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ORIGINAL PAPER

Genetic diversity and population differentiation of traditional fonio millet (*Digitaria* spp.) landraces from different agro-ecological zones of West Africa

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Abstract Fonio millets (*Digitaria exilis* Stapf, *D. iburua* Stapf) are valuable indigenous staple food crops in West Africa. In order to investigate the genetic diversity and population differentiation in these millets, a total of 122 accessions from five countries (Benin, Burkina Faso, Guinea, Mali and Togo) were analysed by Amplified Fragment Length Polymorphisms (AFLPs). Genetic distance-based UPGMA clustering and principal coordinate analysis revealed a clear-cut differentiation between the two species and a clustering of *D. exilis* accessions in three major genetic groups fitting to their geographical origins. Shannon's diversity index detected in *D. iburua* was low ($H = 0.02$). In *D. exilis*, the most widespread cultivated species, moderate levels of genetic diversity (Shannon's diversity $H = 0.267$; Nei's gene diversity $H' = 0.355$) were detected. This genetic diversity is unequally distributed with the essential part observed in the Upper Niger River basin while a very low diversity is present in the Atacora mountain zone. Analysis of molecular variance (AMOVA) revealed that a large part of the genetic variation resides among the genetic groups (70%) and the country of origin (56%), indicating a clear genetic differentiation within *D. exilis*. Influence of mating system (inbreeding or apomixis), agricultural selection and ecological adaptations as well as founding effects in the genetic make-up of the landraces were visible and seemed to jointly contribute to the genetic structure detected in this species. The genetic variability found between the analysed accessions was weakly correlated with their phenotypic attributes. However, the genetic groups identified differed significantly in their mean performance for some agro-morphologic traits. The results obtained are relevant for fonio millets breeding, conservation and management of their genetic resources in West Africa.

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Introduction

Sub-Saharan Africa is known for its large diversity in various native crop plant species including many types of millet. Two of them, known as fonio millets, belong to the genus *Digitaria* Haller, i.e. white fonio (*D. exilis* Stapf) and black fonio (*D. iburua* Stapf). Fonio millets are small grained, C_4 metabolism cereals with a short life cycle and medium height (Haq and Ogbe 1995). Different hypotheses exist on their reproductive system, ranging from inbreeding (Watson and Dallwitz 1992; Sarker et al. 1993) to outcrossing (Fogg 1976; Hilu et al. 1997). Fonio species are previously reported to be diploid ($2n = 2x = 18$), tetraploid ($2n = 4x = 36$) or hexaploid ($2n = 6x = 54$) (Hunter 1934;

Zeven and de Wet 1982), but only the tetraploidy level has been recently confirmed (Adoukonou-Sagbadja et al. 2007).

Fonio millets are among the oldest cereal crops domesticated by West African farmers, at around 5 millennia BC (Murdock 1959). Besides others such as pearl millet, sorghum, etc., fonio crops have played a central role in the emergence and development of traditional agriculture in the West African savannah (Busson 1965; Haq and Ogbe 1995). In this region, fonio millets supply food for several millions of tribal people either as a staple or as a major part of the diet with a high value in the local food cultures (Vietmeyer et al. 1996; Adoukonou-Sagbadja et al. 2006). Dishes made with fonio (porridge, couscous, paste, etc.) are highly appreciated by traditional farmers, particularly in many tribes such as the Akposso and Lamba in Togo, the Peul and Malinké in Guinea or the Dogon in Mali. Fonio is ranked among the most nutritious grain crops due to its exceptional richness in the human-vital amino acids methionine and cystine, deficient in major cereals like wheat, rice, maize, and sorghum (Jideani 1990). As rain-fed and low input crops, they are highly adapted to marginal land farming, flourish well on poor soils and withstand drought and floods. Due to their large ecological adaptability these plants are believed to have a high potential as key-crops in future agriculture and food security supply in their traditional cultivation zones or beyond (Eyzaguirre and Thormann 1998; Adoukonou-Sagbadja et al. 2006).

Landraces or farmers' varieties constitute valuable resources for crop breeding and conservation of its genetic diversity (Kölliker et al. 2003). In the local agriculture of West Africa, hundreds of fonio landraces exist and derive from traditional selection. Unlike crops of worldwide importance, little effort has been made so far to improve these millets as no modern varieties are currently available. Despite the wide perspectives in utilisation of fonio genetic resources, the crops are still on a primitive production level and feature many drawbacks like poor yield, tiny grain size, seed shattering, plant lodging, pests and diseases, etc. (Kwon-Ndung et al. 1998; Adoukonou-Sagbadja et al. 2006). Additionally, the presence of high flavonoid content in the crude fonio grains with probably anti-thyroid properties (Sartelet et al. 1996) has been reported. Improving the efficiency in breeding strategies as well as conservation management of fonio genetic resources require adequate knowledge on the amount, distribution and structure of genetic diversity. Up to now, the genetic diversity present in these millets and the differentiation of landrace populations remain poorly understood.

Early efforts in studying the agro-morphological variability to assess genetic diversity indicate that fonio types are morphologically variable. In *D. exilis*, Portères (1976) identified a number of botanical varieties (with many cultivars each) based on morpho-botanical characters and geographic origin. However, Sanou (1993) reported divergent agro-morphological classification by studying some fonio ecotypes originating from Burkina Faso and Mali. Thus, it turns out that there is a limitation in using only morphological attributes for population characterization due to genotype × environment interaction and the complexity in genetic control of polygenic morphological and agronomic traits (Smith and Smith 1992). Such limitations have resulted in an early use of biochemical markers and the recently increased development of molecular approaches for assessing genetic diversity (Karp et al. 1997). The use of isozyme electrophoresis for genotype identification is very limited in fonio and no significant genetic variation has been detected (unpublished data). Molecular analyses allowing an accurate characterization of fonio accessions are quite rare. Recently, two molecular studies using Random Amplified Polymorphic DNA (RAPD) have been reported, but they are restricted to very small fonio germplasm samples originating from Togo (Hilu et al. 1997) or Nigeria (Kuta et al. 2005). This indicates the necessity of more comprehensive studies to consider patterns of genetic diversity in relation to its regional distribution.

In absence of SSR markers, Amplified Fragment Length Polymorphisms (AFLPs) have proven to be a powerful and efficient approach in population genetic and diversity analysis, molecular taxonomic classification, gene mapping and marker-assisted breeding in variable crops (Ayele et al. 1999; Carr et al. 2003; Uptmoor et al. 2003). Furthermore, AFLPs are a more stable and reproducible marker system compared to RAPDs (Rafalski and Tingey 1993). Therefore, AFLPs have been used in the present study to investigate the genetic diversity and population differentiation in a large collection of fonio landraces originally collected from diverse producing areas of West Africa. Additionally, landraces were evaluated morphologically in order to investigate the possible relationships of phenotypic variability to molecular attributes. The results are useful for defining strategies in fonio breeding and conservation management of the genetic resources of these indigenous millets in West Africa.

Materials and methods

Plant materials

A total of 122 accessions of *D. exilis* (118) and *D. iburua* (4) representing 89 farmer-named landraces of five West African countries, i.e., Togo (collected by the first author), Benin (partially provided by Niaouli Gene Bank, National Agriculture Research Institute of Benin—INRAB), Burkina Faso, Guinea and Mali (provided by National Agricultural Research Institute of Guinea—IRAG, via the West and Central Africa Office of Bioversity International, ex-IPGRI at Cotonou, Benin) were investigated (Table 1; Fig. 1). The

approximate collection areas of accessions of Burkina Faso, Mali and Guinea were reconstituted following the ORSTOM (actually, Institut de Recherche pour le Développement, France) catalogue (Clement and Leblanc 1984). This collection covers the major centres of diversification of fonio as identified by Portères (1976) and is assumed to be representative of fonio diversity in the region.

