International Journal for Pharmaceutical Research Scholars (IJPRS)

V-2, I-4, 2013 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Pattern of Diversity Using Molecular Marker (nrITS): An Example from Acanthaceae and Euphorbiaceae

Vishwesh R. Potkar*, Satish N. Pardeshi, Pratima S. Jadhav

Department of Biochemistry, Biotechnology, Institute of Science, Mumbai - 400 032, India.

Manuscript No: IJPRS/V2/I4/00248, Received On: 21/12/2013, Accepted On: 28/12/2013

ABSTRACT

Molecular sequence data was used from the nuclear ribosomal internal transcribed spacers (ITS) to study phylogenetic relationship within Acanthaceae and Euphorbiaceae. 8 Species belonging to family Acanthaceae and 2 species belonging to family Euphorbiaceae were collected from different locations of Mumbai for this present study. The ribosomal internal transcribed spacers (ITS1-ITS2) along with 5.8S region from individual member were amplified by polymerase chain reaction (PCR) using universal primers and were sequenced. Sequences were uploaded in NCBI genbank, and were aligned and phylogenetic tree was constructed by neighbor joining method using online tools. ITS length and GC% was calculated using bioinformatics tool. (http://www.bitools.org). ITS length ranges from 524 to 638 base pairs (bp) where as GC% ranges from 53.4 to 61.8 in various Acanthaceae and Euphorbiaceae members. 5.8 S gene was found to be highly conserved and its size ranged from 178 to 259 bp. This combined data set provides a highly resolved hypothesis of relationship. Our analysis provides considerable resolution of relationship within various genera of family Acanthaceae and Euphorbiaceae. So ITS region may be considered as a good marker to study evolutionary history and to resolve controversies if any, in plant systematic.

KEYWORDS

Acanthaceae, Euphorbiaceae, nrITS, Phylogenetic

INTRODUCTION

Taxonomy is the science of identification, nomenclature, description of organisms and classification. Taxonomy is a part of the scientific practice known as systematic, which entails the evolutionary relationships between organisms. The most prevalent nomenclature system is the Linnaeus system, which is binomial system of nomenclature. This system uses two words to classify an organism as genus and specific epithet. These two words are written in Italics in scientific documents.

***Address for Correspondence:**
Vishwesh R. Potkar
Department of Biochemistry, Institute of Science,
15, Madam Cama Road, Fort,
Mumbai - 400032, India.
E-Mail Id: vishweshpotkar@gmail.com

The typical classification system goes from broadest to narrowest as follows: kingdom, class, series, order, family, genus and species. Phylogenetic is the study of evolutionary relatedness among various groups of organisms (e.g., species, populations). Also known as phylogenetic systematics or cladistics, phylogenetic treats a species as a group of lineage-connected individuals over time. Taxonomy, the classification of organisms according to similarity, has been richly informed by phylogenetic but remains methodologically and logically distinct[1].

Acanthaceae is one of the families of dicotyledonous flowering plants containing about 240 genera and more than 2,200 described

species. It is largely a tropical family of plants, which includes perennial herbs, armed/ unarmed shrubs, trees and vines[2]. *Hygrophila*, commonly known as the temple plants or hygros, is a genus of family Acanthaceae. There are about 100 species of *Hygrophila* in the world[3]. In India, about 7 species are generally found in tropical and warm temperate regions[4]. While 3 species were reported from then Presidency of Bombay[5].

Phyllanthus is the largest genus in the family Euphorbiaceae. *Phyllanthus* has a remarkable diversity of growth forms including annual and perennial herbaceous, arborescent, climbing, floating aquatic, pachycaulous, and phyllocladous. *Phyllanthus* has more than 700 species in at least 10 sub-genera[6,7]. Among the herbs of subgenus *Phyllanthus* used medicinally, it was found that aqueous extracts of *P. amarus*, *P. debilis*, *P. fraternus*, *P.niruri* and *P. urinaria* L. all inhibited viral DNA polymerase (DNAp) of hepadnaviruses *in vitro*[7].

The internal transcribed spacer (ITS) has been used in numerous systematic studies at the generic and specific levels of a wide array of plant taxa [8]. The two internal spacers, ITS-1 and ITS-2, are located between genes encoding the 5.8s, 18s and 26s nuclear ribosomal RNA (nrRNA) subunits[8]. Individually, ITS-1 and ITS-2 are around 300 bp in length and the 5.8s subunit is almost invariant in length within angiosperms (163-164 bp), making the entire ITS region approximately 700 bp. Given the short length and the highly conserved nature of the flanking ribosomal subunit genes, the ITS region is easily amplified from small amounts of genomic DNA by the polymerase chain reaction[9]. The two internal transcribed spacers (ITS1 and ITS2) of nuclear ribosomal DNA have become commonly exploited sources of informative variations for phylogenetic analysis among angiosperms[10]. Hence, this technique is widely used for molecular systematic investigations of higher plants.

