Journal of Applied Biosciences 129: 12982 -12995

ISSN 1997-5902

Cross-species amplification of microsatellite loci developed for *Digitaria exilis* Stapf in related *Digitaria* species

Ablaye Ngom[1, 2, 3*], Mame Codou Gueye[4], Mathieu Gueye[5], Claire Billot[6,7], Caroline Calatayud[6,7], Baye Magatte Diop[4], Ndjido Ardo Kane[2, 3], Marie Piquet[3, 8], Yves Vigouroux[8], Leila Zekraoui[3, 8], Mame Samba Mbaye[1], Kandioura Noba[1], Adeline Barnaud[2, 3, 8]

[1] Laboratoire de Botanique et Biodiversité, Département de Biologie Végétale, Université Cheikh Anta Diop, Sénégal.
[2] Laboratoire National de Recherches sur les Productions Végétales, Institut Sénégalais de Recherches Agricoles, Sénégal.
[3] Laboratoire mixte international Adaptation des Plantes et microorganismes associés aux Stress Environnementaux (LMI LAPSE), Centre de recherche de Bel Air, Sénégal.
[4] Centre d'Études Régionales pour l'Amélioration de l'Adaptation à la Sécheresse, Institut Sénégalais de Recherches Agricoles, Sénégal.
[5] Département de Botanique et Géologie, Institut Fondamental d'Afrique Noire, Université Cheikh Anta Diop, Sénégal.
[6] CIRAD, UMR AGAP, F-34398 Montpellier, France.
[7] AGAP, Univ Montpellier, CIRAD, INRA, Montpellier SupAgro, Montpellier, France.
[8] DIADE, IRD, Univ Montpellier, Montpellier, France.
*Corresponding author : ablaye11.ngom@ucad.edu.sn

Original submitted in on 15th May 2018. Published online at www.m.elewa.org on 30th September 2018 http://dx.doi.org/10.4314/jab.v129i1.2

ABSTRACT

Objectives: Digitaria exilis Stapf (white fonio) is a staple crop in West Africa, mainly consumed during food shortage and highly associated to cultural events. The aim of this study was to test the transferability of microsatellite loci developed for *D. exilis* to other species of the genus *Digitaria* in order to further investigate the diversity of species for which there is little genomic resources available.

Methodology and Results : Eight (8) microsatellite loci on 119 accessions from 39 *Digitaria* species were tested. With an average amplification percentage of 62.8% ranging from 25% to 100 %, these SSR markers are transferable to other species of the genus *Digitaria*. The transferability was higher for *D. argyrothrica*, *D. fuscescens*, *D. longiflora*, *D. milanjiana* and *D. ternata*. In some species such as *D. abyssinica*, *D. nuda*, *D. pennata*, *D. pseudodiagonalis* and *D. tisserantii*, the very low percentage of cross-amplification confirmed their distance from *D. exilis*. *D. longiflora*, thought as the most related wild species to *D. exilis*, exhibited 100% level of transferable polymorphic loci.

Conclusion and application of findings : This set of markers will be useful for breeding and studying relationships between *Digitaria* species.

Keywords : Cross-species amplification, *Digitaria*, genetic diversity, microsatellite loci.

INTRODUCTION

Digitaria Haller comprises about 230 species distributed worldwide in tropical, subtropical, and temperate areas (Clayton *et al.*, 2006 ; Sánchez-Ken, 2012). It is the second largest genus after *Paspalum* L. in the tribe *Paniceae*. It includes forage species, minor cereals, turf plants, and soil binders, as well as some weeds (Watson and Dallwitz, 1992). In West Africa, only *D. exilis* (white fonio) (Figures 1, 2 and 3) and *D. iburua* (black fonio) are cultivated. Presenting all amino acids and low gluten content, seeds of *D. exilis* can be a strategic source for nutrition and food security. However, it is still neglected and underutilized in this region. Its wild related species represent a potentially important genetic reservoir of genes controlling natural resistance to biotic and abiotic stresses and other characters for improvement (Monteiro *et al.*, 2013). Despite the work of Vega *et al.* (2009), the relations between *Digitaria* species are still poorly explored. However, *D. longiflora* appears to be the closest relative of *D. exilis* (Hilu *et al.*, 1997; Adoukonou-Sagbadja, 2010). Genetically, the only studies carried on some species (*D. ciliaris*, *D. exilis*, *D. iburua*, *D. lecardii*, *D. longiflora*, *D. sanguinalis* and *D. ternata*) used Randomly Amplified Polymorphic DNA (Hilu *et al.*, 1997) and Amplified Fragment Length Polymorphism (Adoukonou-Sagbadja, 2010) technics. Recently, Simple Sequence Repeat (SSR) markers have been developed in *D. exilis* (Barnaud *et al.*, 2012). This type of highly polymorphic, co-dominant, and easy to score markers are abundant in most studied organisms (Oliveira *et al.*, 2006). They offer great potential for studies on genetic diversity (*e.g.* Allentoft *et al.*, 2009), gene flow between populations (*e.g.* Arens *et al.*, 2007) and parentage analyses (*e.g.* Conte *et al.*, 2011). The objective of this study was to test the transferability of some SSR loci to other species of the genus *Digitaria* in order to further investigate the diversity of species for which there is no genomic resources available.

