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Relationship between *Digitaria Exilis* Stapf and its Wild Relatives Based on Morphological and Genetic Approaches

Ablaye Ngom[1*], Mame Samba Mbaye[1], Oumar Diack[2], Madiop Gueye[1], Kandioura Noba[1]

[1]Laboratoire de Botanique et Biodiversité, Département de Biologie Végétale, Université Cheikh Anta Diop, Sénégal
[2]Centre d'Études Régional pour l'Amélioration de l'Adaptation à la Sécheresse (CERAAS), Institut Sénégalais de Recherches Agricoles, Sénégal

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***Corresponding author:** Ablaye Ngom

Abstract **Original Research Article**

White fonio (*Digitaria exilis* Stapf), a neglected crop in West African countries, is considered as a strategic source for nutrition and food security and a potential source to generate significant financial returns for farmers. Fonio has agronomic potentialities and adaptation to drought conditions that make it deserve special attention as well as its wild relatives which have interesting genetic traits for its improvement. The objective of this study was to evaluate the relationship between white fonio and its wild relatives based on agro-morphological traits and SSR markers. A total of 25 accessions belonging to 10 species and provided from the Herbaria of DAKAR and IFAN and from our collections were analyzed. Morphological and SSR similarity between species was calculated and the correlation between morphological and genetic variation was analyzed by the Mantel test. The results showed three main classes for each method used and a closely relationship between *D. exilis* and *D. longiflora* phenotypically and genetically compared to other studied *Digitaria* species. Mantel test revealed positive correlation between the two marker systems ($r = 0.39$, $p = 0.013$). Therefore, the techniques of molecular biology, such as the use of SSR markers, are effective tools to better understand genetic diversity within te genus *Digitaria*.

Keywords: Genetic diversity, phenotypic traits, SSR markers, *Digitaria longiflora*, fonio.

INTRODUCTION

White fonio (*Digitaria exilis* Stapf) is known for its importance in human nutrition due to its nutritional and therapeutic qualities that make this cereal a strategic source of nutrition [1-5]. These properties generate significant financial returns for farmers and, significantly, for women, who are traditionally involved in processing and marketing [6, 5]. It contributes to food security, a growing concern in West Africa where it is grown. Despite the importance of this crop to traditional agriculture in this region, research efforts to improve the crop are still inadequate. Consequently, the crop remains primitive facing diverse agronomical problems which, contribute notably to grain yield lost [7, 8]. Even so, its wild relatives which are a source of genes for adaptation and resistance can be used to increase the yields of its production through a process of improvement [9]. However, the relationship between fonio and its wild relatives is poorly known despite the existence of a genus Monograph [10] and several taxonomic studies [11-13]. Based on morphological characters [14], have made a significant contribution to understanding phylogenetic relationships within the genus. However, this study did not take into account this important cultivated species as well as wild species, considered until now, as the closest ones. Genetically, various molecular biology techniques such as the use of Randomly Amplified Polymorphic DNA (RAPD) [15], Amplifed Fragment Length Polymorphism (AFLP) [3], Inter-Simple Sequence Repeat (ISSR) [16] markers and cytological studies have allowed to understanding the relationships between cultivated and wild species in the genus *Digitaria*. But, the use of new methods such as SSR markers is valuable for a better understanding of their relationships. In fact, SSR markers are becoming the marker of choice for fingerprinting and genetic diversity studies for a wide range of plants [17]. Microsatellites markers are a powerful tool to quantify genetic diversity within crop species and genetic relationships among species because of their high polymorphism, abundance, and codominant inheritance [18]. By the way, [19] showed a high transferability of microsatellite loci developed for *D. exilis* to other wild for which there is little genomic resources available.

This study aims to evaluate the relationship between white fonio and its wild relatives on the basis of morphological and molecular characters, using SSR

markers, for more targeted use of wild genetic resources in fonio improvement and their better conservation.

