Journal of Applied Horticulture, 20(3): 237-241, 2018

Polymorphism and genetic diversity assessment of some ornamental ferns by microsatellite (ISSR) markers

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Abstract

This study assessed the genetic diversity of six ornamental ferns in parks and gardens in Nigeria using inter-simple sequence repeat (ISSR) markers. Genomic DNA were extracted from the samples and Polymerase Chain Reaction (PCR) was performed using seven ISSR markers. The elcetrophoregram showed amplicon size ranged from 100bp-1Kb among the ferns. A total of 87 bands were generated with 71.26 % polymorphism and 28.73 % monomorphic bands. The average number of band per primer was 12.4 and polymorphism range was between 31.23-100 %, the highest polymorphism was obtained with ISSR2. The mean Nei's genetic diversity was 29 % while the Shannon's index was 43.5 %. The pair of *Adiatum capilus-veneris* and *Pteris acanthoneura* had maximum genetic distance of 0.6592 while *A. capilus-veneris* and *A. furcans* are the closest species. At genetic similarity of 78 %, the cluster analysis revealed two major groups. Group-1 comprised of four species, where *P. acanthoneura* is a distant member, the other three species in the group are closely related. Group-2 on the other hand had two closely related *Adiantum* species. The study concludes that ISSR markers are effective in the genetic study of the ferns and the genetic diversity information provided could be utilized for selection, improvement and conservation of the ornamental plants.

Key words: Diversity assessment, DNA polymorphism**,** genetic diversity**,** ISSR marker, ornamental ferns, PCR

Introduction

Ornamental plants are grown for display of aesthetic features such as the flowers, leaves, scent, and fruits for human pleasure, environmental management and conservation. Ornamental plants commonly used for landscaping and aesthetic purposes include various herbs, shrubs, trees and even lower plants such as algae. Ferns are among preferred ornamental herbs in homes, gardens, parks and public buildings, they consist of 42 genera and about 90 species which are widely distributed mostly in the humid areas of the world (Jim, 1999). Ferns are annual or perennial seedless, flowerless, vascular plants with high aesthetic and cultural values. They could be terrestrial, aquatic and sometimes epiphytic. Some ferns can serve as animal's feed due to low levels of oxalate and cyanid (Babayemi *et al*., 2006) while a number of them are used for medicinal and folkloric purposes (Christensen, 1997; Oloyede *et al.,* 2010). In fact, in New Zealand, much value is placed on ferns that it is imprinted on the national emblem, bank notes and coins (Patrick and John**,** 2000). In Nigeria, ornamental plants are grossly underutilized, however, in recent years, the growing of these plants have increased in the cities. Such ornamental ferns are now used as a strategy to conserve unused and marginal land which previously served as a dumping ground for refuse and some unwanted materials.

Ferns identification is difficult because many species often look very similar. Attempts made so far to characterize ferns in Nigeria were based on morphological (Oloyede and Odu, 2011; Oloyede, 2012) and phytochemical features (Oloyede *et al*., 2010). The use of morphology and phytochemical features in delimiting genetic resources is limited and inadequate as the features used could be influenced by the environment. There is a wide gap in the knowledge of genetic diversity and molecular characterization of ornamental plants and there are no available literatures on genetic diversity among ornamental ferns in Nigeria using molecular markers.

Molecular markers such as random amplified polymorphic DNA (RAPD), inter-simple sequence repeats (ISSR) are easy to use with high level of polymorphism and reproducibility. These markers have been reportedly used in genetic diversity studies of ornamental species such as bamboo (Das *et al*., 2005**),** willow-leaved foxglove (Nedauer *et al.,* 1999), *Orychopgragmus violaceus* (Zhang and Dai, 2010**)** and Rose (Mohapatra and Rout, 2005) among others. However**,** none has been reported on ferns in Nigeria. The present study therefore assessed polymorphism pattern and genetic diversity among six common ornamental ferns in Nigeria. The findings from this work would provide insight into the genetic background as a reference for enhanced utilization of ferns for ornamental, landscaping and designing programmes for the species breeding and conservation.

