Fonio Millets: Ethnobotany, Genetic Diversity and Evolution

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True fonio (*Digitaria exilis* (Kipp.) Stapf) and black fonio (*D. iburua* Stapf) are two domesticated millets of West Africa. These cereals are used as food and fodder as well as in brewing. They are persistent crops that do not require careful cultivation, and thrive under a range of difficult agricultural conditions. Fonio millets are amongst the least studied cereal crops, and there has been no study on the genetic diversity and evolution of these two millets. Random Amplified Polymorphic DNA (RAPD) approach was used in this study to assess the genetic diversity in fonio millets and to evaluate proposed hypotheses on their presumed wild progenitors. The results point to a very high genetic diversity in true fonio and to the possibility of multiple domestication or/and strong ecological or agricultural differentiation. The genetic diversity in black fonio could not be assessed because of the availability of only one accession for the crop. The molecular data substantiate previous hypotheses that point to the morphologically allied species *D. longiflora* (Hatz) Pers. and *D. ternata* (A. Rich.) Stapf as possible progenitors of true and black fonios, respectively, and do not support the proposed genetic affinities between *D. fuscescens* (Presl) Henr. and true fonio. The study underscores the need for concerted effort to collect and conserve genetic resources of fonio millets and their wild progenitors since they are poorly represented in world gene banks.

Key words: Fonio millets, *Digitaria*, RAPD, evolution, genetic diversity, ethnobotany.

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Introduction

Millets; small-grain cereals belonging to different grass genera; rank 14 among the 30 leading crops that feed the world (Hilu 1994a). Although millets represent a small portion of the total world food production, they are crops of local importance in semi-arid regions of the world. They provide reliable yield in low rainfall areas (Dogget 1989), especially in drought-plagued parts of Africa and India. Millets also provide an inexpensive source of nutrition for rural peoples (Rao 1989). Nutritional studies of finger millet and barnyard millet (Malleshi 1989; Barbaeu & Hilu 1993; Mandelbaum *et al.* 1995) have demonstrated their competitiveness with, or even superiority to, the more commonly consumed grains such as wheat and rice. For instance, Barbaeu & Hilu (1993) reported that the calcium content of finger millet was 63 times that of corn meal and about 12 times that of brown rice and flour wheat. Furthermore, millets provide quality fodder of excellent nutritional value. Thus, these cereal crops have the potential to play a larger future role in world agriculture, especially with the predicted global warming and its consequent droughts. Both basic and applied research on millets is needed for the improvement of their agronomic traits.

Fonio millets are amongst the least studied cereal crops in general and millets in particular. There has been no study focusing on the genetic diversity and evolution of these two millets. Evaluation of genetic diversity of crops and determination of their gene pools represent basic and valuable practical information in their breeding programs. In this paper the Random Amplified Polymorphic DNA (RAPD) approach is used to assess the genetic diversity in fonio millets and to evaluate proposed hypotheses on the presumed wild progenitor of these crops. This paper is one in a series of papers that focus on genetic diversity and gene pools of millets (Hilu 1994b, 1995; M'Ribu & Hilu 1994, 1996).

Ethnobotanical overview of fonio millets

Two of the about 230 species of the cosmopolitan genus *Digitaria* (Clayton & Renvoize 1986) are domesticated millets of west Africa: *D. exilis* (Kipp.) Stapf and *D. iburua* Stapf. *Digitaria exilis* is commonly known as fonio, true fonio, or hungry rice. It is only known from western Africa, where it has been cultivated throughout the Savanna zone from Cape Verde to Lake Chad (Lewicki 1974; Portères 1976; Purseglove 1972). Fonio is a morphologically very variable crop in which numerous botanical varieties have been recognized (Portères 1955). Harris (1976) noted that the greatest varietal diversity of fonio occurs in the region of the Fouta Djallon plateau and in the valley of the upper Senegal and Niger, and Portères (1976) noticed that the number of varieties decrease sharply eastwards towards Chad. The cultivation of the crop covers approximately 721,000 acres annually, and during the ecologically most difficult months fonio supplies food to 3–4 million people (Portères 1976). Fonio is a staple crop on the Fonta-Djallon in Guinea and the Bauchi Plateau in Nigeria (Purseglove 1972). This millet, although not a staple crop in the Sudanic zone, is widely grown there as a complementary cereal where the rainfall exceeds 400 mm (Purseglove 1972). *Digitaria exilis*, as well as *D. iburua*, are sown during May or June and harvested in September (de Wet 1989).