Figure 1: Field of *Digitaria exilis* (Barnaud©IRD).

Figure 2: Leaf and racemes of *Digitaria exilis* (Piquet©IRD).

Figure 3: Mature grains of *Digitaria exilis* (Barnaud©IRD).

MATERIALS AND METHODS

An overall sample of 119 accessions was selected from thirty-nine *Digitaria* species. These accessions were acquired from collections of the Herbaria DAKAR and IFAN (Senegal), the CIRAD Herbarium (France) and from personal collections (fresh material) (Table 1). DNA from fresh material was extracted following the protocol of Doyle and Doyle (1987). For dry material, some modifications were performed (2% of sodium bisulphite in the lysis buffer). DNA were quantified on a spectrophotometer and diluted to a working concentration of 25 ng/µl. A set of eight SSR loci (Barnaud *et al.*, 2012) were selected based on their polymorphism (Table 2). Forward primers were tagged with a 5'-M13 universal sequence (Schuelke, 2000). PCR was conducted in a 10 µl final volume with a buffer (10X), MgCl2 (50 mM), dNTP (2 mM), forward primer (10 mM), reverse primer (10 mM), BSA (10 mg/ml), Taq (2 U/µl), DNA (3.5 ng) and H_20. PCR conditions were as follows: 4 min at 94°C, 10 *Touch down* cycles (94°C/30 s, 60°C down to 50°C per cycle allowing specific annealing/90 s, 72°C/30 s), followed by 30 classics cycles (94°C/30 s, 58°C /90 s, and 72°C / 30 s) and final extension for 10 min at 72°C. PCR products were run on an ABI Prism 3500 (Applied Biosystems) with GS600LIZ as size standart. Genotyping data were scored and checked using GeneMapper software (version 5., Applied Biosystems). Number of alleles per locus (Na), as well as amplification percentage and percentage of polymorphic loci for each species were calculated using GenAlEx version 6.5 (Peakall and Smouse, 2012).

Table 1: Accessions of *Digitaria* evaluated using *D. exilis* microsatellite primers.

Species	Extraction code	Country of origin	Collection number	Collection
Digitaria abyssinica (A. Rich) Stapf	CIRAD-76	Djibouti	60409 b	Herbarium CIRAD
Digitaria abyssinica (A. Rich) Stapf	CIRAD-63	Somalia	42457 b	Herbarium CIRAD
Digitaria acuminatissima Stapf	IF-Dacu1	Chad	1528	Herbarium IFAN
Digitaria acuminatissima Stapf	CIRAD-49	Mali	34716	Herbarium CIRAD
Digitaria acuminatissima Stapf	CIRAD-16	Niger	5879 bis	Herbarium CIRAD
Digitaria argillacea (Hitchc. et Chase) Fern.	CIRAD-72	Mali	56529	Herbarium CIRAD
Digitaria argillacea (Hitchc. et Chase) Fern.	Dk-Dlec1	Burkina Faso	5309	Herbarium DAKAR
Digitaria argillacea (Hitchc. et Chase) Fern.	Dk-Dlec2	Senegal	3230	Herbarium DAKAR
Digitaria argyrotricha (Andersson) Chiov.	CIRAD-102	Liberia	69864	Herbarium CIRAD
Digitaria aristulata (Steudel) Stapf	CIRAD-33	Senegal	15042	Herbarium CIRAD
Digitaria aristulata (Steudel) Stapf	IF-Dari1bis	Senegal	14301	Herbarium IFAN
Digitaria aristulata (Steudel) Stapf	IF-Dari2	Senegal	14865	Herbarium IFAN
Digitaria atrofuscae (Hack.) A. Camus	IF-Datr-2	Guinea	14667	Herbarium IFAN
Digitaria atrofuscae (Hack.) A. Camus	IF-Datr-1	Guinea	5390	Herbarium IFAN
Digitaria atrofuscae (Hack.) A. Camus	CIRAD-66	Ivory Coast	46608	Herbarium CIRAD
Digitaria atrofuscae (Hack.) A. Camus	CIRAD-20	Nigeria	8856	Herbarium CIRAD
Digitaria brazzae (Franch.) Stapf	CIRAD-68	Democratic Republic of Congo	54703	Herbarium CIRAD
Digitaria brazzae (Franch.) Stapf	IF-Dbra-1	Sierra - Leone	9784	Herbarium IFAN
Digitaria brazzae (Franch.) Stapf	CIRAD-90	Zimbabwe	67250	Herbarium CIRAD
Digitaria ciliaris (Retz.) Koel.	CIRAD-36	Netherlands Antilles	16567	Herbarium CIRAD
Digitaria ciliaris (Retz.) Koel.	S-Fs-I103	Senegal	-	Personal collection
Digitaria ciliaris (Retz.) Koel.	S-Fs-I107	Senegal	-	Personal collection
Digitaria ciliaris (Retz.) Koel.	S-Fs-I111	Senegal	-	Personal collection
Digitaria debilis (Desf.) Willd.	IF-Ddeb5	Ivory Coast	-	Herbarium IFAN
Digitaria debilis (Desf.) Willd.	CIRAD-67	Mali	50569 c	Herbarium CIRAD
Digitaria debilis (Desf.) Willd.	IF-Ddeb1	Senegal	2784	Herbarium IFAN
Digitaria delicatula Stapf	Dk-Ddel1	Senegal	16907	Herbarium DAKAR
Digitaria delicatula Stapf	Dk-Ddel2	Senegal	16898	Herbarium DAKAR
Digitaria delicatula Stapf	CIRAD-31	Senegal	13715	Herbarium CIRAD
Digitaria diagonalis (Nees) Stapf	IF-Ddiag5	Guinea	14538	Herbarium IFAN
Digitaria diagonalis (Nees) Stapf	CIRAD-11	Centrafrican Republic	3653	Herbarium CIRAD
Digitaria diagonalis (Nees) Stapf	CIRAD-89	Zimbabwe	67246	Herbarium CIRAD
Digitaria eriantha Steud.	IF-Deri-1	-	846	Herbarium IFAN