Materials and methods

Six species of common ferns were collected from gardens and parks in South-West Nigeria. Permission were sought and approval granted prior to samples collection from the garden and parks facilities. The collected species were identified at the herbarium, Department of Plant Biology, University of Ilorin, Nigeria where the voucher details were deposited. The names of the fern species are presented in Table 1 and their photographs shown in Fig 1. The ferns were grown in the screen house at the

Fig. 1. Morphology of the six ornamental ferns assessed for genetic diversity. a= *Nephrolepis exaltata;* b = *Ploystichum acrostichoides*; c = *Pteris acanthoneura;* d = *Nephrolepis furcans;* e = *Adiantum caudatum*; f = *Adiantum capilus-veneris*

botanical garden, University of Ilorin, Ilorin, Nigeria to produce leaves for DNA extraction and molecular characterization.

DNA Isolation: Young fully expanded leaves were collected from the samples and washed in distilled water. Genomic DNA was extracted from the leaves using DNeasy Mini Plant Kit. (QIAGEN, USA) based on the manufacturer prescribed procedure. The quality of the DNA was checked on 0.8 % agarose gels and the concentrations determined with a Nanodrop 8000 spectrophotometer (Thermo Scientific).

PCR Amplification with ISSR primers: PCR-amplification of the genomic DNA by ISSR primers (Eurofins, Germany) was performed on 96 well thermal cycler (Eppendorf, USA). The final reaction volume consisted of 50 ng of genomic DNA, 1 X Reaction buffer which composed of 2.0 mM $MgCl_2$, 10 pM primer, 200 µM dNTPs, 1 unit of *Taq* polymerase (Fermentas, Life Technologies) and made up to 25 µL with nuclease free water (Ambion). The cycling conditions started with an initial denaturation at 94 ˚C for 5 min; followed by 35 cycles of 94 ˚C for 30 s, 44-54 ˚C for 45 s and 72 ˚C for 90 s. The final extension was at 72 ˚C for 20 min and the setup was put on hold at 4 ˚C for 10 min. The PCR products were resolved on 1.5 % agarose gel, electrophoresed for 90 minutes at 110 Volt. A 1kb DNA ladder (Thermo Fishaers) was used as a gene ruler. The gel was visualized on UV transmullator and the image captured with gel documentation system (Bio-rad, USA).