True fonio is known for its short growing season that ranges from 40 days to three and a half months. This agronomic attribute is probably due to the quick germination (3–4 days after sowing) and the early rapid growth (Purseglove 1972). Among the large number of varieties grown by the Temne and other northern tribes in Sierra Leone, long and short season cultivars are recognized and are known as *Pa-sacta* (longer season cultivars) and *Apeindei-pufundf* (short season cultivars). Fonio is a persistent crop that does not require careful cultivation, and its varieties thrive under a range of difficult agricultural conditions, including sterile or unproductive soils, difficult terrains, and water stress (Lewicki 1974; Portères 1976; Purseglove 1972). In addition, herbarium collections examined indicated that the crop

grows on shallow sandy soil in open grass, forest, and savanna communities.

Several local names exist for true fonio millet. It is known as *fundi, fonde*, and *findi* in Wolof; *Pene* in the language of the Sierra Leone Temne; *ansi* in the Songhai language; *achcha* or *atcha* in the Hausa areas of the Nigeria (Lewicki 1974); *kputi* in the Mender and *kpeindei* in the Temne. Portères (1955) studied the common appellations of fonio in detail and found out that these names can be generally translated as 'things to eat' or 'foods'. *Digitaria exilis* also has the common name 'hungry rice', however, Harlan (1977) indicated that the grain is frequently grown as a delicacy food. The grains are made into couscous, boiled like rice either alone or used in stews, ground into a flour which is eaten as gruel seasoned with butter or fat, and used in brewing. In the Niger, the seeds are used as a delicacy where they are ground and made into a sauce. True fonio is also used as fodder, mixed with building clay, and in some areas of Africa utilized for ceremonial purposes (Clark 1976; Dalziel 1937).

The first mention of fonio was in the geographical work of Al-Omari, who wrote of West Africa from 1342–1349 (Lewicki 1974). Al-Omari reported that fonio is one of the basic foods in the western Sudanic region of Africa (Portères 1976). Another report of the cultivation and use of fonio comes from Ibn Batuta (1353–1354), who tells of local peoples selling the grain to travelers in Mali (Portères 1976). However, the domestication of the crop is more ancient. Munson (1976) suggested that fonio was domesticated around the headwaters of the Niger River, at around 4500 B.C. (Murdock 1959). The names for fonio come from linguistic groups originating from the middle Niger and Senegalic areas, providing evidence for its domestication in the vicinity of the central Nigerian delta (Portères 1976). The ancestral species of *Digitaria exilis* is not conclusively known. The closest wild relative of true fonio is *Digitaria longiflora* (Hatz) Pers., which grows in hot regions throughout Africa and Asia (Harlan 1992). *Digitaria fuscescens* (Presl) Henr., a species morphologically related to *D. longiflora*, has also been proposed as a possible putative ancestor for true fonio (Portères 1955).

The other domesticated species, *Digitaria iburua* (black fonio), is cultivated by the Hausa tribe and by the Pagans on the Jos plateau of Nigeria, in the Atacora Mountains of Dahomey, in Guinea, Cameroun, and Zaire (Dalziel 1937; de Wet 1989; Harris 1976; this study). The name is derived from the dark color of the spikelet. This millet is considered as the principal grain crop for the Pagan people. Being cultivated between 400 and 1300 m, black fonio is considered to be a sub-alpine plant (Portères 1976). Black fonio is often found in cultivation with true fonio, and is frequently planted between rows of sorghum and pearl millet (Portères 1976; de Wet 1989). The crop is drought tolerant and often yields a harvest when the major cereal it accompanies fails to survive (de Wet 1989). It is used to prepare a couscous called *Wusu-Wusu* in parts of western Africa; however, difficulty in removing the chaff of black fonio gives true fonio an edge over black fonio as a food crop in that region (Portères 1946, 1976). Black fonio is typically grown as a beer grain, making the beverage known as *Tchapulo* (Harris 1976; Portères 1976).

