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Flow cytometric analysis reveals different nuclear DNA contents in cultivated Fonio (*Digitaria* spp.) and some wild relatives from West-Africa

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Abstract. Nuclear DNA amounts of 118 cultivated fonio accessions representing 94 landraces collected from the major growing areas of West-Africa (Benin, Burkina Faso, Guinea, Mali and Togo) and eight accessions of four wild relatives were investigated by Laser flow cytometry. In cultivated species, average 2C-values ranged from 1.848 ± 0.031 pg for *Digitaria iburua* to 1.956 ± 0.004 pg for *D. exilis*. In *D. exilis* landraces the chromosome number was determined at 2n = 36. The closely related wild species *D. longiflora* and *D. ternata* showed similar 2C DNA contents of 1.869 ± 0.035 pg and 1.775 ± 0.070 pg, respectively. Distinctly larger genomes were identified for more distant species *D. lecardii* and *D. ciliaris* with 2.660 ± 0.070 pg and 2.576 ± 0.030 pg per 2C nucleus, respectively. Intra-specific variations were found to be slight and insignificant, suggesting genome size stability mainly within the cultivated gene pool. These results support the distance of cultivated fonio species *D. exilis* and *D. iburua* from *D. lecardii* and *D. ciliaris* as well as their close relationships with *D. longiflora* and *D. ternata*. Relevance of the results for ploidy level considerations in fonio millets is discussed.

Key words: Fonio, *Digitaria* spp., 2C-values, genome size, flow cytometry, chromosome number, West-Africa.

Introduction

The genus *Digitaria* Haller comprises 230–325 annual and perennial grass species with a wide geographic distribution in the tropics and subtropics (Henrard 1950, Clayton and Renvoize 1986). Many *Digitaria* species are important worldwide or regionally mainly as fodder but also as food crops. In West-Africa, *D. exilis* (Kipp.) Stapf and *D. iburua* Stapf are native millets cultivated as major staple food since five millennia BC (Murdock 1959). White fonio (*D. exilis*) is the most diverse

and widely cultivated species in the region. Conversely, *D. iburua* (black fonio) cultivation is restricted to northern Nigeria, Benin and Togo. In addition to this cultivated gene pool, there are a number of wild relatives that can provide potentially valuable resources for the improvement of fonio crops. They are aggressive weeds widely distributed in West Africa and some of them are considered by local farmers as "wild fonio" or "bird fonio" and were in the past harvested for food during long hunting trips or for fowl feeding (Adoukonou-Sagbadja et al. 2006). Based on botanical descriptions, several wild *Digitaria* species were proposed to be progenitors of cultivated fonio (for an overview cf. Haq and Ogbe 1995). However, using RAPD markers, Hilu et al. (1997) showed that only *D. longiflora* (Retz.) Persoon and *D. ternata* (A. Rich) Stapf were genetically closely related to white and black fonio, respectively.

According to Vietmeyer et al. (1996), fonio millets supply food to several millions of people. The special richness of their grains in methionine and cystine, two human-vital amino acids deficient in major cereals such as wheat, rice, maize, sorghum or barley, ranks fonio among the most nutritious of the grain crops (Jideani 1990). However, despite its important role in household food security the crop is still on a primitive production level and features many drawbacks, such as tiny seeds, poor yield, pests, diseases, plant lodging, laborious farming practices, difficult seed processing, etc. (Kwon-Ndung et al. 1998, Adoukonou-Sagbadja et al. 2006). During the last decade, important germplasm of fonio genetic resources was collected and conserved in the National Agricultural Research Centres of the main producing countries in West-Africa. For an efficient use of such germplasm in basic research and crop breeding programmes, information on chromosome numbers and genome size (DNA content) is very useful (Tuna et al. 2001). But, available information on ploidy level in fonio millets is still confusing. Hunter (1934) reported the unique chromosome count in *D. exilis* with 2n = 54 chromosomes. Since the basic chromosome number of the *Digitaria* is thought to be x = 9, as in most of the Paniceae (Avdulov 1931, Hunter 1934), many authors assumed that this species is hexaploid with 2n = 6x = 54 (Portères 1976, Wanous 1990, Haq and Ogbe 1995). In contrast, Zeven and de Wet (1982) suggested that *D. exilis* may be diploid with 2n = 2x = 18 chromosomes or tetraploid having 2n = 4x = 36 chromosomes. Both tetraploid (Zeven and de Wet 1982) and hexaploid (Wanous 1990) levels were proposed for *D. iburua*. To our knowledge, information on the nuclear DNA contents for both species does not exist until now. Furthermore, genome size documentation exists only for two species (*D. ascendens* Rendle and *D. sanguinalis* L.) in the genus *Digitaria* (Bennett et al. 2000).

Analysis of nuclear DNA content can be performed by microdensitometry or by flow cytometry. Nowadays, flow cytometry is the method of choice because of its ease, quickness, precision and accuracy in detecting small differences in DNA content (Rayburn et al. 1989). This technique has been successfully used in various ways in determining nuclear DNA content of major crop plants (Arumuganathan and Earle 1991), the ploidy level of grass species (Arumuganathan et al. 1999) or for taxonomical and evolutionary studies (Koopman 2000, Doležalová et al. 2002, Price et al. 2005).

In the present work, flow cytometric analysis was used to estimate the nuclear DNA content in a large and representative cultivated fonio germplasm and some wild related species. The study aims to investigate the possible correlations of genome size variations with taxonomic and ancestral relationships of these species or with ecological and geographic features. The study offers also a comparatively large and representative view on the ploidy level of fonio millets.

