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Genetic diversity of the two cultivated rice species (*O. sativa & O. glaberrima*) in Maritime Guinea. Evidence for interspecific recombination

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Abstract In Maritime Guinea, the interpenetration of upland, lowland and mangrove rice growing ecosystems has found expression in the cohabitation of the two rice cultivated species. Recent changes in cropping practices may lead to the replacement of local varieties by modern high-yielding varieties. In the framework build-up of a strategy for the preservation of local varieties, we analysed the extent, the organisation and the specificities of the rice genetic diversity. One hundred seventy accessions collected in farmers' fields were genotyped with 11 SSR markers and phenotyped with 26 morpho-physiologic descriptors. The general organisation of rice genetic diversity in Maritime Guinea, and its tight relationship with the rice growing ecosystems were similar to the one observed elsewhere. The two major subspecies of *O sativa—indica* and tropical *japonic*—as well as the two major ecotypes of *O. glaberrima*—''floating'' and ''upright''—were present. Moreover, an original genetic compartment was detected, highlighting the occurrence of *glaberrima* × *sativa* hybridisation. Allelic diversity was found to be comparable to that noted worldwide for *indica* and *japonica* groups of *O. sativa*, but not as large for *O. glaberrima*. Given its extent, its original compartment, and its potential for inter-specific and inter-subspecific *indica* × *japonica* recombination, the preservation of rice genetic diversity in Maritime Guinea deserves special attention.

Keywords Conservation · Diversity · Guinea · *O. glaberrima* · *O. sativa* · Rice

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Introduction

Rice cropping is a very ancient tradition in Guinea. Accounts of Eustache de la Fosse ''travelling to the occidental coasts of Africa'' 1479–1480, attest large-scale rice cropping in Guinea well before the Portuguese introduced the Asian rice, *Oryza sativa*, varieties. The rice sample observed by Steude in 1885 to create and describe the African cultivated rice species *Oryza glaberrima* originated from Guinea (Godon 1991;

Portères 1955). Thanks to the diversity of rice growing ecosystems, Guinea is a large reservoir of rice genetic diversity in West Africa (Ghesquière ans Second 1983). The country is considered centre of diversification of *O. glaberrima* (Portères 1950) and the most relevant place for in situ conservation of African rice species (Bezançon 1995).

Maritime Guinea, also called ''lower Guinea'', is the most emblematic natural region of Guinea regarding the diversity of rice cropping systems. Almost all types of rice cropping are present: rainfed upland rice in slash-and-burn system, rainfed lowland rice in a continuum of fluvial plain, inland valley and mangrove rice with different degrees of crop exposure to soil and water salinity (Beavogui et al. 2000). The interpenetration of different rice growing ecosystems has found expression in the cohabitation of the two cultivated species which may have given rise to an original genetic diversity through interspecific recombination. In another respect, recent public efforts to improve regional rice productivity have led to the introduction of new cropping practices, including modern rice varieties. This process may lead to the replacement of local varieties by modern high-yielding varieties and, thus, endanger the conservation of local genetic resources.

The importance of the genetic diversity of rice in this region has prompted the Institut de Recherche Agronomique de Guinée (IRAG) to develop conservation strategies. In the framework of developing such a strategy for rice genetic resources, the aim of this study was to document the extent, the organisation and the specificities of the rice genetic diversity in Maritime Guinea.

Materials and methods

Plant collection

The rice plant material was collected in 2001 by B. Barry, a Guinean scientist, as part of a broad study involving an analysis of farmers' management of rice varieties and seeds in Maritime Guinea, on a regional, village and farm scale. Fourteen villages were chosen to account for the agro-ecological diversity in the region on the basis of current agro-ecological zoning data (Beavogui et al. 2000). In each village an inventory of rice varieties was carried out. For each variety, a seed sample was collected in the field in the presence of a group of farmers who identified the variety by consensus agreement. For each variety, 15 panicles from 15 different plants, all belonging to the predominant phenotype recognised by the farmers, were collected in the same field. Before sampling, the research team determined whether the variety belonged to *O. glaberrima* or *O. sativa*, on the basis of the degree of panicle ramification. The ecosystem in which the variety was collected was also recorded. A total of 170 accessions were collected, 144 of which belonged to *O. sativa* and 26 to *O. glaberrima*. Ninety-three accessions were collected in a lowland ecosystem (LLE) and 77 in an upland ecosystem (ULE).

Genotyping

Eleven unlinked simple sequence repeats (SSR) loci were chosen on the basis of the polymorphism they had revealed in recent rice genetic diversity studies (Luce et al. 2001). Each accession was represented by a sample composed of a blend of young leaves from four plantlets derived from four different panicles. Total DNA was extracted according to the method described by Risterucci et al. (2000). PCR amplification was performed using Mastercycler 384-well plates (Eppendorf). The PCR products migrated in multiplex (two primer pairs) on acrylamide gel (7 or 8%) on a LiCor IR^2 DNA sequencer (genotyping platform at Génopole Montpellier Languedoc Roussillon, URR, PIA, CIRAD). Allele sizes were determined using the SAGA (version 3.2) software package, which encodes genes in base pairs using size markers deposited in one per eight wells for each gel. The *indica* IR 36 rice variety was used as control for all gels.