MATERIALS AND METHODS

Morphological Characterization

In this study, a total of 27 accessions belonging to 10 *Digitaria* species was analyzed. Accessions are consisted of herbarium samples conserved in the Herbaria of DAKAR and IFAN (Senegal) and also fresh material from our collections (Table-1). Morphological data were obtained from direct study of specimens and, when information was not available, from literature sources [20, 21]. A total of 25 traits (Table-2) were scored including 7 vegetative traits, 15 from the inflorescence and 3 traits from the seed. All characters are recorded in a summary table (presence by 1 and absence by 0) comprising 56 modalities and the 10 species. The binary matrix obtained was used to calculate the morphological similarity matrix between species using Jaccard's coefficient [22], with PAST software, version 2.17c [23]. A dendrogram was generated from the similarity matrix by the unweighted pair-group method using arithmetic averages (UPGMA) [24] in XLSTAT software, version 2018.7 [25]. The cophenetic correlation coefficient was calculated in order to estimate how well the dendrogram represents its corresponding similarity matrix. Correlation coefficient was used to estimate the genetic distance between species.

Table-1: Accessions of *Digitaria* used for morphological and molecular characterization

Species	Extraction code	Country of origin	Collection number	Herbarium/ Collection
Digitaria acuminatissima Stapf**	CIRAD-16	Niger	5879 bis	CIRAD
Digitaria acuminatissima Stapf**	CIRAD-49	Mali	34716	CIRAD
Digitaria acuminatissima Stapf	IF-Dacu1	Chad	1528	IFAN
Digitaria acuminatissima Stapf*	IF-Dacu2	-	-	IFAN
Digitaria aristulata Stapf**	CIRAD-33	Senegal	15042	CIRAD
Digitaria aristulata Stapf	IF-Dari1bis	Senegal	14301	IFAN
Digitaria aristulata Stapf	IF-Dari2	Senegal	14865	IFAN
Digitaria ciliaris Koel.	S-Fs-I103	Senegal	-	Personal collection
Digitaria ciliaris Koel.	S-Fs-I107	Senegal	-	Personal collection
Digitaria ciliaris Koel.	S-Fs-I113	Senegal	-	Personal collection
Digitaria delicatula Stapf**	CIRAD-31	Senegal	13715	CIRAD
Digitaria delicatula Stapf	Dk-Ddel1	Senegal	16907	DAKAR
Digitaria delicatula Stapf	Dk-Ddel2	Senegal	16898	DAKAR
Digitaria delicatula Stapf*	Dk-Ddel3	Senegal	16951	DAKAR
Digitaria exilis Stapf**	CIRAD-30	Senegal	13501	CIRAD
Digitaria exilis Stapf**	CIRAD-38	Senegal	19408	CIRAD
Digitaria exilis Stapf*	IF-Dex5	Mali	2126	IFAN
Digitaria exilis Stapf*	IF-Dex6	Guinea	2795	IFAN
Digitaria exilis Stapf	IF-Dex9	Guinea	2796	IFAN
Digitaria horizontalis Willd.**	CIRAD-22	Nigeria	9523 a	CIRAD
Digitaria horizontalis Willd.**	CIRAD-50	Ivory Coast	35500	CIRAD
Digitaria horizontalis Willd.	Dk-Dho3	Senegal	584	DAKAR
Digitaria horizontalis Willd.*	DK-Dho5	Senegal	285	DAKAR
Digitaria horizontalis Willd.*	IF-Dho3	Senegal	283	IFAN
Digitaria longiflora Pers.**	CIRAD-26	Ivory Coast	11404	CIRAD
Digitaria longiflora Pers.*	DK-Dlon5	Senegal	17858	DAKAR
Digitaria longiflora Pers.	IF-Dlon11	Mali	6829	IFAN
Digitaria longiflora Pers.	S-Fs-I46	Senegal	-	Personal collection
Digitaria perrottetii Stapf	Dk-Dper1	Senegal	17900	DAKAR
Digitaria perrottetii Stapf	Dk-Dper2	Senegal	10056	DAKAR
Digitaria perrottetii Stapf	Dk-Dper3	Senegal	3623	DAKAR
Digitaria ternata Stapf**	CIRAD-15	Cameroon	5354	CIRAD
Digitaria ternata Stapf**	CIRAD-85	Zimbabwe	66950	CIRAD
Digitaria ternata Stapf**	CIRAD-92	Zimbabwe	67460	CIRAD
Digitaria ternata Stapf*	DK-Dter1	Senegal	2293	DAKAR
Digitaria ternata Stapf*	IF-Dter1	Mali	15005	IFAN
Digitaria velutina Beauv.**	CIRAD-60	Democratic Republic of Congo	41357	CIRAD
Digitaria velutina Beauv.**	CIRAD-91	Zimbabwe	67457	CIRAD
Digitaria velutina Beauv.*	IF-Dvel-4	Senegal	171	IFAN
Digitaria velutina Beauv.	IF-Dvel-5	Sierra - Leone	200	IFAN
Digitaria velutina Beauv.*	IF-Dvel-6	Senegal	244	IFAN