Materials and methods

Experimental material. One hundred and eighteen fonio accessions (six accessions of *D. iburua* and

112 accessions of *D. exilis*) representing 94 farmer-named landraces and eight accessions of wild *Digitaria* species originating from five West-African countries (Benin, Burkina Faso, Guinea, Mali and Togo) were used in this study (Table 1, Fig. 1). *D. exilis* and *D. iburua* accessions from Togo were collected from farmers' fields (Adoukonou-Sagbadja et al. 2004), while accessions from Benin were obtained from the Gene Bank of the Benin National Agricultural Research Institute based at Niaouli. Guinean, Malian, and part of Burkina Faso accessions were provided by the National Agricultural Research Institute of Guinea via the West and Central Office of Bioversity International (ex IPGRI) based at Cotonou, Benin. The second part of accessions from Burkina Faso came from Niaouli Gene Bank. Wild species recognized by local farmers as wild types of fonio were collected by the first author from different areas in these countries and taxonomically identified during the present study as *D. longiflora*, *D. ternata*, *D. ciliaris* (Retz.) Koeler (syn. *D. adscendens* (H. B. K.) Henrard), and *D. lecardii* (Pilg.) Stapf. Voucher specimens of the majority of the accessions analysed in this study are deposited in the Herbarium Gatersleben (GAT) at the Gene Bank of the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK Gatersleben, Germany). All plants were grown under greenhouse conditions at approx. 22°C and 12 hours light.

Flow cytometry measurement of nuclear DNA content. Nuclear DNA content was determined at IPK with a FACSAria flow cytometer (Becton Dickinson, San Jose, CA, USA) using the WinMDI 2.8 analysis programme (Joseph Trotter 1993–1998, http://facs.scripps.edu/). *Glycine max* (2C value: 2.72 pg) or *Raphanus sativus* (2C value: 1.38 pg) were used as internal standards (Doležel et al. 1998). Nuclear suspensions were prepared and flow cytometry analysis was performed following Barow and Meister (2002). Approximately 50 mg of fresh and young leaf tissue was excised from individual plants and used for sample preparation. To release nuclei, leaf fragments of *Digitaria* and of the reference plant(s) were placed together in a pre-cooled Petri dish and chopped with a sharp razor blade in 1 ml ice-cold Galbraith's buffer (Galbraith et al. 1983) supplemented with 50 $\mu g\ ml^{-1}$ propidium iodide (PI) and 50 $\mu g\ ml^{-1}$ RNase (DNA-free). The suspension of isolated nuclei was filtered through a nylon mesh with a pore size of 35 μm and analysed immediately. If ever possible, four individual plants were separately analysed per accession, each of them was considered as one replicate. The mean DNA content per measurement was based on at least 10,000 scanned nuclei. The 2C DNA content of the sample was calculated as the sample peak mean, divided by the reference peak mean, and multiplied with the amount of DNA of reference plant ($2C_{Digitaria} = [Peak_{Digitaria}/Peak_{reference}] \times 2C_{reference}$).

Statistics: Genomes size data were analysed using the SAS system for Windows software, release 8.02 (SAS Institute, Cary, NC, USA). Differences in DNA content were tested by one-way analysis of variance (ANOVA), and the Scheffé test was used to discriminate dissimilar groups within and between the studied species.

Chromosome counting. Fonio grains were germinated on moist filter paper at 24°C. About 1 cm long root tips were fixed in ethanol: acetic acid (3:1). After hydrolysis in 1N hydrochloric acid at 60°C for 15 min the roots were stained in Schiff's reagent according to the standard Feulgen method. Chromosome spreads were prepared in propion orcein. Because chromosomes could not be spread in one focus layer an epifluorescence microscope (Zeiss Axiophot) integrated into a Digital Optical 3D Microscope System (Schwertner GbR, Jena, Germany) was used to take image stacks to produce 3D images for chromosome counting. The image stacks were also used for karyogram establishment via the Ikaros software (MetaSystems GmbH, Altlussheim, Germany).

Results and discussion

The flow cytometric measurements yielded DNA histograms with standard deviations of DNA content measurements in most cases lower than 5%, regardless of the internal standard used. Histograms representing single plants of all species analysed are visible in Fig. 2. Table 1 shows the nuclear DNA contents of all 126 accessions of the six species investigated and Table 2 exhibits the results of Scheffé's test conducted on the average DNA contents by pairwise comparisons and the 1C genome sizes calculated for each taxon. Fig. 3 illustrates the major botanical characteristics of the spikelet of the six species investigated.

Table 1. Origin and DNA content of fonio landraces and wild species accessions (voucher numbers: Herbarium Gatersleben, GAT), with the number of determinations per accession (n), standard deviation (SD)