Phenotyping

In 2002, the 170 accessions and the control variety IR36 were cropped in the field under rainfed upland conditions for upland varieties and under

irrigated conditions for lowland varieties at the Koba research station in Maritime Guinea. A complete randomised block experimental design was used with three replications. Each basic plot included three 5 m long rows with 50 cm row spacing. Fourteen qualitative characters and 12 quantitative characters were monitored (Table 1) using IRRI's standard evaluation system for rice (http://www.knowledgebank.irri.org/ses/SES.htm).

Data analysis

Genotypic data were first assessed to detect and eliminate double accessions, i.e. accessions with different names but the same genotype. No accession was eliminated since no case of complete (11 loci) genotypic identity was detected. Then, data were analysed by correspondence analysis (hereafter called CAg) using GENETIX 4.04 software (Belkhir et al. 2001). The genetic distance between accessions was calculated using the Dice similarity index (Saitou and Nei 1987), and accessions were grouped using the neighbour-joining method (NJg) with DARWIN version 5.0 (Perrier et al. 2003). Phenotypic data were analysed by correspondence analysis (hereafter called CAp) using STATISTICA 5.5 software. Quantitative data were transformed into qualitative data by dividing their distributions into three classes of comparable range. Using the same data, the Dice similarity index was also calculated and presented as a tree display using the neighbour-joining method (NJp) with DARWIN version 5.0.

Table 1. List of morpho-physiological descriptors

Qualitative descriptors	Quantitative descriptors
Colour of seedling sheath	Duration of vegetative growth
Presence of ligules	Total duration
Leaf pilosity	Number of vegetative tillers
Angle of flag leaf	Number of fertile tillers
Lodging resistance	Length of flag leaf
Panicle exertion	Plant height
Panicle erectness	Panicle length
Panicle compactness	Number of spikelets per panicle
Secondary ramification	Sterility
Resistance to shattering	1,000 grain weight
Aristation	Grain length
Apex colour	Grain width
Spikelet colour	
Caryopsis colour	

Results

Extent of the genetic diversity

A total of 129 alleles were detected (Table 2). The mean number of alleles per locus was 12 (range 9–15). The mean PIC per locus was 0.81 (range 0.68–0.88). The heterozygosity rate ranged from 1 to 16%, depending on the accession, with a mean of 7%, but these figures include both the heterozygosity and the intra-accession variability as each accession was actually represented by a blend of DNA from four individuals. The mean number of alleles per locus was 11 for *O. sativa* and 4 for *O. glaberrima.*

Genotypic diversity

The projection of the 170 accessions and control variety IR36 on the first plane of CAg (Fig. 1) revealed five subsets. Three of these subsets (E1, E2 and E3) corresponded to the well-known dual-species structure of cultivated rice, i.e. *O. sativa* and *O. glaberrima*, and the subdivision of *O. sativa* into the two subspecies *indica* and *japonica.* The two others (E4 and E5) seemed to be intermediate forms specific to Maritime Guinea.

The E1 subset indiscriminately pooled upland and lowland *O. glaberrima* accessions. E2 pooled 89 *O. sativa* accessions cultivated in LLE as well as the *indica* control IR36. E3 pooled 39 *O. sativa* accessions cultivated in ULE. E4 consisted of five *O. glaberrima* accessions and one *O. sativa* accession, both cropped in ULE. E5 was midway between E2 and E3 and pooled six accessions with the name Djou Kémé, five accessions with a name synonymous to Djou Kémé and three accessions derived from mass selection within Djou Kémé or from a cross with this variety.

The 5 subsets revealed by the CAg were not characterised by any strictly specific allele but rather by the high frequency of some alleles or/and by the presence of a specific allelic association. For instance the subset E4 differs

Table 2. Diversity at 11 SSR loci

Marker	Total (N = 170)			*O. sativa*		*O. glaberrima*	
	Na	PIC	Ho	Na	PIC	Na	PIC
RM001	13	0.86	0.07	13	0.83	2	0.36
RM007	11	0.83	0.04	10	0.79	6	0.40
RM011	11	0.85	0.04	11	0.81	4	0.50
RM021	13	0.84	0.11	13	0.86	6	0.48
RM122	9	0.75	0.01	8	0.69	3	0.29
RM164	15	0.88	0.05	15	0.88	3	0.54
RM168	12	0.73	0.04	12	0.67	3	0.35
RM222	12	0.83	0.04	11	0.81	4	0.21
RM224	13	0.85	0.16	13	0.83	6	0.74
RM229	9	0.79	0.04	9	0.77	4	0.40
RM332	11	0.68	0.14	7	0.57	7	0.68
Total	129			122		47	
Mean	12	0.81	0.07	11	0.77	4	0.45

N: Number of accessions; Na: mean number of alleles per locus; H_o: mean Heterozygosity rate per locus; PIC: Polymorphism Information Content

from the other subsets, including E1, by the very high frequency of one allele at eight loci: RM7, RM11, RM21, RM122, RM164, RM168, RM224 and RM332 (Table 3); similarly, E5 differed from the other subset cropped in ULE, E3, by an allelic association involving seven loci: RM1, RM7, RM11, RM122, RM164, RM229 and RM332 (Table 4). The *O. sativa* accession (Messemesse) classified into E4 displayed an allelic association at loci (RM001, RM021 and RM168) present only in *O. glaberrima* subsets, E1 and E5 (Table 5).

