*Annals of West University of Timişoara, ser. Biology, 2018, vol. 21 (1), pp.21-28*

# GENETIC DIVERSITY IN POPULATIONS OF *DIPCADI FILAMENTOSUM* MEDIK. USING ISSR MOLECULAR MARKERS

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Received 5 March 2018; accepted 7 May 2018

**ABSTRACT**

*Genetic diversity was estimated among 13 populations of Dipcadi filamentosum Medik. Inter Simple Sequence Repeat (ISSR) was used to generate data to examine the patterns of genetic differentiation between and within the populations. A total of ten primers used generated 146 amplicon of which 97.9% of loci detected revealed polymorphism. The Shannon's indices (I) and Nei's genetic diversity (h) among the studied populations from the various regions were estimated at 0.6216 (SD = 0.1199) and estimate of gene flow (Nm) in the population was 5.0555. Cluster analysis (neighbor-joining, NJ) revealed that the North-West populations (KAT) are genetically distinct from the North-central populations (SOB, KAB, KAM and KAS). Ordination by Principal Component Analysis (PCA) supported the findings of NJ. The SOB and YOB; MAI and UNI populations clustered as closely related are probably as a result of correlation between the geographical locations and gene flow.*

**KEY WORDS:** *Dipcadi filamentosum*, *genetic diversity, polymorphism, ISSR, population.*

## INTRODUCTION

*Dipcadi filamentosum* is a bulbous species in genus *dipcadi* of Hyacinthaceae family, formerly known as Liliaceae found in Africa, the Mediterranean region, Africa, Socotra, Madagascar and India. The greatest diversity is found in South Africa where about 13 species are present (Stedje *et al.,* 1987) and India, where about 9 species were recorded (Deb & Dasgupta, 1976). The Hyacinthaceae includes about 700-900 species of bulbous plant which are mainly distributed through Europe, Africa and South-West Asia, with small genus in South America (APG 11, 2003).

The plant is a perennial herb. The family is mostly found in seasonal climates with a pronounced dry season and is rare in tropical lowlands and forested areas. It grows well in sandy – loam soil. It has one to many leaves. The leaves are long and narrow arising from the base. In Nigeria it is found growing in regions where shady trees are absent (Taye *et al*., 2013). The plant has medicinal uses due to the presence of phytochemicals such as tannin, saponin, flavanoids and alkaloids. The bulb is used in the treatment of various illness in Borno state in the north- eastern region of Nigeria. Three species are identified in Nigeria and the plant is found wild (Adelanwa, 2008). The objectives of the study is to evaluate the genetic diversity of *D. filamentosum* using the molecular tool ISSR, to see the intraspecific relationship among

and within the populations under study and to provide a molecular base assessement of the Nigerian species of *Dipcadi*.

## MATERIALS AND METHODS

The bulbs of the plant were collected from eleven locations in eight States in South-Western, North – Eastern, North – Central and North – Western geographical zones of Nigeria (Table 1). These were transplanted in polythene bags.

**Genomic DNA Extraction.** The fresh leaves of *Dipcadi filamentosum* from 13 samples were used for DNA extraction. 0.2 g of tissue was frozen with liquid nitrogen and ground into fine powder. Total DNA was isolated using Qiagen Dneasy mini plant extraction kit. The quantity and quality of the DNA were checked using the spectrophotomter/Nanodrop and 0.8% agarose gel electrophoresis respectively. Absorbance ratio of the DNA material at a wavelength of 260 nm and 280 nm was recorded to ascertain the quality o f the genomic DNA. The DNA sample was stored at -4

**Polymerase Chain Reaction (PCR) Amplification.** The ISSR assay was performed with screened seven di- and three tri- nucleotide repeats ISSR primers (Table 2). Polymerase chain reaction (PCR) was carried out in a volume of 25 µl containing 1 X Reaction buffer (pH 8.3) with 3.0 mM $MgCl_2$, 10µM primer, 400 µM each of deoxynucleotides (dNTPs), 1 unit of *Taq* polymerase (Premix Taq Version 2.0, Cat.No.:XG334A, Xcelris, India), and 50 mg of genomic DNA. The PCR mixture was subjected to initial denaturation at 94°C for 4 minutes. The reaction was subjected to a 35 cycles of denaturation at 94°C for 1 sec, annealing at 50 - 60°C for 1 sec and extension at 72°C for 2 min with a final extension at 72°C for 7 min. The products of amplification were stored at 4°C till further use.

**ISSR Gel Electrophoresis.** Amplified products along with external size standard were separated in a horizontal gel electrophoresis unit using 1.5% agarose gel in 1 X TAE buffer at 110 volt for one and half hours (run 2/3 of gel) and stained with EtBr (10 mg/ml). The banding patterns were visualized under UV light and photographed using a Gel Documentation System (Bio-Rad, India).

