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RESEARCH ARTICLE

# Rice genetic diversity at farm and village levels and genetic structure of local varieties reveal need for in situ conservation

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**Abstract** Rice genetic diversity partitioning between farms, varieties and, within-variety diversity, were analysed in two villages of Maritime Guinea with contrasted agroecological conditions. One thousand and two hundred individual plants belonging to 45 accessions collected in eight farms were genotyped using 10 SSR markers. The molecular variance was evenly shared between and within accessions, while the farm effect was almost nil. Local varieties had a multi-line genetic structure. The number of multilocus genotypes was proportional to the utilisation rate of the variety in the village. The $F_{ST}$ values between different accessions of each variety were significant which indicated low genetic consistency in the variety names. This varietal structure could mainly be explained by the migration phenomenon and the high varietal turnover. Compared to allelic diversity, multilocus genotypic diversity seemed to be the most suitable indicator of the quantitative distribution of diversity at different management scales (accession, farm and village). The within- and between-farm $F_{ST}$ values were in the same order of magnitude. The within-farm diversity was not farm-specific but quantitatively high, i.e. up to 50% of the total genotypic diversity of a given village. Given the relative importance of the within-variety diversity, the in situ approach stands out as the most effective solution. As farms do not host specific diversity the in situ approach could be implemented by working with a small number of farms.

**Keywords** Diversity partition · Guinea · In situ conservation · *Oryza* · Rice · Within-variety diversity

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## Introduction

The genetic diversity of crop plants has been recognised as crucial since the early 1970s, which has prompted public authorities to invest in the creation and maintenance of genebanks or ex situ conservation, especially for major food crops such as rice (Hawkes 1983; Bellon et al. 1998). In situ and on-farm conservation concepts (Altieri and

Merrick 1987; Maxted et al. 2002) have recently emerged. They involve continuous cultivation of target species or varieties by farmers in the agrosystem within which the plant have evolved (Bellon et al. 1997). It is particularly recommended for out-crossing crops but it was also demonstrated to be beneficial for adaptation to the prevailing cropping conditions even in a self-crossing crop like rice (Tin et al. 2001). Many authors recommend benefitting from the complementarity of ex situ and in situ conservation strategies by implementing them jointly (Olfield and Alcorn 1987; IPGRI 1993; Brush 1999; Wood and Lenné 1999; Maxted et al. 2002).

To develop an in situ conservation strategy for crop plant, a prime methodological prerequisite is to gain insight into the extent, structuring and spatial distribution of genetic diversity to be conserved, as well as the extinction hazards (Bellon et al. 1997). It could provide a basis for choosing genetic entities to conserve, and eco-geographical and social organisation units (farm, village, group of villages, agricultural region, etc.) upon which conservation initiatives should be focussed. Although the farm is the smallest social unit where decisions on selection and maintenance of crop diversity are made, diversity evolution analyses should be undertaken at the village level (Bellon et al. 1997; McKey et al. 2001). Accurately defining the ''local variety'' to be conserved is another important methodological concern. Many studies have reported that morphological variability occurs within local varieties, but few studies have assessed the genetic component of this variability. Within-variety variability—largely shaped by farming practices—is, however, a key in situ genetic evolution factor (Pham et al. 2000). Within-variety polymorphism also accounts for the fact that the same local variety name can be used in reference to populations that do not have exactly the same phenotypic and genotypic composition.

We focussed on these methodological questions with the aim of developing a strategy for conservation of rice genetic resources in Guinea considered as a centre of diversification of the African cultivated rice species *O. glaberrima* Steud. (Portères 1956) and an interesting area for in situ conservation of indigenous African rice species (Bezançon 1995). Analysing the structure and the eco-geographical distribution of rice genetic diversity at the scale of a natural region, i.e. Maritime Guinea, Barry et al. (2006a, b) identified two main differentiation levels to be considered in any initiatives for rice genetic diversity preservation: the ecosystem and the village. The two large ecosystems, i.e. lowland and upland, represented 23% of the regional rice molecular diversity and accounted for the differentiation of two cultivated species *O. sativa* L. /*O. glaberrima* Steud. and the one of the two sub-species of *O. sativa*, *indica*/*japonica*.The genetic differentiation between varieties from the same village accounted for 70% of the regional molecular variation, while differentiation associated with differences between villages within the same ecosystem was accounted for only 7%.

The present study was designed to define methodology for the sampling of farms and rice varieties within villages targetted for conservation initiatives. We thus analysed: (1) the extent and structure of genetic diversity within local rice varieties; (2) the extent of diversity managed by individual farms; and (3) the partitioning of genetic diversity within villages, between farms varieties, and the within-variety diversity. These analyses also enabled us to assess the genetic consistency of variety names and the diversity within minor and major varieties.

## Materials and methods

### Choice of villages to be studied and their main features

The choice of the study village was carried out in two steps. First 14 villages (Fig. 1) were chosen to account for the agro-ecological diversity of Maritime Guinea on the basis of agro-ecological zoning data (Beavogui et al. 2000). In each village, a public survey was conducted on the history of rice growing over the previous 20 years, rice cropping practices, and farmers' management of rice variety and seed (Barry 2006). Inventory of rice varieties cultivated in each village was also carried out and a seed sample was collected. Eco-regional distribution of the genetic diversity was analysed using molecular markers. These studies

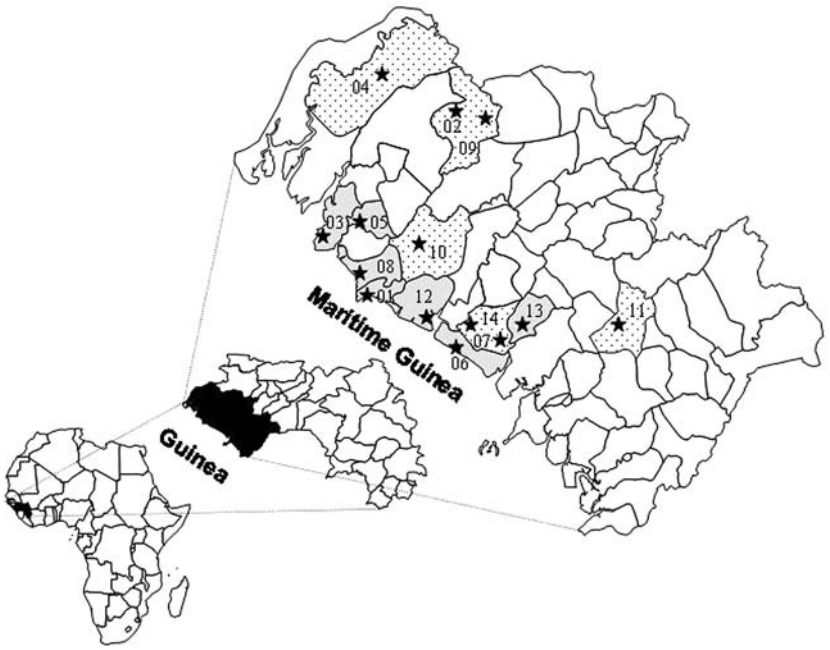


**Fig. 1** Eco-geographical distribution of the 14 research villages in Maritime Guinea. The 08: Kifinda village situated in lowland ecosystem; 04: Kancthrott village situated in upland ecosystem

