for 20 varieties of 2003, and this difference was significant (Table 5). Conversely, the  $H_o$  and  $H_s$  values were not significantly different. The allelic diversity for the village was thus higher in 2003 than in 1979, due to the higher number of varieties and also the higher intra-varietal diversity in 2003.

The overall genetic differentiation ( $F_{ST}$ ) between varieties present in the village in 1979 and those present in 2003 was very high (0.690) and significant ( $P < 0.001$ ) when considering all varieties, while it was low (0.071) but not quite significant ( $P < 0.001$ ) when only varieties common to the two collection periods were considered. In contrast, levels of genetic differentiation between 1979 varieties (0.623) and that between 2003 varieties (0.737) did not differ (Table 5).

Comparison of the allelic composition between homonymous accessions for the two collection dates indicated that a mean 43% of the inventoried alleles were common, 11% were specific to 1979 and 46% were specific to 2003 (Fig. 2). For cv. *Kinsmpéna*, the proportion of alleles common to 1979–2003 was the same as that of alleles common to the three accessions belonging to varieties collected in 2003. For cv *Katako*, the proportion of alleles common to 1979–2003 was much lower than that of alleles common to the two accessions of the variety collected in 2003. These results confirmed the gain in intra-varietal diversity between 1979 and 2003 in the village of Kifinda.

## Discussion

The evolution of rice molecular diversity in Maritime Guinea was studied in six villages through a comparison of accessions collected in 1979/1982 (preserved *ex situ*) with accessions collected in 2003.

Relations between genetic diversity, number of varieties collected and number of varieties inventoried

The comparison of the lists of varieties collected in 1979/1982 and in 2003 revealed a temporal increase in the number of *O. sativa* varieties cultivated per village, while the number of *O. glaberrima* varieties had decreased. Only one of the six villages surveyed showed a decrease in the number of varieties cultivated due to a shift from rice-based to groundnut-based farming systems in response to a decline in soil fertility.

The increase in varietal diversity noted here was in line with the results of a study carried out in four natural regions of Guinea, which also revealed that the number of varieties cultivated per village had increased by around 20% between 1996 and 2001 (Barry 2006). This increase could be explained by the increase in the number of rice growers, the increase in the rice-growing area, the need for more diverse varieties, and the opportunities to obtain new diversity. The need for new diversity is related to the evolution of rice cropping systems: extension of rice cultivation to new or marginal areas, decrease of soil fertility in upland ecosystem, development of improved water management systems, need for short duration varieties,.... The new opportunities to obtain new diversity is related to the overall opening of Guinea to foreign influx and more efficient extension services after the change of political regime in Guinea in 1984. Nevertheless this increase should be quantified with caution. We actually demonstrated a substantial difference between the number of varieties collected and the number inventoried in a retrospective survey. Two non-exclusive hypotheses could be put forward to explain this difference.

First, the retrospective surveys are not very reliable. Remembering varieties that were cultivated

**Table 5** Genetic diversity of rice varieties collected in Kifinda village in 1979/1982 and 2003

Collection periods	Number of accessions	Mean number of plants per accession	Na	$H_o$	$H_s$	Mean $F_{ST}$
1979/1982	8	19	1.7	0.03	0.275	0.623
2003	20	27	2.5	0.03	0.202	0.737
Probability			0.025*	0.955	0.265	0.210

Na, number of alleles per locus;  $H_o$ , degree of heterozygosity observed;  $H_s$ , degree of heterozygosity expected

\*The difference between the 1979/1982 and 2003 values is significant

some 20 years earlier is not an easy task, even for communities with a long-standing rice-growing tradition. It is even harder when considering the rapid varietal turnover, as shown by the low number of rice varieties cultivated in 1979/1982 that were still present in 2003, which was already reported by Barry (2006). It is not known whether this problem led to an increase or a decrease in the number of varieties mentioned. Note, however, that in the 2003 surveys most farmers declared that there were more varieties than in 1979/1982. It thus seems likely that rice varietal diversity has actually increased over the last 20 years.