Like true fonio, black fonio has no traces in the archeological records to verify its area of domestication, but is considered an endemic of West Africa (Harlan 1992). Black fonio was first mentioned outside Africa through the reports of Dudgeon in 1911 (Portères 1976), which mentioned its cultivation in northern Nigeria. Portères (1976) suggests that this cereal was first domesticated in Northern Nigeria, whereas Harris (1976) speculates that it may have originated as a cultivated plant in the Aïr region of the Southern Sahara. The wild progenitor of the crop is not known. Stapf (1915) pointed out *Digitaria ternata* (A. Rich.) Stapf as the closest wild species to *D. iburua*. Hernard (1950) proposed *D. barbinodis* Henr. as species closely allied to black fonio. *Digitaria barbinodis*, although overlaping in geographical distribution with both fonio millets, differs morphologically from black fonio in several characters such as the shape of the rachis, the relative length of the upper glume to the lemma, and the number of nerves on the glume (Clayton 1972).

Material and Methods

Plant material: Twelve collections for true fonio (*D. exilis*), one collection each for black fonio (*D. iburua*), *D. longiflora*, *D. ternata*, and *D. fuscescens* (Presl) Henr. were examined for the RAPD study. Table 1 lists the accessions used, their area of origin and the sources of plant material. The representation of the species in this study was constrained by the scarcity of the collections for these crops and their related wild species in the various world germplasm banks. Collections for true fonio were limited in geographic origin and found in the International Livestock Center for Africa (ILCA) germplasm bank only. Similarly, there was only one collection available for each of the other four species in the germplasm banks. For the ethnobotanical and morphological aspects of the study, herbarium specimens for the above species were examined from the herbaria of the Royal Botanical Garden, Kew, England and the Natural History Museum in Paris.

DNA isolation: Plants were grown from seeds in the greenhouse and leaf samples were harvested from four-week old plants and stored at -80°C. Total cellular DNA was isolated from leaf material of a single individual plant following M'Ribu & Hilu (1996). We

Table 1 Species, accessions, sources of material, and geographic origin of the material used in the study. For uniformity of labeling, the collection numbers of the senior author (KH) were used throughout. *Digitaria exilis* accession NIAR 27737 of South Africa is marked as *Gambia M/67/125* cultivar which implies a possible origin from Gambia

Species	Accessions	Source of material	Geographic origin
D. exilis	KH2730	[a]ILCA15610	Togo
D. exilis	KH2734	ILCA15615	Togo
D. exilis	KH2735	ILCA15617	Togo
D. exilis	KH2736	ILCA15618	Togo
D. exilis	KH2738	ILCA15620	Togo
D. exilis	KH2740	ILCA15622	Togo
D. exilis	KH2741	ILCA15624	Togo
D. exilis	KH2742	ILCA15625	Togo
D. exilis	KH2743	ILCA15627	Togo
D. exilis	KH2745	[c]NIAR27737	South Africa
D. iburua	KH2760	[b]PI238288	Nigeria
D. longiflora	KH2770	PI364523	South Africa
D. ternata	KH2800	PT299841	South Africa
D. fuscescens	KH2756	PI196479	Brazil

[a]ILCA: collection numbers of the International Livestock Center for Africa

[b]PI: Plant Introduction numbers of the U. S. Department of Agriculture Plant Introduction Stations.