Digitaria eriantha Steud.	CIRAD-05	Madagascar	747	Herbarium CIRAD
Digitaria eriantha Steud.	CIRAD-86	Zimbabwe	66952	Herbarium CIRAD
Digitaria exilis (Kippist) Stapf	IF-Dex6	Guinea	2795	Herbarium IFAN
Digitaria exilis (Kippist) Stapf	IF-Dex9	Guinea	2796	Herbarium IFAN
Digitaria exilis (Kippist) Stapf	CIRAD-70	Mali	56061	Herbarium CIRAD
Digitaria exilis (Kippist) Stapf	IF-Dex5	Mali	2126	Herbarium IFAN
Digitaria exilis (Kippist) Stapf	CIRAD-30	Senegal	13501	Herbarium CIRAD
Digitaria exilis (Kippist) Stapf	CIRAD-38	Senegal	19408	Herbarium CIRAD
Digitaria fuscescens (Presl) Henr.	CIRAD-95	Guinea	67972	Herbarium CIRAD
Digitaria fuscescens (Presl) Henr.	CIRAD-21	Nigeria	8863	Herbarium CIRAD
Digitaria gayana (Kunth) Stapf	Dk-Dgay6	Burkina Faso	5311	Herbarium DAKAR
Digitaria gayana (Kunth) Stapf	CIRAD-18	Chad	5951	Herbarium CIRAD
Digitaria gayana (Kunth) Stapf	CIRAD-61	Ouganda	42454	Herbarium CIRAD
Digitaria gayana (Kunth) Stapf	Dk-Dgay9	Senegal	3428	Herbarium DAKAR
Digitaria gazensis Rendle	CIRAD-14	Chad	4711	Herbarium CIRAD
Digitaria gazensis Rendle	CIRAD-65	Malawi	42482	Herbarium CIRAD
Digitaria horizontalis Willd.	CIRAD-50	Ivory Coast	35500	Herbarium CIRAD
Digitaria horizontalis Willd.	CIRAD-22	Nigeria	9523 a	Herbarium CIRAD
Digitaria horizontalis Willd.	Dk-Dho5	Senegal	585	Herbarium DAKAR
Digitaria horizontalis Willd.	Dk-Dho6	Senegal	54	Herbarium DAKAR
Digitaria humbertii A. Camus	CIRAD-03	Madagascar	68	Herbarium CIRAD
Digitaria humbertii A. Camus	CIRAD-06	Madagascar	777	Herbarium CIRAD
Digitaria iburua Stapf	CIRAD-45	Nigeria	33509	Herbarium CIRAD
Digitaria lehmanniana Henrard	CIRAD-51	Bolivia	37513	Herbarium CIRAD
Digitaria lehmanniana Henrard	CIRAD-52	Bolivia	37536	Herbarium CIRAD
Digitaria leptorhachis (Pilger) Stapf	CIRAD-47	Ivory Coast	33942 a	Herbarium CIRAD
Digitaria leptorhachis (Pilger) Stapf	Dk-Dlep1	Senegal	5555	Herbarium DAKAR
Digitaria leptorhachis (Pilger) Stapf	IF-Dlep1	Senegal	1501	Herbarium IFAN
Digitaria leptorhachis (Pilger) Stapf	IF-Dlep4	Senegal	18131	Herbarium IFAN
Digitaria longiflora (Retz.) Pers.	IF-Dlon5	Chad	1906	Herbarium IFAN
Digitaria longiflora (Retz.) Pers.	CIRAD-19	Chad	6597	Herbarium CIRAD
Digitaria longiflora (Retz.) Pers.	CIRAD-96	Guinea	67996	Herbarium CIRAD
Digitaria longiflora (Retz.) Pers.	CIRAD-26	Ivory Coast	11404	Herbarium CIRAD
Digitaria longiflora (Retz.) Pers.	IF-Dlon11	Mali	6829	Herbarium IFAN
Digitaria longiflora (Retz.) Pers.	CIRAD-17	Niger	5886 bis a	Herbarium CIRAD