The length and sequences of ITS regions of rDNA repeats are believed to be fast evolving and therefore may vary. Universal PCR primers designed from highly conserved regions flanking the ITS and its relatively small size (600-700 bp) enable easy amplification of ITS region due to high copy number up to 30000 per cell of rDNA repeats[11].

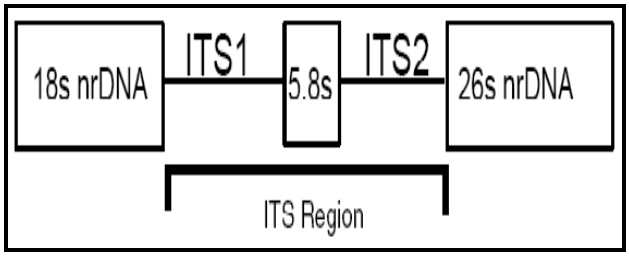


Figure 1: Internal transcribed spacer region of ribosomal RNA

MATERIALS AND METHOD

Collection of Plant Material

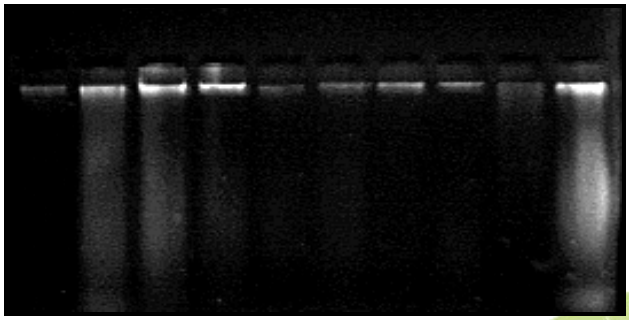
8 Species belonging to family Acanthaceae and 2 species belonging to family Euphorbiaceae were collected from different locations of Mumbai such as Sanjay Gandhi National Park, Veermata Jijabai Bhonsale Udyan, Maharashtra Nature Park, Kanheri caves, Kalina campus, Vihar lake, Manori.

Table 1: Species names and its family

No.	Species	Family
1	Hygrophila schulli	Acanthaceae
2	Hygrophila seryphyllum	Acanthaceae
3	Hygrophila ringens	Acanthaceae
4	Hygrophila phlomoides	Acanthaceae
5	Ruellia brittoniana	Acanthaceae
6	Pseudoeranthemum atropurpurium	Acanthaceae
7	Crossandra infundibuliformis	Acanthaceae
8	Dipteracanthus prostratus	Acanthaceae
9	Phyllanthus fraternus	Euphorbiaceae
10	Phyllanthus tenellus	Euphorbiaceae

Isolation of Genomic DNA

Total genomic DNA was isolated from fresh leaves from single individual using a modified genomic DNA isolation protocol for used for amplification and sequencing [12]. Tissue was collected during winter season. 0.5-1 gm of leaf tissue was used to isolate genomic DNA from respective individuals. The DNA was then resuspended in TE buffer (10 mM: 1 mM) and stored at -20 °C



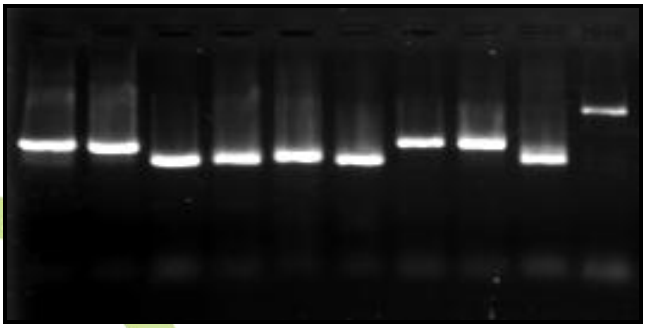
1 2 3 4 5 6 7 8 9 10

Figure 2: Agarose gel electrophoresis of isolated genomic DNA. Numbers to lanes are given according to table no. 1

Quality and quantity of DNA was checked by loading 2µl genomic DNA and 3 µl of gel loading dye on 1.5% agarose gel. The amount of DNA obtained ranged from 20 µg to 60 µg.