[c]NIAR: National Institute of Agrobiological Resources of Japan

found this procedure to be most effective in producing DNA for the *Digitaria* species and it can be used successfully for amplification by the polymerase chain reaction method. Approximately 50–100 mg of tissue was macerated inside a 1.5 ml Eppendorf tube containing 300 µl of CTAB extraction buffer (2% hexadecyltrimethyl-ammonium bromide, 0.7 M NaCl, 10 mM EDTA, 50 mM Tris-HCl pH 8 and 0.1% mercaptoethanol). Following maceration, another 300 µl of buffer was added, and the suspension was incubated in a water bath at 60°C for 10 min. The suspension was spun lightly to pellet the plant debris, and the aqueous portion was mixed with an equal volume of chloroform and centrifuged for 1 min. The DNA in the aqueous phase was precipitated with isopropyl alcohol, pelleted by centrifugation, and dissolved in TE buffer (Tris-EDTA, pH 8.0).

DNA amplification and electrophoresis: Eleven 10-base oligonucleotide primers (Operon Technologies, primers OPA 2, 3, 5, 9, 10, 11, 12, 16, 17, 18, 19) were selected on the basis of their ability to generate informative data. PCR amplification followed the procedure of Williams *et al.* (1990) with some modifications (Hilu 1995). Each reaction mixture (25 µl) contained 2.5 µl of 20 × reaction buffer, 2 mM magnesium chloride, 10 mM each of dATP, dCTP, dGTP and dTTP, 0.047 µg of single PCR primer, 0.9 unit of 6 Taq DNA polymerase (Promega), and approximately 15 ng of genomic DNA template. Master mixes for reaction mixtures were prepared to standardize the reactions in each experiment. Amplification was carried out in a Perkin Elmer Cetus thermal cycler following Stewart & Via (1993): one initial denaturation cycle at 95°C for 5 min followed by 75 cycles of 94°C for 10 seconds, 36°C for 10 seconds and 72°C for 2 min. Twenty µl of the amplification product samples were loaded on 1.5% agarose gel and run in Trisacetate-EDTA (TAE) buffer at 100 V for 4.5–5 hours. A one-kilobase DNA ladder marker (Bethesda Laboratories) was used as a molecular standard. DNA was stained with ethidium bromide and photographed in UV light. This PCR amplification protocol is routinely used in our molecular studies of millets (Hilu 1994b, 1995; M'Ribu & Hilu 1994, 1996). Different DNA concentrations were examined to assess the optimal amount required for successful amplification. To examine the reproducibility of the RAPD experiments, six representative DNA samples were amplified with six primers. Controls that contain all the components of the PCR amplification reactions except for the template DNA were included.

Data analysis: Accessions were scored for the presence or absence of shared RAPD fragments. DNA bands that were present at lower intensities were scored as present. RAPD markers that were shared by all accessions were excluded from the data analysis since they do not provide information on the genetic relationships among the species. In cases where template DNA of an accession was not well amplified, missing values were used in the scoring. To calculate the similarity co-efficients among accessions and species, the data were analyzed with the Dice algorithm (Dice 1945). The Dice coefficient is equivalent to equation 21 of Nei & Li (1979); both calculate similarities on the basis of shared presence of a DNA band and exclude shared absence of fragments from the analysis. The matrix of similarity was used to group accessions via the Unweighted Pair Group Method (UPGMA). The co-phenetic co-efficients for the clusters was computed by using normalized Mantel statistics z. The Dice matrix of similarity was used in a principal coordinate analysis (PCO) to resolve patterns of variation among the accessions. The principal co-ordinate has advantages over the principal component analysis since the DNA bands represent qualitative data points (i.e. scored as present-absent) and the original matrix contains missing values (see Sneath & Sokal 1973). The minimum spanning tree (MST) was computed from the Dice similarity matrix and was projected onto the plot of the first three factors of the PCO. The MST links nearest neighbor accessions (Rohlf 1993). The NTSYS-PC computer program (version 1.80; Rohlf 1993) was used in analyzing the data.