Digitaria longiflora (Retz.) Pers.	CIRAD-25	Centrafrican Republic	10807	Herbarium CIRAD
Digitaria longiflora (Retz.) Pers.	DK-Dlon-04	Senegal	17875	Herbarium DAKAR
Digitaria longiflora (Retz.) Pers.	Dk-Dgen1	Senegal	309	Herbarium DAKAR
Digitaria longiflora (Retz.) Pers.	Dk-Dgen2	Senegal	323	Herbarium DAKAR
Digitaria longiflora (Retz.) Pers.	Dk-Dlon5	Senegal	17858	Herbarium DAKAR
Digitaria longiflora (Retz.) Pers.	IF-Dlon20	Senegal	294	Herbarium IFAN
Digitaria longiflora (Retz.) Pers.	S-Fs-I46	Senegal	-	Personal collection
Digitaria longiflora (Retz.) Pers.	S-Fs-I48	Senegal	-	Personal collection
Digitaria longiflora (Retz.) Pers.	S-Fs-I52	Senegal	-	Personal collection
Digitaria longiflora (Retz.) Pers.	S-Fs-I68	Senegal	-	Personal collection
Digitaria longiflora (Retz.) Pers.	S-Fs-I81	Senegal	-	Personal collection
Digitaria longiflora (Retz.) Pers.	S-Fs-I88	Senegal	-	Personal collection
Digitaria longiflora (Retz.) Pers.	S-Fs-I89	Senegal	-	Personal collection
Digitaria longiflora (Retz.) Pers.	CIRAD-46	Zambia	33531	Herbarium CIRAD
Digitaria macroblephara (Hack.) Paoli	CIRAD-56	Ethiopia	38615	Herbarium CIRAD
Digitaria macroblephara (Hack.) Paoli	CIRAD-58	Ethiopia	39650 a	Herbarium CIRAD
Digitaria maitlandii Stapf & C.E. Hubb.	CIRAD-74	Burundi	56664	Herbarium CIRAD
Digitaria maitlandii Stapf & C.E. Hubb.	CIRAD-82	Zimbabwe	66947	Herbarium CIRAD
Digitaria maniculata Stapf	CIRAD-41	Chad	28379	Herbarium CIRAD
Digitaria maniculata Stapf	CIRAD-87	Zimbabwe	67109	Herbarium CIRAD
Digitaria milanjiana (Rendle) Stapf	CIRAD-93	Zimbabwe	67550	Herbarium CIRAD
Digitaria milanjiana (Rendle) Stapf	CIRAD-84	Zimbabwe	66949	Herbarium CIRAD
Digitaria nitens Rendle	IF-Dnit-1	Belgian Congo	4763	Herbarium IFAN
Digitaria nodosa Parl.	CIRAD-77	Djibouti	60512	Herbarium CIRAD
Digitaria nodosa Parl.	CIRAD-39	Saudi Arabia	21716	Herbarium CIRAD
Digitaria nuda Schumach	CIRAD-32	Niger	14095	Herbarium CIRAD
Digitaria nuda Schumach	IF-Dnud1	Senegal	239	Herbarium IFAN
Digitaria parodii Jacq.-Fél.	CIRAD-42	Chad	28520	Herbarium CIRAD
Digitaria pennata (Hochst.) T. Cooke	CIRAD-75	Djibouti	60287	Herbarium CIRAD
Digitaria pennata (Hochst.) T. Cooke	IF-Dpen-1	Ethiopia	853	Herbarium IFAN
Digitaria pennata (Hochst.) T. Cooke	CIRAD-54	Ethiopia	38488	Herbarium CIRAD
Digitaria perrottetii (Kunth) Stapf	CIRAD-59	Chad	41091	Herbarium CIRAD
Digitaria perrottetii (Kunth) Stapf	Dk-Dper1	Senegal	17900	Herbarium DAKAR
Digitaria perrottetii (Kunth) Stapf	Dk-Dper2	Senegal	10056	Herbarium DAKAR
Digitaria perrottetii (Kunth) Stapf	Dk-Dper3	Senegal	3623	Herbarium DAKAR