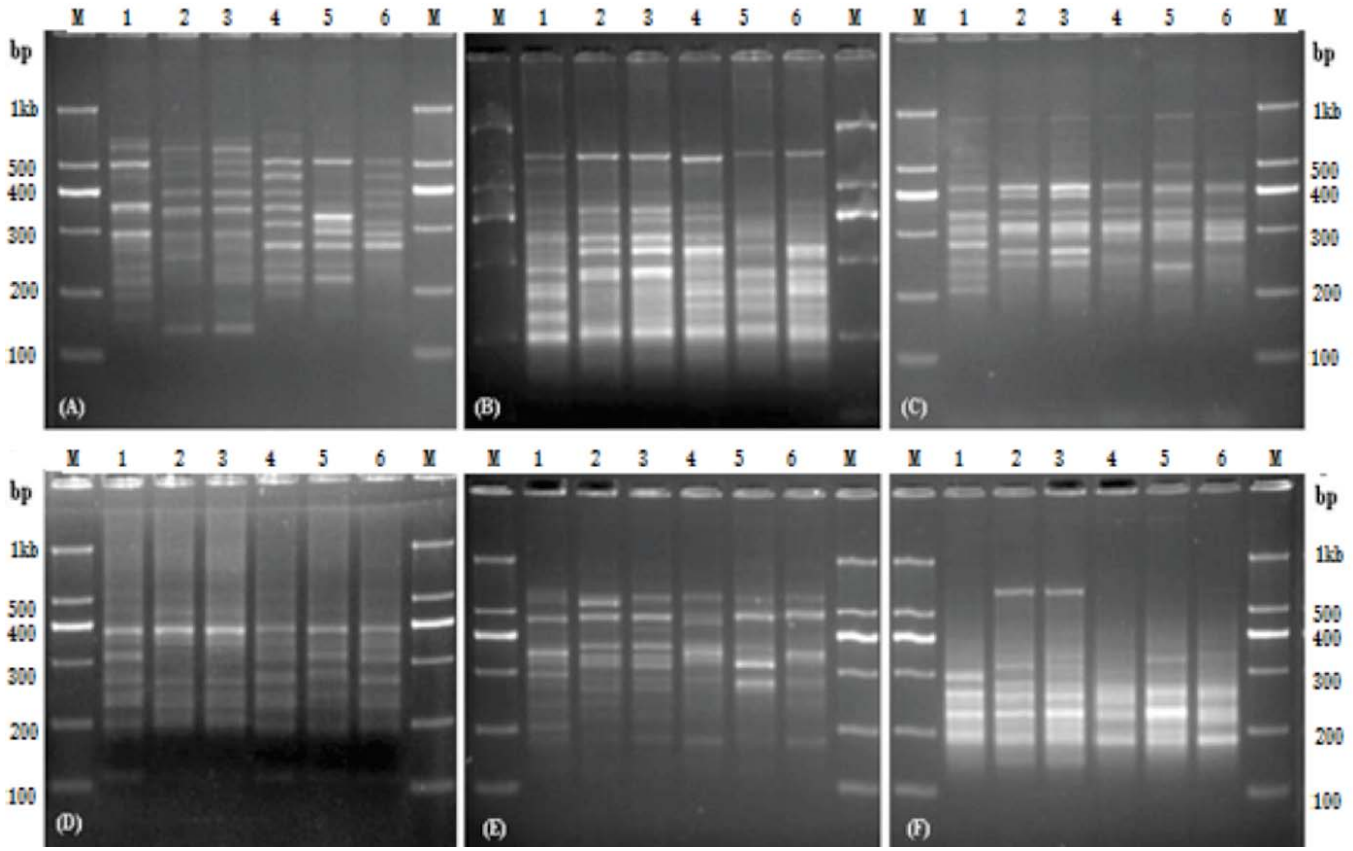
Data Analysis: The analysis only considered clear and reproducible bands which were visually scored as either present (1) or absent (0). All data were scored in the form of a binary matrix and each band was interpreted as one allele. Bands with the same mobility were assumed to be homologous. Each marker was treated as an independent unit character. The genetic distance and genetic similarity were calculated among the species using Nei's coefficient. Cluster analysis based on Un-weighed Pair Group Method using Arithmetic Averages (UPGMA) and relationships between species were used to construct a dendrogram. The genetic divergence analysis was performed with POPGENE 1.32 software.

Results and discussion

Ferns are diverse and versatile ornamental and medicinal plant. They are of great importance in landscaping, environmental management of erosion and various medicinal purposes (Chritensen, 1997; Jim, 1999; Oloyede, 2012). The use of molecular markers is most reliable in studies of species and germplasm genetic diversity, identification of individual genotype and conservation genetic materials. In the present study, seven out of eleven ISSR primers tested produced distinctive and reproducible loci amplifications on the ferns. The electrophoregram of the amplified loci by the seven primers revealed that amplicon size ranged from 100 bp to 1 kb (Fig.

Table 1. Nomenclature of ornamental ferns assessed for genetic diversity and relationship

S/N	Fern family	Genus	Species name	Common name
1	Nephrolepidaceae	Nephrolepis	*Nephrolepis exaltata*	Sword fern
2	Dryopteridaceae	Ploystichum	*Ploystichum acrostichoides*	Christmas fern
3	Pteridaceae	Pteris	*Pteris acanthoneura*	Brake fern
4	Nephrolepidaceae	Nephrolepis	*Nephrolepis furcans*	Fishtail sword fern
5	Pteridaceae	Adiantum	*Adiantum caudatum*	Tailed maidenhair fern
6	Pteridaceae	Adiantum	*Adiantum capilus-veneris*	Maidenhair fern



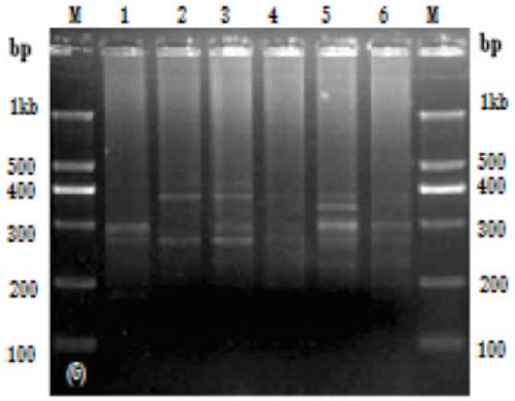


Fig 2. Electrophoregram profile of six ornamental ferns in Nigeria as amplified by seven primers. A = ISSR1; B = ISSR2; C = ISSR3; D = ISSR4; E = ISSR5; E = ISSR6; and G = ISSR7

2). The species *Nephrolepis exaltata* and *Adiantum caudatum* had 1kb fragment size amplified by ISSR3 while other primers produced less fragment size on the ferns. A similar pattern of fragment size variation by RAPD markers was reported for rose cultivars (Mohapatra and Rout, 2005).