Acc. N°	Local name	Voucher	Origin			n	2C DNA content (pg)
			Country	District	Coll. site		Mean ± SD
D. exilis							
III-3	M'balia 2	—	Guinea	-	Unknown	4	1.660 ± 0.053
II-4	Kansambaran	GAT5304-5305	Guinea	-	Unknown	4	1.861 ± 0.086
I-7	Kokountèrè	GAT5268,5269	Guinea	-	Unknown	2	1.903 ± 0.179
III-2	Farmali	GAT5320	Guinea	Tougué	Tougué	2	1.911 ± 0.050
II-6	Litty	GAT5300,5301	Guinea	Mali district	Near Mali city	4	1.922 ± 0.163
IV-1	Siragbè	—	Guinea	-	Unknown	1	1.933
I-4	Fomba	GAT5274,5275	Guinea	-	Unknown	4	1.939 ± 0.089
III-1	Siragbé	GAT5321,5322	Guinea	-	Unknown	4	1.942 ± 0.111
IV-3	Siragbé	GAT5355,5356	Guinea	-	Unknown	4	1.942 ± 0.045
I-6	Foundelen	GAT5270,5271	Guinea	Lélouma	Lélouma	3	1.950 ± 0.104
I-2	Mamanden	GAT5278,5279	Guinea	-	Unknown	4	1.955 ± 0.055
II-7	Bassamba 2	GAT5298,5299	Guinea	Labé	Labé	3	1.960 ± 0.131
III-7	Fayahè	GAT5319	Guinea	Koundara	Koundara	1	1.961
IV-4	Konson	GAT5351-5354	Guinea	-	Unknown	4	1.970 ± 0.047
IV-6	Hothio 2	GAT5347,5348	Guinea	-	Unknown	4	1.972 ± 0.061
IV-11	Gblingbè	GAT5340,5341	Guinea	-	Unknown	4	1.972 ± 0.033
IV-12	Mora 2	GAT5336-5339	Mali Republic	Mopti	Unknown	4	1.983 ± 0.051
III-5	Momo	—	Guinea	Dalaba	Dalaba	1	1.992
II-8	Niougou	GAT5296,5297	Guinea	-	Unknown	4	2.002 ± 0.018
III-15	Dalaman	GAT5311,5312	Guinea	-	Unknown	4	2.005 ± 0.014
I-11	Mora	GAT5260,5261	Mali Republic	Mopti	Unknown	4	2.006 ± 0.016
I-16	Mossogbè	GAT5250,5251	Guinea	-	Unknown	4	2.013 ± 0.084
I-12b	Yaoukö	GAT5280,5281	Guinea	-	Unknown	4	2.019 ± 0.011
I-1	Konso	GAT5276,5277	Guinea	Mandiana?	Unknown	4	2.020 ± 0.033
I-3	Sèkètè	—	Guinea	Mali	Mali	4	2.022 ± 0.039
III-12	Dibon	GAT5285-5290	Guinea	-	Unknown	4	2.022 ± 0.013
II-10	Tobbhérè	GAT5282-5284	Guinea	Lélouma	Lélouma	4	2.024 ± 0.017
II-11	Koundara	—	Guinea	Labé	Labé	3	2.024 ± 0.027
III-16	Kouroussa	GAT5266,5267	Guinea	-	Unknown	4	2.026 ± 0.032
I-8b	Saara	GAT5264,5265	Guinea	-	Unknown	4	2.029 ± 0.030
I-8a	Saara	GAT5272,5273	Guinea	-	Unknown	4	2.031 ± 0.016

I-5	Werura	GAT5315,5316	Guinea	-	Unknown	4	2.034 ± 0.022
III-8	Raného	GAT5315,5316	Guinea	Tougué	Tougué	4	2.040 ± 0.036
I-10	Yéléboui	GAT5262,5263	Guinea	Kindia ?	Unknown	4	2.043 ± 0.035
I-12a	Yaoukö	GAT5254-5255	Guinea	Lélouma	Lélouma	4	2.058 ± 0.022
I-13	Hothio 1	GAT5252,5253	Guinea	Kita	Unknown	4	2.054 ± 0.018
IV-14	Oulè oulè	GAT5232,5233	Mali Republic	Bougouni	Dossola	4	1.871 ± 0.066
IV-8	Tama	GAT5346	Mali Republic	-	Unknown	4	1.974 ± 0.023
IV-13	Pon - Biré	GAT5334,5335	Mali Republic	Mopti	Biré	4	1.981 ± 0.073
IV-10	Kansambara	GAT5342,5343	Mali Republic	Kénieba	Kénieba	4	1.991 ± 0.033
II-9	Prépéazo	GAT5291-5295	Mali Republic	Kénieba	Kénieba	4	1.994 ± 0.022
IV-5	Dierry	GAT5349,5350	Mali Republic	-	Unknown	4	2.007 ± 0.021
IV-15	Pon - Madongon	GAT5330,5331	Mali Republic	Mopti	Madongon	4	2.025 ± 0.072
IV-9	Prépéazo 2	GAT5344,5345	Mali Republic	-	Unknown	4	2.031 ± 0.048
TAB 92a	Naman	GAT5481	Benin	Boukoumbé	Near Nadoba (Togo)	4	1.886 ± 0.014
BEN 38	Ipoya	—	Benin	Natitingou	Moupémou	1	1.925
BEN 34	Tontonga	GAT5427	Benin	Boukoumbé	Koutcheta	4	1.926 ± 0.063
BEN 21	Ipodapiah	GAT5430,5431	Benin	Boukoumbé	Koutchatahongou	4	1.941 ± 0.040
BEN 01	Ikantoni	—	Benin	Boukoumbé	Kouya	1	1.946
BEN 30	Ipodawon	GAT5428	Benin	Boukoumbé	Kountchéhégou	4	1,948 ± 0.032
BEN 49	Ipoaga	—	Benin	Natitingou	Kouaba	4	1.950 ± 0.038
BEN 48	Afiyo	GAT5404,5405	Benin	Copargo	Koutchanti	4	1.951 ± 0.021
BEN 32	Iponi	—	Benin	Boukoumbé	Koutchatahongou	1	1.956
BEN 39a	Pei (precocious)	GAT5417,5418	Benin	Natitingou	Kotopounga	4	1.958 ± 0.042
BEN 43	Poigui	—	Benin	Tanguiéta	Hantanguéri	4	1.961 ± 0.036
BEN 13	Kpatinafa	GAT5436	Benin	Boukoumbé	Kouya	4	1.962 ± 0.015
BEN 16	Ikoumga	GAT5433,5434	Benin	Boukoumbé	Kountchougou	4	1.962 ± 0.055
BEN 103	Tamaou	—	Benin	Boukoumbé	Korontière	1	1.966
BEN 08	Tentenga	GAT5441,5442	Benin	Boukoumbé	Kountchougou	4	1.973 ± 0.062
BEN 15	Ipoda	—	Benin	Boukoumbé	Koutchagou	4	1.974 ± 0.031
BEN 05	Ipomoan	GAT5443,5444	Benin	Boukoumbé	Kouya	4	1.975 ± 0.024
BEN 40	Ipohaga (Ipoaga)	—	Benin	Natitingou	Tigniti	4	1.978 ± 0.056
BEN 22	Tentepera	GAT5429	Benin	Boukoumbé	Kountchougou	4	1.980 ± 0.014
BEN 11	Dipodawon	GAT5437,5438	Benin	Boukoumbé	Kounacogou	4	1,985 ± 0.037
BEN 09	Ipodapieh	GAT5439,5440	Benin	Boukoumbé	Kodogou	4	1.995 ± 0.021
BEN 110	Iponouda	—	Benin	Boukoumbé	Koussétiengou	1	1.995
BEN 47	Cafera	—	Benin	Copargo	Koutchanti	4	1.997 ± 0.037
BEN 03	Tontonga	GAT5445,5446	Benin	Boukoumbé	Koudogou	4	1.998 ± 0.067