The hierarchical classification of accessions based on genetic distances per pair, using the Dice similarity index, fully confirmed the identified subsets (Fig. 2). The CAg and hierarchical classifications conducted separately with *O. sativa* and *O. glaberrima* accessions (results not shown) confirmed their subdivision into three and two subsets, respectively.

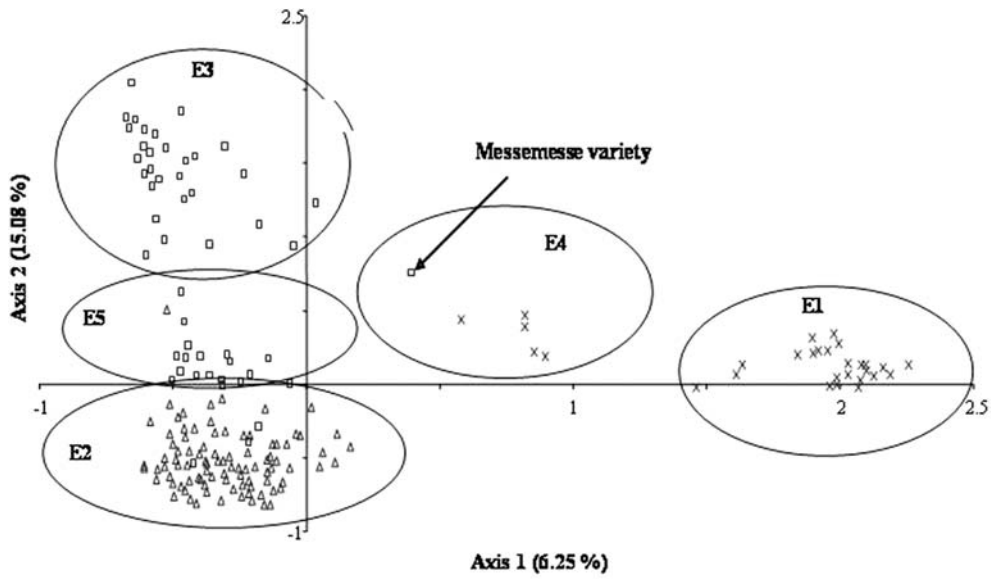

Fig. 1 First plane of the correspondence analysis based on 171 accessions and 11 SSR loci. □ and Δ: upland and lowland *O. sativa;* x: *O. glaberrima*

Table 3. Frequency of the most frequent alleles of E4 subset in other subsets identified by Correspondence analysis (CAg)

	E4 most frequent allele	Subset identified by CAg				
		E1 (21)	E2 (89)	E3 (39)	E4 (6)	E5 (15)
RM001	103*	0.75	0.01	0.06	0.20	0.00
RM007	202	0.00	0.03	0.05	0.60	0.00
RM011	144	0.00	0.00	0.05	0.60	0.00
RM021	152	0.07	0.13	0.05	0.60	0.00
RM122	256	0.00	0.00	0.02	0.80	0.00
RM164	264	0.02	0.00	0.01	0.80	0.00
RM168	111	0.05	0.02	0.05	0.60	0.00
RM222	000	0.95	0.02	0.07	0.83	0.00
RM224	159	0.07	0.02	0.06	0.80	0.47
RM229	140	0.77	0.17	0.03	0.80	0.07
RM332	188	0.02	0.01	0.03	0.80	0.00

(*x*): Number of accessions; *: number of base pairs

Phenotypic diversity

The phenotypic traits that most contributed to determining the CAp axes were panicle and grain characteristics and growth duration. Axis 1 was determined by panicle compactness (13%), secondary panicle branching (10%), caryopsis colour (9%) and grain width (7%). Axis 2 was determined by the grain length (17%), the duration of the sowing-heading cycle (14%), of the sowing-maturity cycle (14%) and the panicle length (13%). Axis 3 was determined by the grain width

Table 4. Frequency of the most frequent alleles of E5 subset in other subsets identified by Correspondence analysis (CAg)

	E5 most frequent allele	Subset identified by CAg				
		E1 (22)	E2 (89)	E3 (39)	E4 (6)	E5 (15)
RM001	99*	0.00	0.31	0.10	0.00	0.73
RM007	186	0.00	0.41	0.09	0.00	0.53
RM011	160	0.00	0.33	0.11	0.00	0.83
RM021	168	0.05	0.05	0.45	0.00	0.47
RM122	250	0.00	0.02	0.03	0.00	0.87
RM164	272	0.00	0.21	0.16	0.00	0.43
RM168	115	0.05	0.33	0.71	0.20	0.87
RM222	222	0.00	0.00	0.59	0.00	0.66
RM224	222	0.10	0.00	0.58	0.00	0.60
RM229	159	0.07	0.02	0.06	0.80	0.47
RM332	142	0.00	0.07	0.05	0.00	0.80
	198	0.00	0.25	0.19	0.00	0.87

