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Short Communication

EST-SSR development for *Digitaria exilis* and its relatives *D. iburua* and *D. longiflora* from transcriptome sequences

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Abstract

Digitaria exilis is an important indigenous cereal in West Africa. The first fonio reference transcriptome was released and became a key tool for developing new molecular markers contributing to a better understanding of its genetic diversity. A total of 126 new putative primer pairs were successfully designed in 37,327 unigenes from the *D. exilis* transcriptome. Thirty-seven primer pairs were randomly selected and tested for their ability to cross-amplify to related species. Clear amplification patterns were observed on 24 primer pairs. Of these, 71, 74 and 35% showed polymorphism in three species: *D. exilis*, *D. longiflora* and *D. iburua*. The transferability from *D. exilis* was 96% to *D. longiflora* and 71% to *D. iburua*. The new SSR markers confirmed the close genetic proximity of *D. exilis* with *D. longiflora* and its stronger genetic difference of *D. exilis* from *D. iburua*. These markers will be valuable for completing future knowledge on *Digitaria* evolutionary history, and for testing gene flows between related species.

Keywords: cross-amplification, *Digitaria*, diversity, EST-SSR, microsatellite, Poaceae

Introduction

The *Digitaria* genus in the Poaceae family comprises over 300 species. Of them, two species are cultivated as key components for food and nutritional security in western Africa: *Digitaria exilis* Stapf (white fonio) and *Digitaria iburua* Stapf (black fonio). *D. exilis* exhibits wide distribution from Senegal to Lake Chad, while *D. iburua* is restricted to Nigeria and Togo (Blench, 2012). Genomic data resources have been developed for *D. exilis*: 21 polymorphic microsatellites (Barnaud *et al.*, 2012, 2017),

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chloroplast SNPs (Scarcelli *et al.*, 2011), full chloroplast genome sequences (Mariac *et al.*, 2014) and recently transcriptomic sequences (Sarah *et al.*, 2016). The polymorphic SSRs currently available for white fonio are still insufficient for accurate genetic studies of the population. Moreover, *D. iburua* and wild relatives such as *D. longiflora*, which are potential sources of diversity and adaptation, have no genomic resources available. The recently published transcriptome for white fonio amounts to a unique library, which can be used for *in silico* microsatellite development. SSR markers generated in this way can be directly associated with traits and are more transferable between related species (Varshney *et al.*, 2005). Our study explored this asset to develop new SSR markers for *D. exilis* and provides the first set of SSR markers for its relatives.

Experimental

D. exilis transcriptome sequences (FASTA format, GFF3 format freely available at http://arcad-bioinformatics.southgreen.fr/), representing 37,327 unigenes, were screened for SSR presenting at least five repeats. A total of 159 SSRs were detected (one SSR per 219 kb). Based on a PCR Tm of 60°C, a primer size of 20 bp, and specific amplification size ranges (80–180, 180–280 and 280–380 bp), a total of 126 primer pairs were successfully designed using SAT software (SouthGreen platform, http://galaxy.southgreen.fr/galaxy/, Dereeper *et al.*, 2007). A subset of 37 was randomly selected, synthesized and tested on 44 accessions: 29 *D. exilis* from Benin, Burkina Faso, Mali, Guinea, Niger, Senegal and Togo (Clément and Leblanc, 1984), five *D. iburua* from Nigeria and 10 *D. longiflora* from Senegal (online Supplementary Table S1). Accessions were selected to provide a good representation of available genetic diversity (Barnaud and Billot, pers. comm.). Genomic DNA was extracted from fresh leaves as described by Barnaud *et al.* (2012). Forward primers were tailed with a 5′-M13 universal sequence [CACGACGTTGTAAAACGAC] (Schuelke, 2000) and the corresponding M13-tail primer was labelled with one of the four dyes FAM, NED, PET or VIC and added to the PCR mix. Accessions were genotyped as described in Barnaud *et al.* (2012). PCR products were run in a multiplexed ABI Prism 3500 and sized with GS600LIZ (regional genotyping platform, http://www.gptr-lr-genotypage.com). Genotyping data were checked and scored by three different persons using GeneMapper 5.0 software and generated a dataset of 100 alleles in the three species.

Using GenAlEx 6.5 (Peakall and Smouse, 2012) for markers showing a maximum of two alleles in genotypic profiles of individuals, we calculated observed heterozygosity (H_o), expected heterozygosity (H_e) and the inbreeding coefficient (F_{IS}) (online Supplementary Table S4). The distribution of genetic diversity within and between species was estimated through: (i) a principal component analysis performed using the package FactoMineR (Husson *et al.*, 2007) and FactoExtra (Kassambara and Mundt, 2017), (ii) a discriminant analysis of principal components (Jombart *et al.*, 2010) implemented in the adegenet package (Jombart, 2008), (iii) an analysis of molecular variance, carried out with GenAlEx 6.5 (Peakall and Smouse, 2012) and (iv) a neighbour-joining tree implemented in the ape package (Paradis *et al.*, 2004). All analyses were performed using R statistical software (R Core Team, 1995–2015).

