EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 3
Issue: 7
328-332
Year: 2016

GENETIC DIVERSITY ANALYSIS OF *SELAGINELLA BRYOPTERIS* (L.)BAK. GENOTYPES IN TELANGANA, INDIA.

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Article Received on 26/04/2016 **Article Revised on 17/05/2016** **Article Accepted on 08/06/2016**

ABSTRACT
The present study is aimed to evaluate the genetic diversity among the eight genotypes of *Selaginella bryopteris* (L.)Bak. belonging to Telangana state, India. Genetic variation among the genotypes was analyzed with PCR based Random amplified polymorphic DNA (RAPD) markers.12 RAPD primers OPA-01, OPA-02, OPA-03, OPA-04, OPA-05, OPA-09, OPA-10, OPA-13, OPA-16, OPA-19, OPC-05 and OPC-10 were used in this study. Amplified DNA fragments were separated in 1.5% agarose gel for RAPD analysis. A total 443 DNA fragments were amplified with an average of 6.81 RAPD markers per primer. A maximum of 9 fragments were amplified with primer OPC-05 and a minimum of 4 bands with primer OPA-09.The PIC ranged from 0.06-0.672. The dendrogram based on similarity coefficient showed two major clusters. Cluster-I consisted of Medak-1 and Medak-2. Cluster-II is divided into four sub classes including the remaining six genotypes. Maximum similarity was observed between RR-1 and RR-2 genotypes with 0.85% similarity.

KEYWORDS: *Selaginella bryopteris* (L.)Bak.,Telangana., Genetic diversity and RAPD markers.

INTRODUCTION
Selaginella bryopteris(L.)Bak. is an endemic, resurrect and Pteridophytic species of India. It is a lithophyte that can be found on the hills of tropical areas. This herb can grow well in xeric conditions as an indicator of atmospheric humidity (Singh and Singh, 2015).The fronds are curled, become dry and look as folded legs of birds, in water availability the plant gets rehydrate and turns as green. It shows high desiccation tolerance. Hence it is called magic herb. Because of this unique character the plant is sold as an ornamental in various markets of India.

Genetic diversity is the part of the bio diversity. Genetic diversity includes variability of DNA or genes from one species to another species (variation within species) and among the population. It plays a vital role in the polygenetic and evolutionary studies of various crop varieties, medicinal plants and endangered species. In recent years many researchers have been used various molecular markers like RFLP, RAPD, AFLP, ISSR and SSRs to evaluate the genetic combinations and variability among the different species.

Random amplified polymorphic DNA (RAPD) analysis is PCR based technique which is used for genome mapping, gene tagging and studies of phylogenetic relationships (Williams et al., 1990, and Pratibha Devi, 2005). RAPD primers are universal primers and random decamer oligonucleiotides. In the case of RAPD (Rapid) short oligonucleiotide primers are arbitrarily selected to amplify a set of DNA fragments randomly distributed throughout the genome. Hence this technique also known as arbitrarily primed PCR (AP-PCR).

MATERIALS AND METHODS
Plant Collection
Eight genotypes of *Selaginella bryopteris* (L.) Bak. were obtained from various hilly regions of Telangana, India especially in the regions of Rangareddy, Medak, Mahaboobnagar, Karimnagar and Warangal districts in various seasons of the year respectively. Collected plants were wrapped in aluminum foils and stored at 4^{o}C of temperature for future usages. For the present study fresh as well as frozen plant materials were used. The sampling locations and their GPS (Global Position System) are provided in the Table-1.

Table -1: The sampling locations and their GPS (Global Position System)

Sampling site no.	Sampling site code	Location of Sampling	Latitude	Longitude
1	KNR-1	Shanigharam,Karimnagar(D)	$18^0$11'32.84"N	$79^0$00'32.57"E
2	KNR-2	Lingannapet, Karimnagar(D)	$18^0$17'10.87"N	$78^0$35'05.48"E
3	MBNR-1	Ippalpalle,Mahaboobnagar(D)	$16^0$40'59.43"N	$77^0$58'34.08"E
4	MDK-1	Pullur,Medak(D)	$18^0$10'33.95"N	$78^0$48'44.77"E
5	MDK-2	Chandlapur,Medak(D)	$18^0$10'07.13"N	$78^0$49'25.43"E
6	R.R-1	Ekambbari,Rangareddy(D)	$17^0$82'09.31"N	$77^0$27'10.22"E
7	R.R-2	Kulakcharla,Rangareddy(D)	$17^0$01'13.37"N	$77^0$54'44.12"E
8	WL-2	Komaravelli,Warangal(D)	$17^0$55'51.41"N	$78^0$53'51.04"E

DNA Isolation Method

Total genomic DNA of eight genotypes of *Selaginella bryopteris*(L.)Bak. was extracted by CTAB method given by Murray & Thompson (1980) with some modifications. Quantity and quality of genomic DNA of *Selaginella bryopteris*(L.)Bak. assessed by spectrophotometre and gel electrophoresis methods. After quantification the DNA was diluted with $T_{10}E_1$ buffer (Tris 10 mM and EDTA 1Mm, pH 8.0) to perform PCR analysis.