(*x*): Number of accessions; *: number of base pairs

Table 5. Frequency of the Messemesse variety alleles in the 5 subsets identified by Correspondence analysis (CAg)

	Messeme-sse allele	Subsets identified by CAg				
		E1 (21)	E2 (89)	E3 (39)	E4 (5)a	E5 (15)
RM001	103*	0.75	0.01	0.06	0.20	0.00
RM007	202	0.00	0.03	0.05	0.60	0.00
RM011	142	0.00	0.18	0.20	0.20	0.33
RM021	148	0.79	0.00	0.05	0.20	0.00
RM122	256	0.00	0.00	0.03	0.80	0.00
RM164	264	0.02	0.00	0.01	0.80	0.00
RM168	113	0.86	0.01	0.01	0.60	0.00
RM222	222	0.00	0.00	0.59	0.00	0.66
RM224	159	0.07	0.02	0.06	0.80	0.47
RM229	134	0.07	0.52	0.08	0.20	0.07
RM332	186	0.05	0.60	0.56	0.20	0.00

(*x*): Number of accessions; a: Messemesse data were excluded *: number of base pairs

(13%), panicle compactness (11%), panicle leaf habit (10%), panicle habit (9%) and grain length (9%).

The plane axes 1 and 3 of CAp (Fig. 3) helped best to meet again the subsets identified through CAg. The *O. glaberrima* subset (E1), which had quite dispersed accessions, was located near the positive side of axis 1 and characterised by loose and barely ramified panicles, mainly with narrow grains and a red caryopsis. The subsets *indica* (E2) and *japonica* (E3) diverged along axis 1 by the grain width and colour and along axis 3 by the grain length. Upland varieties, which were mainly grown for self-consumption, had short wide grains, often with a red caryopsis. The intermediate subset between *indica* and *japonica* (E5) was also highlighted but not the one between *O. sativa* and *O. glaberrima*.

Interestingly, the *O. sativa* variety Messemesse, which was classified in the CAg as belonging to the intermediate subset E4, was found to be close to *O. glaberrima* on the basis of phenotypic characters. In order to turn down any doubt about the status of this variety, Messemesse plants were grown in glasshouse in Montpellier France. The presence of secondary ramification of the panicle confirmed the classification of Messemesse as an *O. sativa* variety.

The hierarchical classification of accessions (Fig. 4) confirmed the CAp results. Upland

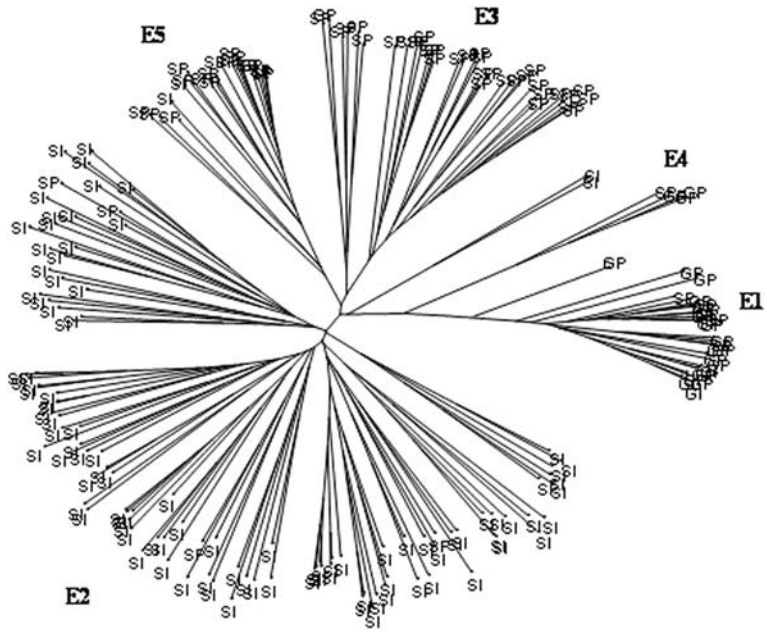

Fig. 2 Unrooted neighbour-joining tree based on 171 accessions and 11 SSR loci. SI: lowland *sativa*; SP: upland *sativa*; GI: lowland *glaberrima*; GP: upland *glaberrima*

O. glaberrima and *O. sativa japonica* accessions were quite close, and lowland *O. glaberrima* were classified amongst *indica* accessions with long duration. The Djou Kémé group, despite the fact that it was mainly grown in ULE, was classified with medium-cycle *indica* accessions.