showed that the most important determinant of rice genetic diversity at the village level was its belonging to the two contrasted ecosystems, lowland or upland (Barry et al. 2006a, b). Second, two villages, Kifinda and Kancthrott, were selected out of 14 to represent the two contrasted ecosystems (Table 1) and the related eco-geographical distribution of rice genetic diversity: number of varieties, number of alleles per locus at the village level, genetic differentiation ($F_{ST}$) among pairs of villages of the same ecosystem.

The Kifinda village, located in the coastal area is representative of the lowland ecosystem where lowland rice is cultivated in a continuum ranging from highly saline ocean-side mangroves to freshwater floodplains (Table 1). The Kancthrott village is located far from the coastal strip on a glacis at 20–100 m elevation with wooded savanna ecosystem where rice is grown in the uplands in a shifting slash-and-burn cropping system. These villages are hereafter referred to as lowland ecosystem village (LEV) and upland ecosystem village (UEV), respectively.

## Farm sampling

The farm sampling was carried out in two steps. First, in each of the two villages an inventory of farms was carried out. Then 12 farms were randomly chosen and a farm typology was established on the basis of the status of production factors, especially the area under rice crops and the farm labour force. In each farm, the management of rice varieties and seeds was also surveyed. The 12 farms were divided in two types, A and B. The A type farms have the highest cultivation area and labour force (Table 2). The number of varieties apart, no other difference for varieties and seed management was observed among the farms surveyed. Second, in each village, among the 12 farms surveyed, two A type farms and two B type farms were randomly chosen for analysis of rice diversity. The number of A and B type farm chosen at this step was not proportional to the total number of A and B type farm. The number of farms chosen at different steps was a compromise between the representativeness and feasibility of the study.

## Rice variety sampling

In 2002, in both study villages, an in-depth inventory of cultivated rice varieties was conducted on the basis of the rice variety names. The extent of utilisation of each variety in the village was then determined through a survey of all farms

**Table 1** Main characteristics of the two study villages

| Characteristics | Village no 1 (LEV) | Village no 2 (UEV) |
|---|---|---|
| Name | Kifinda | Kancthrott |
| Geographical location | | |
| Prefecture | Boffa | Boké |
| Sub-prefecture | Tougnifily | Dabis |
| Ecosystem | Lowland<br>Mangrove area crossed by a wide-mouthed river and under a tidal regime in its downstream part | Upland<br>Coastal plateau and glacis on ferralitic soil—savanna with African locust bean tree cover |
| Accessibility | Good | Very bad |
| Farming systems | | |
| Number of inhabitants | 1600 | 350 |
| Number of farms | 193 | 40 |
| Main ethnic groups | Baga; Soussou | Landouma |
| Intensity of landuse | Low | High |
| Main crops | Mangrove rice (1) and rainfed lowland rice | Upland rice and peanut |
| Secondary crops | Cassava, sweet potatoes | Fonio, cassava |
| Other activities | Livestock breeding, salt and palm oil extraction, fishing | Livestock breeding |
| Extension services | Present | Absent |
| Rice cropping systems | | |
| Crop rotation | Rice monoculture | Slash-and-burn, rice, peanut, fonio, fallows |
| Crop establishment | Transplanting | Direct broadcasting |
| Chemical input | No | No |
| Workforce | Manual | Manual |
| Spatial distribution of rice plots | Widespread | Grouped |
| Rice varieties and seed management | | |
| Species | *O. sativa* | *O. sativa* and *O. glaberrima* |
| Cultivated varieties | 22 | 19 |
| Major varieties (2) | 4 | 1 |
| Origin of new varieties | Extension services and neighbouring villages | Neighbouring villages |
| Origin of seeds | Previous harvest, neighbours and family | Previous harvest, neighbours and family (3) |
| Seed selection | Very rare | Rare |

LEV: Lowland ecosystem village; UEV: Upland ecosystem village. (1): located on the seaside these paddy fields are highly saline despite embankment; (2): variety cultivated by at least 50% of the village farmers; (3): recourse to neighbours and family may have two different causes: (a) quantitative shortage of seed at the farm level; (b) deterioration of seed purity after several years

in the village. The varieties were subsequently classified in two categories, i.e. major varieties used by over 50% of the farms in the village, and minor varieties used by less than 50% of the farms. Each variety was assessed on the basis of the panicle traits in the field to determine whether they belonged to one of the two cultivated rice species, i.e. *Oryza sativa* L. or *O. glaberrima* Steud. Finally, each variety was classified as a traditional or improved type (Barry 2006). For each variety, a

**Table 2** Main characteristics of farms where plant material was collected

| Village type | Lowland ecosystem | | | | Upland ecosystem | | | |
|---|---|---|---|---|---|---|---|---|
| Farm type | $A^2$ | $B^2$ | $A^4$ | $B^8$ | $A^2$ | $B^2$ | $A^3$ | $B^9$ |
| Age of farmer | 58.5 | 56.5 | 57.8 | 59.5 | 50.5 | 46.0 | 46.0 | 47.0 |
| Household | 11.0 | 9.0 | 13.0 | 8.4 | 17.0 | 7.0 | 17.3 | 7.1 |
| Active household | 5.5 | 4.5 | 6.5 | 4.4 | 8.5 | 3.5 | 7.7 | 4.0 |
| Number of rice plots | 6.7 | 3.5 | 6.5 | 4.0 | 2.0 | 1.0 | 2.0 | 1.0 |
| Rice cultivated area (ha) | 3.7 | 1.8 | 3.4 | 1.6 | 1.5 | 0.8 | 1.8 | 0.7 |
| Number of rice varieties | 10.5 | 2 | 9.3 | 2.1 | 4.5 | 2.5 | 4.3 | 2.6 |

A and B: Large and small farms; $A^2$, $B^2$: means values for the two randomly chosen farms in each village; $A^4$, $B^8$: $A^3$, $B^9$: mean values for the total number of A and B type of farms in each village

four-plant sample was collected in the field for subsequent molecular analysis (Barry et al. 2006a).