Table 1. (Continued)

Acc. N°	Local name	Voucher	Origin			n	2C DNA content (pg)
			Country	District	Coll. site		Mean ± SD
TKD 58	Djibiga	GAT5399	Togo	Doufelgou	Koré	4	1.833 ± 0.012
TKD 60	Fig'm	GAT5393,5394	Togo	Doufelgou	Baga	2	1.858 ± 0.004
TKD 75	Sèmbre	GAT5387	Togo	Doufelgou	Broukou	4	1.868 ± 0.032
TKK 85	Sèmbre	GAT5489,5490	Togo	Kéran	Ataloté	4	1.871 ± 0.011
TKD 59	Namba	GAT5395-5398	Togo	Doufelgou	Koré	4	1.887 ± 0.037
TSO 88	Ounfissa	GAT5491-5494	Togo	Oti	Gando-Djèbouri	4	1.892 ± 0.075
TKD 62	Tchabigô	GAT5390	Togo	Doufelgou	Amadi-Paha	4	1.896 ± 0.043
TKB 72	Fôlôm	—	Togo	Bassar	Koundoum	4	1.898 ± 0.035
TKB 74	Sèmbre	GAT5363-5365	Togo	Bassar	Didoudikpre	4	1.900 ± 0.042
TPA 26	Trikpa	GAT5499-5501	Togo	Amou	Mouna	4	1.901 ± 0.058
TKK 83	Ayôrô	GAT5487,5488	Togo	Kéran	Adjéidé	4	1.902 ± 0.071
TSO 86	Ounvonikpa	GAT5495,5496	Togo	Oti	Okparobôsso	1	1.902
TKD 61	Lanfig'm	GAT5391,5392	Togo	Doufelgou	Baga	4	1.907 ± 0.049
TKD 89a	Tchapionga	GAT5371,5372	Togo	Doufelgou	Massédéna	3	1.915 ± 0.039
TPW 42	Ougniva	—	Togo	Wawa	èkèto	2	1.916 ± 0.050
TKD 81	Yôlôm	GAT5373,5374	Togo	Doufelgou	Kadjalla	4	1.920 ± 0.029
TPW 32	Egniva	—	Togo	Wawa	Klabé-Akpéganmè	4	1.937 ± 0.032
TKK 69	Iporlapiah	GAT5486	Togo	Kéran	Warango	4	1.962 ± 0.032
TKD 56	Fig'm	GAT5400,5401	Togo	Doufelgou	Koka	4	1.966 ± 0.052
TKK 66	Kopordagou	GAT5482,5483	Togo	Kéran	Bassamba	2	1.969 ± 0.039
TKK 70	Itamali*	—	Togo	Kéran	Nadoba	1	2,012
TPW 52	Oufakpôh	—	Togo	Wawa	Yalla	1	2.048
TPW 54	Trikpa	—	Togo	Wawa	Kabanyi	1	2.048
TPA 27	Ezio	—	Togo	Amou	Mouna	1	2.049
TKB 71	Kiwo	—	Togo	Bassar	Koundoum	1	2.050
TPA 38	Ova	—	Togo	Amou	Amoutsi	4	2.060 ± 0.02
TPW 41	Dikaba	—	Togo	Wawa	Ekèto	1	2.071
TPA 23	Vitchi	—	Togo	Amou	Ougbo-Ali	4	2.074 ± 0.01
TPW 29	Vafoo	—	Togo	Wawa	Klabé-Akpéganmè	1	2.074
TPW 50	Gnimimbi	—	Togo	Wawa	Vhé-Nkougna	1	2.093
BUF 64	Fii (Cfv 533)	GAT5506	Burkina Faso	Orodara	Samogohiri	4	1.878 ± 0.028
BUF 74	Fomou (Cfv 413)	—	Burkina Faso	Banfora	Toumousseni	4	1.921 ± 0.052
BUF 69	Foni Femba	GAT5503	Burkina Faso	Nouna	Soin	4	1.942 ± 0.067