Second, the 2003 survey was more comprehensive than that of 1979/1982. Considerable time was indeed devoted to the 2003 survey, and an exhaustive inventory of varieties cultivated in each village preceded the collection of genetic material. This inventory was done in two or three rounds in order to obtain a sample of each listed variety. The 1979/1982 survey was rapid, so each village was only visited once by the survey team, which sometimes visited as many as eight villages in 1 day, e.g., on 7 December 1982 (De Kochko and Koffi 1982). A variety collected in one village could not be sampled in another village, and improved varieties were not sampled (Charrier, pers. comm.). In other words, the 1979/1982 surveys were geared more towards highlighting varietal diversity on the regional or national scale than on the village scale.

This situation dictated the limits for analysis of the temporal evolution of genetic diversity. Our results in the Kifinda village must be considered only as an indication of the trends as the diversity of the 1979 accessions was probably down-weighted. However, the increase in allelic diversity noted at Kifinda was also generally observed in all villages on an intra-accession scale.

#### Temporal genetic differentiation of varieties

High genetic differentiation was noted between homonymous accessions collected in 1979/1982 and 2003. Accessions collected in 2003 generally had higher allelic diversity than those collected in 1979.

From a methodological standpoint, it would have been better to collect homonymous accessions on the same farms at the two collection times, which were

two decades apart. However, just the fact that the compared accessions were from different farms could not fully explain the overall increase in intra-accession genetic diversity over time. Our results also revealed lower genetic differentiation between homonymous accessions collected in 2003 relative to those collected in 1979/1982.

Differences of genetic differentiation may have been associated with differences in sampling method and accession handling. In a study on wild species related to cultivated potato, del Rio et al. (1997) suggested that the significant genetic differentiation they observed between samples maintained in a genebank and samples recollected in farmers' fields could be explained by differences in collection methods during the periods compared. Samples analyzed in our study from the 2003 collections were all derived from unsorted bulk seed in farmers' granaries. However, it was unknown whether the samples from the 1979/1982 collection were obtained via the same process or if they were derived from seeds shed from panicles that had been screened for morphotype during the survey. If this was how the samples were collected in 1979/1982, the allelic diversity actually present at the time of the collection might have been underestimated. It is, however, unlikely that the allelic diversity difference noted for most of the homonymous accession pairs was due to this possible sample purification during the first collection since such a process would eliminate only off-type plants, which are generally present with low frequency.

The increased intra-accession allelic diversity observed in 2003 could be explained by the rise in the number of rice varieties cultivated over the last few decades. Rice variety and seed management practices noted in the region support this hypothesis. There is actually considerable potential for migration since, for instance, farmers crop several varieties side-by-side every year in the same field, or in nearby fields, and they thresh different varieties in the same area, while also planting ratoons generated from previous crops (Barry 2006). Seed selection is very uncommon and the size of the population (seed quantity used) is relatively high, thus avoiding substantial loss of intra-varietal diversity from generation to generation through genetic drift. In such a process, the allelic diversity of a population should

increase with its age, which seems to have been the case here (Barry 2006).

## Conclusion

Our study highlighted the methodological problems that can arise when assessing genetic diversity changes *in situ*. The main problem we encountered was that the rice genetic diversity situation reflected by the collections undertaken 25 years ago was likely not very accurate.

However, these genetic resource collections are valuable since they often still provide a unique evidence of the past diversity of crop plants. It is therefore essential to develop methods to utilize this historical data for diversity monitoring. The results of the present study indicated that the scale aspect should be carefully considered. Our analysis of diversity on a village scale (Kifinda) did not provide any clues as to the stability of the diversity situation, but the intra-accession molecular variability analysis conclusively indicated that there had been an increase in allelic diversity that was in line with the observed increase in varietal diversity.

These results suggest that the genetic diversity of *O. sativa* rice varieties cultivated in Maritime Guinea has been maintained or even enhanced. This conclusion opposes the doomsday opinions on genetic erosion, but it concurs with the conclusions of a microsatellite marker study carried out in Niger on the diversity of cultivated millet and sorghum varieties, which also indicated that the diversity situation had remained stable overall between 1976 and 2003 (Bezaçon et al. 2005). As in this study, it would now be interesting to conduct a comparative analysis of changes in agromorphological traits that could potentially be altered by natural and human selection processes.