* *Accessions not used for the molecular characterization*

** *Accessions not used for the morphological characterization*

Table-2: Morphological traits and their descriptions used for Digitaria' species characterization

Traits	Description
Stubble height	< 100 cm; x ≥ 100 cm
Stubble nodes pubescence	glabrous; hairy
Stubble internodes pubescence	glabrous; hairy
Sheath pubescence	glabrous; hairy
Ligule height	between 0 and 1.5 mm; > 1.5 mm
Ligule pubescence	glabrous; hairy
Limb pubescence	glabrous; hairy
Racemes disposition	digitate; subdigitate; whorled
Racemes rhachis	triquetrous winged; triquetrous not winged
Spikelets grouping	by 2; by 3; by 4
Spikelets pubescence	glabrous; hairy
Spikelets' hair type	appressed; clavate; verrucose
Pedicels roughness	scabrous; smooth
Pedicels pubescence	glabrous; hairy
Lower glume presence	absent; present
Relative length of upper glume	shorter than the spikelet; equal to the spikelet
Number of veins of the upper glume	3-veined; 5-veined
Upper glume pubescence	glabrous; hairy
Relative length of the lower lemma	shorter than the spikelet; equal to the spikelet
Number of veins of the lower lemma	5-veined; 7-veined; 9-veined
Lower lemma pubescence	glabrous; hairy
Relative length of the upper lemma	shorter than the spikelet; equal to the spikelet
Fruit shape	elliptic to linear; oblong; egg-shaped
Fruit length	≤ 1 mm; between 1 and 2 mm; > 2 mm
Fruit color	brown; other colors

Molecular Characterization

An overall sample of 30 accessions was selected including 6 accessions from the Herbarium of DAKAR, 6 accessions from the Herbarium of IFAN, 14 other accessions acquired from collections of the Herbarium of CIRAD in France and 4 accessions from personal collection (Table 1). Each species is represented by 3 accessions.

DNA from fresh material was extracted following the protocol of [26]. 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 [27] were selected on the basis of their polymorphism (Table 3). Forward primers were tagged with a 5'-M13 universal sequence [28]. 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 *Touchdown* 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 standard. 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 [29].

Table-3: Characteristics of the 8 single amplification site SSR markers developed in Digitaria exilis [27]

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

For molecular analysis, matrix of binary data was constructed with rows equal to species, and columns equal to alleles found in the different loci. The body matrix contained zeros and ones, corresponding to the absence or presence of alleles. As for the morphological analysis, the binary matrix obtained was used to calculate the SSR similarity matrix between species using Jaccard's coefficient (Jaccard 1908), with PAST software, version 2.17c [23]. A dendrogram was also generated from the similarity matrix using the UPGMA clustering method in XLSTAT software. A cophenetic correlation coefficient was measured as indicated. As far as that goes, correlation coefficient was used to estimate the genetic distance between species.

The levels of correlation between the morphological and SSR similarity matrices were determined using the Mantel test with 10000 permutations (Mantel, 1967) using XLSTAT. This procedure examines the matrix correspondence by taking the 2 matrices together and plotting one against the other, element by element.