Discussion

Of the 37 primers, 24 showed clear amplification profiles (Table 1). The observed amplification rates were 65% (24/37), 62% (23/37) and 46% (17/37) for *D. exilis*, *D. longiflora* and *D. iburua*, respectively. Transferability of the 24 *D. exilis* amplified loci to its relatives was estimated at 96% (23/24) and 71% (17/24) for *D. longiflora* and *D. iburua*, respectively. Polymorphism rates of 71% (17/24), 74% (17/23) and 35% (6/17) were observed in *D. exilis*, *D. longiflora* and *D. iburua*, respectively.

Depending on loci and species, genotypes exhibiting over two alleles is expected for putative tetraploid genomes (Adoukonou-Sagbadja *et al.*, 2007, online Supplementary Fig. S1, Tables S2 and S4). We found genotypes exhibiting over two alleles and a high level of observed heterozygosity (H_o) (online Supplementary Table S4). This is likely associated with duplicated SSR loci. Data were thus recorded as a binary data type (0 = absence, 1 = presence of allele, online Supplementary Table S3) as described by Roullier *et al.* (2011) for polyploid species.

Structure analyses were congruent and showed that the new markers were useful in distinguishing the three species (Fig. 1, online Supplementary Figs. S2 and S3). PCA1 (26.1%) separated *D. iburua* from *D. exilis* and *D. longiflora* and PCA2 (14.1%) clearly distinguished *D. exilis* from *D. longiflora*. This separation was confirmed by the AMOVA with 77% of genetic variance found between species (PhiPT = 0.77; $P < 0.001$). In spite of the relatively low geographical distribution of *D. longiflora* accessions, the level of genetic diversity observed was striking, as shown by the accession dispersion in Fig. 1. The organization of genetic diversity, the higher polymorphism, the transferability and the amplification rate observed for *D. exilis* and *D. longiflora* may suggest strong proximity between their genomes. This is in accordance with the assertion that *D. longiflora* is the wild progenitor parent of *D. exilis* (Adoukonou-Sagbadja *et al.*, 2007). The lower transferability of these EST-SSRs on *D. iburua* and the structure observed suggests that this species is more distant. As expected for EST-SSRs, a smaller number of alleles (1–11) were observed (online Supplementary Table S2) compared

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Table 1. Characteristics of 24 SSR markers designed from *Digitaria exilis*

Locus	Forward sequences (5′–3′)	%GC	Reverse sequences (5′–3′)	%GC	Repeat motif	Allele range	Ta
De-ARC 003	GTTGTGACCTGACTCGTCCA	55	GCTCTGCACTGGGAAGTTCT	55	(CCA)5	191–194	60–55
De-ARC 004	TTCAGCATATTCCCCTCAGC	50	AACTGGAAGTCCACATGGAAA	43	(CTG)6	134–140	60–55
De-ARC 005	TTCAGCATATTCCCCTCAGC	50	AGCCATACAGCAAGCACAAA	45	(CTG)6	221–230	60–55
De-ARC 007	CGTACTACGTGCGCAACATT	50	GCGTTGGTTCGTCTACCTCT	55	(GA)10 (CCAT)3	159–179	55–50/60–55
De-ARC 008	TACACTTGGGGCAAACATCA	45	ACTAGCGAAGCCATCCAGAA	50	(CTG)7	217–232	60–55
De-ARC 010	CAACCGTGGAGCGATAAGTA	50	GTGCTACGACACCTGCACAT	55	(GT)6 (GTGTAC)4	199–209	60–55
De-ARC 011	TCATCAATGCCAGATTTCCA	40	GGTGTGTTGGTCTGGGGATA	55	(AT)6	190–202	60–55
De-ARC 013	CCTTTTGCCACCATCATTCT	45	GAGGGCAATGAAGTGGACAT	50	(TCT)5	186–219	60–55
De-ARC 014	GGTGATACAACTGCAACGACA	48	ACCTTCTACCGCTCCTTCGT	55	(AC)6 (CCGTT)3 (CGGGCC)3	173–239	55–50
De-ARC 015	TCAGGTCTGGACACTTGAGC	55	ACGCGTCCAACATAACCATA	45	(CGC)5	174–217	55–50/60–55
De-ARC 016	GAACGCCTGGACATTCACAT	50	TGGTGGAGGAGCTAAGGATG	55	(CGA)5	210–224	60–55
De-ARC 017	ACACGGTGCTTCATGTACGA	50	CGATACAGCAGGGACGTGTA	55	(CT)6 (GTCA)3	217	60–55
De-ARC 018	TCTGCAAGAGCTGTCAATCG	50	TTGTGTGCCATCATTCACCT	45	(GA)6 (TCAA)3	300–308	60–55
De-ARC 019	TGCCAACCAGAAAACCATTT	40	TACCAGAGGAACAAGCAGCA	50	(GCA)5	154–169	60–55
De-ARC 021	CGCTCATACTCTGACCACCA	55	GCAGTTGAGTGAGGTCATGC	55	(GCC)5 (CCA)6	310–319	60–55
De-ARC 024	ACCGTCGTGCTCAGCTTTTA	50	AGGACCTGTCGTGAGCTACC	60	(CA)6 (CAGTA)4	168–198	55–50 /60–55
De-ARC 025	GCTGAACCAGATAGGGCAAC	55	GCTCTCTGCAGATGGTTGAA	50	(CCA)5	213–216	55–50/ 60–55
De-ARC 026	AAAAAGTTAAGCGCGCACAC	45	TCCCGTTATTTTGAAACACCA	38	(GC)6 (CACG)4	124–158	60–55
De-ARC 028	TAAACTTTGCCTTCCGTGGT	45	TTGGACCAAACAATCCAGGT	45	(TGG)6 (GTGCTG)3	200–224	55–50/60–55
De-ARC 029	AGGGTGGATCACTTCGTCAG	55	GCATGCAGTTGAATGCCTTA	45	(CTT)5 (ATTC)3	300–326	60–55
De-ARC 030	CAACAGGCAGCATGACCTAA	50	AGAGGGTTCATGTGGATTGG	50	(CTG)6	333	60–55
De-ARC 031	CCACGATGTGTTGAATCAGG	50	CTGAAGGAGATCCCCACTTG	55	(AGC)5	207–216	60–55
De-ARC 033	AGACTCGCTGAACAACCACA	50	CCGTGGTTGAACTTTGAAGG	50	(AACC)5	146–154	60–55
De-ARC 034	CCGCTTCAGGAGTCTGGTCT	60	GAGAAGAAGGCCCTGATTGA	50	(ACC)5	313–317	55–50/60–55