Assessment of molecular variability

DNA isolation and AFLP analysis

Fonio accessions were grown in the greenhouse (Plant Breeding Department, Giessen, Germany). In order to take into account possible genetic variability within each accession, total genomic DNA was extracted from bulked young leaves (100–200 mg per accession) of ten 4- to 5-week-old plants following the CTAB procedure according to Doyle and Doyle (1990). After RNAse treatment, DNA content was fluorometrically quantified (DynaQuant 200 Hoefer Scientific Instruments) and diluted to 25 ng μl^{-1} working solution.

AFLP analysis was performed according to Vos et al. (1995) by using the Invitrogen AFLP® Core Reagent Kit following the manufacturer's instructions. Here, 125 ng of genomic DNA (i.e., 5 µl of working solution) were digested using *Eco*RI and *Mse*I restriction enzymes, and generated fragments were ligated with double-stranded site-specific

Table 1 Countries of origin, local names and number (*N*) of fonio accessions used

Origin	Total	Local names (number of accessions)	
		D. exilis	*D. iburua*
Benin	35	Afiyo (1), Caféra (1), Ikantoni (1), Ikounga (1), Ipodawon/Dipodawon (5), Ipoeda/Ipoda/Ypoda (3), Ipohaga/Ipoaga (8), Iponi (1), Iporlapieh (2), Kpatinafa (1), Naman (1), Poigui (1), Tentepera (1), Tentenga (2), Tontonga (4)	Péi (2)
Burkina Faso	8	Feningué (1), Fii (1), Fomou (1), Foni (1), Foni Femba (1), Fonibâ (1), Péri (1), Pongwé (1)	–
Guinea	25	Dalaman (1), Dibon (1), Fannali (1), Fomba (1), Foundélin (1), Hothio (1), Kansambaran (1), Konso (1), Konson (1), Kokountèrin (1), Kouroussa (1), Mamanden (1), Mossogbé (1), Momo (1), Mora (1), Niougou (1), Ranéhô (1), Saara (1), Sèèkètè (1), Siguiridon (1), Siragbé (1), Tobbhéré (1), Werura (1), Yaoukô (1), Yéléboui (1)	–
Mali	8	Kansambahon (1), Pon-Madongon (1), Dierry (1), Tama (1), Oulè-Oulè (1), Pon-Biré (1), Prépézo (2)	–
Togo	46	Afiohoun (1), Ayôrô (1), Dikaba (1), Djibiga (1), Egniva (2), Eziô (1), Fig'm (3), Fôlom (1), Gnimimbi (1), Ipibim (1), Iporlapiah (2), Itamali (1), Kayara (1), Kiwo (2), Kopordagou (1), Lanfig'm (1), Namba (1), Ougniva (1), Ounfissa (1), Ounvoenikpa (2), Ova (7), Sèmbre (5), Tchabigô (1), Tchapionga (1), Trikpa (1), Vafoo (1), Vitchi (1), Yôlôm (1)	Tchibam (2)

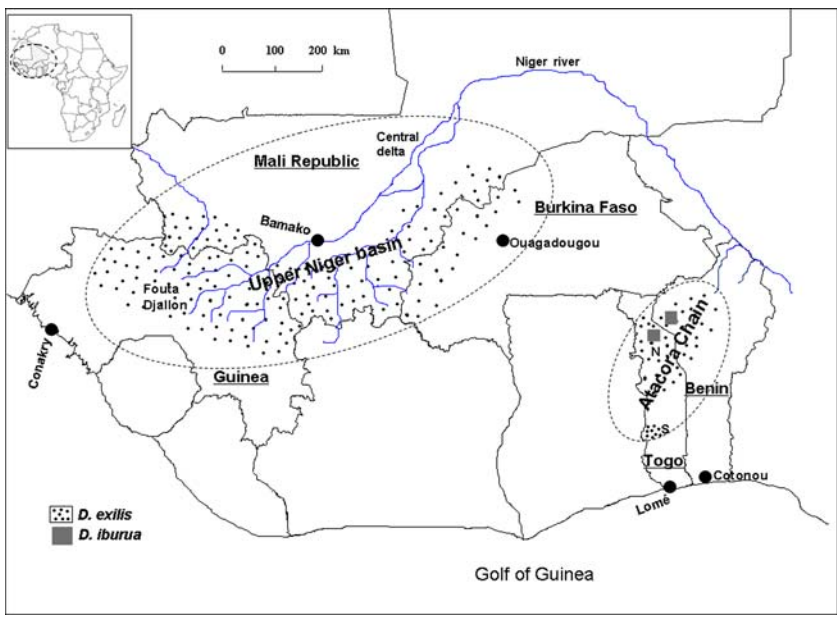

Fig. 1 Countries of origin and approximate collecting areas of the 122 accessions of *D. exilis* and *D. iburua* investigated in this study. *S* and *N* stand respectively for southern and northern growing areas of Togo

adapters using T4 DNA ligase. Ligation was followed by two pre-amplifications (+0, +1) prior to the final amplification phase performed by using primer combinations having three selective nucleotides. The selective amplification mixture (total volume of 25 µl) consisted of 7.5–12.5 ng fluorescent dye-labelled *Eco*RI primer, 30 ng *Mse*I primer, 0.2 mM of each dNTPs, 2 µl PCR buffer, 0.5 U *Taq*-polymerase (Qiagen, Germany) and 5 µl of pre-amplified PCR-product in deionised distilled water. Details of the PCR reactions programme were described by Scheurer et al. (2001). Twenty-four (Table 2) out of 72 primer combinations tested on a set of eight accessions were selected on the basis of their ability to generate informative data and further used for the total germplasm analysis. Selective amplification products were separated on 8% denaturing polyacrylamide gels using a Li-Cor 4200 DNA Analyzer. Fragments size was estimated in comparison to a 50–750 bp labelled DNA-ladder.

Scoring and analyses of AFLP data

AFLP fragments were detected using the RFLPScan 2.1 software package (Scananalytics, Fairfax, USA). Clear and unambiguous fragments were scored as present (1) or absence (0) to generate a binary data matrix. The total number of fragments scored, the number of polymorphic fragments and percentage of polymorphic fragments were determined for each primer pair used. Only polymorphic fragments were used for further data analysis.