PCR Amplification and DNA Sequencing

The entire ITS region of nuclear ribosomal DNA which comprises both internal transcribed spacers ITS 1 and ITS 2 and the 5.8 S sub unit was PCR amplified in a thermal cycler (Helena Biosciences, U.K) using the G1 (GGAAGTAAAAGTCGTAACAAGG) and C2 (TCCTCCGCTTATTGATATGC) primers, which are complimentary to 18S and 26S rDNA near the ITS1 and ITS2 borders respectively [12]. Reaction volumes were 50 µL and contained, 1X Taq DNA Polymerase buffer (Bangalore genei, India), 1.5 mM $MgCl_2$ (Bangalore genei, India), 200 µmolar each deoxynucleotide triphosphate (Bangalore genei, India), 10 pmol oligonucleotide primers (Bangalore genei, India), 1.0 unit of Taq DNA polymerase (Bangalore genei, India), and ~25–60 ng of genomic DNA. PCR was performed in a thermal cycler (Helena Biosciences, U.K) and consisted of initial denaturation of 5 min at 94°C, 35 cycles of 1 min at 94°C for template denaturation, 1 min at 50°C for primer annealing, 1 min at 72°C for primer extension, followed by a final extension of 5 min at 72°C. PCR products were subsequently visualized on a 1.5% agarose gel. Purified products were sequenced, using the same conditions as the PCR. ITS sequences of all species were obtained using G1 primers and sequencing was carried out on ABI Sequencer (Chromous Biotech, Bangalore) with minor manual adjustments.



1 2 3 4 5 6 7 8 9 10

Figure 3: Agarose gel electrophoresis of PCR amplified ITS region. Numbers to lanes are given according to table no. 1

Each ITS DNA sequence was compared by using the BLAST alignment program with data available from GenBank at the National Institutes of Health.

RESULTS AND DISCUSSION

The length of all ITS regions and GC % of were calculated by using online bioinformatics tools. ITS length ranges from 524 to 638 base pairs (bp) where as GC% ranges from 53.4 to 61.8 in various *Acanthaceae* and *Euphorbiaceae* members. 5.8 S gene found to be highly conserved and size ranges from 178-to259 bp.

Sequence Alignment and Phylogenetic Tree

The regions studied obtained from the NCBI GenBank and 18S and 26S regions were edited manually. All nrITS sequences were aligned using were aligned using Clustal computer program with Gap Open Penalty 15 and Gap Extension Penalty 6.66[13].

Table 2: Accession Numbers, Length and GC content of ITS sequences uploaded to NCBI genbank

Species	Accesion No.	ITS length	GC %	Species	Accesion No.	ITS length	GC %
Hygrophila schulli	EU489060.1	625 bp	61.8	Pseudoeranthemum atropurpurium	JF346166.1	626 bp	60.4
Hygrophila seryphyllum	FJ784753.1 FJ784751.1 FJ784752.1	633 bp	59.9	Crossandra infundibuliformis	JF346168.1	610 bp	60.7
Hygrophila ringens	EU621916.1 EU621918.1 EU621917.1	565 bp	58.4	Dipteracanthus prostratus	JF346169.1	638 bp	61.4
Hygrophila phlomoides	FJ625832.1 FJ625834.1 FJ625833.1	633 bp	60.5	Phyllanthus fraternus	EU580532.1 EU580530.1 EU580531.1	567 bp	55.6
Ruellia brittoniana	FJ607442.1 FJ607440.1 FJ607441.1	638 bp	61	Phyllanthus tenellus	EU580529.1 EU580527.1 EU580528.1	524 bp	53.4

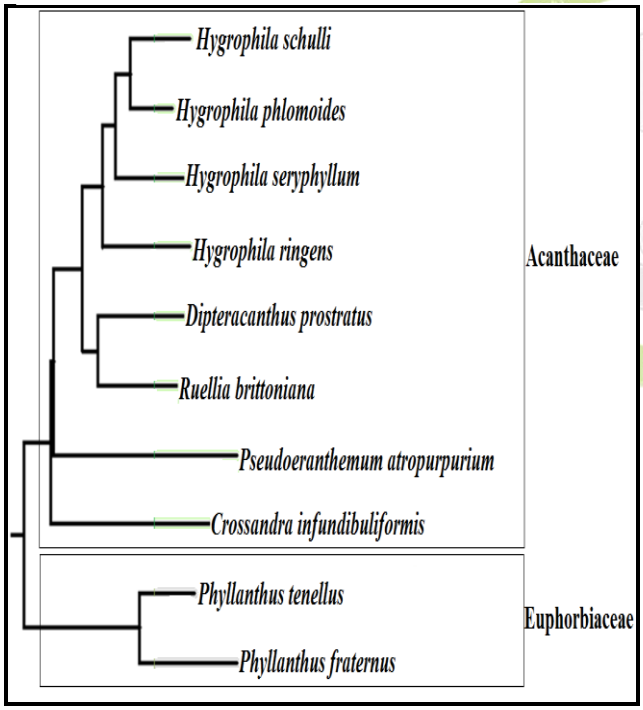


Figure 4: Tree obtained with neighbor- joining method

In the present study it is revealed that all Acanthaceae members very closely related and Euphorbiaceae members are distantly related to Acanthaceae.