Table 2 Primers used in the RAPD study of the *Digitaria* species. The primer number, its nucleotide sequence, and statistics on the number of bands amplified are reported

Primers	Sequences	Total bands amplified	Variable bands	Percentages
OPA 2	TGC CCA GCT G	11	11	100
OPA 3	AGT CAG CCA C	14	14	100
OPA 9	GGG TAA CGC C	13	12	92
OPA 10	GTG ATC ACA G	13	13	100
OPA 12	TCG GCG ATA T	15	14	93
OPA 16	AGCCAG CGA A	18	18	100
OPA 17	GAC CAC TTG T	16	15	94
OPA 19	CAA ACG TCG G	14	14	100

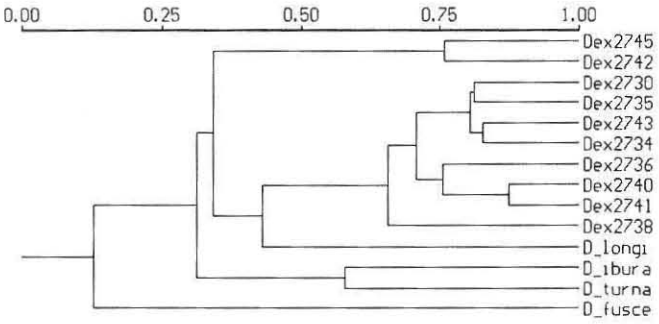

Figure 1 The clustering of the 14 accessions that represent *D. exilis*, *D. iburua*, *D. longiflora*, *D. ternata* and *D. fuscescens*. The ten accessions of *D. exilis* are designated with the first letter of the generic name and the first two letters of the specific epithet (Dex) and by the KH numbers cited in Table 1. *Digitaria longiflora* is abbreviated as 'D_longi', *D. ternata* as 'D_terna' and *D. fuscescens* as 'D_fusce' due to the limited number of characters allowed by the NTSYS program.

Results

Out of the eleven primers initially used in this study, eight amplified the majority of the DNA samples on a consistent basis. Consequently, data from these eight primers alone were used in the analysis to minimize the amount of missing data. Two DNA samples of *D. exilis* showed poor amplification with the majority of the primers and were, thus, eliminated from the analysis. The eight primers amplified a total of 114 DNA bands (Table 2) of 0.2–2.5 kb in size, of which only two were common to all species examined. The number of amplified bands varied from 11 (OPA 2) to 18 (OPA 16), with a mean of 14.1 (Table 2). The majority (92–100%) of the bands were variable across all five species (Table 2).

The genetic similarity as measured by the Dice algorithm (Table 3) ranged from zero (*D. fuscescens*, two accessions of *D. exilis*, *D. fuscescens* - *D. longiflora*) to 0.88 (between two accessions of *D. exilis*). Within true fonio, the 10 representative accessions showed a wide range of similarities (0.27–0.88). The Dice similarity between the ten accessions of true fonio and its proposed ancestor varied from 0.29–0.60, and between black fonio and its suspected wild species was 0.58 (Table 3). The co-phenetic correlation between the Dice matrix and the UPGMA clustering was r = 0.95, reflecting strong agreement between the two.

Table 3 The similarity matrix of 10 accessions of *Digitaria exilis* (numbered KH) and 4 accessions belonging to four other species examined in this RAPD study. The similarity values are calculated from the Dice algorithm using the NTSYS-pc computer program

Accessions or species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. KH2745	1.00													
2. KH2730	0.31	1.00												
3. KH2742	0.76	0.28	1.00											
4. KH2743	0.35	0.82	0.33	1.00										
5. KH2734	0.33	0.80	0.28	0.83	1.00									
6. KH2735	0.38	0.81	0.33	0.78	0.82	1.00								
7. KH2736	0.34	0.65	0.27	0.69	0.70	0.72	1.00							
8. KH2738	0.33	0.68	0.35	0.63	0.63	0.63	0.61	1.00						
9. KH2740	0.41	0.74	0.37	0.66	0.75	0.70	0.76	0.70	1.00					
10. KH2741	0.41	0.70	0.35	0.72	0.77	0.71	0.75	0.72	0.88	1.00				
11. *D_longiflora*	0.36	0.36	0.36	0.29	0.31	0.40	0.67	0.57	0.44	0.40	1.00			
12. *D_ibura*	0.30	0.32	0.35	0.29	0.32	0.30	0.26	0.45	0.34	0.36	0.17	1.00		
13. *D_ternata*	0.28	0.33	0.29	0.31	0.33	0.33	0.33	0.38	0.35	0.37	0.00	0.58	1.00	
14. *D_fuscescens*	0.11	0.10	0.21	0.09	0.10	0.11	0.15	0.18	0.00	0.00	0.00	0.27	0.35	1.00