Pairwise relatedness based on genetic similarity (Dice 1945) was estimated between all fonio accessions using the SIMQUAL module of NTSYS pc software version 2.20e (Rohlf 2000). UPGMA (unweighted pair-grouped method using arithmetic averages) cluster analysis was performed following GenDist and NEIGHBOR programs available in the software package PHYLIP 3.6 (Felsenstein 1985). Reliability and robustness of the clustering were based on 1,000 random re-sampling prior conducted on the datasets through the bootstrap procedure of this software package. The goodness of fit of the clustering compared to the basic data matrix was also tested by computing the co-phenetic correlation coefficient using normalized Mantel statistics *Z* test (Mantel 1967) via the COPH and MXCOMP procedures of NTSYS-pc version 2.20e (Rohlf 2000). Additionally, principal coordinate analysis (PCoA) was carried out

Table 2 AFLP primer-combinations, the number of DNA fragments generated and the number of uniquely identified accessions

No	Primer combination	Total markers	Polymorphic markers	Percentage polymorphism	No. of uniquely identified accessions
1	E-ACA/M-CCT[a]	82	53	64.6	65
2	E-AAT/M-CCG	78	49	62.8	54
3	E-CTC/M-GTC	55	36	65.4	36
4	E-CCT/M-GAC	48	30	62.5	41
5	E-CCC/M-GAC[a]	71	46	64.8	85
6	E-CGC/M-GGG	54	27	50.0	36
7	E-ACC/M-CAG	70	36	51.4	37
8	E-CCA/M-GCA	46	34	73.9	62
9	E-AGA/M-CGG[a]	59	31	52.5	36
10	E-AGG/M-CGT	50	32	64.0	37
11	E-CAT/M-GAG[a]	77	46	59.7	50
12	E-CTT/M-GTA[a]	79	45	57.0	55
13	E-ACC/M-CCC	61	38	62.3	66
14	E-AAG/M-CCA	103	67	65.0	47
15	E-CAG/M-GAC	49	28	57.1	52
16	E-ACT/M-CAA	101	63	62.4	34
17	E-AGG/M-CTG	91	58	63.7	43
18	E-CCT/M-GAA	51	33	64.7	43
19	E-CTC/M-GTA[a]	43	22	51.1	27
20	E-ATT/M-CTG[a]	106	76	71.7	85
21	E-ATG/M-CAC	74	49	66.2	56
22	E-AAT/M-CCC	70	46	65.7	29
23	E-ACA/M-CCA[a]	109	86	78.9	82
24	E-CAA/M-GAA	55	34	61.8	26
	Total	1682	1065	63.3	–
	Mean	70.1	44.3	–	–

[a] Indicates those primer-pairs that are uniquely able to differentiate the two fonio species

based on the pairwise genetic similarity matrix using the DCENTER and EIGEN procedures of NTSYS pc software package (Rohlf 2000).

Genotypic diversity was estimated using Shannon's phenetic index (Shannon and Weaver 1949) following Yeh et al. (1995) and Lacerda et al. (2001): $H = -\Sigma P_i \log_2 P_i/N$; where P_i is the frequency of a particular AFLP fragment and N is the total number of loci. Shannon's diversity index was used because it is recognized to be more insensitive to the bias related to the inability of differentiating heterozygous from homozygous loci when using a dominant marker system like AFLP (Dawson et al. 1995). Further on, Shannon's diversity index does not rely on prior knowledge of the mating system of the relevant species (Sun and Wong 2001) and is therefore well-suited for fonio millets since their mating system is not completely understood.

Analysis of molecular variance (AMOVA; Excoffier et al. 1992) was used to calculate variance components within and among the groups inferred from PCoA. Due to limiting information available on collection sites of fonio landraces originating from Burkina Faso, Mali and Guinea, genetic diversity among and within countries and specifically among and within the two producing areas (northern vs. southern agro-ecology) of Togo was estimated to reflect the geographic and agro-ecologic relevance in diversity shaping of *D. exilis*. Because *D. iburua* was only represented by four accessions, this species was excluded from AMOVA. The variance components' estimation was performed based on the presence/absence matrix using the software ARLEQUIN 3.0 (Schneider et al. 2000). The AMOVA-derived Φ_{ST} (Weir and Cockerham 1984) is analogous to Wright's F statistics differing only in their assumption of heterozygosity (Paun et al. 2006). Φ_{ST} provides an effective estimate of the amount of genetic divergence or structuring among populations (Excoffier et al. 1992). Significance of variance components was tested using a non-parametric procedure based on 1,000 random permutations of individuals using the software ARLEQUIN 3.0 (Schneider et al. 2000).

Genetic diversity (H') and population differentiation (F_{ST}) were examined in parallel following the method of Lynch and Milligan (1994) based on allelic frequencies using the software package AFLP-SURV 1.0 (Vekemans et al. 2002). Estimates of alleles' frequencies were performed using the Bayesian approach for dominant data types such as AFLP markers developed by Zhivotovsky (1999). A non-uniform prior distribution of allelic frequencies was assumed with its parameters derived from the observed distribution of fragment frequencies (Zhivotovsky 1999). Because of hints that fonio seems to be a self-pollinating species (further discussed below), estimates were made by using an hypothetical inbreeding (F_{is}) value of 0.9, assuming thus deviation from Hardy–Weinberg genotypic proportions. The between populations differentiation level was tested by using pairwise F_{ST} distance comparisons with 1,000 random permutations of individuals among populations using the software package AFLP-SURV 1.0 (Vekemans et al. 2002).

Phenotypic evaluation and data analysis

In order to investigate the relationship of molecular attributes with phenotypic traits, all accessions were morphologically evaluated in the 2005 growing season at the Laboratory of Genetics and Biotechnology Experimental field, University of Abomey-Calavi (Benin). This location is 6N24 and 2E20 at 15 m above sea level with ferralitic soil and has received 1,160 mm rainfall in 2005. Each fonio accession was grown in a one-row plot (five plants per plot) in a randomised complete block design with three replications under traditional rain-fed conditions supplemented by moderate occasional irrigations when needed. Approx. 30 kg/ha NPK fertilizer was applied. Row and plant spacing was 40 and 20 cm, respectively. Phenotypic data were recorded on 16 different traits (Table 6) and averaged across three plants per plot.

To access trait variability and significance levels, an analysis of variance (ANOVA) followed by a multiple means' comparison was performed using the SAS system for Windows software, release 8.02 (SAS Institute, Cary, NC, USA). Principal component analysis (PCA) was carried out in order to reveal a two-dimensional grouping pattern of fonio accessions using the software package PC-ORD 4.41 (McCune and Mefford 1999). Prior to PCA computation, z-transformation of the variables was performed to reduce the effects of different scales. Associations of phenotypic traits with molecular attributes of fonio accessions were tested by evaluating the relatedness between genetic and Euclidean distance matrices, pairwise computed for all accessions based on AFLP data and morphological variables, respectively. Genetic distance (G_d) was calculated as $G_d = 1 -$ Dice's similarity. Mantel statistics (Mantel 1967) calculated with NTSYS pc 2.20e (Rohlf 2000) was used to test the goodness of fit and significance was tested by 1,000 permutations. Furthermore, the identified genetic groups were additionally compared by ANOVA to determine whether there were any significant differences regarding the mean of the 16 evaluated traits. Pairwise linear contrasts between the genetic groups were calculated and tested at 0.05 level of significance using the Bonferroni correction.

Results

AFLP polymorphisms

The 24 selected AFLP primer-pairs yielded a total of 1,682 scorable bands of which 1,065 (63.3%) were polymorphic

(Table 2) ranging in size from 50 to 460 bp. The number of polymorphic bands generated by each primer-combination varied from 27 (E-CGC/M-GGG) to 86 (E-ACA/M-CCA) with a mean of 44.3. The level of polymorphism ranged from 50% (E-CGC/M-GGG) to 78.9% (E-ACA/M-CCA). All the AFLP primer-combinations used were suitable to fingerprint the 122 accessions while the number of individual accessions uniquely identified by a given primer combination ranged from 27 (E-CTC/M-GTA) to 85 (E-ATT/M-CTG). Marker specificity to a single accession was rare (6.2% of polymorphic bands) and most of the polymorphic bands appeared to be either frequently or infrequently present in all accessions (Fig. 2). However, high marker-specificity was detected at the species level: 27 AFLP markers were strictly associated with black fonio while 557 were specific to white fonio; the remaining 481 bands (45.2%) were shared between both species. Out of the 24 selected primer-pairs, eight were found efficient to uniquely differentiate between the two species (Table 2).