CAp carried out on *O. sativa* accessions only (results not shown) did not lead to further details

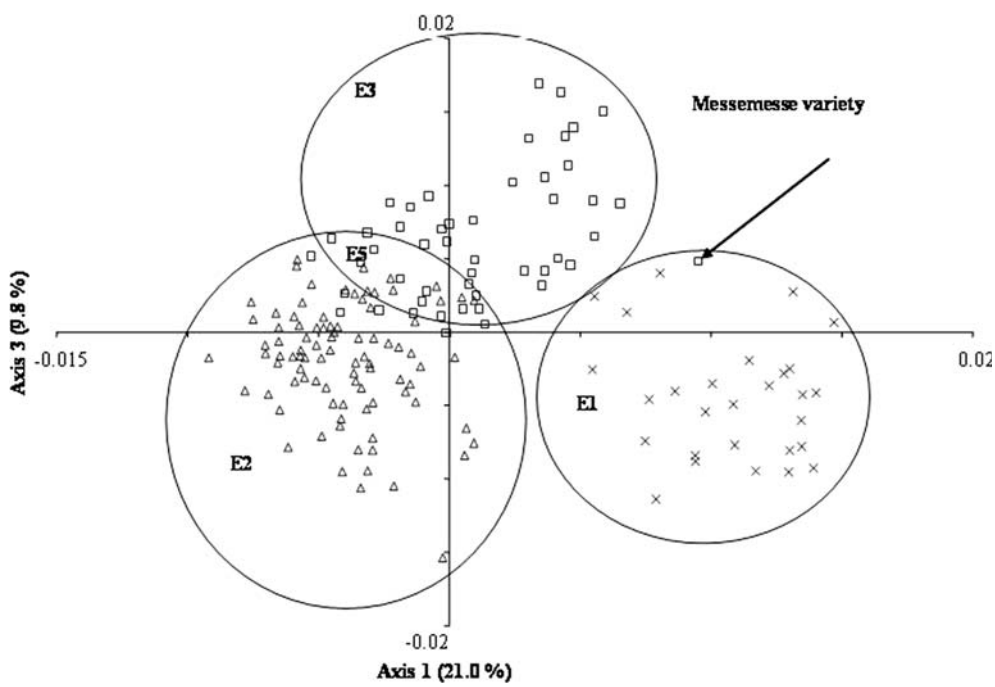

Fig. 3 Second plane of the Correspondence analysis based on 171 accessions and 26 morpho-physiological characters. □ and Δ: upland and lowland *O. sativa;* x: *O. glaberrima*

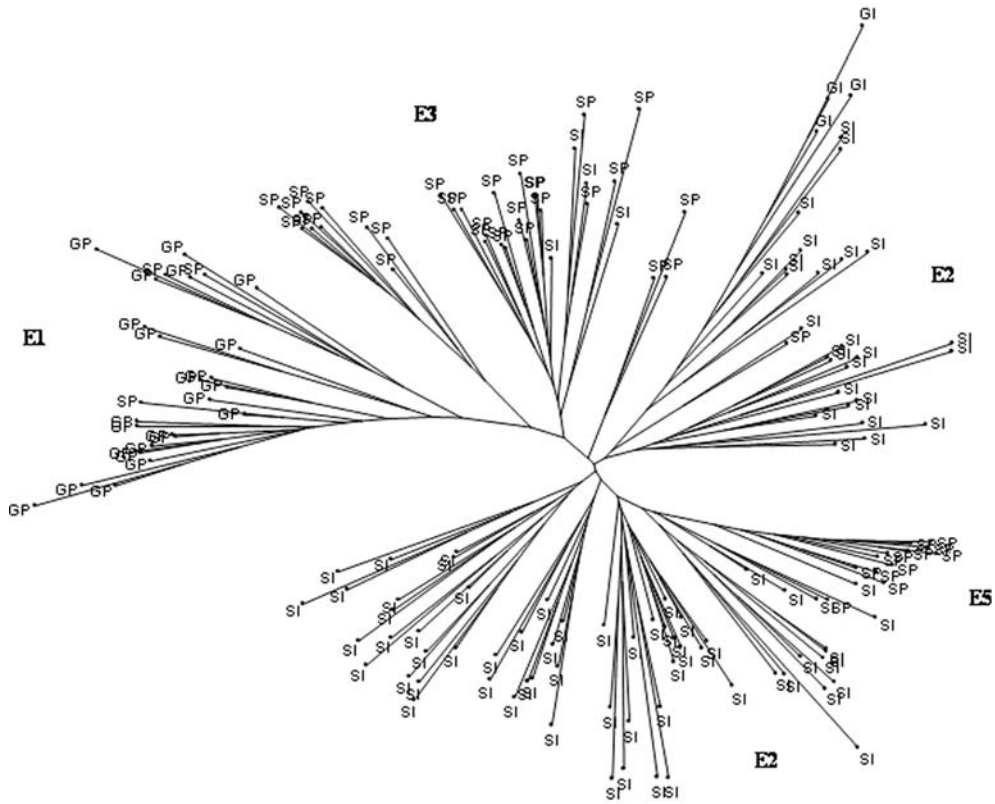

Fig. 4 Unrooted neighbour-joining tree based on 171 accessions and 11 SSR loci. SI: lowland *sativa*; SP: upland *sativa*; GI: lowland *glaberrima*; GP: upland *glaberrima*

on the organisation of phenotypic diversity. CAp on *O. glaberrima* accessions revealed four subsets (Fig. 5). Og1 consisted of 17 upland accessions characterised by small grains and short duration. Og2 included three upland accessions with intermediate-size grains. Og3 consisted of four upland accessions with wide grains and intermediate duration. While Og4 included four lowland accessions with long duration, and very tall plants producing long wide grains. The hierarchical classification (results not shown) confirmed the CAp results.