BUF 56	Foni (Cfv 453)	GAT5510	Burkina Faso	Nouna	Kouro	4	1.949
BUF 57	Pongwé (Cfv 411)	GAT5508,5509	Burkina Faso	Tougan	Séné	4	1.936 ± 0.041
BUF 65	Péri Maoulè	—	Burkina Faso	Nouna	Komanbira	1	1.957
BUF 66	Foni Maloulè	—	Burkina Faso	Nouna	Soin	1	1.960
IV-18	Feningué	GAT5324,5325	Burkina Faso	Orodara	Ouéléni	1	1.989
IV-19	CVF 107	GAT5323	Burkina Faso	-	Unknown	1	2.001
IV-16	Fonibâ	GAT5327-5329	Burkina Faso	Sideradougou	Degué	1	2.014
IV-17	Peri	GAT5326	Burkina Faso	Nouna	Towkorowi	1	2.024
BUF 70	Pogwôn	—	Burkina Faso	Titao	Ban	1	2.030
BUF 71	Peri	—	Burkina Faso	Nouna	Sanaba	1	2.045
BUF 67	Kiyu	—	Burkina Faso	Tibo	Fulse	1	2.080
D. iburua							
BEN 36b*	Péi (long cycle)	GAT5423-5426	Benin	Natitingou	Koudengou	4	1.792 ± 0.094
BEN 39b*	Péi (long cycle)	GAT5423-5426	Benin	Natitingou	Kotopounga	4	1.749 ± 0.054
BEN 40b*	Ipoaga	GAT5408-5411	Benin	Natitingou	Tigniti	4	1.836 ± 0.079
TKD 75b	-	GAT5380-5386	Togo	Doufelgou	Broukou	2	1.964 ± 0.041
TKD 63b	Tchibam	GAT5388,5389	Togo	Doufelgou	Défalé	2	2.002 ± 0.031
TKD 89b	-	GAT5367-5370	Togo	Doufelgou	Massédéna	4	1.856 ± 0.086
D. ternata							
BEN 36c*	Wild type	GAT5419-5422	Benin	Natitingou	Koudengou	4	1.775 ± 0.070
D. longiflora							
TAB 92b	Wild type	GAT5479,5480	Benin /Togo	-	Nadoba border	4	1.870 ± 0.035
D. ciliaris							
MAL 01	Wild type	GAT5523-5529	Rep. Mali	Sikasso	Niéno	4	2.472 ± 0.063(A) **
GUI 02	Wild type	GAT5511-5516	Guinea	Tougué	Kolé	4	2.593 ± 0.035(A) **
TKD 75c*	Wild type	GAT5378,5379	Togo	Doufelgou	Broukou	1	2.929 (B)**
D. lecardii							
SMB 06*	Wild type	GAT5456-5458	Benin	Matéri	Pingou	4	2.504 ± 0.172
STB 02	Wild type	GAT5459-5463	Benin	Toukountouna	Kouba	4	2.787 ± 0.036
SCB 08	Wild type	GAT5450,5451	Benin	Cobly	Touga	1	2.785

*indicates *Raphanus sativus* as internal standard, *Glycine max* was used for the other genotypes, **Scheffé's grouping in *D. ciliaris*; homogeneity was observed within the other species with more than one accession

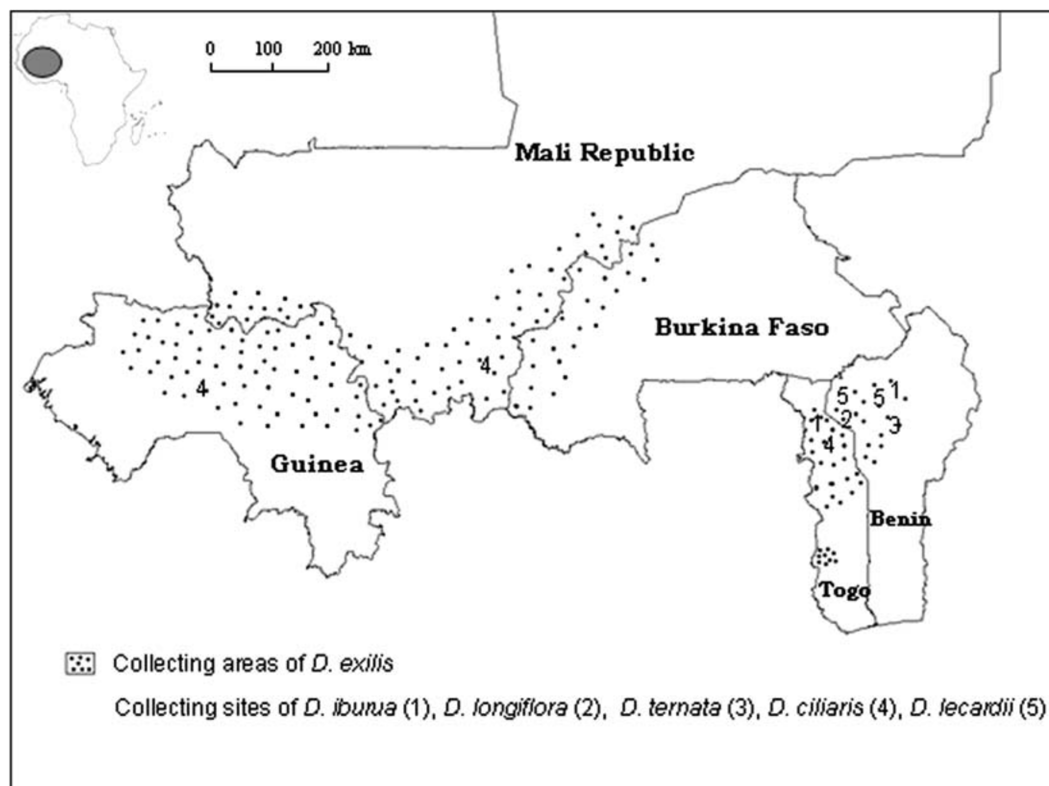

Fig. 1. Collecting areas/sites of fonio landraces and wild species in Benin, Burkina Faso, Guinea, Mali and Togo