Genetic relationships and cluster analyses

Dice genetic similarity (GS_D) for all accessions under investigation varied from 0.41 (landraces Dibon vs. Oulè-Oulè) to 1.00 (two accessions of landrace Ova) with an overall mean of 0.79 (data not presented). At the species level, mean GS_D values of 0.77 (0.41–1.00) and 0.97 (0.96–0.99) were observed between accessions within *D. exilis* and *D. iburua*, respectively. In general, the lowest GS_D in the whole germplasm analysed was estimated between white and black fonio landraces indicating interspecific divergence between the two fonio species. Within species, GS_D values were generally much lower between accessions from different origins compared to within estimates.

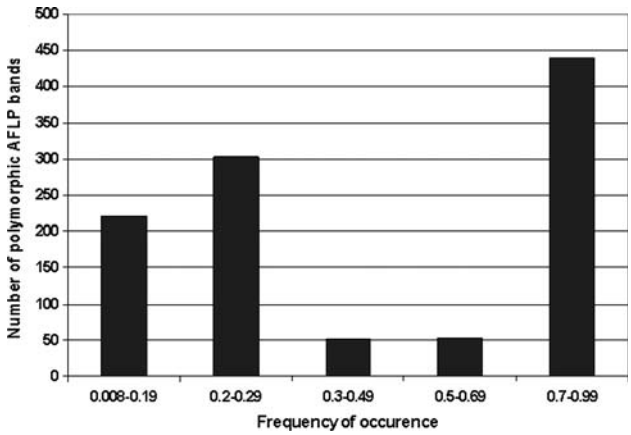

Fig. 2 Number of AFLP polymorphic markers in relation to their frequencies of occurrence in all fonio accessions

UPGMA cluster analysis revealed the genetic relatedness among the fonio accessions (Fig. 3). In general, the main clusters of fonio genotypes were supported by high bootstrap values, indicating the reliability and stability of the relationships as well as the robustness of the AFLP dataset. The high co-phenetic correlation coefficient obtained ($r = 0.91$) confirmed also this trend. The dendrogram revealed four main clusters (Fig. 3). The first node separated *D. iburua* accessions (cluster I) from *D. exilis* ones that split afterwards into three clusters fitting largely to the geographic origin of the fonio accessions. Cluster II comprised the vast majority of Guinean accessions (24 of 26 analysed) and four Malian accessions (landraces Tama, Dierry, Kansambahon, Pon-biré). Cluster III concerned essentially the eight accessions of Burkina Faso and the remaining four accessions from Mali (two Prépéazo, Pon-Madongon and Oulè-Oulè) and two Guinean landraces (Mora, Yeleboui). Cluster IV essentially comprised Benin and Togo accessions differentiated in two sub-groups (A, B) independent of the accessions' origin (country) or their agro-ecologic cultivation area. Focussing on each sub-group, further separation is visible with the landraces from the southern agro-ecologic area clustering distinctly from those of the northern area. Regarding each cluster, accessions grouped very closely at a mean similarity coefficient ranging from 0.84 to 0.95. Associations of minor sub-clusters of the introduced major clusters to particular morphological traits such as plant habit, plant cycle, grains' colour, etc., traits mainly used by farmers in traditional classification and naming system, were in general weak. However, many accessions with the same (Ova, Prépéazo, etc.) or equivalent names (Fig'm/Kiwo, Egniva/Vitchi/Vafoo, etc.) clustered almost together, supporting the hypothesis of their identity or common genetic origin.

Further on, the results of a principal coordinate analysis (PCoA, Fig. 4) support the ones of UPGMA cluster analysis (Fig. 3). The PCoA revealed that the first two axes, explaining almost 59% of the total variation, clearly separated the black and white fonio gene pools and strictly differentiated the white fonio accessions into three major genetic groups, i.e., groups 1, 2 and 3 corresponding to the UPGMA clusters II, III and IV, respectively. Geographically, the genetic groups 1 and 2 reflect approximately the same areas, i.e. a zone covering the whole Upper basin of Niger River from Guinea (predominance of group 1) to Burkina Faso (exclusively group 2). Conversely, the group 3, containing Beninese and Togolese accessions, is geographically isolated from the first ones and covers the cultivating areas along the Atacora Massif Chain which crosses the two countries from Northern Benin to Southern Togo. They are hereafter referred to as Upper Niger group 1 (UNIG 1), 2 (UNIG 2)