RESULTS

Morphological Analysis

Out of the 25 qualitative traits observed, ten characters were found to be almost constant. They are shared by nine out of ten species studied and are related to the internodes pubescence, ligule height, the limb pubescence, racemes disposition, spikelet pubescence, pedicels pubescence, number veins of the upper glume and its pubescence, relative length and the pubescence of the lower lemma. Indeed, the internodes are often glabrous, the ligule not exceeding 1.5 mm, the limb pubescent, the racemes digitated, the spikelets pubescent, the pedicels glabrous, the upper glume 3-veined, the upper glume pubescent and the lower lemma as long as the spikelet and pubescent. After these, the most commonly recorded characters are the absence of hair in the sheath (except in *D. aristulata* and *D. velutina*), the upper glume which is shorter than the spikelet (except in *D. exilis* and *D. longiflora*) and the lower lemma 7-nerved (except for *D. delicatula* and *D. ternata*).

For similarities among the *Digitaria* species, the generate dendrogram (UPGMA) grouped the species sharing similar phenotypic features. The cluster analysis revealed three main classes in which the species are grouped on the basis of characters from the vegetative and reproductive system (Fig-1). The first class was distinguished by two groups: one comprising *D. aristulata* and the other represented by *D. delicatula* and *D. ternata*. The second main class was represented by *D. exilis* and *D. longiflora*. The third main class grouped all of the remaining species into two groups: one consisted of *D. acuminatissima* and *D. perrottetii* and the other represented by *D. ciliaris*, *D. horizontalis*, and *D. velutina*. The cophenetic correlation coefficient of this cluster analysis was r = -0.931. Table 4 shows the results of correlation coefficient between species. The correlation coefficient ranged from -0.14 to 0.79 for all pair-wise combinations, confirming the wide morphological diversity of species for the traits under study (Table 4). The minimum correlation coefficient of -0.14 was recorded between *D. exilis* and *D. perrottetii* while the highest of 0.79 was observed between *D. ciliaris* and *D. horizontalis*. It is remarkable to note a weak linear relationship, whether positive or negative, between *D. exilis* and the other species. Despite this weakness, the correlation between *D. exilis* and *D. longiflora*, which was 0.25, was stronger compared to the relationship between the cultivated species and the other wild relatives.

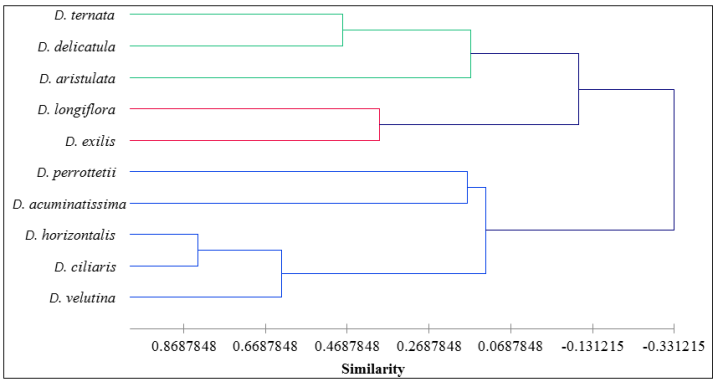

Fig-1: UPGMA cluster analysis on the basis of the morphological data

Table-4: Correlation matrix based on phenotypic traits for all pair-wise comparisons of *Digitaria exilis* and its wild species

		1	2	3	4	5	6	7	8	9	10
1	*D. acuminatissima*	1									
2	*D. aristulata*	0.32	1								
3	*D. ciliaris*	0.43	0.18	1							
4	*D. delicatula*	0.36	0.46	0.29	1						
5	*D. exilis*	-0.04	-0.07	0.04	-0.04	1					
6	*D. horizontalis*	0.57	0.25	0.79	0.36	0.18	1				
7	*D. longiflora*	0.21	0.11	0.14	0.22	0.25	0.28	1			
8	*D. perrottetii*	0.47	0.07	0.40	0.32	-0.14	0.47	0.18	1		
9	*D. ternata*	0.14	0.11	0.00	0.50	-0.04	0.14	0.07	0.25	1	
10	*D. velutina*	0.50	0.32	0.64	0.14	-0.11	0.71	0.07	0.47	0.21	1