A total of 87 bands were amplified by the primers. The least number of bands per primer (6 bands) was recorded with ISSR7 while the highest were generated by ISSR1 and ISSR2 which produced 16 bands each (Table 2). The average of 12.4 band/ primer showed the primers were effective for the diversity study because, effectiveness of the primers are determined by the number of loci amplified per sample. Tripathi *et al*. (2012) obtained loci amplification between 5-16 bands in *Bacopa monnieri* with ISSR primers and the authors elucidated the usefulness of the markers in revealing genetic variation in the plant. Out of 87 bands amplified by the ISS primers in this study, 62 (71.26 %) were polymorphic and 25 (28.73 %) were monomorphic. The percentage polymorphism per primer ranged from 31.25 % to 100 %. While 100 % polymorphism was obtained

Table 2. Amplification and polymorphism pattern of ISSSR markers used in the diversity study of ornamental fern species

Marker	Sequence (5'- 3')	NF	NB	NMB	NPB	P(%)
ISSR1	GAGAGAGAGAGACC	45	16	0	16	
ISSR2	CTCTCTCTCTCTCTCTAC	39	16	11	5	31.25
ISSR3	CACACACACACAAG	36	14	5	9	64.25
ISSR4	CAGCACACACACACACA	32	9	1	8	88.88
ISSR5	GTGTGTGTGTGTCC	38	13	2	11	84.62
ISSR6	CTCTCTCTCTCTCTCTGC	25	13	3	8	61.54
ISSR7	GAGAGAGAGAGAGG	19	6	3	4	66.67

TNF = Total number of fragment; TNB = Total number of bands; NMB = Number of monomorphic bands; NPB = Number of polymorphic bands; P(%) = Percentage polymorphism

with ISSR1, only 31.25 % (5) of the 16 bands generated by ISSR2 are polymorphic. The high percentage polymorphism indicates high genetic diversity in the species, The percentage polymorphism obtained in the present study was similar to that of Rose cultivars (Mohapatra and Rout, 2005), but less than the values reported for *Calibrachoa caesia* (Perez de la Torre *et al.,* 2012) and *Bacopa monnieri* (Tripathi *et al,* 2012).

Major advantages provided by molecular markers are their selective neutrality, availability in unlimited numbers and high resolution that may display genotypic differences down to single base pair (Paterson *et al.,* 1991). Selection and improvement of plant genetic resources depends greatly on its available genetic information. To achieve sustainable and efficient use of plant materials, existing variability in different species and varieties should be taken into consideration. Thus knowledge