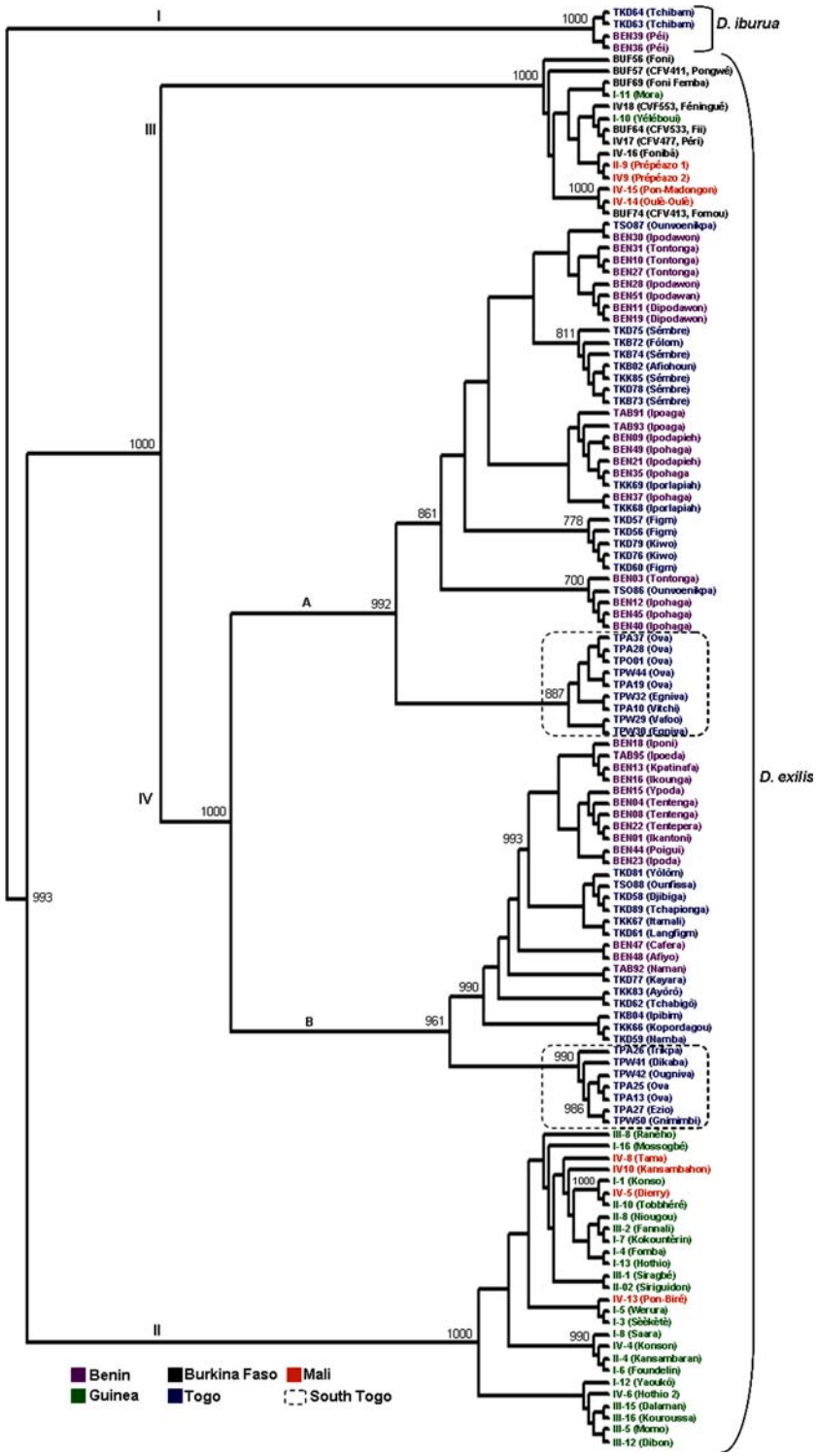

Fig. 3 UPGMA dendrogram showing relationships among 122 accessions of fonio millets based on 1,065 AFLP markers. Bootstrap values obtained from 1,000 re-samplings higher than 70% are indicated. The local names of landraces preceded the accession numbers. I, II, III, IV, A and B indicate landrace clusters and sub-clusters; circled landraces originated from southern agro-ecologic zone of Togo

and Atacora group (ATAC), respectively. Basically, all fonio accessions of the same origin clustered in identical group, with the exception of Guinean and Malian accessions, which split in two different groups (UNIG 1 and 2).

Genetic diversity and differentiation

Estimates of Shannon's index of phenetic diversity (*H*), calculated for each species as well as the different *D. exilis* populations are summarized in Table 3. The total genotypic

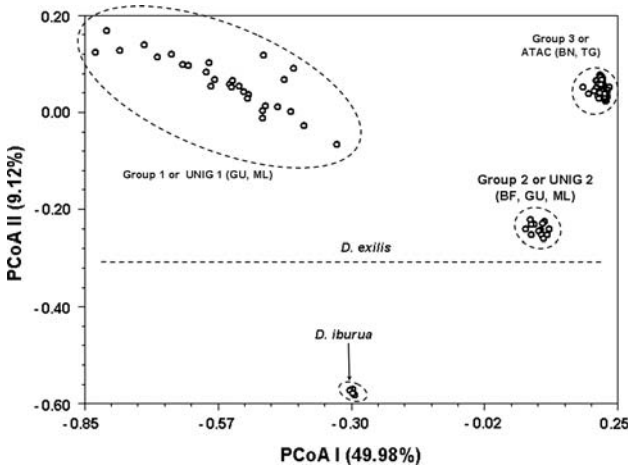

Fig. 4 Diagram showing the relationships among 122 accessions of fonio millets based on Principal Coordinate Analysis (PCoA) using AFLP markers. *D. exilis* accessions formed three genetic groups occupying broadly two geographic areas: UNIG (Upper Niger basin) groups 1 or 2; ATAC (Atacora group); BN (Benin), TG (Togo), GU (Guinea), ML (Mali) and BF (Burkina Faso)

diversity of *D. exilis* was estimated at $H = 0.267$ while a diversity of $H = 0.020$ was detected in the four *D. iburua* accessions. Regarding the *D. exilis* populations, the highest diversity ($H = 0.172$) was observed within UNIG 1, which held also the highest number of polymorphic loci (80.9%) and specific AFLP fragments (159). Conversely, a lower genetic diversity was found in UNIG 2 and ATAC groups with $H = 0.083$ and 0.060, respectively. The mean pairwise genetic similarities within the groups varied in a similar manner from UNIG 1 ($GS_D = 0.84$) to UNIG 2 and ATAC ($GS_D = 0.94$ and 0.95, respectively). At the country level, fonio diversity was found to be concentrated in Guinea ($H = 0.205$) and Mali ($H = 0.203$) followed respectively by Burkina Faso ($H = 0.076$), Togo ($H = 0.060$) and Benin ($H = 0.050$). Average GS_D values between landraces were 0.69, 0.74, 0.93, 0.94 and 0.96 for Guinea, Mali, Burkina Faso, Togo and Benin, respectively. The number of innate loci also varied among origins with the highest present in Guinean producing areas. For both genetic groups and countries of origin, Nei's genetic diversity (H') obtained according to Lynch and Milligan (1994) was ordered in the same way as Shannon's index estimates with slightly different values (Table 3).

AMOVA estimates revealed that most of the genetic variation was attributable to differences among genetic groups ($\Phi_{ST} = 0.71$) or among origins ($\Phi_{ST} = 0.56$); the remaining at each level (29 and 44% of total variation, respectively) being found within populations (Table 4). The variance components for each source of variation were highly significant ($P < 0.001$). Significant differentiation ($P < 0.001$) with approx. 33% of the total variation ($\Phi_{ST} = 0.33$) was also detected among the two Togolese agro-ecologies, attesting that within countries substantial differentiation may exist among diverse agro-ecological areas.

Allele frequency-based F_{ST} estimates based on Bayesian approach under the hypothesis of high self pollination were in the same order of magnitude as those of AMOVA Φ_{ST}, though always somewhat lower (Table 4). The pairwise F_{ST} test (Table 5) revealed significant differentiation ($P < 0.001$) among all pairs of genetic groups with ATAC and UNIG 2 less differentiated ($F_{ST} = 0.52$) than other pairwise comparisons ($F_{ST} = 0.69$ for ATAC/UNIG 1 and 0.64 for UNIG 1/2), fitting then well to the results of the UPGMA cluster analysis. Considering the geographic ori-

Table 3 Genetic variability within each fonio species and *D. exilis* populations (genetic groups, country of origin) based on AFLP data

Populations	*N*	P_{loci}	S_{loci}	GS_D	*H*	*H′*
D. iburua	4	44	28	0.97	0.020	–
D. exilis	118	1021	557	0.77	0.267	0.355
Upper Niger group 1 (UNIG 1)	27	862 (80.9)	159	0.84	0.172	0.231 ± 0.005
Upper Niger group 2 (UNIG 2)	14	603 (56.6)	6	0.94	0.083	0.088 ± 0.004
Atacora group (ATAC)	77	268 (25.2)	28	0.95	0.060	0.058 ± 0.004
Benin	33	175 (16.3)	7	0.96	0.046	0.040 ± 0.020
Burkina Faso	8	210 (19.7)	5	0.93	0.076	0.070 ± 0.040
Mali	8	599 (56.2)	49	0.74	0.203	0.210 ± 0.130
Guinea	25	921 (86.5)	111	0.69	0.205	0.240 ± 0.140
Togo	44	238 (22.3)	21	0.94	0.060	0.055 ± 0.030
Northern Togo	28	233 (21.9)	13	0.95	0.050	0.046 ± 0.022
Southern Togo	16	141 (13.2)	8	0.95	0.050	0.043 ± 0.022

[a] By assuming self pollination rate of 0.95; in brackets, proportion to total diversity in *D. exilis*

Calculations were additionally computed for each agro-ecology of Togo. *N* number of different genotypes, P_{loci} number of polymorphic loci, S_{loci} number of specific loci, GS_D mean genetic similarity (Dice 1945), *H* Shannon index, *H′* Nei's gene diversity (Lynch and Milligan 1994)

Table 4 Analysis of molecular variance (AMOVA) and *F*-statistics (Lynch and Milligan 1994) for the 118 *D. exilis* accessions assembled from five West-African countries (origins) and assigned to three genetic groups (see text) based on 1,065 AFLP fragments