PCR based Random Amplified Polymorphic DNA (RAPD) Analysis

RAPD Markers are random decamer oligonucleiotide primers. A set of 12 random decamer oligonucleiotides (OPA01,OPA-02,OPA-03,OPA-04,OPA-05, OPA-09, OPA-10,OPA-13, OPA-16,OPA-19,OPC-05 and OPC-10) were purchased from *Operon Technolgies* (Almanda,USA) used as primers for the amplification of RAPD fragments. The list of RAPD primers and their sequences are presented in Table-2. PCR amplification was performed in an ependroff thermal cycler (MJ Research, Inc, Watertown, MA. USA) with a final volume of 25 µl. The components of PCR reaction mixture was given in Table-3. The PCR amplification for RAPD analysis was performed according to Williams et al. (1990) with certain modifications within three steps of denaturation, primer annealing and prime extension. The thermo profile of PCR was presented in Table-4. Amplified DNA products were stored at 4°C until the gel electrophoresis is carried.

Agarose gel electrophoresis

The amplified DNA fragments were separated in 1.5% agarose gel, visualized under UV light and photographed. The gel is documented by Gel Doc 2000 for scoring the bands. The amplicon size is determined by comparing with the ladder (Genie 1kb ladder).

Data scoring and analysis

The frequency of RAPD polymorphism is calculated based on presence (taken as 1) or absence (taken as 0) of common bands (Ghosh et al., 1997). The binary data is used to compute pair wise similarity coefficient (Jaccard, 1908) on NTSYS-PC (Numerical taxonomy and multivariate analysis system) version 2.02e statistical package (Rohlf, 1997). A dendrogram based on similarity coefficient is generated by using the un-weighted pair group of arithmetic means (UPGMA).

RESULTS AND DISCUSSION

Genomic DNA of eight *Selaginella bryopteris*(L.)Bak. genotypes were amplified with 12 different random primers. Different primers produced a different level of polymorphism and the polymorphic index content among the eight genotypes was presented in Table(5). In gel documentation, the amplified DNA banding pattern with different RAPD Primers was presented in Figs 1, 2 and 3. A total of 443 DNA fragments were amplified, with an average of 6.81 RAPD markers per primer. Out of 443 amplified fragments 184 were found to be monomorphic and the remaining 259 amplicons were polymorphic. The amplitude of polymorphisms was high and not a single primer (out of 12 studied) could differentiate clearly all the varieties. The size of the amplified fragments also varied with different primers. The approximate size of the largest fragment produced is 3.0 kb and the smallest fragment produced as 0.5 kb. A maximum of 9 fragments were amplified with primer OPC-5 and a minimum of 4 bands with primer OPA-9. The PIC ranged from 0.06 to 0.672 (Table-5).

To estimate the genetic similarities of the *Selaginella bryopteris*(L.)Bak. genotypes, a similarity matrix is obtained using Jaccard's similarity coefficient (1908) and presented in Table-6. These similarity coefficients were used to generate a dendogram (Figure-4) by UPGMA analysis in order to determine the grouping of different varieties. The dendrogram clearly depicted the genetic similarity ranging between 0.60 to 0.84. The dendrogram showed two major clusters. Cluster-I with Medak-1 and Medak-2, revealed 0.66% similarity. Cluster-II is divided into four sub class. Sub class-I comprised of KNR-1, RR-1 and RR-2. Sub class-II contained only KNR-2 with 0.75% similarity to sub class-I. Sub class-III with MBNR-1 showed 0.71% similarity to sub cluster-II. Sub class-IV contained only WL-1 with a 0.67% similarity to sub cluster-III. Maximum similarity was observed between RR-1 and RR-2 with 0.85% similarity among the eight *Selaginella bryopteris*(L.)Bak. genotypes studied from different regions of Telangana.

Table-2: List of random decamer primers used for PCR amplification.

