International Scholars Journals

African Journal of Tropical Agriculture ISSN 2375-091X Vol. 7 (8), pp. 001-004, August, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Optimization of protocols for DNA extraction and RAPD analysis in West African fonio (*Digitaria exilis* and *Digitaria iburua*) germplasm characterization

Danladi Dada KUTA[1], Emmanuel KWON-NDUNG[2] Stephen DACHI[1], Oladele BAKARE[2], and Liasu Adebayo OGUNKANMI[3]

[1]Biotechnology Advanced Laboratory, Sheda Science and Technology Complex (SHESTCO), Km 32 Abuja-Lokoja road, Sheda, P.M.B. 186 Garki, Abuja, Nigeria.
[2]Acha Research Programme, National Cereals Research Institute, Badeggi, PMB 8 Bida, Niger State, Nigeria.
[3]Department of Cell and molecular biology, University of Lagos, Lagos State, Nigeria.

Accepted 11 April, 2019

Fonio is an important indigenous grain crop of West Africa, but the extent of genetic diversity in fonio, its origin and phylogeny are not well understood. DNA markers allow precise characterization of plant germplasm accessions, but there is no literature report of their use in fonio. This paper reports the result of protocol optimization research for DNA isolation and RAPD analyses in fonio. High quality DNA was successfully isolated and RAPD was effectively used to study genetic similarity among fonio accessions. This result might stimulate the application of DNA markers to investigate the origin and phylogeny of fonio in Africa.

Key words: *Digitaria* spp., DNA markers, RAPD, germplasm characterization, fonio**.**

INTRODUCTION

Fonio [*Digitaria exilis* (Kippist) Stapf, and *Digitaria iburua* Stapf, Poaceae] is sometimes regarded as “grain of life” in several communities of West Africa, as it provides food early in the farming season, when other crops are yet to mature for harvest (Ibrahim 2001). Fonio grains are also considered as one of the best tasting and nutritious of all grains (Vietmeyer et al., 1996), with about 7% crude protein that is high in leucine (9.8%), methionine (5.6%) and valine (5.8%) (Temple and Bassa, 1991). The grains are also reported to have high brewing and malting potentials (Nzelibe and Nwasike, 1995). The enormous traditional and technological uses of fonio have earlier been reviewed elsewhere (Misari et al., 1996; Jideani, 1999).

Despite the importance of fonio, especially in West Africa, then crop has not received adequate research attention. Constraints like low harvest index, weeds, shattering, lodging, insect pests and diseases are yet to be addressed. In Nigeria, for example, there is no single registered variety of fonio: farmers continue to grow their landraces. National fonio genetic improvement programme has just been established in Nigeria in year 2003. To develop new improved fonio varieties, breeders must have access to diverse fonio accessions with a broad range of agronomic traits like maturity, grain size, plant height, weed competitiveness, and yield potential. The collection and conservation of local and exotic germplasm of fonio in a gene bank is therefore the key to successful genetic improvement program.

However, germplasm collections can only be exploited in breeding programs after they are properly analyzed. Fonio germplasm are traditionally analyzed using several phenotypic traits that demand extensive observations. Moreover, phenotypic traits are often affected by environmental conditions. It has earlier been suggested (Kuta et al., 2003) that biotechnology can contribute in enhancing genetic characterization of fonio germplasms. DNA markers, like RFLP, RAPD, AFLP, SSR, are now

*Corresponding Author. E-mail: Drkuta2002@yahoo.com.

available to make gene pool analysis more precise. DNA markers generated by random amplified polymorphic DNA (RAPD) have been extensively used for genetic mapping, molecular taxonomy and molecular diagnostics (Williams et al., 1990) . In rice (*Oryza sativa* L,), RAPD and AFLP (amplified Fragment length polymorphism) have been shown to be the effective DNA markers for characterization of diverse rice germplasm (Jackson, 1999). These markers might be effective for DNA fingerprinting of fonio accessions, collected from diverse fonio growing regions of Africa. However, we are yet to come across any literature report on DNA isolation and RAPD analysis of fonio germplasm accessions. This paper attempts to bridge the gap by optimizing protocols for DNA isolation and RAPD analysis of selected fonio germplasm accessions.

Table 1. List of fonio accessions used for the study.

NCRI accession no.	Cultivar local name	Village located
Nde 001	Kumbe	Gborong
Nde 015	Nkpwos	Tse
Nde 006	Gongerandong	Danto
Ndi 001	Aburu	Mushere
Nde 004	Exsum I	Boi
Nde 005	Exsum II	Bauchi
Nde 009	Harat Seng	Dantse
Nde 017	Tsala	Kwakwi
Ndi 007	Guzuksar	Kaper/Jipal
Ndi 002	Babudama	Ban

MATERIALS AND METHOD

Plant materials

A total of ten accessions (Table 1) of fonio were grown in glasshouse in pots containing topsoil. Young leaves were harvested from 2 to 3 weeks old seedling, placed in a sealable plastic bag and appropriately labeled. The collected leaves were used immediately for DNA extraction, while excess leaf materials were stored in –80°C freezer for future use.