**Data analysis.** The amplification products were scored across the lanes comparing their respective molecular weights. Scoring of bands was done from gel photographs. Each band was treated as one ISSR marker. Homology of bands was based on distance of migration in the gel. Each amplification fragment was named by the source of the primer (Xcelris, India) kit letter or number, and its approximate size in base pairs. The ISSR bands were scored 1 for present or 0 for absent across the genotypes and only those bands which were well defined and consistently reproducible in three independent amplifications were included in the final analysis. The data were scored in an excel sheet and was converted manually in PAST. Cluster analysis was performed using the unweighted pair group method with arithmetic averages (UPGMA). Dendrograms were constructed using the UPGMA. The binary matrix was used to determine the genetic diversity, genetic differentiation and gene flow using the software POPGENE (Glaubitz, 2004) ver. 3.2. (Nei, 1973) and the Shannon's index (I) were estimated within and among the populations and genetic diversity (h) using corrected allele frequency. Gene flow (Nm) was estimated from Nm = 0.5 (1 _ GST)/GST (Nei, 1973).

**RESULTS AND DISCUSSIONS**

The 10 ISSR primers used of which only 6 amplified in this study generated a total of 146 amplicon in 13 populations. A total of 41 loci were detected by the 6 primers with a total of 34 loci represented by 82.92% polymorphic bands. Averages of six to eight bands were produced by each primer per population. The banding patterns are shown in Plates 1a-1f. The sizes of the amplicon in all loci ranged from 100 to 1500 bp (Table 3). The highest number of amplicon was produced in ISSR 4 while the least was in ISSR 1 (Table 3). The principal component analyses for correlation of the performance of the various loci on the alleles revealed that the first three principal components accounted for approximately 80.02% of the total variation among the 13 populations (Table 4).

The highest genetic variation observed was explained by component one (32.93%), followed by the 28.92% in component two and the third component (18.17%) (Table 4).The highest positive loading (0.6714) in principal component 1 of locus contributing to detection of genetic variations was in ISSR 1 while least negative loading (0.082) was in ISSR 6. In component 2, the highest (0.687) was in ISSR 1 with the least (0.1177) in ISSR 7, however the highest positive loading (0.7626) in component 3 was in ISSR 5 with the least recorded value (0.1343) in ISSR 2 (Fig 1). The Biplot analysis showed the populations from OGN and EKT which are far from the centroid and MAI very close to the centroid in quadrant I and ISSR 1 primer accounts for its distribution in the quadrant. Five populations were shown in quadrant II, these are SOB, YOB, KBA, KAB and TNK. In quadrant III, KAM and KAS populations are overlapping and OYO is close to the centroid while KAT was far from it. The markers delimiting the distribution of the populations were ISSR 4 and ISSR 5. Similarly the population from UNI which are located far away from the centroid are marked by 3 ISSR markers (Fig. 1).

The Shannon's indices (I) and Nei's genetic diversity (h) among the studied populations from the various regions were estimated at 0.6216 (SD = 0.1199) and estimate of gene flow (Nm) in the population was 5.0555.

The neighbour joining cluster analysis using Jaccard similarity matrix indicated that populations from OYO and KAT are closely related from which other populations arose (Fig.2). The KBA Population has gathered more amino acids probably due to a higher rate of mutation. The MAI and UNI are clustered together as close relatives while the populations from Kaiama i.e. KAB, KAM and KAS are however clustered as related. SOB and YOB populations are also clustered together showing a consistency in clustering of north central population with the north eastsern region together. Populations from TNK are shown to have mutated at a higher rate than OGN and EKT which are closely related.

The ISSR markers used revealed genetic diversity and relatedness among the populations of *D. filamentosum* in this study. The assessment of intra specific genetic variability is significant in the conservation of germplasm and genetic resources in plant breeding programmes. A total of 41 loci were detected by 6 ISSR primers with 34 of these loci representing 82.92% as polymorphic loci. This shows that the markers used are effective in detecting the intraspecific genetic variations present in the populations.

A high number of alleles and polymorphism are very important in assessment of genetic diversity. The highest number of amplicon (65) was generated from the ISSR 4 marker which is an indication that the marker was an effective locus in this study. This is in agreement

with the work of Ajibade *et al*. (2000) who opined that a high number of alleles and polymorphism are very important for correct estimation of genetic diversity of a germplasm.

ISSR markers used in this study were rapid and highly reproducible due to its primer length and the high stringency achieved by the annealing temperature. This was evident in the reproducible bands obtained. This is in agreement with the work of Esslink *et al*., (2004) who opined that microsatellite profile in diversity studies are commonly interpreted in terms of allele phenotypes.