Source of variation	AMOVA estimates						*F*-statistics [b]	
	df	Sum of squares	Variance components	% of variance	Φ_{ST}	*P*-value[a]	F_{ST}	Std
Among groups	2	7,478.845	123.117	70.66	0.71	<0.001	0.64	0.09
Within groups	115	5,879.087	51.122	29.34		<0.001		
Among origins (countries)	4	7,343.225	80.813	56.55	0.56	<0.001	0.44	0.10
Within origins	113	7,234.663	62.367	43.45		<0.001		
Among agro-ecology (Togo)	1	322.917	11.7854	32.8	0.33	<0.001	0.27	0.05

[a] Probability of obtaining a more extreme random value computed from non-parametric procedures (1,000 permutations)

[b] Under self pollination rate $F_{is} = 0.90$ (conclusions do not change significantly when assuming 0.80 (1.3% decrease) or complete inbreeding (5.77% increase) as compared with $F_{is} = 0.9$); Std standard deviation

Estimates were additionally computed for the two Togolese agro-ecologies

Table 5 Pairwise genetic differentiation (below diagonal) and Nei's genetic distance (above diagonal) between *D. exilis* populations (groups and origins) based on Bayesian approach (Lynch and Milligan 1994) by assuming self pollination rate of $F_{is} = 0.90$ using AFLPsurv 1.0

Genetic groups				Origins					
	UNIG 1 (*x*)	UNIG 2 (*y*)	ATAC (*z*)		Benin (1)	Togo (2)	Burk. Faso (3)	Mali (4)	Guinea (5)
x	–	0.42	0.46	1	–	0.04	0.09	0.26	0.33
y	0.64[a]	–	0.09	2	0.07[a]	–	0.09	0.26	0.33
z	0.69[a]			3	0.52[a]	0.50[a]	–	0.19	0.28
–	–	–	–	4	0.53[a]	0.52[a]	0.42[a]	–	0.01
–	–	–	–	5	0.58[a]	0.57[a]	0.51[a]	0.03[ns]	–

[a] Values are all significant at $P < 0.0001$; ns not significant

gins, all pairwise differentiations were found to be significant ($P < 0.001$), except between Guinea and Mali (Table 5). The lowest level of pairwise differentiation was observed for Guinea/Mali and Benin/Togo while other combinations indicated a higher level of differentiation. Nei's genetic distances (Lynch and Milligan 1994) highlighting the genetic relationships between the three groups or five origins are also presented in Table 5.

Morphological variation, relationship with molecular data and comparison of genetic groups

ANOVA of 16 quantitative traits revealed large and significant morphological differences between the 122 fonio accessions (Table 6). The phenotypic relationships among fonio accessions as assessed by the first two principal components (PCs) are presented in Fig. 5. Contrary to the AFLP data, *D. iburua* accessions could not be clearly differentiated from *D. exilis* accessions. Furthermore, no meaningful morphological groups were formed within *D. exilis* accessions (Fig. 5) in contrast to the results obtained at the molecular level.

The relationship of morphological traits with AFLP markers was assessed by the Mantel matrix correspondence test. This test revealed no correlation between Euclidean and genetic distances matrix ($r = 0.04$, $P > 0.05$). However, pairwise linear contrasts between the genetic groups for phenotypic traits followed by Bonferroni correction for multiple testing indicated significant differences for nine of the 16 traits used (Table 6). Among them, dry biomass, panicle and grain yields were found to be particularly useful to discriminate the genetic groups. On average, landraces from UNIG 1 showed a significantly better performance regarding these traits as compared to ATAC and UNIG 2. Furthermore, the mean number of grains per cm of raceme and fresh biomass significantly differentiated UNIG 1 and UNIG 2. Leaf morphology (length of the leaf and leaf sheath) and panicle length significantly discriminate ATAC accessions from UNIG 1 and UNIG 2. The harvest index, days to heading, days to maturity, plant height, number of internodes and tillers seemed to be non-distinctive traits for discrimination of genetic groups. In summary, these results indicate that genetically differentiated groups are obviously characterised by distinct phenotypic characteristics.

Table 6 Morphological characteristics showing the mean traits for all genotypes (including *D. iburua*) and mean traits differences (linear contrasts) among the three genetic groups of *D. exilis*

Traits	Total genotypes		Group means			Linear contrasts between pairs of groups (*P*-values)		
	Mean	SD	ATAC (1)	UNIG 1 (2)	UNIG 2 (3)	1 vs 2	1 vs 3	2 vs 3
Days to 50% heading	82.9[a]	7.7	82.6	84.7	80.2	0.370	0.584	0.181
Days to physiological maturity	108.2[a]	9.4	107.6	111.6	104.8	0.121	0.597	0.076
Plant height (cm)	97.9[a]	14.9	97.6	103.2	89.5	0.106	0.410	0.682
Number of internodes	8.8[a]	1.6	8.8	9.0	8.3	0.648	0.339	0.255
Number of tillers	171.3[a]	54.9	167.7	179.0	176.9	0.272	0.437	0.153
Leaf length (cm)	17.0[a]	3.0	17.3	16.2	17.1	***<0.001***	***0.001****	0.500
Flag leaf sheath (cm)	15.7[a]	2.9	16.5	14.1	14.0	***<0.001***	***<0.001***	0.606
Fresh biomass weight (g/plant)	128.6[a]	22.2	132.4	123.7	116.8	***0.003***	0.685	***0.015***
Dry biomass weight (g/plant)	39.5[a]	5.8	38.9	41.6	38.9	***0.017***	***<0.001***	***0.025***
Panicle exertion (cm)	14.6[a]	4.4	14.0	16.1	15.3	0.507	0.511	0.914
Panicle length (cm)	12.3[a]	2.0	12.7	11.7	11.5	***<0.001***	***<0.001***	0.880
Panicles yield/plant (kg/ha)	1705.9[a]	234.1	1684.2	1803.9	1650.4	***0.006***	***<0.001***	***0.001***
Grains yield/plant (kg/ha)	874.3[a]	162.5	861.2	953.6	804.5	***<0.001***	***<0.001***	***0.006***
Harvest index	25.5[a]	2.7	25.5	26.3	24.2	0.06	0.213	0.799
Mean number of grains/cm of raceme	9.0[a]	1.5	9.1	9.2	8.1	0.141	0.017	***0.002***
1,000-grains mass (mg)	822.8[a]	189.0	793.7	865.4	905.4	***<0.001***	***<0.001***	0.118

[a] Indicates significant mean difference ($P < 0.05$) between genotypes

* Bold italic indicates significant mean differences on an experiment-wise confidence level of significance of 0.05 (after Bonferroni Correction) between pair of groups

Discussion

This study represents the first approach that comprehensively investigates the genetic diversity within a large collection of fonio millets and assesses its structure and regional pattern of distribution based on AFLP markers. Despite its drawback of being dominant markers, the major advantage of AFLPs is their capacity to generate a large number of markers comparative to other molecular marker systems, making it an important tool for population genetic investigations. In the present study, AFLP analysis offered the possibility for screening a large number of polymorphic loci (Table 2) allowing an adequate assessment of the genetic diversity of fonio millets and particularly detecting highly informative genetic population structure within *D. exilis*. In the absence of SSR markers as is still the case in fonio millets, AFLPs appear to be highly suitable for genetic diversity studies of these crops.