The morpho-physiological diversity of *O. glaberrima* was thus found to be structured along two gradients: (i) an ecological gradient that distinguished lowland accessions from the upland ones; and (ii) a growth duration gradient that classified upland accessions in three subsets.

Agreement between genotypic and phenotypic structure patterns

The organisations of genetic diversity revealed by the genotypic and phenotypic data were in agreement with respect to the worldwide components (species, subspecies) and local (Maritime Guinea) components. Both datasets highlighted the intermediate position of the Djou Kémé group between *indica* and *japonica*. However, the two structures did not match in terms of the genetic diversity of *O. glaberrima*, i.e. the genotypic structure did not account for the phenotypic structure according to the ecosystem and growth duration. The phenotypic structure did not show the intermediate E4 subset between *glaberrima* and *sativa* that was highlighted by the CAg. The genotypic and phenotypic data were thus

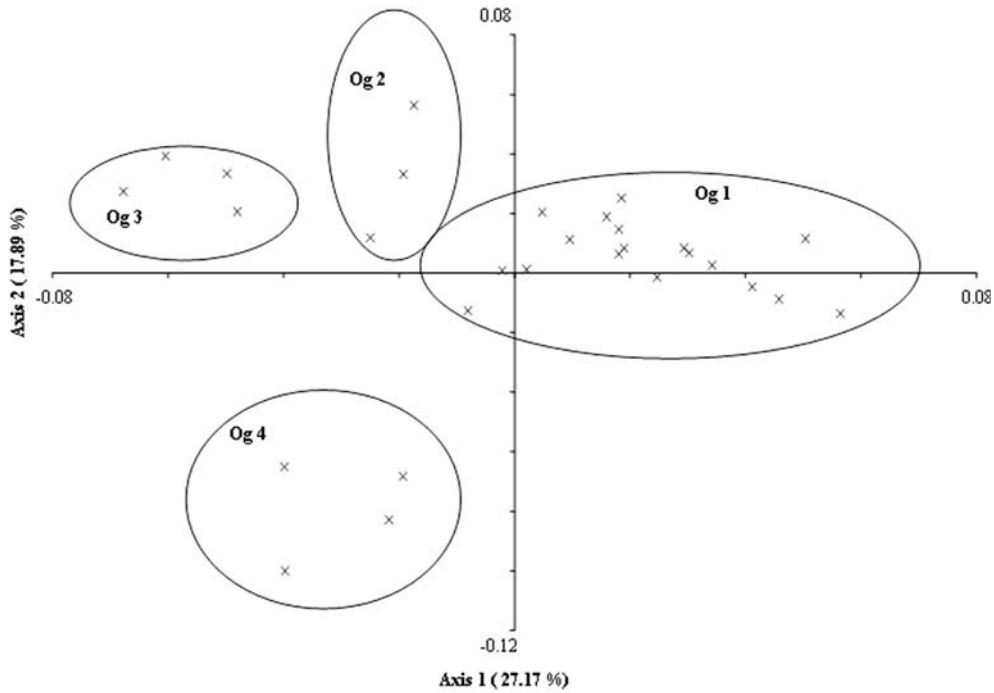

Fig. 5 First plane of the Correspondence analysis based on 29 *O. glaberrima* accessions and 26 morpho-physiological characters

complementary in describing the genetic diversity prevailing in our rice samples.

Discussion

Direct comparison of the extent of rice genetic diversity in Maritime Guinea with those observed elsewhere is risky as it may be biased by the number and the type of accessions used to evaluate genetic diversity as well as by the number and the choice of the SSR loci. For instance, we noted very important differences in the polymorphism of some SSR loci depending on the species. The RM164 and RM1 loci were amongst the most polymorphic in *O. sativa* and the least polymorphic in *O. glaberrima*. Conversely, RM332 and RM7 were highly polymorphic in *O. glaberrima* but not very polymorphic in *O. sativa.* In the same way, though direct comparison of the diversity of *O. sativa* and *O. glaberrima* classify the first one far beyond the other, with 11 alleles per locus against 4, a comparison which takes into account the number of accessions dramatically reduces the difference: the mean number of alleles per locus within each species calculated on the basis of subgroups of 10 accessions obtained by 100 independent permutations was 3.6 in *O. sativa* and 2.6 in *O. glaberrima*. This difference was still significant and suggests that, irrespective of the sample size, the allelic diversity of *O. glaberrima* was not as high as that of *O. sativa*.