In 2003, a sample of all the varieties cultivated by each of the four farms (two A-type and two B-type) in both study villages was collected in the field. Some varieties found to be present in many farms were sampled several times and were thus represented by several accessions. Each accession consisted of 27 panicles sampled on 27 randomly chosen plants—with 27 being a tradeoff between the laboratory genotyping procedure capacity and adequate representation of the within-variety diversity. Plantlets (hereafter termed ''individuals'') derived from a seed sampled on each of the 27 panicles were used for the molecular analyses.

In UEV, the number of minor varieties present in the four study farms was considered insufficient for the comparison of intra-accession diversity of minor varieties. To rectify this situation, one or two additional samples of four minor varieties were collected (Table 3) in five other farms chosen for farm sampling.

## Genetic diversity analysis

### *Markers*

Genetic diversity was analysed via 10 microsatellite loci chosen for their high polymorphism in *O. sativa*, i.e. RM1, RM7, RM11, RM21, RM122, RM164 and RM168 (Luce et al. 2001), or *O. glaberrima*, i.e. RM229, RM224 and RM332 (Simon Mande personal communication) as well as for their ability to reveal the structure of the two species (Barry et al. 2006a).

### *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 $IR^2$ automatic sequencer (genotyping platform of Génopole Montpellier Languedoc Roussillon, hosted by CIRAD's IRU Polymorphisms of Interest in Agriculture).

### *Data analysis*

Using the Power Marker version 3.20 software package (Liu and Muse 2001–2004), the diversity of each accession was analysed on the basis of four statistical parameters: number of alleles per locus (Na), heterozygosity rate ($H_o$), polymorphism information content (PIC), which measures the genic diversity (Bostsstein et al. 1980), and the number of multilocus genotypes (Ng).

Genetic differentiation between accession pairs was evaluated by $F_{ST}$ values (Wright 1931, 1978) calculated using ARLEQUIN version 2.000 software (significance assessed with 1023 permutations).

The hierarchical distribution of the molecular variance between different sampling levels was

**Table 3** Inventory and main characteristics of rice varieties in the two study villages

| | Lowland ecosystem village | | | | | | | | Upland ecosystem village | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Varieties | | | | Collect | | | | Varieties | | | | Collect | | | |
| | Name | Type | | | Farms | | | | Name | Type | | | Farms | | | |
| *N* | | 1 | 2 | 3 | A1 | A2 | B1 | B2 | | 1 | 2 | 3 | A1 | A2 | B1 | B2 |
| 1 | Djogoya | Mj | Os | LV | x | x | x | | Djou Kèmè | Mj | Os | LV | x | x | x | x |
| 2 | Kinsampéna | Mj | Os | LV | x | x | x | | B-Djou Kèmè | Mi | Os | LV | x | | | |
| 3 | B38D2 | Mi | Os | IV | x | x | | | Conakry (2) | Mi | Os | LV | | x | | |
| 4 | BA8A | Mi | Os | IV | | | | | Fafendé | Mi | Os | LV | | x | | |
| 5 | Balanta | Mi | Os | LV | | | | | Kondon (1) | Mi | Og | LV | | | | x |
| 6 | Barkamadina | Mi | Os | LV | x | | | | Mawapou | Mi | Os | LV | | x | | |
| 7 | Dissi | Mi | Os | LV | | | | | Messè Messè | Mi | Os | LV | | x | | |
| 8 | Fodè Linsény | Mi | Os | LV | | x | | | Moromi (1) | Mi | Os | LV | x | | | x |
| 9 | Guinè Kobi | Mi | Os | LV | | x | | | Saagnakhi | Mi | Og | LV | x | | | |
| 10 | Kaoulaka | Mi | Os | LV | x | x | | | Samanden (1) | Mi | Og | LV | | | x | |
| 11 | Kaoulaka | Mi | Os | LV | | | | | Dépa | Mi | Os | LV | | | | |
| 12 | Katako | Mi | Os | LV | x | | | | Toundébo | Mi | Os | LV | | | | |
| 13 | Khobè | Mi | Os | LV | | x | | | Kimbéli | Mi | Og | LV | | | | |
| 14 | Kissosso | Mi | Os | LV | x | x | | | Yimbaya | Mi | Os | LV | | | | |
| 15 | Koba | Mi | Os | LV | | x | | | Mawouyon | Mi | Os | LV | | | | |
| 16 | Manènè | Mi | Os | LV | | x | | | Massékou | Mi | Os | LV | | | | |
| 17 | Rok5 | Mi | Os | IV | | | | | Lissi Lissi | Mi | Og | LV | | | | |
| 18 | Tambayéguéty | Mi | Os | LV | | | | | Massouba | Mi | Os | LV | | | | |
| 19 | Tankoro | Mi | Os | LV | | | | x | Wassoulon | Mi | Os | LV | | | | |
| 20 | War73 | Mi | Os | IV | x | x | | | | | | | | | | |
| 21 | WAR77 | Mi | Os | IV | | x | | | | | | | | | | |
| 22 | Yampony | Mi | Os | LV | x | | | x | | | | | | | | |
| Total | | | | | 9 | 12 | 2 | 2 | | | | | 4 | 5 | 2 | 3 |

Mj: Major variety; Mi: Minor variety; LV: Local variety; IV: Improved variety; Os: *O. sativa*; Og: *O. glaberrima*; (1): additional accessions collected outside the four study farms

assessed by the analysis of molecular variance (AMOVA) (Excoffier et al. 1992) using ARLEQUIN version 2.000 software (Schneider et al. 2000).

The genetic structure of each accession was analysed via tree diagrams using the neighbour joining (NJ) method with a similarity matrix, between the 27 accession plants, based on the Dice index (Saitou and Nei 1987). The tree diagrams were obtained using the DARwin.5 software package (Perrier et al. 2003). The number of multilocus genotypes of each accession was obtained from the accession tree diagram.