The UPGMA-based phenogram resolved three subclusters that corresponded to 1) *D. exilis–D. longiflora* 2) *D. iburua–D. ternata*, and 3) *D. fuscescens* (Figure 1). The two fonio millets (*D. exilis* and *D. iburua*) were very distinct, separated at 0.31 similarity level. The 10 accessions of true fonio, *D. exilis*, grouped into two clusters separated at a low similarity (0.34), (Figure 1). One cluster was comprised of a *Gambia M/67/125* cultivar collected from South Africa and one of the Togo cultivars. The second cluster included the remaining eight accessions as well as the wild species *D. longiflora*; the latter grouped with these true fonio accessions at a similarity value of 0.43. The black fonio accession grouped with *D. ternata* at the 0.58 level. *Digitaria fuscescens* was last to cluster with the other four wild and domesticated species of *Digitaria* (Figure 2).

The three co-ordinates of the PCO accounted for 60% of the variation. The PCO separated the species and the accessions of *D. exilis* in a pattern that was supportive of the grouping generated by the UPGMA clustering method (Figure 2). *Digitaria longiflora* appeared within the group of eight accessions of true fonio, while the other two true fonio accessions were removed on the second co-ordinate (Figure 2). Similarly black fonio (*D. iburua*) and *D. ternata* appeared in a very close proximity on the three co-ordinates, while *D. fuscescens* was distinct, especially on the third co-ordinate. The minimum spanning tree demonstrated the lack of distortion in the PCO as neighboring units were first connected prior to their linkage to other groups. The overall pattern of neighbor joining depicted by the MST resembles the clustering pattern obtained by the UPGMA method.

Discussion

Harlan (1992) considered true and black fonio millets as endemic crops in his classification of crops according to geographic distribution. Both are west African, with true fonio grown from Nigeria to Senegal and black fonio from Nigeria to Togo. The limited distribution of true fonio, however, is accompanied by an extensive degree of morphological variability (Harris 1976) and by the presence of a considerable number of varieties and cultivars. The information from RAPD reflected this feature of true fonio at the genetic level. The ten accessions of the crop displayed about 20% variability in the amplified RAPD bands. The grouping of the ten accessions in two clusters that show resemblance at 0.34 is an indication of genetic differentiation in the crop that is either associated with multiple domestication events or a broad genetic diversity associated with selection, possibly for different ecological conditions or agricultural practices. This finding gains support from the proposed presence of three centers of diversity for true fonio in west Africa and the adaptation of