Nevertheless, compared to the literature data, the extent of rice genetic diversity in Maritime Guinea, as evaluated by the total number of alleles and the mean number of alleles per locus can be considered as quite important. In *O. sativa*, the mean number of alleles per locus was 10 in the *indica* group and 8 in the *japonica* group from Maritime Guinea, whereas it was found to be only 7.3 and 6.1, respectively, in the *indica* and *tropical japonica* groups in a sample of 79 and 44 accessions representative of the genetic diversity of these groups worldwide, as genotyped using 169 SSRs (Garris et al. 2005). This higher number of alleles per locus could be partially due to the fact that the SSR loci used in our study were

chosen for their high PIC levels. However, a comparison with the mean number of alleles detected (Na = 9.5) at the same SSR loci in a population of more than 400 Mediterranean rice varieties (Luce et al. 2001) revealed that the extent of allelic diversity that we observed in Maritime Guinea could not be solely explained by the choice of SSR loci used. In *O. glaberrima*, the mean number of alleles per locus noted in the 26 accessions from Maritime Guinea was much lower than reported by Semon et al. (2004). However, these authors used 93 SSR loci and analysed 198 accessions derived from all rice-growing ecosystems throughout Africa, whereas our *glaberrima* sample was mainly from upland ecosystems.

The general organisation of the rice genetic diversity in Maritime Guinea and its tight relationship with the rice growing ecosystems are similar to the one observed elsewhere. Regarding *O. sativa*, the two subspecies *indica*, grown in LLE, and tropical *japonica*, grown in ULE, have been described with morpho-physiological characters (Jacquot and Arneaud 1979), enzymatic characters (Glaszmann 1987) and molecular markers (Garris et al. 2005; Second and Zy 1992). Regarding *O. glaberrima*, several authors (Bezançon 1995; Portères 1956; Second 1985) reported on a ''floating'' ecotype cultivated in flooded ecosystems and an ''upright'' ecotype cultivated in ULE. Semon et al. (2004), using SSR markers, identified five groups, two of which were close to the two *O. sativa* subspecies *indica* and *japonica* and three reflected an eco-geographical adaptation. Our results refined the morpho-physiological description of the ''floating'' and ''upright'' ecotypes and highlighted high diversity for growth duration in the ''upright'' ecotype.

Our results also revealed the existence of two original subsets, one (E5) intermediate between the two *O. sativa* subspecies *indica* and *japonica*, and the other (E4) intermediate between the two species *O. sativa* and *O. glaberrima*.

The E5 subset consisted mainly of accessions under the name Djou Kémé or a synonym. Its intermediate position between *indica* and *japonica* suggests that outcrossing could have occurred between the two subspecies since they have a long history of cohabitation in Maritime Guinea. However, it is known that the Djou Kémé variety was introduced in Maritime Guinea in the mid-1980s and disseminated within the framework of a development operation. Moreover, there is no mention of this name in the records of surveys undertaken in Maritime Guinea in the early 1960s (Portères 1966). The most credible hypothesis to explain the intermediate position of E5 would be that it belongs to a diversity compartment of the *indica* subspecies that is not very well represented in Guinea. The *indica* subspecies has very broad genetic diversity (Glaszmann 1987). Some discontinuity was noted between the different compartments of this subspecies since all of this diversity is not present in Guinea.

The E4 subset only differed from *O. glaberrima* genotypically. The CAp did not make a distinction between the E1 and E4 subsets identified by the CAg. At collection time in the field, five of the six E4 accessions were classified as *glaberrima* and one was assigned to *sativa*. This classification was confirmed by the results of a new assessment of the panicle architecture in these accessions, grown in glasshouse. This suggests that the genotypic differentiation of the E4 *glaberrima* accessions could be explained—as for the E5 subset—by the fact that they belong to an *O. glaberrima* diversity compartment that is not very well represented in Maritime Guinea. The facts that a single allele is predominant at 8 of the 11 loci studied (cf., Table 3) and that varieties belonging to E4 are mainly present in the southern part of the region suggest that these forms have evolved from the same initial introduction.

The *sativa* variety Messemesse, which was classified in the E4 subset, was a different case. This variety bears at three loci an allelic association specific to *O. glaberrima*, but it mainly has a *sativa japonica* genetic base as well as a *sativa*-type panicle with secondary branching. This suggests that it may be the result of interspecific *sativa* × *glaberrima* hybridisation. It is known that F1 plants generated via such crosses are completely sterile, but also that the backcrossed plants are sometimes highly fertile (Bougerol and Pham 1989). The use of inter-specific *sativa* × *glaberrima* hybridisation by Jones et al. (1997) in an upland rice breeding program and

by Ghesquière et al. (1997) for the creation of chromosome segment substitution lines is a proof of the occurrence of fertile progeny. F1 sterility, which is not absolute in the female gametes, is therefore not an impassable barrier to recombination between these two species. This has very likely taken place in Maritime Guinea, especially since the two species are often cropped by farmers in neighbouring fields, and some farmers even intercrop the two species in the same upland rice field.