The $F_{ST}$ data per accession pair and the genetic structure of each accession were used to document the ''name consistency'' issue concerning traditional rice varieties, i.e. the genetic identity between accessions of the same variety but sampled on different farms.

## Results

### Plant material collected

The inventory of rice varieties cultivated in the two villages revealed the presence of 22 varieties in LEV and 19 in UEV. LEV had two major varieties (cultivated by more than 50% of the farmers), while UEV just had one and, similarly, LEV had six improved varieties but UEV had none. Finally, LEV only had *O. sativa* varieties, while UEV also had five *O. glaberrima* varieties. The major varieties in both villages were traditional *O. sativa* varieties (Table 3).

**Table 4** Characteristics of varieties in each of the eight study farms

| Village | Lowland ecosystem | | | | | Upland ecosystem | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Farms | Variety type | | | | | Variety type | | | | |
| | Mj | Mi | LV | IV | Total | Mj | Mi | Os | Og | Total |
| A1 | 2 | 7 | 7 | 2 | 9 | 1 | 3 | 3 | 1 | 4 |
| A2 | 2 | 10 | 8 | 4 | 12 | 1 | 4 | 5 | 0 | 5 |
| B1 | 2 | 0 | 2 | 0 | 2 | 1 | 1 | 1 | 1 | 2 |
| B2 | 0 | 2 | 2 | 0 | 2 | 1 | 2 | 2 | 1 | 3 |
| Total | 6 | 19 | 19 | 6 | 25 | 4 | 10 | 11 | 3 | 14 |

A and B: Large and small farms; Mj: Major variety; Mi: Minor variety; LV: Local variety; IV: Improved variety; Os: *O. sativa*; Og: *O. glaberrima*

A total of 25 accessions corresponding to 16 varieties were inventoried and collected in the four LEV farms and 14 accessions corresponding to 10 varieties were collected in the UEV farms (Table 4). The number of varieties per farm was much higher in LEV than in VIP, especially on A-type farms. In our farm samples, A-type farms generally had a higher number of minor varieties and they were the only ones to have improved varieties. Data for the two villages were processed separately because of the differences in the number of accessions and the presence of *O. glaberrima* varieties in UEV.

## Within-variety genetic diversity

### *Genetic structure of local varieties*

The genotypes of the 27 individuals of each accession were not identical at the ten studied loci. The mean number of alleles per locus (Na) was 3.1 for LEV accessions and 2.1 for UEV accessions. Each accession consisted of several

**Table 5** Within-accession genetic diversity

| Accessions | | Lowland ecosystem village | | | | | Upland ecosystem village | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | All | Mj | Mi | LV | IV | All | Mj | Mi | Os | Og |
| *N* | | 25 | 6 | 6 | 19 | 6 | 19 | 4 | 9 | 11 | 3 |
| Na | Mean | 3.1 | 3.5 | 1.9 | 3.3 | 2.5 | 2.1 | 2.3 | 1.8 | 2.1 | 2.1 |
| | Min | 1.3 | 3.1 | 1.3 | 1.3 | 1.3 | 1.4 | 1.8 | 1.3 | 1.4 | 1.8 |
| | Max | 4.8 | 4.4 | 2.2 | 4.8 | 3.4 | 2.9 | 2.9 | 2.8 | 2.9 | 2.8 |
| | STD | 0.93 | 0.5 | 0.4 | 0.9 | 0.9 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| $H_o$ | Mean | 0.02 | 0.02 | 0.01 | 0.02 | 0.01 | 0.02 | 0.05 | 0.01 | 0.03 | 0.00 |
| | Min | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 |
| | Max | 0.11 | 0.03 | 0.01 | 0.11 | 0.03 | 0.09 | 0.09 | 0.02 | 0.09 | 0.01 |
| | STD | 0.02 | 0.01 | 0.00 | 0.02 | 0.01 | 0.03 | 0.05 | 0.01 | 0.04 | 0.00 |
| PIC | Mean | 0.257 | 0.336 | 0.093 | 0.288 | 0.155 | 0.190 | 0.221 | 0.121 | 0.169 | 0.176 |
| | Min | 0.100 | 0.252 | 0.037 | 0.010 | 0.037 | 0.039 | 0.136 | 0.100 | 0.039 | 0.115 |
| | Max | 0.510 | 0.456 | 0.155 | 0.510 | 0.364 | 0.326 | 0.268 | 0.300 | 0.326 | 0.252 |
| | STD | 0.147 | 0.074 | 0.045 | 0.140 | 0.130 | 0.089 | 0.091 | 0.086 | 0.094 | 0.079 |
| Ng | Mean | 13.9 | 17.0 | 6.4 | 15 | 10.3 | 9.3 | 13.0 | 7.7 | 8.54 | 12 |
| | Min | 2 | 11 | 3 | 4 | 2 | 2 | 6 | 2 | 2 | 6 |
| | Max | 25 | 21 | 10 | 25 | 19 | 23 | 23 | 13 | 14 | 23 |
| | STD | 6.7 | 3.8 | 2.9 | 6 | 7.2 | 5.2 | 7.4 | 4.1 | 3.8 | 9.5 |

*N*: Number of accessions; Na: mean number of alleles per locus and per variety; $H_o$: mean heterozygosity rate per locus; PIC: Polymorphism information content; Ng: Number of multilocus genotypes; Min: Minimum; Max: Maximum; STD: Standard deviation. All: All accessions; Mj: Major variety; Mi: Minor variety; LV: Local variety; IV: Improved variety; Os: *O. sativa*; Og: *O. glaberrima*

multilocus genotypes. The mean number of genotypes (Ng) per accession was 14.0 in LEV and 9.1 in UEV (Table 5).

The Ng per accession was highly variable—some contained only two to three genotypes, while others contained as many as 25 genotypes among the 27 individuals analysed (Fig. 1). Each accession generally consisted of just one or two frequent or leading genotypes and a set of secondary genotypes that were more or less distant from the leading genotype. However, the most heterogeneous accessions did not have any leading genotype.

The Na and Ng values were very similar for *O. sativa* and *O. glaberrima* accessions, suggesting that the multilocus genetic structure was not species dependent (Table 5). Conversely, the Na and Ng values obtained for accessions belonging to major varieties were much higher than those of minor varieties.

Finally, the Na and Ng values of traditional varieties were substantially higher than those of improved varieties, thus confirming that migration or mixing phenomena are crucial in multilocus structure formation.