In Acanthaceae all four species which are belonging to genus *Hygrophila* formed monophyletic lineage whereas *Dipteracanthus* and *Ruellia* are formed one separate group. *Psedoeranthemum* and *Crossandra* are distantly related to all other Acanthaceae members. Thus in order to classify and trace out evolutionary path among related species, it is important to investigate them at the molecular level for better understanding of evolution of plants.

Biologists are making increasing use of ITS region in phylogenetic to confirm the systematic position of species in classification which is based on morphological characters and also investigating whether biological diversification is because of geographical distribution or ecological adaptation of the species.

The ITS region has served as a remarkable source of data for plant phylogenetic reconstruction. ITS divergence provided phylogenetic information for closely related species as well as distantly related species. These data taken together provided support for the utility of the ITS region for genetic inference.

Thus in order to classify and trace out evolutionary path among related species, it is important to investigate them at the molecular level for better understanding of evolution of plants.

CONCLUSION

Biologists are making increasing use of ITS region in phylogenetic to confirm the systematic position of species in classification which is based on morphological characters and also investigating whether biological diversification is because of geographical distribution or ecological adaptation of the species.

The ITS region has served as a remarkable source of data for plant phylogenetic reconstruction. ITS divergence provided phylogenetic information for closely related species as well as distantly related species. These data taken together provided support for the utility of the ITS region for genetic inference.

Thus in order to classify and trace out evolutionary path among related species, it is important to investigate them at the molecular level for better understanding of evolution of plants.

ACKNOWLEDGEMENT

Authors are grateful to the Honorable Director, Institute of Science, Dr. N. Javali (Head, M.M.G.E.S, M.B.D, B.A.R.C.) and Dr. Ajay Saini (Scientific Officer, M.M.G.E.S, M.B.D, B.A.R.C.) for providing necessary facilities.

REFERENCES

1. Edwards, AWF, Cavalli-Sforza LL, "Phenetic and Phylogenetic Classification: Reconstruction of evolutionary trees", Systematics Association, 1964, 6, 67-76.
2. Benton W, "Anonymous", Encyclopedia Britannica, Chicago, USA, 1965, 1, 61-62.
3. Mazumdar UK, Gupta M, Maiti S, "Chemical and Pharmacological evaluation of Hygrophila spinosa root", Indian journal of Pharmaceutical Sciences, 1999, 61, 181-183.
4. Hooker JD, Flora of British India. L. Reeve & Co. Ltd., Ashford, Kent – 1879- 1885, 4, 406-409.
5. Cooke, T, "The Flora of Presidency of Bombay", London, S leng three Saraswati Press Ltd., Calcutta, India, 1958, 4, 353-354.
6. Holm-Nielsen LB, "Comments on the distribution and evolution of the genus Phyllanthus (Euphorbiaceae)", In: Larsen K, Holm-Nielsen LB Tropical botany, Academic Press, New York, 1979, 277-290.
7. Webster GL, "A monographic study of the West Indian species of Phyllanthus", Journal of the Arnold Arboretum, 1957, 38, 51-80.
8. Baldwin BG, Sanderson MJ, Porter JM, Wojciechowski MF, Campbell CS, Donoghue MJ, "The ITS region of nuclear ribosomal DNA: A valuable source of evidence on angiosperm phylogeny. Annals of the Missouri Botanical Garden", 1995, 82, 247-277.
9. Baldwin BG, "Phylogenetic utility of the internal transcribed spacers of the nuclear ribosomal DNA in plants: an example from the Compositae", Molecular Phylogenetics and Evolution, 1992, 1, 3-16.
10. Hershkovitz MA, Zimmer EA, "Conservation patterns in angiosperm rDNA ITS 2 sequences", Nucleic acid research, 1996 24(15), 2857-2867.
11. Dubouzet JG, Shinoda, K, "Relationships among old and new world Alliums according to ITS DNA sequence analysis", Theoretical and Applied Genetics, 1999, 98, 422-433.
12. Saini A, Krishna TG, Reddy KS, Jawali N, "The BamHI site in the Internal Transcribed Spacer Region of Mungbean ribosomal RNA gene is partially methylated", Euphytica, 2000, 114(1), 55-59.
13. Higgins D, Thompson J, Gibson T, Thompson JD, Higgins DG, Gibson TJ, "CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice", Nucleic Acids Research, 1994, 22, 4673-4680.