In cultivated species, the nuclear DNA contents of *D. exilis* and *D. iburua* landraces were found to be very similar. In white fonio (*D. exilis*), the lowest mean 2C DNA content (1.660 pg) was documented for landrace M'balia 2 (III-3) collected from the Fouta-Djallon highlands in Guinea while the highest (2.093 pg) was observed in Gnimimbi (TPW 50), a landrace cultivated by the Akébou tribe in southern Togo. The DNA content of the six black fonio (*D. iburua*) accessions ranged from 1.792 pg in landrace Péi cultivated by Wama farmers in Benin to 2.002 pg in landrace Tchibam especially cultivated by Lamba tribe in northern Togo for brewing local beer (Adoukonou-Sagbadja et al. 2006). The overall average DNA content calculated for white fonio was 1.956 ± 0.004 pg while in black fonio a slightly lower average (1.848 ± 0.031 pg) was observed. The present results corroborate some morpho-botanical resemblance reported between the two cultivated fonio millets (Portères 1975, Haq and Ogbe 1985). However, evidence of genetic differentiation of the two species has been proven by molecular markers such as RAPDs (Hilu et al. 1997) and AFLPs (Adoukonou-Sagbadja, unpubl. res.).

Among the wild species, the nuclear DNA contents of *D. longiflora* and *D. ternata* were found very close to those of cultivated fonio species. In fact, mean 2C DNA content values of 1.869 ± 0.035 pg and 1.775 ± 0.070 pg, respectively, were observed in these two wild species that are far the most cited by local farmers as wild fonio types due to their high morphological resemblance with cultivated fonio. Botanically, *D. longiflora* and *D. ternata* resemble effectively in many ways white and black fonio,

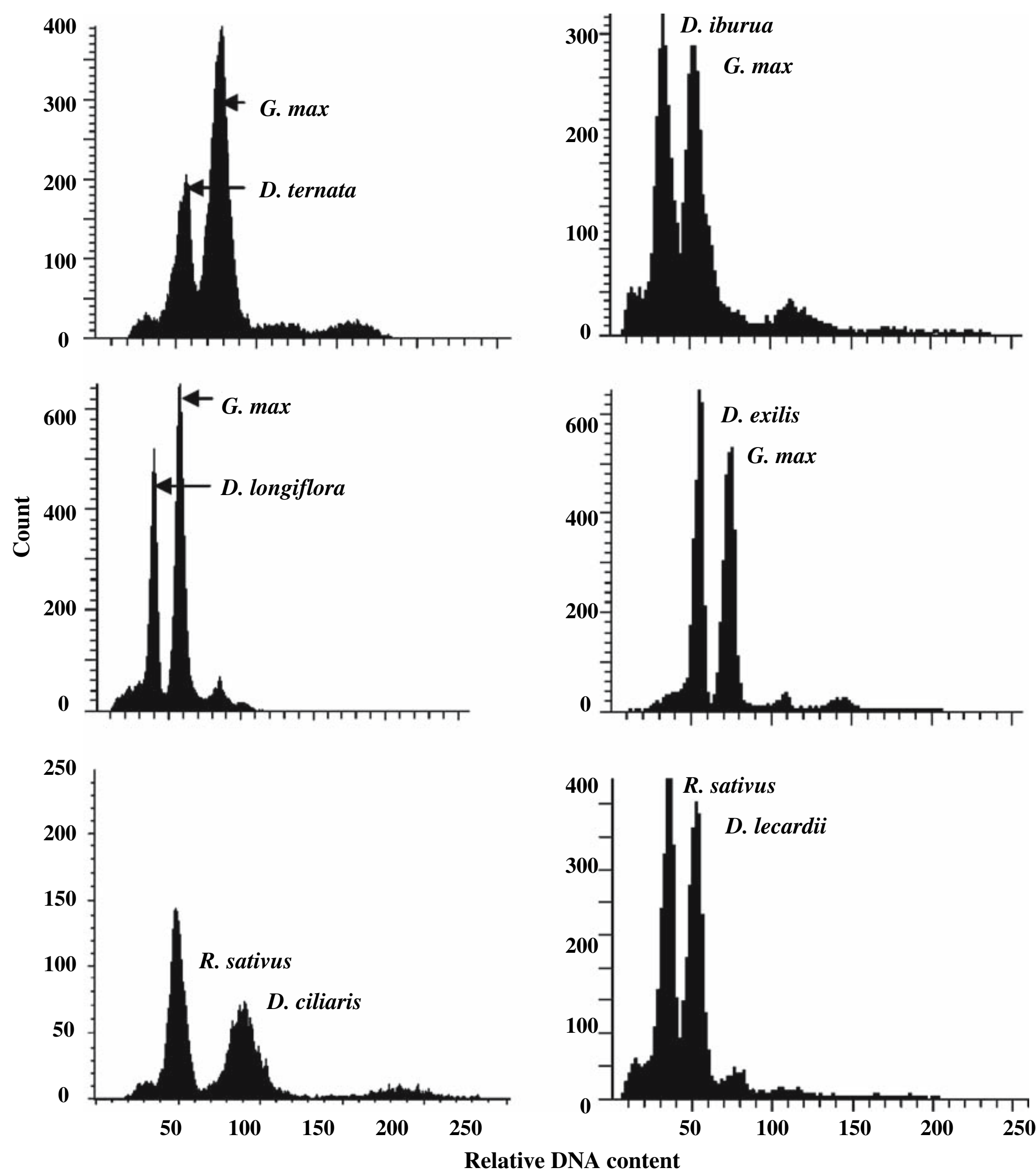

Fig. 2. Relative DNA content of different cultivated fonio and wild species in comparison to the reference plants *Glycine max* or *Raphanus sativus*