### *Consistency between the accession name and genotype*

The sampling in the two study villages provided an opportunity to analyse the consistency of one name involving four accessions, of five names each involving three accessions and six names each involving two accessions (Table 5).

None of the accession pairs studied had a perfect genotypic identity. The degree of name consistency was then assessed by two different approaches. One consisted of an assessment of the genetic differentiation between accessions with the same name by calculating the $F_{ST}$ per accession pair, while the other was based on an analysis of the position of individuals of the different homonym accessions on the neighbour-joining tree built from between-individual Dice indices.

The $F_{ST}$ values per accession pair were highly variable between varieties and for a given variety, depending on the accession pair (Table 6). Many $F_{ST}$ values were around 0.5 or above, especially in UEV. However, according to Wright (1978), when the $F_{ST}$ between two populations is above 0.50, these two populations should not be considered as a single genetic entity. In some cases, e.g. the name Djou Kème in UEV, the observed genetic differentiation did not seem to be due to standard genetic drift phenomena during the transfer of a variety from one farm to another, and/or to migration between different varieties from the same farm—it seems instead that completely distinct genetic entities, thus varieties, were involved. There was no apparent direct correlation between the $F_{ST}$ values and the variety type in terms of the major or minor status

**Table 6** Genetic differentiation between different accessions of the same variety

| Village | Varieties | | Pairwise $F_{ST}$ | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|
| | Names | Type | Pair1 | Pair2 | Pair3 | Pair4 | Pair5 | Pair6 | |
| LEV | Djogoya (3) | Mj | 0.36 | 0.14 | 0.11 | | | | 0.15 |
| | Kinsampéna (3) | Mj | 0.48 | 0.38 | 0.56 | | | | 0.47 |
| | Kissosso (2) | Mi | 0.13 | | | | | | 0.13 |
| | Kaoulaka (2) | Mi | 0.33 | | | | | | 0.33 |
| | B38D2 (2) | Mi | 0.42 | | | | | | 0.42 |
| UEV | Djou Kèmè (4) | Mj | 0.6 | 0.16 | 0.11 | 0.7 | 0.69 | 0.05 | 0.38 |
| | Moromi (2) | Mi | 0.66 | | | | | | 0.66 |
| | Conakry (3) | Mi | 0.02 | 0.05 | 0.03 | | | | 0.03 |
| | Kondon (2) | Mi | 0.61 | | | | | | 0.61 |
| | Samanden (2) | Mi | 0.43 | | | | | | 0.43 |

LEV: Lowland ecosystem village; UEV: Upland ecosystem village; Mj: Major variety; Mi: Minor variety; (x): Number of accessions for the given variety

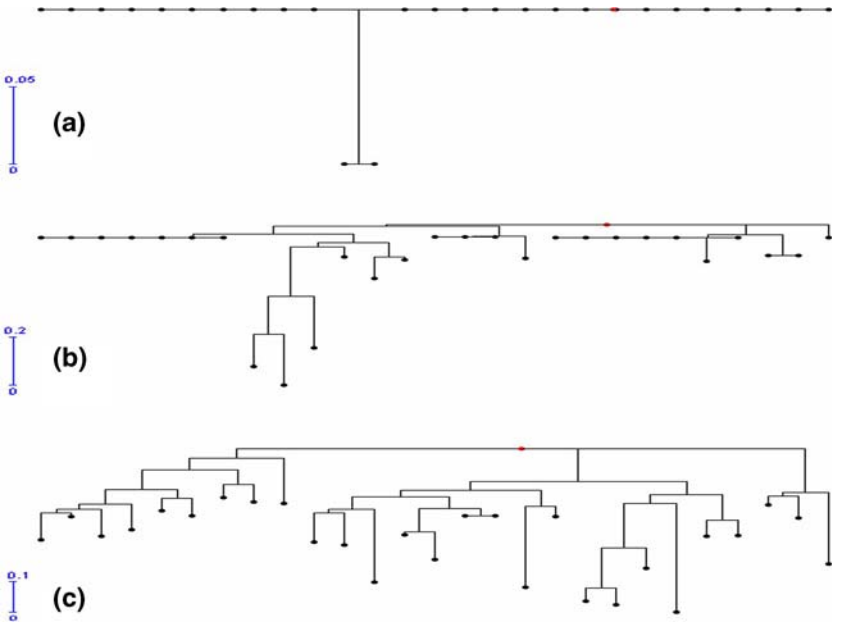


**Fig. 2** Neighbour-joining diagrams using the Dice similarity index between the 27 individual analysed of an accession. (**a**) the Mawapou variety with one leading genotype and one secondary genotype; (**b**) the Samanden variety with 2 leading genotypes and several secondary genotypes; (**c**) the Kaoulaka variety, accession from A1 farm, with no leading genotype

of the variety or of whether or not it belonged to either of the two cultivated species.

The neighbour-joining trees of the Dice similarity indices confirmed the name consistency variability measured by pairwise $F_{ST}$ (data not presented). They also showed that most accessions of one variety sampled on different farms had the same leading genotype(s) of the variety but the frequencies of this (or these) genotype(s) varied between accessions (Fig. 2). Moreover, secondary genotypes were generally specific to each accession. The Kissosso variety (Fig. 3a) illustrated a low genetic differentiation situation ($F_{ST}$ = 0.13). The leading genotype represented 89% of the individuals in the accession sampled in farm A1, and 81% in the accession sampled in farm A2. Most secondary genotypes of the two accessions differed. The Djogoya variety (Fig. 3b) illustrated a high genetic differentiation situation ($F_{ST}$ = 0.45) between three accessions. The frequency of the leading genotype was below 50% and most of the secondary genotypes were also different.

The diversity within one variety was thus markedly higher than that within each accession of this variety used by different farms in the village.

Within-farm genetic diversity

*Extent and variability of genetic diversity*

The ANOVA comparison (Table 7) of Na and PIC calculated for each farm highlighted significant differences between category A and B farms within the same village. Conversely, no significant differences were noted between allelic diversity levels of farms of the same category. Diversity differences highlighted by a comparison of A- and B-type farms were more marked in LEV, where farmers on A-type farms cultivated a much higher number of varieties than those on B-type farms. However, the number of varieties was not the sole explanatory factor, i.e. the per-farm Na and PIC values integrated the between- and within-variety diversity.