S. No.	Primer Name	Sequence
1	OPA-01	5'CAGGCCCTTC3'
2	OPA-02	5'TGCCGAGCTG3'
3	OPA-03	5'AGTCAGCCAC3'
4	OPA-09	5'GGGTAACGCC3'
5	OPA-05	5'AGGGGTCTTG3'
6	OPA-04	5'AATCGGGCTG3'
7	OPA-10	5'GTGATCGCAG3'
8	OPA-13	5'CAGCACCCAC3'
9	OP C-05	5'GATGACCGCC3'
10	OP A-16	5'AGCCAGCGAA3'
11	OP A-19	5'CAAACGTCGG3'
12	OP C-10	5'TGTCTGGGTG3'

Table-3: The components of PCR reaction mixture.

Sl. No.	Components	Quantity (µl/Reaction)
1.	10x assay buffer with 15 mM $MgCl_2$	2.5
2.	$MgCl_2$	1.0
3.	DNTPs mix (2.5 mM each)	1.0
4.	Primer (5 pM/µl)	1.0
5.	Template (15 ng/µl)	2.0
6.	Sterile distilled water	17.16
7.	*Taq*DNA polymerase (3U/µl)	0.34

Table-4: Thermo profile of PCR.

Initial Denaturation	94^0C for 5min
Denaturation	94^0C for 45Sec
Primer Annealing	37^0C for 45Sec
Primer Extension	72^0C for 1min
Final Primer Extension	72^0C for 10min
No. of Cycles	30

Table-5: List of RAPD primers and their polymorphism information content (PIC)

S.No.	Primer name	PIC	No.of alleles	No. of monomorphic alleles
1	OPA-01	0.18	8	5
2	OPA-02	0.53	7	2
3	OPA-03	0.41	7	2
4	OPA-09	0.06	4	3
5	OPA-05	--	--	--
6	OPA-04	0.67	6	0
7	OPA-10	0.40	8	0
8	OPA-13	0.36	8	2
9	OP C-05	0.41	9	2
10	OP A-16	0.15	7	5
11	OP A-19	0.62	6	1
12	OP C-10	0.57	6	1

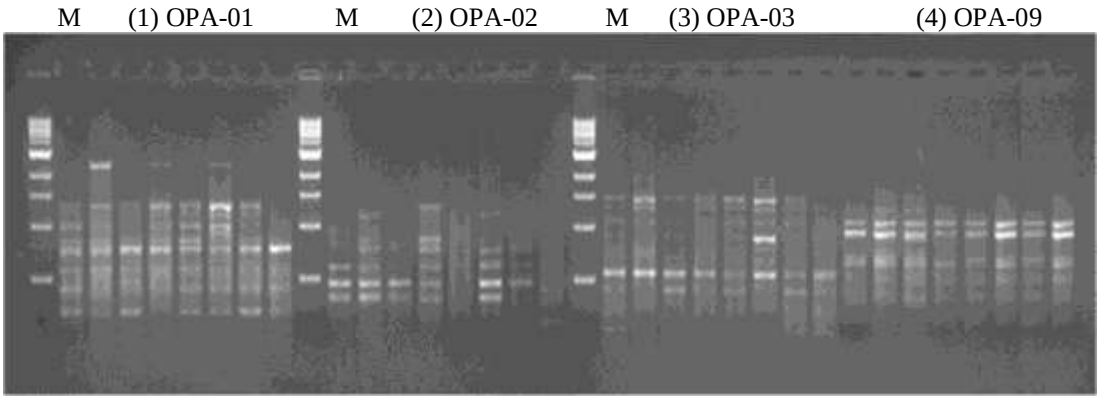

Fig-:1 Amplified profile of *Selaginella bryopteris* (L.)Bak. genotypes with RAPD primer of (1) OPA-01 (2) OPA-02 (3) OPA-03 (4) OPA-09 : Lane M=1kb size marker