Genetic diversity and pattern of distribution

Unlike *D. exilis*, the most widely cultivated fonio species in the region, *D. iburua* is actually grown by very few farmers in some restricted areas of Northern Benin, Togo and Nigeria. Four *D. iburua* accessions from Benin and Togo were included in this study. The diversity indices estimated in this sample were found to be very low (Shannon index $H = 0.02$, $GS_D = 0.96–0.99$), suggesting less genetic diversity and differentiation within this species. Although further conclusions are avoided due to the small number of accessions analysed, this result is in concordance with the residual state of this crop and the low varietal diversity known for this species in its growing areas (Portères 1946; Adoukonou-Sagbadja et al. 2004).

Regarding all 1,065 polymorphic loci investigated, the total genetic diversity estimated in *D. exilis* (Shannon index $H = 0.267$, Nei's gene diversity $H' = 0.355$, $GS_D = 0.41–1.00$) is quite moderate when considering the comparably small effort invested into the breeding of landraces and the large area of origin that comprises the main centres of fonio diversity in West Africa (Portères 1976). However, the level of diversity detected in this species is comparable to that reported in Tunesian and West African (Nigeria, Ghana, Mali, Mauritania) pearl millet cultivars ($H = 0.283$, Ibrahima et al. 2005) but higher than that found in *Eragrostis tef*, an African millet endemic to Ethiopia with a mean GS_D of 0.89 (Ayele et al. 1999), African and Indian finger millet ($GS_D = 0.64–0.92$, Hilu 1995) or at regional level for Southern African Sorghum landraces ($H = 0.169$, Uptmoor et al. 2003). Regarding its geographical distribution, our results reveal that the genetic diversity in *D. exilis* is concentrated in the Upper Niger basin with an eastwards

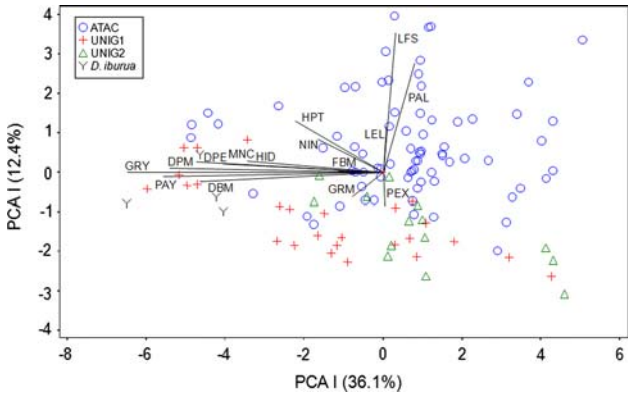

Fig. 5 Diagram showing the relationships among 122 accessions of fonio millets based on Principal Component Analysis (PCA) using phenotypic data. ATAC (Atacorian group), UNIG (Upper Niger group), GRY (grain yield), PAY (panicle yield), DPM (days to physiological maturity), DPE (days to 50% heading), DBM (dry biomass), MNC (mean number of grains per cm of racemes), HID (harvest index), HPT (plant height), NIN (number of internodes), FBM (fresh biomass), GRM (grain mass), LEL (leaf length), LFS (flag leaf sheath), PAL (panicle length), PEX (panicle exertion)

decrease from Guinea/Mali to the Atacora Montain zone, i.e. the Benin and Togo growing areas, where the genetic diversity was found to be particularly low (Table 3). These findings strongly confirm the Upper Niger basin as a major centre of white fonio genetic diversity in the region as described by Portères (1976) based on the varietal distribution pattern. The low genetic diversity found in the Atacorian zone may therefore be related to a narrow genetic make-up of the founding genotypes introduced in this area. However, focussing on Togolese germplasm, our results contrast the report of Hilu et al. (1997) who detected approximately similar range of genetic variability (0.27–0.88, RAPD) in only ten accessions of *D. exilis* compared to the present study with 122 accessions of diverse origin. Because our Togolese *D. exilis* material was directly collected from farmers in the entire growing area (Adoukonou-Sagbadja et al. 2004), it is assumed that the diversity detected in the present study is representative for Togo.

Inter-specific differentiation and genetic structure within *D. exilis*

Morphologically, *D. iburua* resembles *D. exilis* in many ways (Portères 1976; Haq and Ogbe 1995). The 16 morphological traits used in this study seem to confirm this observation because no clear-cut separation of both species was possible. The morphological discrimination of the two species is mostly based on limited discrete morpho-botanical traits mainly related to the structure of their inflorescence and spikelet (Stapf 1915; Henrard 1950). In the present study, UPGMA and PCoA based on AFLP data revealed a clear separation of the two species demonstrating their high genetic differentiation at the DNA level. This finding supports the results of a previous molecular investigation by Hilu et al. (1997) using RAPD markers and confirms the botanical distinction of the two species as described by Stapf (1915) and Henrard (1950). Furthermore, regarding the high number of species-specific markers detected in *D. iburua* (28) and *D. exilis* (557), the AFLP technique appears to be a tool to strengthen the resolution of morpho-botanical approaches (cf. Stapf 1915; Henrard 1950; Portères 1976; Sanou 1993; and the present study) for fonio species' identification and should be useful for taxonomic investigations in the genus *Digitaria*.

The relatedness of the two species as observed in this study seems to be substantial. Out of 1,065 AFLP markers used, 480 (i.e. 45%) were shared by the two species. This high degree of shared genome, supported by their almost substantial morphological resemblance (Portères 1976; Haq and Ogbe 1995), suggests a relatively late evolutionary separation of the two species. Further on, recent work revealed a tetraploid genome of white and black fonio landraces with very similar genome sizes (Adoukonou-Sagbadja et al. 2007). The genetic relatedness between the two species observed here is slightly higher than that reported by Hilu et al. (1997) but the discrepancy may be due to the much larger number of loci and genotypes investigated in the present study.

In this study, a large phenotypic variability was detected among *D. exilis* accessions. However, no distinct morphological group could be ascertained. Although morpho-botanical (racial) groups have been identified by Portères (1976), it has been observed that these races have no geographic unity and grade morphologically one into another (Zeven and de Wet 1982), supporting thus the present findings. The absence of clear racial differentiation was already reported for many grass species like Kodo millet (*Paspalum scrobiculatum* L., de Wet et al. 1983) and saltgrass (*Distichlis spicata* L., Ram et al. 2004). Lem and Lallemand (2003) stated that the lack of reliable and highly discriminative traits is common in many grass species and hamper their morphological characterisation and discrimination. Traditionally, farmers classify and name their fonio landraces merely based on the growth cycle but also on other traits such as plant habit, coloration of shoot or seeds, organoleptic characteristics, etc. (Adoukonou-Sagbadja et al. 2006). In general, our results of AFLP analysis did not conform to the morphological classification. The clustering of white fonio accessions into three main genetic groups (Figs. 3, 4) follows mostly the geographic origin with two groups overlapping in the Upper Niger basin and the last isolated in the Atacora Mountain. The substantial genetic structure obtained after UPGMA and PCoA demonstrates the high degree of differentiation within this species, probably due to limited gene flow primarily restricted by the

mating system. This finding is further supported by AMOVA which shows that the vast proportion of AFLP variation detected in *D. exilis* was present rather among than within groups (Table 5). Furthermore, an important part of the total variation was found among origins (countries), indicating genetic divergences among landraces across the growing areas of *D. exilis* as evidenced by the substantial differentiation detected between the two agro-ecologies in Togo.