*Genetic differentiation associated with farms*

The genetic differentiation between farms of the same village was estimated by comparing the genetic differentiation between varieties on the same farm to that between these varieties and those present on other farms in the village. We thus calculated $F_{ST}$ values for all accession

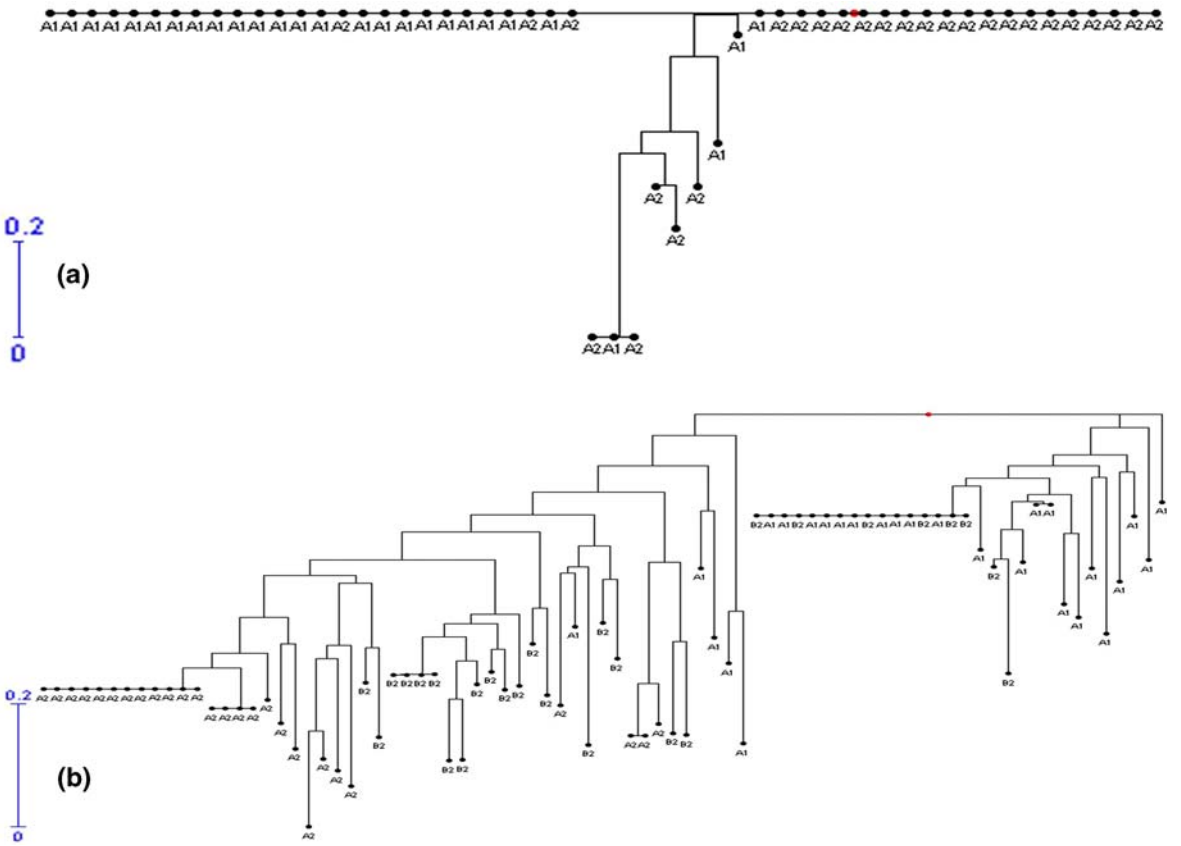


**Fig. 3** Neighbour-joining diagrams using the Dice similarity index between the 27 individual of 2 or more accessions of the some variety (**a**) the Kissosso variety with 2 rather identical accessions (A1 and A2); (**b**) the Djogoya variety with 3 very diverse accessions (A1, A2 and B2)

**Table 7** ANOVA results on different diversity parameters

| Village | Farms | Nac | Na | $H_o$ | PIC |
|---|---|---|---|---|---|
| LEV | A1 | 9 | 8.3 a | 0.027 a | 0.603 a |
| | A2 | 12 | 8.5 a | 0.011 a | 0.605 a |
| | B1 | 2 | 6.1 ab | 0.002 b | 0.534 ab |
| | B2 | 2 | 5.3 b | 0.006 b | 0.432 b |
| | Mean | – | 7.05 | 0.012 | 0.544 |
| UEV | A1 | 4 | 5.7 a | 0.032 a | 0.597 a |
| | A2 | 5 | 5.6 a | 0.019 a | 0.556 a |
| | B1 | 2 | 3.6 b | 0.005 a | 0.481 a |
| | B2 | 3 | 3.5 b | 0.003 a | 0.480 a |
| | Mean | – | 4.6 | 0.015 | 0.529 |

LEV: Lowland ecosystem village; UEV: Upland ecosystem village; A and B: Large and small farms; Nac: Number of accessions; Na: Mean number of alleles per variety; $H_o$: Mean heterozygosity rate per locus; PIC: Polymorphism information content

pairs of each village and assessed the significance of each $F_{ST}$. In both villages, more than 90% of the pairwise $F_{ST}$ values were above 0.25 and, apart from one exception, the 391 $F_{ST}$ values were significantly different from zero (Table 8). Since Wright (1978) considered that each pairwise $F_{ST}$

**Table 8** Frequency of pairwise $F_{ST}$ in different genetic differentiation classes

| Villages | Varieties | Npac | Degree of genetic differentiation* | | | | Mean pairwise $F_{ST}$ |
|---|---|---|---|---|---|---|---|
| | | | Low <0.05 | Moderate 0.05–0.15 | High 0.16–0.25 | Very high >0.25 | |
| LEV | All | 300 | 0 | 5% | 3% | 92% | 0.54 |
| | LV | 190 | 0 | 4 | 3 | 93 | 0.54 |
| | IV | 10 | 0 | 20 | 10 | 70 | 0.34 |
| | LV/IV | 100 | 0 | 5 | 3 | 92 | 0.58 |
| UEV | All | 91 | 0 | 1 | 2 | 97 | 0.73 |
| | Os | 55 | 0 | 2 | 2 | 96 | 0.68 |
| | Og | 3 | 0 | 0 | 0 | 100 | 0.45 |
| | Os/Og | 33 | 0 | 0 | 0 | 100 | 0.83 |

Npac: Number of pairs of accessions; All: All accessions in the village; LV: Local variety; IV: Improved variety; Os: *O. sativa*; Og: *O. glaberrima*; *Wright (1978)