Isolation of fonio genomic DNA

DNA isolation procedure as described by Dellaporta et al. (1983) with some modifications was used to extract total genomic DNA from different fonio accessions. Young leaves were collected from 2 to 3 weeks old single plants of each accession into 1.5 ml eppendorf tube. The samples were ground in liquid nitrogen with konte pestles into fine powder. The volume of the ground leaves was adjusted to about a third of the tube and 700 l of hot (65°C) extraction buffer (100 mM tris-HCL, 50 mM EDTA, 500 mM NaCl, 1% SDS and 10 mM β-mercaptoethanol, pH 8.0). The tubes were incubated at 65°C in water bath for 10 min with occasional gentle shaking and then allowed to cool at room temperature for 5 min.

Next, about 250 l ice- cold potassium acetate (5 M) was added and the tubes incubated on ice for 20 min. The suspension was centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant transferred into clean 1.5 ml eppendorf tubes. Then, 700 l of chloroform/isoamyl alcohol was added and spinned at 10,000 rpm for 10 min and supernatant transferred to new tube. Ice-cold isopropanol (0.7 volumes) was added for the precipitation of DNA at -20°C for 60 min. DNA was recovered by centrifugation at 14,000 rpm for 10 min at 4°C. The supernatant was discarded and the tube was kept inverted for 10 min to allow complete draining. The DNA pellet was washed twice in 100 l 70% cold ethanol and allowed to air -dry completely. After drying, 60 l of TE (50 mM Tris-HCL, 10 mM EDTA, pH 8.0) was added to the pellet, followed by 2 l of RNAse (10 ng/ml) to remove RNA. The mixture was incubated for 40 min at 65°C with gentle shaking at 10 min intervals.

Table 2. Sequences of the 10-base nucléotides primers used for the fonio characterization **and** number of bands generated.

Primer code	Nucleotide sequence 5' to 3'	No of bands
OPAE 10	CTCAAGCGCA	3
OPAG 20	TGCGCTCCTC	3
OPAF 20	CTCCGCACAG	6
OPAC 20	ACGGAAGTGG	2
OPAE 01	TGAGGGCCGT	2
OPAE 03	CATAGAGCGG	2
OPAF 02	CAGCCGAGAA	4
OPAC 15	TGCCGTGAGA	4
OPAE 02	CTCTCGGCGA	3
OPAE 15	CACGAACCTC	3
OPAE 02	TCGTTCACCC	3
OPAC 07	GTGCCCGATC	3
OPAC 1	GACGCGATTG	2
OPAD 2	TCTTCGGAGG	4
OPAE 0	TGTCAGTGGC	2
OPAC 10	AGTCCGCCTG	3
Total		**49**

PCR amplification and electrophoresis

The extracted fonio DNA was used for PCR amplification. A fluorometer (TD-700) was used to measure DNA concentration. RAPD amplification was conducted in a total volume of 25 l consisting of 2.5 l of 10X buffer, 50 ng DNA, 2 l MgCl2, 1 l mixture of 10 mM dNTPs (dATP, dCTP, dGTP and dTTP), 11.1 l of ultra pure water, 2 l of 0.5% Tween 20, and 0.4 l red hot Taq polymerase (Promega), 1 l of RAPD primer. The PCR amplification consists of one cycle of 94°C for 3 min (pre heating), followed by 44 cycles of 94°C for 0.20 min (denaturation), 37°C for 0.40 min (annealing) and 72°C for 1 min (extension). A further extension at 72°C for 7 min was added followed. The RAPD primers used for DNA amplification were ten-mer sequences from AD, AC, A E, AF and AG kits (Operon Technologies Inc.) and are given in Table 2.

RAPD fragments were separated by electrophoresis on 1.4% agarose gel in 0.5M tris borate buffer at 120 V for 2 h. The 1 Kb ladder DNA from GIBCO BRL (New York, USA) was used as a standard molecular weight size marker. The gel was stained in