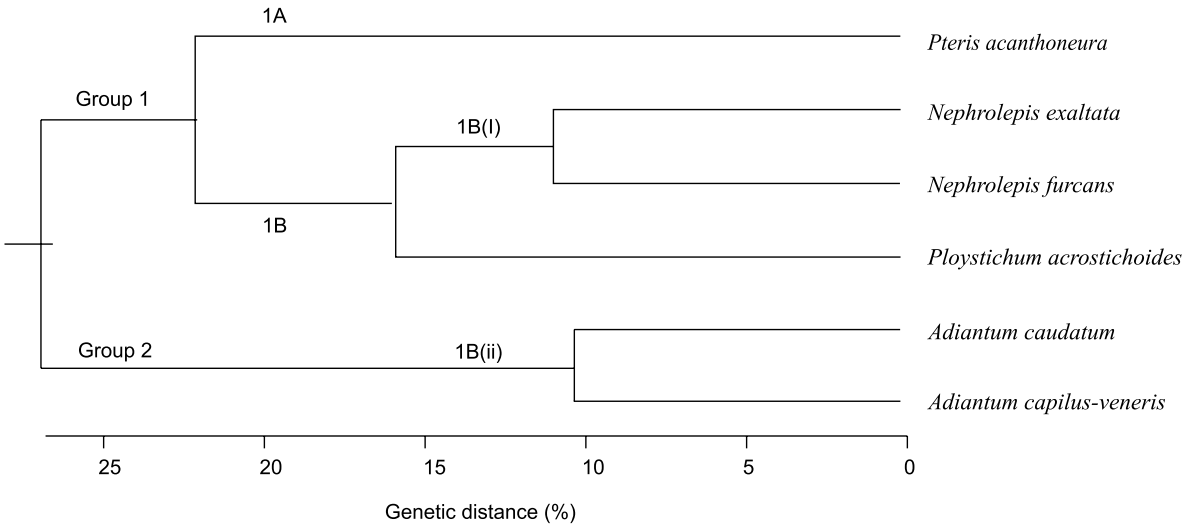


Fig. 3**.** Dendrogram based on the amplification loci of six ornamental ferns by ISSR markers.

of genetic stability of ornamental plants is necessary to determine the functionality in a given framework and to ensure selection of right species for the right purpose and environment. Consequently, morphological characterization (Oloyede and Odu, 2011; Oloyede, 2012) may not be too reliable for selection and horticultural improvement of the ferns because, the feature used to delimit the species could be influenced by environment. Due to multiloci fingerprinting profiles of ISSR, the marker is important in the studies involving genetic diversity, parentage, strain identification, and taxonomic studies of closely related species (Godwin *et al.*, 1997; Gupta *et al.,* 1994). Hence, the result of the present study provide information on analysis of genetic diversity and relationship on which selection of ornamental ferns could be based.

The mean Nei's genetic diversity across all loci was about 29 % and Shannon Index was about 43.5 % (Table 3). This showed that remarkable diversity exits in the gene pool of the studied ferns. The genetic distances for all possible pairs based on Nei's coefficient using ISSR data revealed that the species pair of *Adiantum caudataum* and *A. capilus-veneris* had minimum genetic distance (GD = 0.2032) and the pair *Pteris acanthoneura* and *Adiantum capillus-veneris* exhibited maximum genetic distance (GD = 0.6592). It can be deduced from the results that the former pairs were the most distant of all the six species while the latter pair were the closest relations. Of course, the morphological structures of the pairs to an extent support their marker revealed relationship. This information may be relevant for breeding or improvement of ferns with wider applications.

The dendrogram of the genetic relationship separated the six ornamental ferns into two groups at 73 % similarity index (Fig. 3). The first group (Group 1) further divided into two sub groups (1A 1B). The subgroup 1A had only a member (*Pteris acanthoneura*) which was distinct from other members of the group, while the subgroup 1B comprised of three ferns in two clusters that are similar at 78 % genetic similarity index. Oloyede (2012) earlier elucidated that ferns with different morphological features are likely different species, but he did not elaborate the degree of divergence for the species partitioning.

The results from the present study showed that among the four species that constituted group 1, *N. exaltata* and *N. furcans* the two species from the genus *Nephrolepis* are the most similar and they formed cluster 1B(i). Cluster 1B(ii) only member (*Polystichum acrostichoides*) was about 22 % dissimilar to the 1B(i) group. On the other hand, the second major group (group 2) consisted of two closely related ferns *Adinatum caudatum* and *A. capilus-veneris* which both belong to the family Pteridaceae. This conform to the findings of Mohapatra and Rout (2005) who demonstrated that genetically similar cultivars of ornamental rose clustered together. However, the genetic structure of populations of species is not always reflected in the geographical distribution of individuals. Populations that are not discretely distributed may not be genetically structured, due to unidentified barriers to gene flow. In addition, species with different geographical or habitat requirement, or phenotypes are not necessarily genetically different.

High genetic diversity were detected among different species of ornamental ferns studied. The study showed that ISSR markers are effective for genetic diversity among the species of ornamental ferns. The pairs of *Adiantum caudatum* and *Adiatum capilus-veneris* are the closest species while *A. capilus-veneris* and *Pteris acanthoneura* exhibited the maximum genetic diversity. These results suggest that heterogeneity existed among ornamental ferns, in particular those from different genera. Such genetic diversity could be explored to broaden the gene pool of the available horticultural ferns or selection of ferns with desire ornamental, environmental and economic importance.