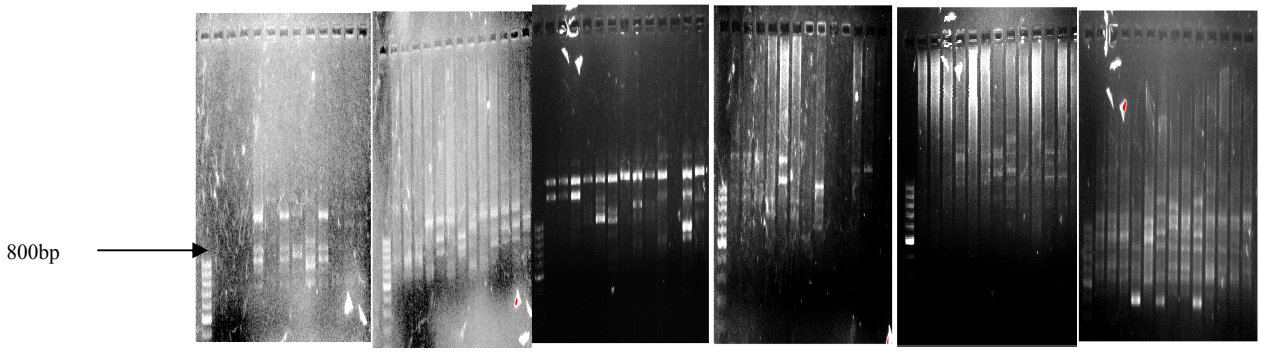
The formation of unique bands at 1000bp and 200bp of 7 alleles in the ISSR 2 loci and at 1500bp in 1 allele in ISSR 1 loci indicated that these populations are different from other populations.

146 reproducible amplicon were generated by the 6 ISSR primers used on 13 genotypes and 6 to 8 allelic richness per loci showed the effectiveness of the markers in detecting the extent of genetic diversity in *D. filamentosum*. This was also asserted by Pfeifer *et al.,* (2011) in their study that the degree of polymorphism and distinct reproducible bands showed the extent of diversity and effectiveness of the markers.

All primers used in this study amplified and produced polymorphic bands, however the ability of primers ISSR 5 and ISSR 6 to produce the highest percentage polymorphism in *D. filamentosum* showed their efficacy on the genotypes. According to Harris *et al*., (2009), the effectiveness of a marker is expressed in the amplified loci polymorphism and this varies for markers and this is in consonance with the above report from the current study.

The principal component analysis revealed that three components are responsible for the observed variations in the study. The occurrence of populations KAM and KAS in the same quadrant showed their relatedness. The presence of the populations from north central and north east in quadrant II is an indication of the relatedness that exist between the populations in terms of gene flow and genetic hybridization. The populations from OYO and KAT are in quadrant IV and close to the centroid. This showed that they are closely related and are a close ancestors from which other populations might have originated (Maniruzzaman *et al*., 2010). The species close to the centroid are closely related while those far from it are distantly related. The markers delimiting the populations are found dispersed in the quadrants. This is an indication that this marker is not affected by the environment and is in accordance with the work of Ozrenk *et al.* (2009).

In neighbour joining cluster analysis, the KAT and OYO populations were shown to be closely related and KBA was a cluster on its own and shown to have evolved more than MAI and UNI because it has sequenced more amino acid. Populations of KAB, KAM and KAS selected on the basis of their sizes were clustered together as same based on their genetic variability. The duo of SOB and YOB from different geographical zones were shown to be closely related. According to Abel (2007) the variation in selection pressure results into inherent variations among different populations of the same species. In the work of Alege (2013), it was asserted that the clustering of sesame from different geographical regions together is an indication of seed flow via market exchange or more specifically the result of gene flow among some regions. This explains the closeness of populations from different geographical regions as recorded in this study.




**PLATES1a-lf: Banding pattern of the ISSR primers.**

**TABLE 1. Population of *D. filamentosum* collected from various sites in Nigeria.**

| Geographical location | Place of collection | State of collection | Code for each location | *Annual rainfall (mm) | *Mean temp ($^{o}$C) | Soil type |
|---|---|---|---|---|---|---|
| South Western | Ado Ekiti | Ekiti | EKT | 1,605.40 | 26.87 | Sandy loam |
| | Ogun | Ogun | OGN | 1,126.05 | 25.05 | Sandy loam |
| | Oyo | Oyo | OYO | 1,318.10 | 26.61 | Loamy |
| North East | Maiduguri | Borno | MAI | 745.70 | 28.86 | Sand |
| | Damaturu | Yobe | YOB | 696.60 | 29.70 | Sand |
| North Central | Sobi | Kwara | SOB | 1,046.30 | 26.35 | Sandy |
| | Tanke | Kwara | TNK | 1,046.30 | 26.35 | Sharp sands |
| | Unilorin | Kwara | UNI | 1,046.30 | 26.35 | Sandy loam |
| | Kaiama (big) | Kwara | KAB | 1,046.30 | 26.35 | Loamy |
| | Kaiama (medium) | Kwara | KAM | 1,046.30 | 26.35 | Clay loamy |
| | Kaiama (small) | Kwara | KAS | 1,046.30 | 26.35 | Loamy |
| | Kabba | Kogi | KBA | 1,05730 | 26.40 | Loamy clay |
| North West | Katsina | Katsina | KAT | 527.5 | 27.28 | Sandy loam |