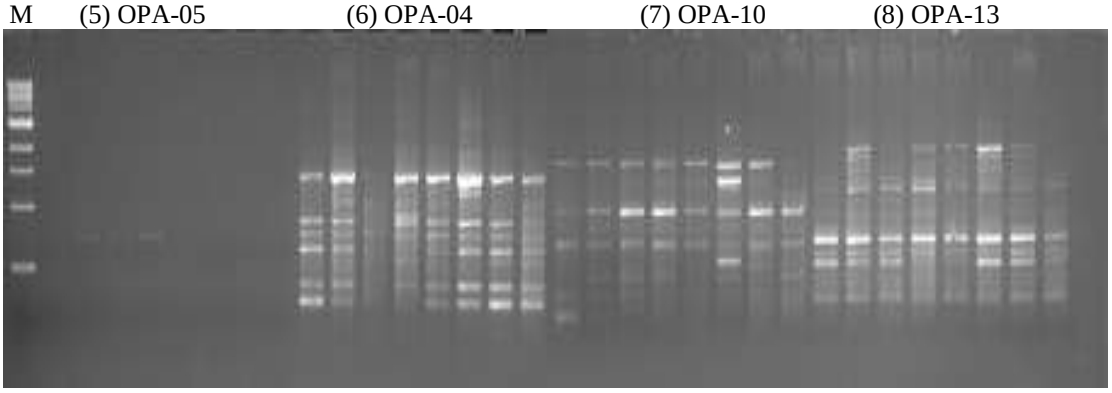

Fig-2: Amplified profile of *Selaginella bryopteris* (L.)Bak. genotypes with RAPD primers of (5) OPA-05 (6) OPA-04 (7) OPA-10 (8) OPA-13 : Lane M=1kb size marker

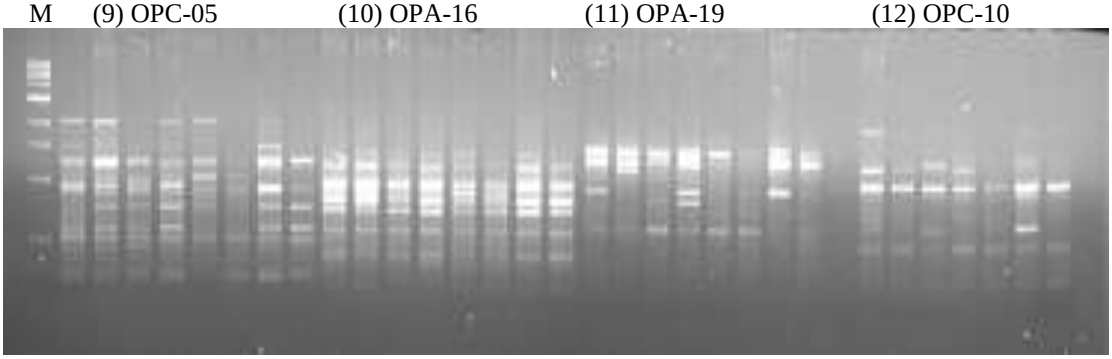

Fig-3: Amplified profile of *Selaginella bryopteris*(L.)Bak. genotypes with RAPD primers of (9) OPC-05 (10) OPA-16 (11) OPA-19 (12) OPC-10 : Lane M=1kb size marker.

Table-6: Similarity co-efficient values among eight genotypes of *Selaginella bryopteris*(L.)Bak.

	KNR-1	KNR-2	RR-1	RR-2	WL-1	MBNR-1	Medak-1	Medak-2
KNR-1	1.000							
KNR-2	0.767	1.000						
RR-1	0.757	0.746	1.000					
RR-2	0.818	0.754	0.845	1.000				
WL-1	0.677	0.690	0.667	0.697	1.000			
MBNR-1	0.667	0.652	0.800	0.732	0.672	1.000		
Medak-1	0.651	0.607	0.667	0.672	0.574	0.574	1.000	
Medak-2	0.597	0.554	0.662	0.554	0.500	0.594	0.650	1.000

CONCLUSION

In the past, genetic diversity studies on plants have been grossly inadequate especially ferns are very poorly studied due to the lack of proper instrumentation and technique. Molecular markers are valuable tools in the characterization and evaluation of genetic diversity within and among the species and population. Marker system depends on polymorphism which has been used to define genetic variation in a population (Mukherjee etal., 2010). Random amplified polymorphic DNA (RAPD) is PCR based technique, commonly used to screen genetic variability among the species and population (Williams etal., 1991). The molecular analysis as an integral component in the conservation of rare and endangered species (N.Kingstone etal.,2004), that can be extended for conservation of the present studied genotypes of *Selaginella bryopteris* (L.)Bak. from various regions of Telangana, India. In the present study maximum similarity was observed between RR-1 and RR-2 (0.85%) among the eight *Selaginella bryopteris*(L.)Bak. genotypes studied from different regions of Telangana. The remaining six genotypes showed variation and are genetically distant, indicating the genetic diversity among the genotypes studied from different regions of Telangana.