**Table 9** Comparison of means of within- and between-farm pairwise $F_{ST}$

| Villages | Mean within-farm $F_{ST}$ | | | | Mean | Mean between-farm $F_{ST}$ | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A1/A1 | A2/A2 | B1/B1 | B2/B2 | | A1/A2 | A1/B1 | A1/B2 | A2/B1 | A2/B2 | B1/B2 | |
| LEV | 0.58 | 0.60 | 0.35 | 0.42 | 0.49 | 0.59 | 0.41 | 0.45 | 0.40 | 0.48 | 0.27 | 0.43 |
| UEV | 0.68 | 0.76 | 0.75 | 0.7 | 0.72 | 0.75 | 0.59 | 0.66 | 0.78 | 0.76 | 0.71 | 0.71 |
| Mean | 0.63 | 0.68 | 0.55 | 0.56 | 0.61 | 0.67 | 0.50 | 0.56 | 0.59 | 0.62 | 0.49 | 0.57 |

A and B: Large and small farms; LEV: Lowland ecosystem village; UEV: Upland ecosystem village

value above 0.25 was ''high'', it could be concluded that the genetic differentiation between accessions from the same village was generally high. Interestingly the genetic differentiation between traditional and improved type accessions in LEV was, on average, higher than that between accessions of the same type. Similarly, in UEV, the genetic differentiation between *O. sativa* and *O. glaberrima* accessions was, on average, higher than that between accessions of the same species.

Concerning between-farm genetic differentiation, the pairwise $F_{ST}$ values (Table 9) highlighted that, on average, varieties from the same farm were not closer than they were with accessions or varieties belonging to other farms in the village. Moreover, in both villages, the mean $F_{ST}$ between variety pairs from a farm did not significantly differ from the mean $F_{ST}$ between accession pairs from the four farms in the village (Table 9). Hence, varieties from the same farm were not closer than varieties from different

**Table 10** Summary of molecular variance analysis results for each study village

| Source of variation | Lowland ecosystem village | | | Upland ecosystem village | | |
|---|---|---|---|---|---|---|
| | *df* | Variance | *P* | *df* | Variance | *P* |
| Between farms | 3 | –0.9 | ns | 3 | 0.1 | ns |
| Between varieties within a farm | 21 | 19.4 | a | 10 | 2.9 | a |
| Within variety | 1325 | 14.4 | a | 742 | 0.8 | a |
| Total | 1349 | 32.9 | | 755 | 3.8 | |

*df*: Degrees of freedom; *P*: Probability; ns: $P > 0.5$; a: $P < 0.0001$

**Table 11** Relative extent of genetic diversity at different diversity management scales

| | | Region(1) | Village | | Farm | | Accession | |
|---|---|---|---|---|---|---|---|---|
| | | | (1) | (2) | A | B | Mj | Mi |
| LEV | Nv | 113.0 | 19.0 | 16.0 | 10.5 | 2.0 | – | – |
| | Na | 12.8 | 5.8 | 13.7 | 8.4 | 5.7 | 3.5 | 1.9 |
| | Ng | 113.0 | 19.0 | 241.0 | 117.0 | 30.0 | 17.0 | 6.4 |
| UEV | Nv | 113.0 | 20.0 | 10.0 | 4.5 | 2.5 | – | – |
| | Na | 12.8 | 6.2 | 10.5 | 5.7 | 3.6 | 2.3 | 1.8 |
| | Ng | 113.0 | 20.2 | 84.0 | 40.0 | 20.0 | 13.0 | 7.7 |

LEV: Lowland ecosystem village; UEV: Upland ecosystem village; Region: Maritime Guinea, mean for 14 villages; A and B: Large and small farms; Nv: Number of varieties; Na: number of alleles; Ng: Number of multilocus genotypes; (1): Data of the inventory of all varieties in each village, not including within-variety diversity; (2): Data from four study farms including within-variety 27 individual diversity

farms—indicating that there was no farm-dependent genetic differentiation.

## Genetic diversity distribution between sampling levels

In both villages, AMOVA decomposition of the total molecular variance, between the three sampling levels (farm, accession and individual), showed that: (1) the between-farm molecular variance was not significant; and the total genetic diversity was mainly distributed between and within accessions; and (2) the between-accession molecular variance was higher than that between individuals of the same accession (Table 10). In LEV, the molecular variance between varieties on the same farm represented 59% of the total variance and that between individuals of the same accession represented 44%. In UEV, the variance associated with varieties on the same farm was even higher, reaching 76%.

Our analysis of the quantitative distribution of genetic diversity present at different sampling levels indicated that, in terms of allelic diversity (Na), each level contained a very high share of the diversity of the immediately higher level (Table 11). Conversely, this share was generally much smaller in terms of the number of genotypes (Ng).

On average, a major variety represented 40% of the allelic diversity of A-type farms, and 60% of that of B-type farms, which had a lower number of varieties. Similarly, an accession of a major variety contained, on average, almost 25% of the allelic diversity of the village, while a accession of a minor variety contained more than 15%. Conversely, in terms of genotype numbers (Ng), a major variety represented only 15–30% of the diversity of an A-type farm and 7–15% of that of the village.

An A-type farm contained more than 55% of the allelic diversity of the village, whereas a B-type farm contained about 35%. The Ng diversity of an A-type farm represented almost 50% of that of the village, while that of a B-type farm was only 10–20%, depending on the village.

According to our regional survey results (Barry 2006), we were also able to evaluate the relative extent of the villages and the region (Maritime Guinea) diversity, i.e. each village contained 17% of the total number of varieties of the region and more than 45% of the its allelic diversity; the allelic diversity of the two study villages represented about 90% of the regional allelic diversity (Table 11).

Finally, Table 11 also shows that the Na values of each village, estimated via the within- and between-variety diversity of accessions from the four study farms, were 1.7- (UEV village) to 2.5-fold (LEV village) higher than those obtained through the analysis of the diversity of consensus samples of all varieties of the village. The Ng values were 4.2- to 12.5-fold higher, thus highlighting the importance of within-variety (and within accession) diversity in the quantitative

assessment of diversity at different diversity management levels.