Digitaria pseudodiagonalis Chiov.	CIRAD-37	Cameroon	17666 bis	Herbarium CIRAD
Digitaria pseudodiagonalis Chiov.	CIRAD-44	Democratic Republic of Congo	33498	Herbarium CIRAD
Digitaria rivae (Chiov.) Stapf	CIRAD-57	Ethiopia	38822	Herbarium CIRAD
Digitaria rivae (Chiov.) Stapf	CIRAD-62	Ethiopia	42455	Herbarium CIRAD
Digitaria ternata (A. Rich.) Stapf	CIRAD-15	Cameroon	5354	Herbarium CIRAD
Digitaria ternata (A. Rich.) Stapf	CIRAD-08	Centrafrican Republic	3116	Herbarium CIRAD
Digitaria ternata (A. Rich.) Stapf	CIRAD-85	Zimbabwe	66950	Herbarium CIRAD
Digitaria ternata (A. Rich.) Stapf	CIRAD-92	Zimbabwe	67460	Herbarium CIRAD
Digitaria tisserantii Jacq.-Fél.	CIRAD-40	Chad	23144	Herbarium CIRAD
Digitaria tisserantii Jacq.-Fél.	CIRAD-43	Centrafrican Republic	30033	Herbarium CIRAD
Digitaria umfolozi D.W.Hall	CIRAD-04	Mali	132	Herbarium CIRAD
Digitaria umfolozi D.W.Hall	CIRAD-07	Mali	2941	Herbarium CIRAD
Digitaria velutina Beauv.	CIRAD-60	Democratic Republic of Congo	41357	Herbarium CIRAD
Digitaria velutina Beauv.	IF-Dvel-5	Sierra - Leone	200	Herbarium IFAN
Digitaria velutina Beauv.	CIRAD-91	Zimbabwe	67457	Herbarium CIRAD
Digitaria xanthotricha (Hack.) Stapf	CIRAD-09	Centrafrican Republic	3300 a	Herbarium CIRAD

Table 2: Characteristics of the 8 single amplification site SSR markers developed in *Digitaria exilis* (Barnaud *et al.*, 2012)

Locus	Forward primers (5'–3')	Reverse primers (5'–3')	Repeat motif	N_a	GenBank accession no.
De-07	TCATGGTGTTTCACTTAATCC	AAATAGATGCCAATCACACC	$(GT)_8$	2	JN587188
De-14	CGAGACCTGATTTGTTTAGC	CAAGTCTTTGATTTCCGTCT	$(TGCG)_3$	3	JN587195
De-17	GTAACGAACATCGGGTGA	CTGATGGCAAGGATGTGT	$(GT)_6$	2	JN587198
De-24	CCTCGATAATGCGTTTGT	CAGCATTTTAATTGTTCACG	$(CT)_{18}$	5	JN587205
De-34	ACTAACAACCAGCGGTGA	CTAGCAGTGTTTCAATGTGC	$(AC)_{11}$	3	JN587215
De-36	GAAGACAGCCCATTGTTAGA	AGACATTGCCAAGAAAATTG	$(CA)_8$	6	JN587217
De-37	TGAACAAATTCCTCTTGCTC	TGGCAATGTTCCATAAAGA	$(TTC)_{29}$	22	JN587218
De-38	AAAACGAAAACCAAATCTCA	AGCCCAAGAAGTATTGCTAA	$(CA)_6$	3	JN587219

RESULTS

The amplification percentage of SSR loci per species ranged from 25% to 100% corresponding to a mean amplification of 63% and among the 8 SSR loci, six (De-07, De-17, De-24, De-36, De-37, De-38) exhibited over 55% of transferability in *Digitaria* accessions (Table 3). *D. argyrothrica*, *D. fuscescens*, *D. longiflora*, *D. milanjiana* and *D. ternata* revealed the highest observed transferability (100%), while *D. abyssinica*, *D. nuda*, *D. pennata*, *D. pseudodiagonalis* and *D. tisserantii* showed the smallest transferability (25%). A total of 107 alleles were found in 119 individuals studied. The number of detected alleles varied considerably depending on the loci. Indeed, the largest number (27) was found at locus De-37 while only seven were found at loci De-14 and De-36. The average number of alleles per locus is 13.38. The percentage of polymorphic loci varied from 0 to 100% with an average of 24.36% (Table 4). *D. longiflora* presented the highest level of polymorphic loci (100%) followed by *D. ciliaris* and *D. ternata* with 75% polymorphism each. *D. fuscescens* and *D. xanthotricha* showed 62.5% polymorphism while 50% of SSR loci were polymorphic in *D. exilis*, *D. gazensis*, *D. leptorhachis* and *D. milanjiana*.