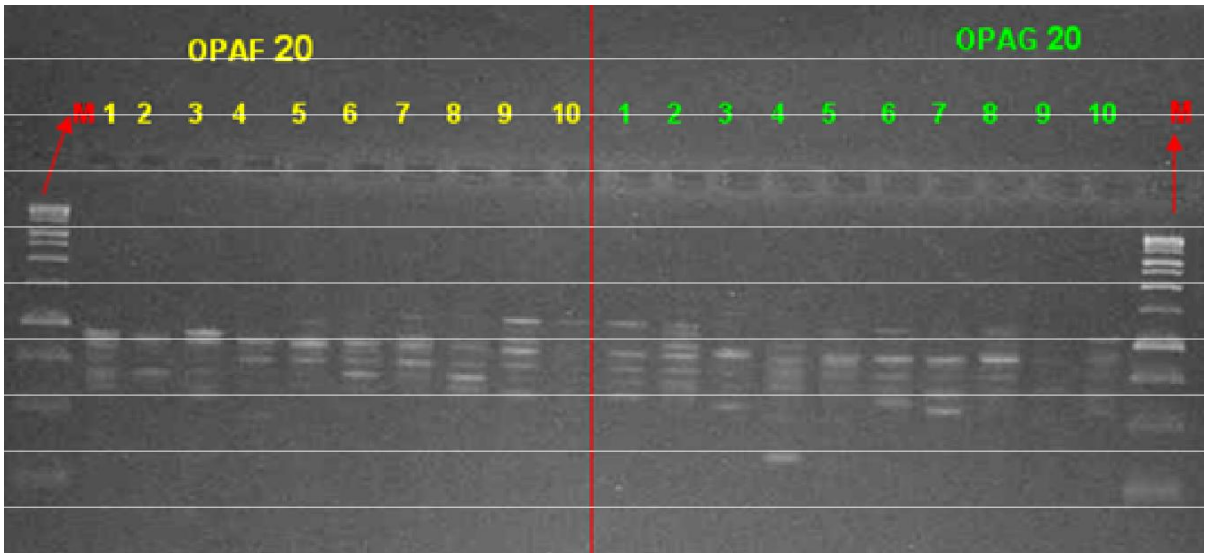

Figure 1. RAPD markers of 10 accessions of fonio amplified with primers OPAF 20 and OPAG 20 (Table 2). Lanes M: 1-kb DNA size marker; 1: Nde-001; 2: Nde-015; 3: Nde-006; 4: Ndi-001; 5: Nde-004; 6: Nde-005; 7: Nde-009; 8: Nde-017; 9: Ndi-007; and 10: Ndi-002.

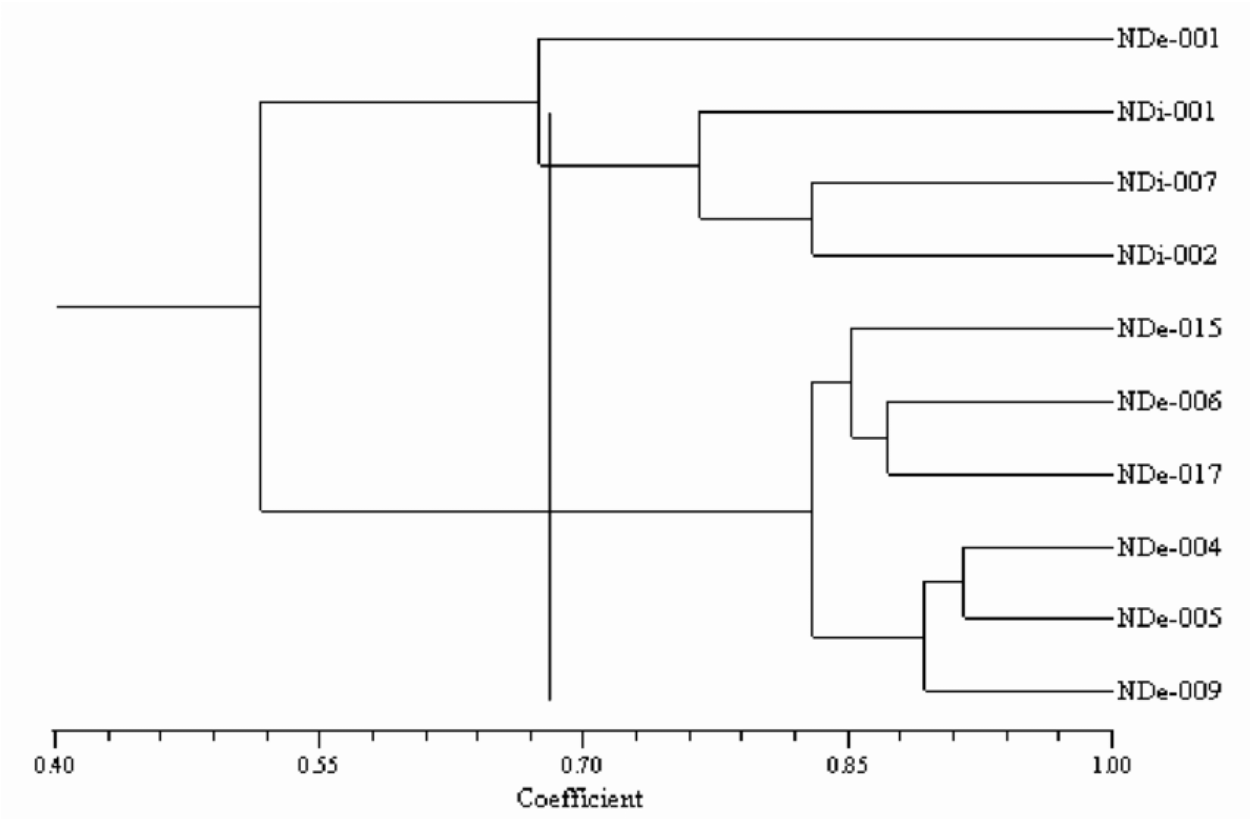

Figure 2. Dendrogram of 10 fonio accessions based on the similarity index by RAPD markers.