Molecular Analysis

A total of 51 alleles are found in the 30 individuals studied. In this study, 13 alleles (25.49% of the total) were found in *D. exilis*, of which 3 alleles (ie 5.88% of the total) are specific to it. 47 alleles (ie 92.16% of the total) were detected in wild species of which 38 alleles (ie 74.51% of the total) are absent in *D. exilis*. Finally, 10 alleles (19.61% of the total) were detected in both *D. exilis* and other wild species. The number of alleles detected was variable between loci. The largest number, 13 alleles, was found at the De-37 locus followed by the De-38 locus with 9 alleles. Two alleles were the lowest number and were found at the De-14 locus. The average number of alleles per locus was 6.38 (Fig-2).

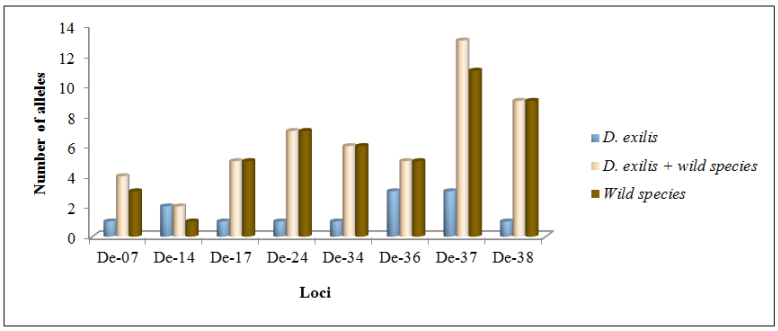

Fig-2: Number and distribution of alleles in SSR loci (De-07 to De-38)

As for phenotypic study, the cluster analysis showed three main classes (Fig-3). The first class included *D. aristulata*, *D. ciliaris*, *D. horizontalis*, *D. perrottetii* and *D. velutina*. The second class was composed by *D. acuminatissima* and *D. delicatula*. The latest was represented by *D. exilis*, *D. longiflora* and *D. ternata*. The cophenetic correlation coefficient of this cluster analysis was r = -0.89. Table 5 shows the results of correlation coefficient between species. The correlation coefficient ranged from -0.16 to 0.57 for all pair-wise combinations, confirming the wide morphological diversity of species for the traits under study. The minimum correlation coefficient of -0.16 was recorded between *D. longiflora* and *D. velutina* while the highest of 0.57 was observed between *D. acuminatissima* and *D. delicatula* followed by *D. horizontalis* and *D. perrottetii*. A weak linear relationship was observed between *D. exilis* and the other species. Indeed, the correlation between *D. exilis* and *D. longiflora* of 0.40 was the strongest.

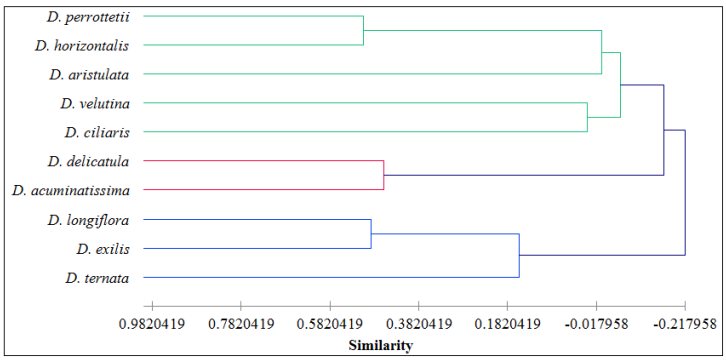

Fig-3: UPGMA cluster analysis on the basis of the molecular data

Table-5: Correlation matrix based on molecular traits for all pair-wise comparisons of *Digitaria exilis* and its wild species