respectively, and were proposed by many scientists to be their probable progenitor(s) (Stapf 1915, Portères 1976). The relationships between *D. longiflora* and white fonio on the one hand and *D. ternata* with black fonio on the other were confirmed genetically by molecular studies using RAPD markers (Hilu et al. 1997). The convergence of their genome sizes with those of cultivated fonio millets, as arisen from this study, seems to support the trends on their ancestral relationships but does not agree with their classification in different taxonomic sections: *D. longiflora* and *D. ternata* in Verrucipilae and Clavipilae, respectively, but fonio species in Atrofuscae (Henrard 1950). This finding argues for the need for taxonomic revision and more emphasis on species relationships in the genus *Digitaria*, as

Table 2. Scheffé's grouping based on the general average 2C nuclear DNA content and calculated genome size (1C) of the cultivated and wild *Digitaria* species.

Species	Status	No. acc.[a]	n	2C nuclear DNA content (pg)			1C genome size (Mbp)*
				Mean Range	Average[b] ±SE	Scheffé $\alpha = 0.05$	
D. exilis	Cultivated	112 (92)	372	1.660 − 2.093	1.956 ± 0.004	B	956
D. iburua	Cultivated	6 (2)	18	1.792 − 2.002	1.848 ± 0.031	BC	904
D. longiflora	Wild	1	4	-	1.869 ± 0.035	BC	914
D. ternata	Wild	1	4	-	1.775 ± 0.070	C	868
D. ciliaris	Wild	3	9	2.472 − 2.929	2.576 ± 0.030	A	1260
D. lecardii	Wild	3	9	2.504 − 2.787	2.660 ± 0.070	A	1301

[a]Number of accessions investigated; in bracket, number of farmer-named fonio landraces used; n = number of measurements; [b]Average over all measurements (SE = Standard error); *conversion factor of 978 Mbp for 1pg of DNA (Doležel et al. 2003)

Fig. 3. Characteristics of the spikelets of the six *Digitaria* species investigated: spikelets with lower lemma (left) and upper glume and upper lemma (right); **1** *D. ternata* (BEN 36 c), **2** *D. iburua* (BEN 40 b), **3** *D. longiflora* (TAB 92 b), **4** *D. exilis* (TKD 56), **5** *D. lecardii* (STB 02), and **6** *D. ciliaris* (TKD 75 c) (Photo H. Ernst)

has been suggested by Haq and Ogbe (1995). Although the difference observed in the average DNA amounts of these four species was slight, it is nonetheless significant ($p<0.05$), indicating that genomic structure variability may be expected among them.

Conversely, the nuclear DNA amounts of *D. ciliaris* and *D. lecardii* were 1.3–1.4 fold higher (significant at $p<0.001$) than that observed for cultivated species and their closely related species. In fact, the overall average nuclear DNA content in *D. ciliaris* was 2.576 ± 0.030 pg while in *D. lecardii*, a value of 2.660 ± 0.070 pg was observed. In contrast to the first two wild species, important botanical divergences of *D. ciliaris* and *D. lecardii* with the cultivated species were reported by Henrard (1950). The differences observed in their genome sizes with fonio millets could then be explained by their phylogenetic distance and highly support their botanical classification in other sections of the genus *Digitaria*. These findings suggest a relationship between genome size and taxonomic distance in the genus, as was earlier reported for many other plant genera such as *Lactuca* (Koopman 2000, Doležalová et al. 2002) and *Pinus* (Hall et al. 2000).

Intra-specific DNA content variations detected in the species with more than one accession were found slight and insignificant, except in *D. ciliaris*. In this species, the statistically significant variation obtained was revealed by Scheffé test to be only due to a comparatively high 2C DNA content (2.929 pg, table 1) detected in the single plant available for analysis in the accession TKD 75c. Minor variations could be attributed to the experimental procedure (day to day variation, use of two different internal standards). But, heterochromatin polymorphisms, chromatin deletions or duplications known to induce small but significant DNA content differences among genotypes (Laurie and