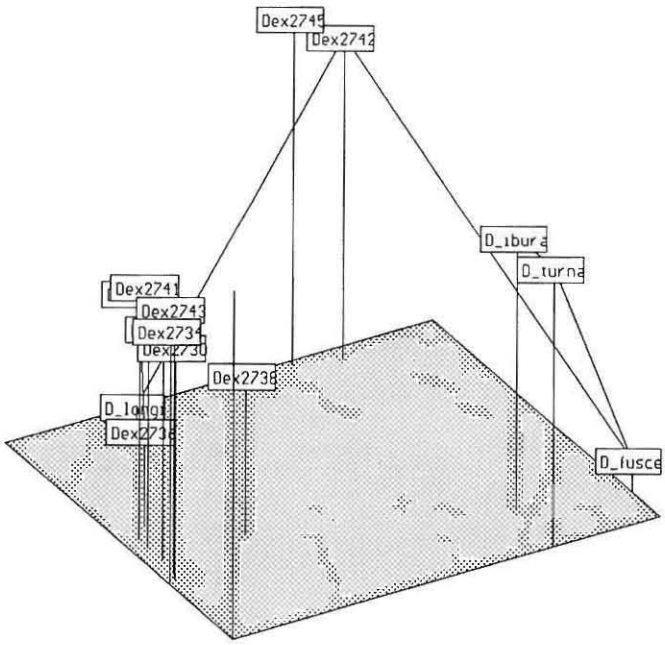

Figure 2 The distribution of the accessions of *D. exilis, D. iburua, D. longiflora, D. ternata* and *D. fuscescens* on the first three axes of the principal co-ordinate. The minumum spanning tree is mapped on the principal co-ordinate plot to show the nearest neighbour connections. Accession and species labels correspond to those cited in Figure 1.

the different varieties to different ecological conditions (Portères 1976). True fonio is thus genetically variable, possibly due to outbreeding mechanisms. The level of genetic variability in true fonio is high when compared with that reported for other millets. For instance, similarities in RAPD among proso millet, *Panicum miliaceum* L., was 0.52–0.91 among accessions examined from Europe, United Kingdom, Asia, and Africa, and was only 0.87 in the Indian and 0.90 in the Iranian collections (M'Ribu & Hilu 1994). In finger millet, [*Eleusine coracana* (L.) Gaertn. subsp. *coracana*], similarities in RAPD among accessions from across India and Africa ranged from 0.64 to 0.92 (Hilu 1995). Therefore, the range of 0.28–0.88 similarity in true fonio is considerably high when the relatively small region from which the accessions originated is considered.

The variable DNA bands in the fonio millets represent molecular markers that can potentially be used in identifying cultivars and tagging genetic traits in breeding programs. The RAPD method thus represents a promising approach for studying genetic variability and genomic relationships in true fonio. Additional molecular markers can be generated with the use of more primers. In true fonio, variation in RAPD bands is high as it is evident from the range of similarities among accessions (0.28–0.88). Unfortunately, detailed background information on the origin of the accessions is not available to assess the pattern of variation at the geographic or population level. The genetic variability in black fonio could not be assessed because of the presence of only one accession for this millet in the international germplasm collections. This lack of genetic resources for such a potentially useful crop in semi-arid regions is a most unfortunate situation that needs to be rectified.

The identification of ancestral species for crop plants is an important step toward their improvement because the wild species is a significant component of the primary gene pool as it possesses much higher genetic diversity than the crop (Hilu 1994a, 1995). Unfortunately, there has not been any cytogenetic or molecular study addressing the ancestral relationships between the fonio millets and other *Digitaria* species. The species proposed as possible progenitors for true and black fonios are selected on the basis of morphological similarities. *Digitaria longiflora* has been proposed on the basis of morphological affinities as a possible ancestral species for true fonio (Dalziel 1937; Stapf 1915). Stapf (1915) indicated that *D. exilis* approaches *D. longiflora* very closely, differing from it mainly by the completely glabrous and somewhat more 'turgid' spikelet. In addition to *D. longiflora*, Henrard (1950) proposed *D. fuscescens* as another putative ancestor for true fonio. *Digitaria fuscescens* was shown to be morphologically close to *D. exilis* (Clayton 1972), with the two appearing in one duplet of the key to tropical west African *Digitaria*. *Digitaria fuscescens* and true fonio are characterized by the glabrous spikelets, a trait that distinguishes them from *D. longiflora* that has spikelets with short appressed hairs. In contrast with these reports, Portères (1976) maintained that the identity of the ancestral species of true fonio remained doubtful.