		1	2	3	4	5	6	7	8	9	10
1	*D. acuminatissima*	1									
2	*D. aristulata*	0,18	1								
3	*D. ciliaris*	0,25	0,04	1							
4	*D. delicatula*	0,57	0,28	0,20	1						
5	*D. exilis*	0,07	0,00	0,13	0,16	1					
6	*D. horizontalis*	0,12	0,28	0,38	0,19	0,16	1				
7	*D. longiflora*	0,17	-0,05	0,02	0,19	0,40	0,04	1			
8	*D. perrottetii*	0,17	0,12	0,07	0,24	0,04	0,55	-0,05	1		
9	*D. ternata*	0,28	-0,06	-0,05	0,27	0,19	-0,04	0,01	0,01	1	
10	*D. velutina*	0,18	0,11	0,17	0,07	0,00	0,28	-0,16	0,12	0,06	1

DISCUSSION

The results from phenotypic data revealed that *D. exilis* is closer to *D. longiflora*. These results corroborate those obtained by [30-36] who considered *D. longiflora* as the species from which *D. exilis* is derived. Moreover [37], includes *D. exilis* in the group of *D. longiflora* because of their resemblance by many characters. *D. longiflora* seems to have the particularity of supporting more varied soil types [38]. As for the proximity of *D. delicatula* and *D. ternata*, it also confirms the infrageneric classification of the genus *Digitaria* [10]. As proof, they belong to the same Section "*Clavipilae*" characterized by ternate spikelets with the presence of appressed clavate hairs [39, 40]. Regarding to *D. acuminatissima*, *D. ciliaris*, *D. horizontalis*, *D. perrottetii* and *D. velutina*, they constitute a fairly complex group sharing many common traits and which are mostly discriminated from reproductive characters [41]. Their grouping in the Section "*Sanguinales*" by [37] in the *Flora of Tropical Africa* attests to this proximity.

In molecular characterization, the very high percentage of alleles (74.51%) specific to wild species coupled to the correlation coefficient between species revealed the high variability and also the complexity of the *Digitaria* species. These results are in concordance with those of [16] highlighting a high genetic divergence between the cultivated *D. exilis* and the other wild species, taxonomically distant. Generally, this great interspecific variability is a guarantee of a great capacity to face up to the variations of the environment. The analysis of the molecular data also reveals proximity between *D. exilis*, *D. longiflora* and *D. ternata*. Findings of [42, 43, 15], based on the use of RAPD markers, consolidate our results since they consider *D. longiflora* as the ancestor of *D. exilis*. As for *D. ternata*, it also has a close relationship with *D. exilis* although it is considered as the species from which *D. iburua* derives. *D. aristulata*, *D. ciliaris*, *D. horizontalis*, *D. perrottetii* and *D. velutina* appear to be distant from the cultivated species. However, as noted with the morphological data, the relationships between them seem to be complex. These species, particularly *D. ciliaris*, *D. horizontalis* and *D. velutina*, have been defined as closely related forming a group in which it is difficult to identify phenotypically.

The results of the Mantel tests (r = 0.39, p = 0.013, 10000 permutations) showed positive relationship between morphological and molecular diversity. However, this value is not very significant probably due to the absence of linkage between the loci that control some of the studied morphological characters and the evaluated markers or the limited number of SSR markers used. It can be explained also by the fact that the morphological characters are determined by a few alleles, whose genotype does not correlate with the overall marker scores for the lines [44].

CONCLUSION

The results of this study have highlighted the genetic diversity within the genus *Digitaria*. With a positive and relatively significant correlation between morphological traits and molecular markers, these findings showed that molecular data could resolve the taxonomic difficulties associated with morphological traits. A better understanding the genetic diversity of *Digitaria* species by analyzing more SSR markers and by considering a larger number of species is important for the identification of species with interesting agronomic traits that are useful for improvement of fonio.

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Conflict of Interest: The authors hereby declare that there is no conflict of interest.

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