Many authors have reported on the likely presence of natural hybrids between these two rice species (Bezançon 1994; Pham 1992; Second 1985). However, to our knowledge, there is no firm evidence of the existence of intermediates between these two cropped species. Semon et al. (2004), in a study of 198 *O. glaberrima* varieties, observed that many African rice varieties are an admixture between *O. glaberrima* and *O. sativa*. They suggested that introgression of *O. sativa* into *O. glaberrima* seems to have created intermediates that cannot be readily distinguished on the basis of phenotypic characters. The Messemesse variety that we found in Maritime Guinea would thus be the first confirmed case of interspecific recombination with introgression of *O. glaberrima* into *O. sativa*.

In conclusion, the diversity and the overlap of rice-growing ecosystems, as well as the presence of the two cultivated rice species, make Maritime Guinea an important reservoir of rice genetic diversity. Given its extent, its original compartment, and its potential for inter-specific and inter-subspecific *indica* × *japonica* recombinations, this genetic diversity deserves special attention to be preserved.

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References

Beavogui L, Diallo A, Dillo M (2000) Affinage du zonage agro-écologique de la Guinée martitime IRAG, Conakry

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2001) GENETIX 4.02, logiciel sous Windows TM pour la génétique des populations, Laboratoire Génome, Populations, Interactions; CNRS UMR 5000; Université Montpellier II, Montpellier France

Bezançon G (1994) Le riz cultivé d'origine africaine Oryza glaberrima Steud. et les formes sauvages et adventices apparentées : diversité, relations génétiques et domestication, Université de Paris-Sud (Centre d'Orsay), Paris-Sud

Bezançon G (1995) Riziculture traditionnelle en Afrique de l'Ouest : valorisation et conservation des ressources génétiques. Journal d'Agriculture Traditionnelle et de Botanique Appliquée 37:3–24

Bougerol B, Pham J (1989) Influence of the *Oryza sativa* genotype on the fertility and quantitative traits of hybrids between the two cultivated rice species *O. sativa* and *O. glaberrima*. Genome 32:810–815

Garris A, Tai T, Coburn J, Kresovich S, McCouch S (2005) Genetic structure and diversity in *Oryza sativa* L. Genetics 169:1631–1638

Ghesquère A, Second G (1983) Polymorphisme enzymatique et évolution d'*Oryza sativa* en Afrique. In: Societé Zoologique de France (ed) Colloque Electrophorère et taxonomie. Société Zoologique de France, Paris, pp 83–90

Ghesquière A, Sequier J, Second G, Lorieux M (1997) First steps towards a rational use of African rice, Oryza glaberrima, in rice breeding through a 'contig line' concept. Euphytica 96:31–39

Glaszmann J (1987) Isozymes and classification of Asian rice varieties. Theor Appl Genet 74:21–30

Godon P (1991) Histoire du riz en Guinée : les variétés. Cirad Presse. CIRAD, Montpellier, France

Jacquot M, Arneaud M (1979) Classification numérique de variétés de riz. Agron Trop 34:157–173

Jones MP, Dinkuhn M, Aluko GK, Semon M (1997) Interspecific Orysa sativa L X O. glaberrima Steud. Progenies in upland rice improvement. Euphytica 92: 237–246

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

Perrier X, Flori A, Bonnot F (2003) Data analysis methods In: Hamon P et al (eds) Genetic diversity of cultivated tropical plants. Enfield, Science Publishers, Montpellier, pp 43–76

Pham J (1992) Evaluation des ressources génétiques des riz cultivés en Afrique par hybridation intra- et interspécifique, Université de Paris XI - Orsay, Paris

Portères R (1950) Vieilles agricultures de l'Afrique tropicale: centre d'origine, de diversification variétale primaire et berceau de l'agriculture antérieure au XVIème siècle. Agron Trop 44:165–178

Portères R (1955) Historique des échantillons d'*O. glaberrima* Stude recueillis en Afrique. JATBA TII, N 10–11:535–537

Portères R (1956) Taxonomie agrobotanique des riz cultivés *O. sativa* Linné et *O. glaberrima* Steudel. Journal d'Agriculture Tropical et de Botanique Appliquées 4

Portères R (1966) Les noms des riz en Guinée. JATBA 13

Risterucci AM, Grivet L, N'Goran JAL, Pierett I, Flament MH, Lanaud C (2000) A high-density linkage map of Theobroma cacao L. Theor Appl Genet 101:1176–1182

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mole Biol Evol 4:406–425

Second G (1985) Relations évolutives chez le genre Oryza et processus de domestication, Orstom, Paris

Second G, Zy W (1992) Mitochondrial DNA RFLP in genus Oryza and cultivated rice. Genet Resour Crop Evol 39:125–140

Semon M, Nielsen R, Jones P, McCouch S (2004) The population structure of cultivated Oryza glaberrima (Steud): Evidence for elevated levels of LD caused by admixture with O. sativa and ecological adaptation. Genetics 169:639–1647