Table 3: Cross-species amplification of height microsatellite loci in thirty-nine *Digitaria* taxa.

Species	Number of specimens	Loci								
		De-07	De-14	De-17	De-24	De-34	De-36	De-37	De-38	Amplification loci (%)
D. abyssinica	2	-	-	+	-	-	-	+	-	25%
D. acuminatissima	3	+	-	+	-	+	+	-	+	62.5%
D. argillacea	3	+	+	+	+	-	+	+	+	87.5%
D. argyrothrica	1	+	+	+	+	+	+	+	+	100%
D. aristulata	3	+	-	+	+	+	+	-	+	75%
D. atrofusca	4	-	-	+	-	-	+	-	+	37.5%
D. brazzae	3	+	+	-	+	-	-	-	+	50%
D. ciliaris	4	+	+	+	+	-	+	+	+	87.5%
D. debilis	3	-	-	+	+	+	+	-	-	50%
D. delicatula	3	+	-	+	-	-	-	-	+	37.5%
D. diagonalis	3	+	-	+	+	+	+	+	-	75%
D. eriantha	3	+	+	+	-	-	+	-	+	62.5%
D. exilis	6	+	+	+	+	+	+	+	+	100%
D. fuscescens	2	+	+	+	+	+	+	+	+	100%
D. gayana	4	-	-	+	+	+	+	-	+	62.5%
D. gazensis	2	+	-	+	+	+	+	+	+	88%
D. horizontalis	4	-	-	+	-	+	+	-	+	50%
D. humbertii	2	+	+	+	+	-	-	+	+	75%
D. iburua	1	+	+	+	-	-	-	+	+	62.5%
D. lehmanniana	2	+	-	+	+	-	+	-	-	50%
D. leptorhachis	4	-	-	+	+	+	+	+	+	75%
D. longiflora	20	+	+	+	+	+	+	+	+	100%
D. macroblephara	2	-	+	+	-	-	+	-	+	50%
D. maitlandii	2	+	-	-	-	+	-	+	-	37.5%
D. maniculata	2	-	-	+	+	-	-	-	+	37.5%
D. milanjiana	2	+	+	+	+	+	+	+	+	100%
D. nitens	1	-	-	+	+	+	-	+	+	62.5%
D. nodosa	2	-	+	+	-	-	+	+	-	50%
D. nuda	2	-	-	+	-	-	-	-	+	25%
D. parodii	1	+	+	-	+	-	+	-	+	62.5%
D. pennata	3	-	-	+	-	-	-	+	-	25%

D. perrottetii	4	-	-	+	-	+	-	+	+	50%
D. pseudodiagonalis	2	-	-	+	+	-	-	-	-	25%
D. rivae	2	+	-	+	-	-	-	+	-	37.5%
D. ternata	4	+	+	+	+	+	+	+	+	100%
D. tisserantii	2	-	-	+	-	-	-	+	-	25%
D. umfolozi	2	-	+	+	+	-	+	+	+	75%
D. velutina	3	+	-	+	+	+	+	+	+	87.5%
D. xanthotricha	1	+	-	+	+	-	+	+	+	75%
Amplification loci (%)		58.97%	41.03%	92.31%	61.54%	46.15%	64.10%	64.10%	74.36%	

+ denotes successful amplification; - denotes no amplification.

Table 4: Allelic diversity per species of *Digitaria* for each microsatellite marker used.

Species	**Number of specimens**	**De-07**	**De-14**	**De-17**	**De-24**	**De-34**	**De-36**	**De-37**	**De-38**	**Polymorphism loci (%)**
D. abyssinica (Franch.) Stapf	2	-	-	218	-	-	-	204, 255	-	12.5%
D. acuminatissima Stapf	3	213	-	218	-	213	186	-	222, 243	12.5%
D. argillacea (Hitchc. et Chase) Fern.	3	211, 213	212	218	224	-	188	186, 252	222	25%
D. argyrothrica (Andersson) Chiov.	1	219, 227	220	218	248	209	186	177	236	12.5%
D. aristulata (Steudel) Stapf	3	213	-	202, 218	240	217	184	-	228, 236	25%
D. atrofusca (Hack.) A. Camus	4	-	-	218	-	-	180	-	216	0%
D. brazzae (Franch.) Stapf	3	213	214	-	224, 230	-	-	-	222, 224	25%
D. ciliaris (Retz.) Koeler	4	223, 225	220	218, 222, 228	220, 230	-	180, 186, 188, 190	159, 243	222, 228	75%
D. debilis (Desf.) Willd.	3	-	-	218	230	213	188, 190	-	-	12.5%
D. delicatula Stapf	3	213	-	210, 218	-	-	-	-	222	12.5%
D. diagonalis (Ness) Stapf	3	213	-	218	230, 232	217	188, 190	243	-	25%
D. eriantha Steud.	3	213, 223	220	218	-	-	188	-	222	12.5%
D. exilis (Kippist) Stapf	6	223	220, 224	218	224	215, 217	180, 188, 190	240, 243, 252, 258, 297	222	50%