Digitaria fuscescens shared very few RAPD bands with most of the true fonio accessions as it is apparent from the low Dice similarity (0.00 and 0.21) between the two (Table 3). *Digitaria fuscescens* did not cluster with the accessions of true fonio; instead, it was last to join the phenogram, clustering with the *D. iburua–D. ternata* group at a very low similarity level of 0.13 (Figure 1). Similar results were also obtained from the PCO and MST analyses (Figure 2). Therefore, the lack of genetic similarities between true fonio and *D. fuscescens* does not support the proposed genomic relationships between the two species. Although true fonio resembles *D. fuscescens* in the glabrous spikelet, the two differ in a number of other morphological traits. *Digitaria fuscescens* is a creeping, up to 20 cm high plant with paired racemes and protruding grain, while true fonio is an erect, 40–80 cm tall plant, with 3–5 racemes per inflorescence and a grain enclosed within the lemma (Clayton 1972). In contrast, true fonio resembles *D. longiflora* in these morphological characters. The high morphological similarities between true fonio and *D. longiflora* parallels the genetic similarities reflected by the RAPD data. *Digitaria longiflora* shared up to 67% of the RAPD bands with the domesticated true fonio and clustered within the accessions of the later. This finding supports Stapf's hypothesis (Stapf 1915) that proposed *D. longiflora* as the putative wild progenitor for true fonio. The main morphological difference between the two is the pubescence of the lemma in *D. longiflora*. Variation in the amount of hairs on the lemmas of *D. longiflora* has been observed in some herbarium specimens and thus the difference in this morphological trait is qualitative and is not significant. The morphological similarities between the two taxa are so high that a number of herbarium specimens of *D. longiflora* were mis-identified as *D. exilis* and some herbarium specimens contained plant parts of both species. In fact, Stapf (1915) placed both taxa in section *Verrucipilae* which is characterized by the pubescent spikelets in spite of the glabrous lemmas of *D. exilis*. Artificial crosses between the two taxa are needed to confirm the chromosome homology and interfertility between the two taxa. Also, additional west African species of section *Verrucipilae* might need to be examined to exclude alternative hypotheses.

Stapf (1915) suspected *D. ternata* to be the progenitor of black fonio on the basis of morphological affinities. He, however, indicated that black fonio differs from *D. ternata* by the crowded, closely imbricated spikelets that tend to be quite glabrous and slightly larger. *Digitaria ternata* differs from black fonio also in the presence of dense lines of appressed hairs between the lemma nerves. The two are similar in the annual habit and the presence of a corona of short hairs on the tip of the pedicel. In this RAPD study, black fonio and *D. ternata* showed a 0.58 Dice similarity. This value is considerably higher than the Dice similarity values obtained between *D. fuscescens* and either black fonio or *D. ternata*. In fact, it is within the range of Dice similarities computed among the accessions of true fonio. Thus, the grouping of *D. ternata* with black fonio presents new evidence in support of the possible origin of black fonio from this wild species as proposed by Stapf (1915). However, cytogenetic studies of these species and the exploration of other *Digitaria* species native to the area of domestication of this millet, especially *D. barbinodes*, are needed for a conclusive decision on the ancestry of black millet.

Both *D. longiflora* and *D. ternata* have very wide geographic distribution as indicated above. In contrast to these wild species, the fonio millets are confined to small regions in tropical west Africa, and have been regarded as endemic crops (Harlan 1992). Therefore, in spite of the genetic variability observed in true fonio, the genetic resources available from the cultivated types are constrained by the endemic nature of the crops. It has been demonstrated that wild species are genetically more variable than their respective crops (Plucknett *et al.* 1983; Hilu 1994a; Hilu 1995). The wide geographic distribution of the wild progenitors over large geographic areas underscores their importance as valuable resources of genetic material. *Digitaria ternata*, for instance, varies from an erect tall to a creeping plant, grows in a variety of soils and associates with a range of plant communities such as grasslands, open shrub savanna, in dry areas or along streams, and as a weed in farmlands and along road sides. Concerted efforts need to be made to collect and conserve the genetic resources of the wild progenitors of fonio millets. In addition, the germplasm collections for the domesticated species, particularly black fonio, also need to be expanded. Fonio millets are important millets in certain parts of Africa, and show promise for agri-

culture in less arable lands and in the dryer regions of the world.

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