Table 3. Summary of genetic diversity of the fern species based on ISSR primers and Nei's Coefficient analysis

TNP	TNF	TNB	TNMB	TNPB	TP(%)	Nei's index (h %)	Shannon's index (I %)	Genetic Distance (GD)	
								Min	Max
7	234	87	25	62	71.26	29	43.5	0.203	0.659

TNP = Total number of primer; TNF = Total number of fragment; TNB = Total number of bands; TNMB = Total number of monomorphic bands; TNPB = Total number of polymorphic bands; TP(%) = Total percentage polymorphism

References

Babayemi, O.J, M.A. Bamikole and A.B. Omojola, 2006. Evaluation of the Nutritive value of two aquatic weeds. (*Nephrolepis biserrata* and *Spirodella polyrhiza*) for feeding West African dwarf goats. *Agroecosystems,* 6: 15-21.

Christensen, D.O. 1997. *Medical Ethnobotany, Phytochemistry and Bioactivity of the Ferns of Mores, French and Polynesia*, Nichole Baltrushes Publishers. Jodpur, India**.**

Das, M., S. Bhattacharya and A. Pal, 2005. Generation and characterization of SCARs by cloning and sequencing of RAPD products: A strategy for species-specific marker development in bamboo. *Ann. Bot*., 95: 835-841.

Godwin, I.D., E.A.B. Aitken and L.W. Smith, 1997. Application of inter simple sequence repeat (ISSR) markers to plant genetics. *Electroph*., 18: 1524-1528**.**

Gupta, M., Y.S. Chyi, J. Romero-Severson and J.L. Owen, 1994. Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple sequence repeats. *Theor Appl Genet.*, 89: 998-1006.

Jim, C. 1999. *Ferns and man in New Guinea*. A paper presented to Papua New Guinea Botany Society Conference.

Mohapatra, A. and G.R. Rout, 2005. Identification and genetic variation among rose cultivars using random amplified polymorphic DNA. *Z. Naturforsch*., 60c: 61-617.

Nebauer, S.G., L. Castillo-Agudo and J. Segura, 1999. RAPD variation within and among natural populations of outcrossing willow-leaved foxglove (*Digitalis obscura* L.). *Theor. Appl. Genet.*, 98: 985-994.

Oloyede, F.A. 2012. Survey of ornamental ferns, their morphology and uses for environmental protection, improvement and management. *Life J. Sci*., 14(2): 245-252.

Oloyede, F.A. and E.A. Odu, 2011. Taxonomic evaluation of homosporous leptosporangiate ferns in southwestern Nigeria. *J. Current Res*., 2(1): 009-017.

Oloyede, F.A., F.M. Oloyede, T.L. Salau and A.A. Ogunwole, 2010. Chemical composition of a tropical fern, *Ceratopteris cornuta* (Parkeriaceae) in Nigeria. *Acta Botanica Hungarica,* 52(1-2): 145-150.

Paterson, A.H., S.D. Tanksley and M.E. Sorrels, 1991. DNA markers in plant improvement. *Adv. Agron*., 46: 39-90.

Patrick, J.B. and C.S. John, 2000. Ferns and allied plant of New Zealand. *Pteridologist*, 4(2): 12-13.

Pérez de la Torre, M, M. García, R. Heinz and A. Escandón, 2012. Analysis of genetic variability by ISSR markers in *Calibrachoa caesia*. *Electronic J. Biotechnol.,* 15(5). Retrieved December 15, 2017, from http://www.ejbiotechnology.info/index.php/ejbiotechnology/article/view/v15n5-8/1510

Tripathi, N., D.S. Chouhan, N. Saini and S. Tiwari, 2012. Assessment of genetic variations among highly endangered medicinal plant *Bacopa monnieri* (L.) from Central India using RAPD and ISSR analysis. *Biotech*., 2: 327-336.

Zhang, L.J. and S.L. Dai, 2010. Genetic variation within and among populations of *Orychophragmus violaceus* (Cruciferae) in China as detected by ISSR analysis. *Genet Resour Crop Evol*., 57:55-64.

Received: March, 2018; Revised: April, 2018; Accepted: June, 2018