D. fuscescens (Presl) Henr.	2	213, 219	220	208, 218	232	217	188, 190	219, 222	240, 243	62.5%
D. gayana (Kunth) A. Chev.	4	-	-	210, 218	220, 252	223	188	-	222	25%
D. gazensis Rendle	2	219, 223, 225	-	218, 222	214, 224	213	180, 186, 188	231	222	50%
D. horizontalis Willd.	4	-	-	218, 236	-	221, 223	180	258	222, 228	12.5%
D. humbertii A. Camus	2	223	220	218, 236	224	-	-	237	222	12.5%
D. iburua Stapf	1	223	220	218, 240	-	-	-	165	222	12.5%
D. lehmanniana Henrard	2	219	-	218	232, 244, 248	-	190	-	-	12.5%
D. leptorhachis (Pilg.) Stapf	4	-	-	218, 224	240	213, 217, 223	184, 190	222, 246, 252	224	50%
D. longiflora (Retz.) Pers.	20	213, 219, 221, 223, 225, 227, 231, 233, 243	214, 218, 220, 226	208, 210, 218, 220, 224	214, 224, 226, 230, 232, 234, 238, 240, 242, 254	209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 233	178, 180, 184, 186, 188, 190, 192	153, 159, 165, 177, 195, 210, 219, 222, 240, 273	222, 226, 228, 229, 231, 233, 236, 238, 240, 242, 243	100%
D. macroblephara (Hack.) Paoli	2	-	216	218	-	-	180	-	243	0%
D. maitlandii Stapf & C.E. Hubb.	2	213	-	-	-	207, 211, 213	-	297	-	12.5%
D. maniculata Stapf	2	-	-	218	230	-	-	-	222, 236	12.5%
D. milanjiana (Rendle) Stapf	2	213, 219, 227	220	218	248, 250	209	180, 186, 190	177	236, 243, 256	50%
D. nitens Rendle	1	-	-	214, 226	222	217	-	258	222	12.5%
D. nodosa Parl.	2	-	218	218	-	-	188	171, 174	-	12.5%

D. nuda Schumach.	2	-	-	218, 222	-	-	-	-	231	12.5%
D. parodii Jacq.-Fél.	1	219	216	-	232	-	180, 190	-	224	12.5%
D. pennata (Hochst.) T. Cooke	3	-	-	218	-	-	-	201, 213, 216, 219	-	12.5%
D. perrottetii (Kunth) Stapf	4	-	-	218, 236	-	217	-	195	222, 224, 229	25%
D. pseudodiagonalis Chiov.	2	-	-	218	240	-	-	-	-	0%
D. rivae (Chiov.) Stapf	2	213	-	218	-	-	-	162	-	0%
D. ternata (A. Richard) Stapf	4	213, 223	220	208, 218	222, 232, 246	223	186, 188, 190	165, 249	222, 226, 233, 240, 243	75%
D. tisserantii Jacq.-Fél.	2	-	-	218	-	-	-	204	-	0%
D. umfolozi D.W.Hall	2	-	220	218	222	-	192	210	224	0%
D. velutina (Forssk.) P. Beauv.	3	219	-	218	236	213	188	165, 255	228	12.5%
D. xanthotricha (Hack.) Stapf	1	213, 223	-	208, 218	222, 250	-	188	177, 222	222, 243	62.5%

DISCUSSION

The transferability of SSR markers developed from a species to another species generally decreases as the phylogenetic distance between species increases (Oliveira *et al.*, 2013). Thus, the very low percentage amplification noted in some species such as *D. abyssinica*, *D. nuda*, *D. pennata*, *D. pseudodiagonalis* and *D. tisserantii* suggested that they are distant from *D. exilis*. For some species such as *D. longiflora*, this study findings (amplification and polymorphism rates) suggested a close relationship with the cultivated *D. exilis*. Moreover, based on shared alleles (Table 4), new information is provided on the close relationships between *D. ciliaris*, *D. eriantha*, *D. fuscescens*, *D. gazensis*, *D. humbertii*, *D. iburua*, *D. longiflora*, *D. ternata*, *D. xanthotricha* and *D. exilis* compared to the studies of Hilu *et al.* (1997) and Adoukonou-Sagbadja (2010).