ethidium bromide, visualised under a UV transluminator, and photographed using a Polaroid MP4 camera (Figure 1).

Data analysis

Fragments that were clearly resolved on the gels were scored as 1 or 0 (i.e., present or absent, respectively) across all the ten accessions of fonio. Bands that could not be confidently scored were regarded as missing data. Pairwise distance (similarity) matrices were computed using sequential, hierarchical and nested (SAHN) clustering option of the NTSYS-pc version 2.02j software package (Rohlf, 1993). The program also generated a dendrogram (Figure 2), which grouped the accessions and species on the basis of Nei genetic distance (Nei, 1972) using unweighted pair group method with arithmetic average (UPGMA) cluster analysis (Sneath

and Sokal, 1973).

RESULTS AND DISCUSSION

A total of 100 RAPD OPERON primers were screened using four (4) of the fonio accessions. Out of these, only fifteen of the primers (Table 2) that showed polymorphisms and were chosen to amplify the whole ten accessions. A typical sample of PCR amplification products is shown in Figure 1.

A dendrogram constructed using neighbour joining method of cluster analysis separated all the ten accessions into 2 clusters at 0.55 similarity coefficient (Figure 2). Cluster 1 consisted of all the NDi group except Nde 001 that grouped among them. The Nde 001 has some resemblances with the NDi groups at 0.67-similarity coefficient. Cluster 2 contained six NDe lines separated into two sub clusters of three accessions each at about 0.83 similarity coefficient level. The first sub cluster consisted of NDe 009, 005 and 004 while the second had NDe 006, 015 and 017

Ten different accessions of fonio plants were analysed in this study. There was no duplication among the accessions. Therefore, the protocols employed were able to yield quality DNA and generate RAPD polymorphic bands. This research has paved the way for intensive research activities in fonio studies using advanced tools like RAPDs and other similar techniques.

ACKNOWLEDGEMENTS

The authors are grateful to the International Plant Genetic Research Institute (IPGRI) for providing financial assistance for this project. We also appreciate the support of National Cereals Research Institute (NCRI) Badeggi, and the National Biotechnology Advanced Laboratory, SHESTCO-Abuja, for making this work possible.

REFERENCES

Dellaporta SL, Wood J and Hicks JB (1983). A plant minipreparation: version II. Plant Mol. Biol. Rep. I: 19-21.

Ibrahim A (2001). Hungry Rice (Fonio): A neglected cereal crop. NAQAS Newslett. Vol 1 No. 4: 4-5.

Jackson M (1999). Managing genetic resources and biotechnology at IRRI's rice genebank. In: Managing Agricultural Biotechnology, Cohen JI (ed.): CABI Publishing, UK, and the ISNAR, the Hague, pp.102-109.

Jideani IA (1999). Traditional and possible technological uses of *Digitaria exilis* (fonio) and *Digitaria iburua* (iburu): A review. Plant Foods Human Nutr. 54: 363-374.

Kuta DD, Kwon-Ndung EH, Dachi S, Ukwungwu M, Imolehin ED (2003). Potential role of biotechnology tools for genetic improvement of "lost crops of Africa": the case of fonio (*Digitaria exilis*; *Digitaria iburua*). Afr. J. Biotechnol. 2 (12): 580-585.

Nei M (1972). Genetic distance between populations. Am. Nat. 106: 283-292.

Nzleibe HC, Nwasike CC (1995). The brewing potential of fonio(*Digitaria exilis*) malt compared to pearl millet (Pennisetum typhoides) malts and sorghum (*Sorghum bicolor*) malts. J. Inst. Brewing 101:345-350.

Rohlf FJ (1993). NTSYS-pc. Numerical taxonomy and multivariate analysis: version 2.02. Applied Biostatistics, New York. pp.???

Sneath PHA and Sokal RR (1973). Numerical taxonomy. W.H. Freeman, San Francisco. Theor. Appl. Genet. 93: 613-617

Temple VJ, Bassa JD (1991). Proximate chemical composition of fonio (*Digitaria exilis*) grain. J. Sci. Food Agric. 56: 561-564.

Williams JKG, Kubelik AR., Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res.18: 6531-6535.