The reproductive system is one of the important life-history characteristics that strongly influence genetic variability (Clegg et al. 1992). In fonio millets, the mating system is not well understood and the available information is mostly speculative. Fogg (1976) as well as Hilu et al. (1997) stated that the *Digitaria* millets are probably cross-pollinated plants. Conversely, describing the reproductive organisation in the genus *Digitaria*, Watson and Dallwitz (1992) indicated that *Digitaria* species are inbreeding. Similarly, regarding the tiny size of their florets, Sarker et al. (1993) assumed that small millets including fonio millets are self-fertilized crops. In their review, Hamrick and Godt (1989) reported that, in contrast to outcrossing species, selfing species have most of their genetic diversity partitioned among populations. The estimated Φ_{ST} values obtained either among genetic groups ($\Phi_{ST} = 0.70$) or among origins ($\Phi_{ST} = 0.56$) are consistent with this pattern. Furthermore, the F_{ST} estimated under high inbreeding hypothesis ($F_{is} = 0.9$) using the approach of Lynch and Milligan (1994) strongly supports these results. Moreover, assuming either predominant (0.8) or complete (1.0) inbreeding values had very little effect on the results (a difference of 1.3% decrease or 5.8% increase respectively, as compared with $F_{is} = 0.9$) and did not affect the general conclusions. Comparable results were reported for rice species such as *Oryza glumaepatula* Steud. (Buso et al. 1998; $F_{ST} = 0.67$, RAPDs) and *Oryza sativa* ssp. *japonica* (Yu et al. 2005; $F_{ST} = 0.746$, RAPDs), known as predominantly autogamous plants. However, Heywood (1991) stated that the genetic architectures observed for predominantly autogamous species are frequently comparable to those of taxa that reproduce by a mixture of apomixis and sexual outcrossing (facultative apomicts). Apomixis prevents sexual recombination within a population and gene flow by pollen among populations, resulting in low genetic variation within but a high differentiation between populations. Because apomixis is common in grass species (Clayton and Renvoize 1986), this would be a different conceivable mating system of fonio which is in accordance to the population structure found. Nonetheless, this hypothesis deserves further specific investigations for finding a definitive conclusion.

In crop plants, farmers and traders play an important role in gene flow between growing areas. In this study, except Burkina Faso, the lowest differentiation was observed between countries within the UNIG basin or the ATAC zone (Table 5), indicating that germplasm exchange may occur frequently rather within than between geographic zones. Although such pattern apparently suggests an isolation by distance process (Wright 1946), this may have an incidental effect since it is obvious that landraces from the ATAC zone are more closely related to those of UNIG 2 landraces than the latter are with their sympatric UNIG 1 landraces (Fig. 1; Table 5). A possible explanation of limitation in seed exchange between these two zones could be due to traditional seed management systems, limited market development towards and within Togo and Benin, marginal fonio cultivation and progressive abandonment of the crop in the Atacora zone (Adoukonou-Sagbadja et al. 2006). Since most of the diversity is present between genetic groups, the differentiation pattern of Burkina Faso landraces from those of other countries in the basin is essentially caused by their clear clustering in one group, i.e. UNIG 2. Because the growing areas of Burkina Faso and Mali are conspicuously linked (Fig. 1) and may allow seed exchange between farmers, a narrow sampling background of the eight accessions originating from Burkina Faso can be hypothetised.

Portères (1976) proposed that the earliest domestication of *D. exilis* occurred in the UNIG basin in the vicinity of central delta. Refering to the large genetic divergence observed in their study, Hilu et al. (1997) speculated that multiple domestication events associated with the different diversity centres may have occurred in *D. exilis*. Similarly, a local domestication of some landraces has been stated by certain Togolese farmers (Adoukonou-Sagbadja et al. 2006). This seems unlikely to be the case considering the low genetic diversity detected in the ATAC group and its close genetic relationship with the cluster UNIG 2 (Table 5). The geographic distribution of the genetic diversity as detected in this study strongly supports the origin of white fonio in the UNIG basin (Portères 1976; Munson 1976). Consequently, the differentiation of two sub-clusters within the ATAC group as detected by UPGMA can be better explained as the result of a secondary diversification (Portères 1976) or of multiple introduction events from the UNIG basin (Adoukonou-Sagbadja et al. 2006). It can be hypothesised that the genetic groups identified represent the major evolutionary groups differentiated over time during the cultivation and dispersal history of the crop. Based on their genetic relatedness and level of diversity, UNIG 1 may well represent the most anciently cultivated fonio group in the basin. From this ancestral group may have evolved UNIG 2 from which the ATAC group differentiated almost recently after the introduction of fonio to Benin and Togo.

Population genetic structures are determined by joint effects of many factors including mating system, selection

and adaptation, mutation, migration and dispersal mechanism, drift, founding effects, etc. (Hamrick and Godt 1989). Most likely, the large differentiation observed within *D. exilis* may be due to strong selection associated with differences in traditional agricultural practices and adaptation of fonio landraces to the contrasting ecological conditions where they have been grown since centuries (Portères 1976; Hilu et al. 1997; Adoukonou-Sagbadja et al. 2006). The substantial differentiation observed among countries and within the Togolese agro-ecological sites as well as the presence of many specific AFLP bands (Tables 4, 5) support this hypothesis. Due to this and because of its geographic isolation, the ATAC group may well represent an ecologically specialized deme. Phenotypically, this group shows more significantly divergent traits from UNIG groups than found between the latter, providing further support of local adaptation (Table 6). Such conclusion may be made with caution for UNIG 1 and 2, though these groups seem nonetheless to be more geographically predominant in southern (humid savannah) and northern (dry savannah) parts of the basin, respectively. Therefore, the possibility that these genetic groups correspond to fonio ecotypes remains an attractive hypothesis that needs further investigation.

In applied breeding, phenotypic or genetic distances between genotypes are expected to provide predictors for high heterosis effects and performance of their hybrids. In the present study, weak correlations were found between genetic and morphological distances of fonio accessions. Discrepancy between molecular and phenotypic distances seems to be a widespread phenomenon in plants (Gerdes and Tracy 1994; Portis et al. 2004, etc.). This is expected to be related to the environmental effects on morphological traits while molecular markers such as AFLPs are neutral and not necessarily linked to genes underlying morphological traits. Furthermore, the number of informative AFLP loci (1,065) compared to the limited number of quantitative traits investigated (16) may also have contributed to this discrepancy. Nevertheless, the statistically significant differences observed between *D. exilis* groups regarding some morphological traits (Table 6) are indicative of genetic determinism. However, additional experiments such as mapping studies are needed to identify specific genes or genomic regions that have an influence on the phenotypic variation observed. Since the germplasm was evaluated in only one location, a more comprehensive view on phenotypic plasticity and adaptive potential of the germplasm has to be developed.

In conclusion, our results on genetic relationships and diversity within some West African fonio accessions revealed a clear separation between the species and the existence of three highly differentiated genetic groups in *D. exilis*. Although essentially present between groups, the genetic diversity observed in *D. exilis* is nonetheless substantial. Many landraces performed well in the field trial and a great variability was observed for most of the agro-morphological traits considered. Detailed knowledge of the genetic population structure, e.g. based on AFLP markers, and its linkage to important agronomic traits will be very useful for future fonio breeding efforts in the region. In addition, the results of this study are relevant for developing effective management and conservation strategies for fonio genetic resources in their traditional growing areas. For this purpose, i.e. breeding efforts and conservation programmes, definitive knowledge on the reproductive system in these valuable but neglected West African native millets is urgently needed.

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