CONCLUSION

With an average of 62.83%, the SSRs used in this study are transferable to other species of the genus *Digitaria*. Therefore, the primary gene pool of the crop, mainly composed of *D. longiflora* considered as the wild progenitor of *D. exilis*, contains thus a valuable source of diversity that could be useful for its improvement. The development of other markers would be an important asset as it would not only increase our understanding of the relationships between the crop and wild relatives but also solve many problems related to the taxonomy of the genus *Digitaria* that is one of the most complex in the tribe Paniceae.

ACKNOWLEDGEMENTS

We are thankful to the West Africa Agricultural Productivity Program (WAAPP/PPAAO 2A) for funding research activities (Grant No CERA 58 ID 06) of A. Ngom. We also thank the curators of DAKAR, IFAN and CIRAD Herbaria who provided the plant materials.

REFERENCES

Adoukonou-Sagbadja, A.H. 2010. Genetic characterization of traditional fonio millets (*Digitaria exilis*, *D. iburua* Stapf) landraces from West Africa: Implications for conservation and breeding. PhD thesis, Justus-Liebig University, Giessen, Germany.

Allentoft, M.E., Siegismund, H.R., Briggs, L. and Andersen, L.W. 2009. Microsatellite analysis of the natterjack toad (*Bufo calamita*) in Denmark: populations are islands in a fragmented landscape. *Conservation Genetics* 10: 15-28.

Arens, P.F.P., van der Slui, T., van't Westende, W.P.C., Vosman, B., Vos, C.C. and Smulders, M.J.M. 2007. Genetic population differentiation and connectivity among fragmented Moor frog (*Rana arvalis*) populations in The Netherlands. *Landscape Ecology* 22: 1489-1500.

Barnaud, A., Vignes, H., Risterucci, A.M., Noyer, J.L., Pham, J.L., Blay, C., Buiron, M., Vigouroux, Y. and Billot, C. 2012. Development of nuclear microsatellite markers for the fonio, *Digitaria exilis* (Poaceae), an understudied West African cereal. *American Journal of Botany* 99: e105-e107.

Clayton, W.D., Vorontsova, M.S., Harman, K.T. and Williamson, H. 2006. GrassBase - The Online World Grass Flora. www.kew.org/data/grasses-db.html. [accessed 10 Nov. 2011].

Conte, M., Zucchi, M.I., Andrade, G.V., Souza, A.P. and Recco-Pimente, S.M. 2011. Study of closely related species within the *Physalaemus cuvieri* group (Anura): contribution of microsatellite markers. *Genetics and Molecular Research* 10 (3): 1434-1444.

Doyle, J.J. and Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11-15.

Hammer, Ø., Harper, D.A. and Ryan, P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4: 1-9.

Hilu, K.W., M'Ribu, K., Liang, H. and Mandelbaum, C. 1997. Fonio millets: Ethnobotany, genetic diversity and evolution. *South African Journal of Botany* 63: 185-190.

Monteiro, F., Romeiras, M.M., Batista, D. and Duarte, M.C. 2013. Biodiversity assessment of sugar beet species and its wild relatives: linking ecological data with new genetic approaches. *American Journal of Plant Sciences* 4: 21-34.

Oliveira, E.J., Pádua, J.G., Zucchi, M.I., Vencovsky, R. and Vieira, M.L.C. 2006. Origin, evolution and

genome distribution of microsatellites. *Genetics and Molecular Biology* 29: 294-307.

Oliveira, G.A., Pádua, J.G., Costa, J.L., Jesus, O.N., Carvalho, F.M. and Oliveira, E.J. 2013. Cross-species Amplification of Microsatellite Loci Developed for *Passiflora edulis* Sims. in Related *Passiflora* Species. *Brazilian Archives of Biology and Technology* 56: 785-792.

Peakall, R. and Smouse, P. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28: 2537-2539.

Sánchez-Ken, G. 2012. A synopsis of *Digitaria* (Paniceae, Panicoideae, Poaceae) in Mexico, including the new species *Digitaria michoacanensis*. *Acta Botanica Mexicana* 101: 127-149.

Schuelke, M. 2000. An economic method for the fluorescent labelling of PCR fragments. *Nature Biotechnology* 18: 233-234.

Vega, A.S., Rua, G.H., Fabbri, L.T. and Rúgolo de Agrasar, Z.E. 2009. A morphology-based cladistic analysis of *Digitaria* (Poaceae, Panicoideae, Paniceae). *Systematic Botany* 34: 312-323.

Watson, L. and Dallwitz, M. 1992. The grass genera of the world. CAB International, Wallingford, UK.