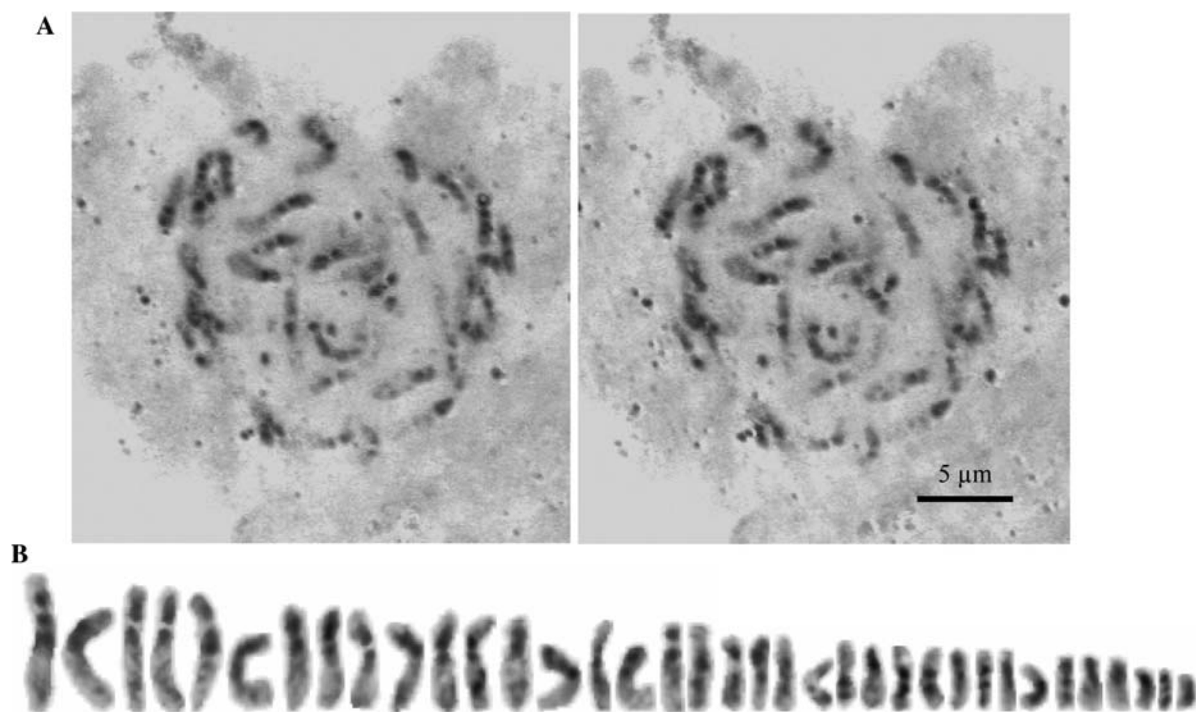

Fig. 4. *D. exillis* chromosomes (2n = 36) of landrace Iporlapieh (Ben21). **A** Spatial somatic metaphase cell (stereo pair can be observed with prism glasses or without glasses at a distance of about 30 cm); **B** Karyogram established from the same cell

Bennett 1985, Greilhuber 2005) could be responsible of the deviating 2C DNA observed in *D. ciliaris*. Although significant intra-specific differences have been reported within other grass species (Murray 2005), the present findings suggest genome size stability in the investigated species, mainly in the cultivated gene pool. Genome size uniformity has been prior proven in many crops such as soybean (Greilhuber and Obermayer 1997) and groundnut (Temsch and Greilhuber 2000). This is in line with a relatively low molecular genetic diversity observed in the cultivated species (Adoukonou-Sagbadja, unpubl. res.).

Fonio millets are grown through contrasting environments in the region and different farming systems (Portères 1976). In contrast to the results found for *Nerine* species (Zonneveld and Duncan 2006), in our study no significant correlation was identified between nuclear DNA content of fonio landraces and the climatic, agro-ecological features or geographic origins (altitude, latitude). These observations could be a consequence of low intra-specific variations detected in their 2C DNA content. Although accessions in *D. ciliaris* had also diverse origins, sample size is too small (only 3) for investigating adequately a possible correlation of 2C DNA variation with eco-geographic parameters. However, authors like Ohri and Pistrick (2001) had a more critical view on the ecological interpretation of genome-size variation in general.

As already reported for many grass genera (e.g. Avdulov 1931, Tuna et al. 2001), determination of chromosome number is difficult in fonio millets due to the small size of their chromosomes. In the investigated wild *Digitaria* species, chromosome counts were not possible since roots tips with good dividing cells were not found. The basic (haploid) chromosome number ($x = 9$) in the genus *Digitaria* and many other *Paniceae* has been reported first by Avdulov (1931) and confirmed by further studies (Hunter 1934, Wipff and Hatch 1994, Bennett et al. 2000). In the present study, $2n = 36$ chromosomes indicating tetraploidy were identified in somatic metaphase cells of *D. exilis,* as shown in Figure 4 established from landrace Iporlapieh (BEN21). The chromosomes are different in size and the mostly median centromeres are mainly surrounded by heterochromatin. Our findings are not in agreement with Hunter (1934) who reported hexaploidy ($2n = 6x = 54$) in *D. exilis*, but they partially support the report of Zeven and de Wet (1982) who suggested tetraploidy ($2n = 4x = 36$) with a possible existence of diploid forms. Although the germplasm analysed in the present study does not cover the whole fonio cultivation areas in West-Africa, it is considered to represent the majority of fonio landrace diversity that exists in the region since it was collected from the most important diversification centres (primary and secondary) where a broad genetic diversity is found (Portères 1976). If diploid and hexaploid types of fonio really exist, their occurrence may be low in comparison to the vast majority of tetraploids exclusively identified in our study. Just like white fonio, black fonio as well as the closely related species *D. longiflora* and *D. ternata* seem to be tetraploids. Expanding the investigations to landraces of Nigeria (another possible secondary centre), but also to minor producing countries like Senegal, Gambia, Côte d'Ivoire and Niger, could help to achieve a definitive conclusion on the existence of diploid or hexaploid fonio. Nonetheless, the present study offers a comparatively large and representative view on the ploidy level of fonio millets which is basic step towards the use of their landraces in fonio breeding programmes. Further investigations on the genomic composition of these crops, but also of the two closely related wild species, remain of practical interest.

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