T_a, annealing temperature (°C).

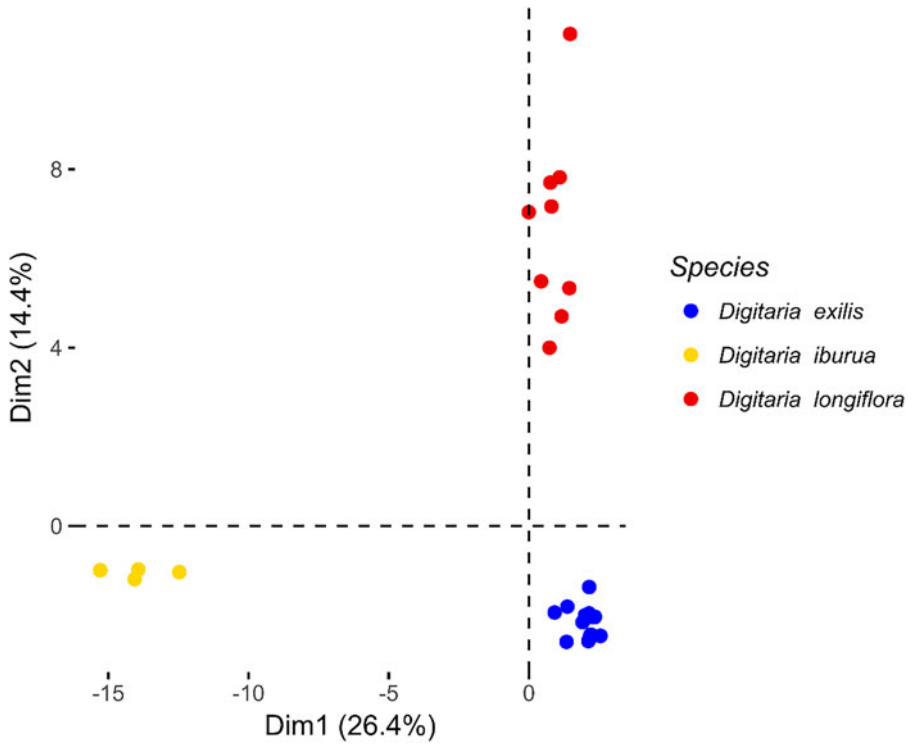


Fig. 1. Population structure based on PCA. The colours correspond to the different species: *D. exilis, D. longiflora, D. iburua*.

with those developed by Barnaud *et al*. (2012), who relied on genomic DNA. EST-SSRs show polymorphism within transcribed or expressed regions and their level of genetic variability might be more constrained.

This study provides fundamental information for implementing genetic diversity assessments, appropriate conservation plans and breeding programmes for *Digitaria* sp. genetic resources.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S1479262118000400

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