**Acknowledgements** The French Ministry of Foreign Affairs and the Centre de Coopération en Recherche Agronomique pour le Développement provided funding for this research.

## References

- Barry MB (2006) Diversité génétique des riz cultivés en Guinée maritime: dynamique des variétés traditionnelles et conservation *in situ* des ressources génétiques. PhD Thesis, ENSAR, Rennes, France, p 174
- Bezaçon G, Koffi G (1979) Prospection des riz africains. Campagne 1979. Mission de prospection en république populaire et révolutionnaire de Guinée, 15 Novembre–30 décembre 1979, p 28
- Bezaçon G, Mariac C, Pham JL, Vigouroux Y, Chantreau J, Deu M, Herault D, Sagnard F, Gérard B, Ndjeunga J, Kapran I, Mamadou MA (2005) How does agrobiodiversity respond to global change? Assessing changes in the diversity of pearl millet and sorghum landraces in Niger between 1976 and 2003. In: First DIVERSITAS open-science conference, integrating biodiversity science for human well-being, Oaxaca, Mexico, 9–12 November 2005
- Brush SB (1999) Genetic erosion of crop populations in centers of diversity: a revision. In: Proc. Techn. Meeting FAO in Prague, pp 34–44
- De Kochko A, Koffi G (1982) Rapport de mission. Prospection des variétés traditionnelles et des espèces sauvages de riz en république populaire et révolutionnaire de Guinée, 17 Novembre–22 décembre 1982, p 16
- del Rio AH, Bamberg JB, Huaman Z, Salas A, Vega SE (1997) Assessing changes in the genetic diversity of potato gene banks. 2. *In situ* vs *ex situ*. Theor Appl Genet 95:199–204
- Fowler C, Mooney P (1990) Shattering: food, politics and the loss of genetic diversity. University of Arizona Press, Tucson
- Goudet J (1995) Fstat version 1.2: a computer program to calculate F-statistics. J Hered 86:485–486
- Hammer K, Knupffer H, Xhuveli L, Perrino P (1996) Estimating genetic erosion in landraces—two case studies. Genet Resour Crop Evol 43:329–336
- Liu K, Muse S (2001–2004) PowerMarker: new genetic data analysis software. Version 3.0. Free program distributed by the author over the internet from <http://www.powermarker.net>
- Luce C, Noyer JL, Tharreau D, Ahmadi N, Feyt H (2001) The use of microsatellite markers to examine the diversity of the genetic resources of rice (*Oryza sativa*) adapted to European conditions. Acta Hort 546:221–235
- Martos V, Royo C, Rharrabti Y, Garcia del Moral LF (2005) Using AFLPs to determine phylogenetic relationships and genetic erosion in durum wheat cultivars released in Italy and Spain throughout the 20th century. Field Crops Res 91:107–116
- Millennium Ecosystem Assessment (2005) Ecosystem and human well-being: biodiversity synthesis. World Resources Institute, Washington, DC
- Ochoa C (1975) Potato collecting expeditions in Chile, Bolivia and Peru, and the genetic erosion of indigenous cultivars. In: Frankel OH, Hawkes JG (eds) Crop genetic resources for today and tomorrow. International biological programme 2. Cambridge University Press, Cambridge, pp 167–173
- Pernes J (1984) Gestion des ressources génétiques des plantes, Tome 1. Ed, Techniques & Documentation, Lavoisier, Paris, p 211
- Peroni N, Hanazaki N (2002) Current and lost diversity of cultivated varieties, specially cassava, under swidden

- cultivation systems in the Brazilian Atlantic forest. *Agric Ecosyst Environ* 92:171–183
- Risterucci AM, Grivet L, N'goran JAK, Pieretti I, Flament MH, Lanaud C (2000) A high density linkage map of *Theobroma cacao* L. *Theor Appl Genet* 101:1176–1182
- Tin HQ, Berg T, Bjørnstad A (2001) Diversity and adaptation in rice varieties under static (*ex situ*) and dynamic (*in situ*) management. *Euphytica* 122:491–502
- Wright S (1931) Evolution in Mendelian populations. *Genetics* 16:97–159
- Wright S (1978) Evolution and the genetics of populations. Vol. 4. Variability within and among natural populations. University of Chicago Press, Chicago