## Discussion

Many authors have analysed rice genetic diversity on a village scale and reported the presence of several dozens of varieties per village and several varieties per farm in different Asian countries (Lambert 1985; Dennis 1987; Vaughan and Chang 1992; Lando and Mak 1994; Kshirsagar et al. 2002; Pham et al. 2000). The results of our study in Guinea confirmed these data, while also generating, for the first time, quantitative genotypic data on the genetic structure of local rice varieties and the distribution of genotypic diversity between different diversity management levels, including: accession or individual farmer's copy of a local variety; local variety defined by a name; farm in which several local varieties are present; and villages where several copies, or accessions, of each variety of the village are present. The SSR molecular markers used have a very high discriminating potential and are very easy to use in rice (Olufowote et al. 1997). The choice of two villages and four farms within each village was based on the results of a preliminary analysis of (1) rice varieties and seed management practices in the region (Barry 2006) and (2) the eco-geographical distribution of rice genetic diversity in the region (Barry et al. 2006b). The two study villages were representative of the two most contrasted rice-growing situations in the region. It could thus be possible to compare the relative weight the genetic diversity present at different scales, e.g. farmers' field, local variety, farm, village and region.

### Extent and structure of within-variety genetic diversity

Accessions of the local rice varieties studied had a high number of alleles per locus (Na > 3), a high genic diversity index (PIC > 0.250), low heterozygosity and a multi-lines genetic structure. Portères (1956) previously mentioned the heterogeneity of local rice varieties in Guinea. Individuals that share a certain number of traits, such as ecosystem adaptation (aquatic/upland) and growth duration, may however differ with respect to many other traits, such as spikelet shape. Similarly, within-variety isozymic diversity has been reported for local *O. sativa* and *O. glaberrima* varieties in Guinea and Côte d'Ivoire (Miézan and Ghesquière 1986), India, Nepal and Thailand (Morishima 1989). Oka (1991) reported that some local rice variety populations were more heterogeneous than populations of the annual wild rice species *O. rufipogon*. The genic diversity observed by Miézan and Ghesquière (1986) was much lower (0.090) than that we observed. However, these authors only analysed a very small number of individuals (about 10) per variety, and it is also well known that microsatellite markers can reveal much higher diversity than isozyme markers (Djè et al. 1999). Thus, our results updated and quantified previous information.

Similar within-variety and composite genetic structure results have been obtained in local sorghum varieties sampled in farmers' fields and assessed with isozyme markers (Ollitrault et al. 1997) or SSR markers (Djè et al. 1999). In sorghum, however, the Na, $H_o$ and PIC had higher value than in rice, despite the small sizes of the population studied. These differences should be compared with the allogamy difference between the two species, which is much higher in sorghum.

The multi-line structure observed is very likely due to migration, genetic drift and selection phenomena caused by farmers' seed management practices. In the absence of a formal seed production system, farmers use plain paddy as seed. They don't implement any seed selection procedure while some of their cropping practices, promote migration or mixing. Selection occurs only when the seed batch becomes extremely mixed or ''dirty''. Off-types plants are eliminated at the field, or the dirty seed is traded with relatives or neighbours in return for a cleaner batch of seed. But these procedures do not ensure the return to perfectly pure-line structures. The most mixture-promoting cropping practices are, in decreasing order (1) threshing of all varieties from one or several farms in the same threshing area; (2) use of ratoons for crop establishment, even if the varieties cultivated in year $n$ and $n - 1$ are not the same; and (3) juxtaposition of plots bearing different varieties, especially common in

UEV. Major varieties are the most prone to mixing as they are cultivated on larger surface areas by many farmers and come into contact with a greater number of other varieties. Due to their recent introduction, improved varieties have undergone less mixing and thus their within- and between-accession diversity is lower than that of traditional varieties.

Another important aspect of farmers' seed management practice is the exchanges which may cause genetic drift when the quantities exchanged are small. Minor varieties, cultivated on small surfaces by a small number of farms are particularly prone to genetic drift.

So, migration and drift phenomena are the main cause of the substantial differences in the genotypic composition of different accessions of a same variety sampled on different farms. But when extremely different genetic entities are classified under a single variety name, e.g. Djogoya, the hypothesis of errors in variety name transmission during variety or seed exchanges should be considered.

## Diversity managed by farms and within-village diversity partitioning

Depending on their size, farms manage a diversity that can be over 50% of the genotypic diversity of a village. However, according to AMOVA, the percentage of diversity shared between farms was almost nil. Likewise, the $F_{ST}$ values, which were not significantly different between pairs of varieties from the same farm or pairs of varieties from different farms, indicated that diversity is not farm-specific. This is in line with the fact that: (1) all farms within a village are under the same agroecological constraints; and (2) the turnover of accessions and varieties is high at the farm level (Barry 2006).

In both villages, genetic diversity was only partitioned within and between accessions. Between-accession diversity was higher in UEV (76%) than in LEV (59%) due to the presence of the two cultivated species *O. sativa* and *O. glaberrima, and their* specific alleles and allele combinations. The $F_{ST}$ values per accession pair were over 0.25 in 90% of cases, indicating a very high genetic differentiation between accessions of a variety, between varieties on a farm, and between varieties in a village. There was relatively little within- and between-variety diversity overlap while the between-farm diversity overlap was high. One accession could contain as much as 40% of the allelic diversity of an A-type farm, and as much as 75% of the allelic diversity of a B-type farm.

## Strategy for the conservation of rice genetic resources in Maritime Guinea

In Maritime Guinea, the two first levels of rice genetic differentiation are the ecosystems, i.e. lowland and upland, and the village accounting respectively for 23% and 70% of the region's total molecular diversity while differentiation associated with differences between villages within the same ecosystem was accounted for only 7% (Barry et al. 2006b). Any conservation strategy should therefore first consider these scales for sampling. A small number of villages per ecosystem will be enough.

At the village level, a few farms would be sufficient to sample the whole allelic diversity (Na). However, given the multi-line structure of the local varieties as well as the extent of the within-accession and within-variety diversity, allelic diversity is not the most suitable indicator of the genetic diversity. Emphasis must be placed on allelic associations or genotypic diversity (Ng). Given the high number of lines or genotypes that compose each individual variety of a village, it would be almost impossible to ensure their conservation by sampling and rejuvenation methods that are commonly used in ex situ conservation projects. Hence, conservation of all inventoried allelic associations or multilines would require an in situ approach. The fact that individual farms do not host specific diversity and that the amount of diversity they manage depends mainly on their size should facilitate the process of farm sampling for in situ conservation. In each village farms sampling should placed emphasis on the complementarities farms to cover the varietal diversity of the village, and the within-variety diversity of each inventoried variety.

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