**TABLE 2. ISSR Markers and Annealing Temperature**

| Oligo name | Sequence | Annealing temperature ($^0$C) |
|---|---|---|
| ISSR 1 | AGCACGAGCAGCAGCGA | 56 |
| ISSR 2 | AGCACGAGCAGCAGCGG | 58 |
| ISSR 3 | AGCACGAGCAGCAGCGT | 56 |
| ISSR 4 | AGCACGAGCAGCAGCGC | 58 |
| ISSR 5 | CACACACACACACAAT | 46 |
| ISSR 6 | CACACACACACACAAC | 48 |
| ISSR 7 | CACACACACACACAGT | 48 |
| ISSR 8 | CACACACACACACAGC | 50 |
| ISSR 9 | CACACACACACACAGA | 48 |
| ISSR 10 | CACACACACACACAAA | 46 |

**TABLE 3: Amplification parameters of ISSR marker in *D. filamentosum***

| DNA Marker | Amplicon Size (bp) | Total Amplicon in Genotype | Amplicon per Genotype | No of Polymorphic Loci | No of Monomorphic Loci | % Polymorphism |
|---|---|---|---|---|---|---|
| ISSR 1 | 1500 – 400 | 12 | 7 | 5 | 2 | 71.45 |
| ISSR 2 | 1400 – 300 | 21 | 6 | 5 | 1 | 83.33 |
| ISSR 4 | 1200 – 100 | 65 | 8 | 5 | 3 | 62.50 |
| ISSR 5 | 1000 – 400 | 20 | 8 | 7 | 1 | 87.50 |
| ISSR 6 | 1200 – 200 | 14 | 6 | 6 | 0 | 100 |
| ISSR 7 | 1100 – 400 | 14 | 10 | 6 | 0 | 60 |

**TABLE 4: Principal Component Analysis (Correlation) using ISSR markers.**

| Principal Component | Eigen value | % Variance |
|---|---|---|
| 1 | 1.97556 | 32.926 |
| 2 | 1.73526 | 28.921 |
| 3 | 1.09039 | 18.173 |

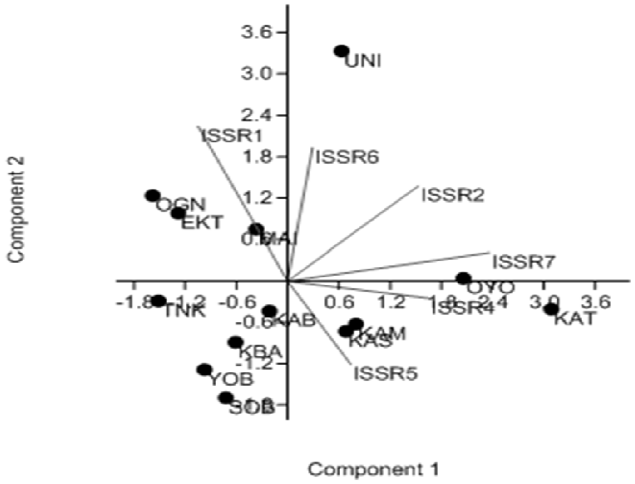



**FIG. 1: Biplot of Principal component analysis (PCA) of *Dipcadi filamentosum* using ISSR markers.**

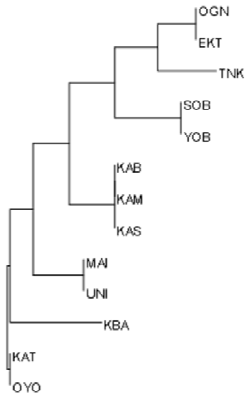



**FIG. 2. Neighbour joining clustering (Jaccard) of 13 populations of *D. filamentosum* using ISSR markers**

## CONCLUSIONS

Based on the phylogenetic results in this study, it can be deduced that the relationships among populations may be based on their basic genetic compositions with certain amount of variations. The genetic diversity present in the 13 populations of *D. filamentosum* was captured in the clustering of populations from different geographical regions together. KAT (from north central) and OYO (from south west) been clustered together at the root of the tree are an indication of the effectiveness of the ISSSR molecular marker in showing the variability in the populations under study.

Acknowledgement
We wish to thank the Federal Government of Nigeria, University of Ilorin, Ilorin, Nigeria for providing the Fellowship and the C.G. Bakhta Institute of Technology, UKA Tarsadia University, Gujurat for their Technical assistant during this study.

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