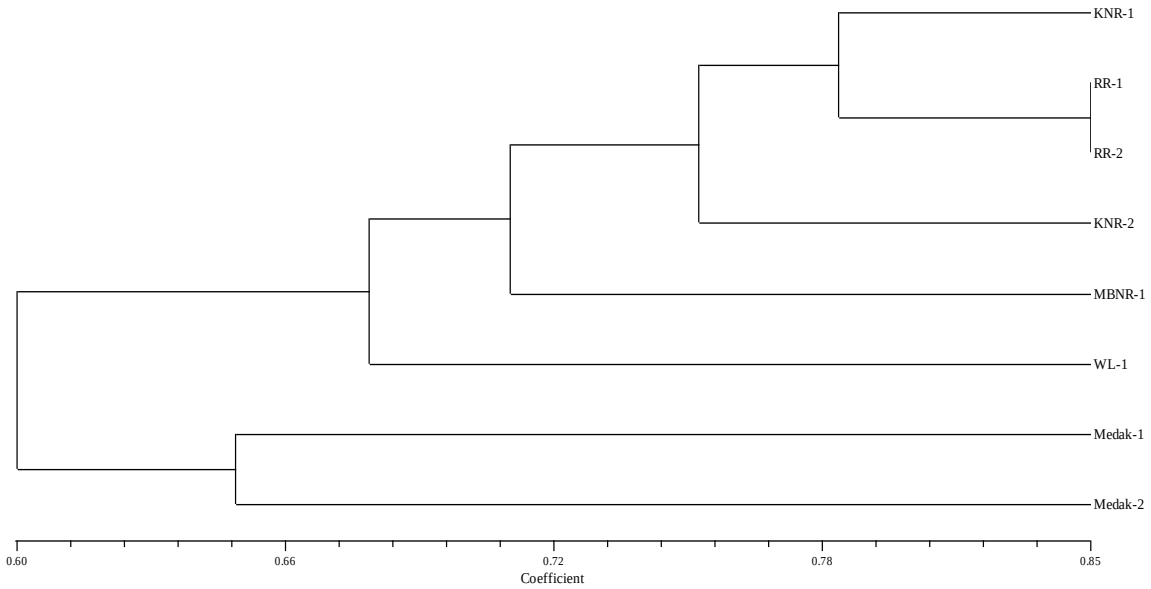

Fig-4: Dendrogram with the genetic similarities among the eight genotypes of *Selaginella bryopteris* (L.)Bak

REFERENCE

1. Ghosh S, Karanjawala ZE, Hauser ER (1997): Methods for precise sizing automated inning of alleles and reduction or error rates in large scale genotyping using fluorescently labeled dinucleiotide markers. Genome Res 7: 165-178.
2. Jaccard P (1908) Nouvelles Researches sur la distribution florale. Bulletin Soc Vand Sci Nat 44: 223-270.
3. Mukherjee AK, Ratha S, Dhar S, Debata AK, Acharya PK, Mandal S, Panda PC, Mahapatra AK (2010): Genetic relationships among 22 taxa of bamboo revealed by ISSR and EST based random primers .BioChem Genet 48: 1015-1025.
4. Murry.M and W.F.Thompson (1980), Rapid isolation of molecular weight plant DNA. Nucleic acid: 8: 4321-5.Cross Ref,PubMed.
5. Naomi Kingstone, Steve Waldren and Noeleen Smyth (2004): Conservation genetics and ecology of *Angiopteris chauliodonia* Copel.(Marattiaceae), a critically endangered fern from Pitcarian Island,SuthCentral Pacific Ocean. Biological Conservation 117: 309-319.
6. Pratibha Devi(2005): Principles and Methods of Plant Molecular Biology, Biochemistry and Genetics. ISBN No.:81-7754-051-3.
7. Rohlf FJ (1997) NTSYS-Pc. Numerical taxonomy and multivariate analysis system version 2.02e.Exeter Software. Newyork.
8. Swetha singh and Rita singh, (2015): A Review an endemic Indian resurrecting herb *Selaginella bryopteris* (L.)BAK.'SANJEEVANI': IJPSR, 6(1): 50-56, 2320-5148.
9. Sayantani Das, Maumita Bandyopadhyay and Subir Bera(2012): Optimization of protocol for isolation of genomic DNA from leaves of *Selaginella* species suitable for RAPD analysis and study of their gene variation, American Fern Journal 102(1): 47-54.2012.
10. Williams, J.G., etal.(1990): DNA polymorphisms amplified by arbitrary primers are useful as genetic markers.