



MOLECULAR SYSTEMATICS OF FLOWERING PLANTS IN INDIA: AN OVERVIEW

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Present communication provides an overview of molecular systematic studies done in India with a brief discussion on the changing perspectives of plant taxonomy. Despite a rich diversity and high degree of endemism in the country, the evolutionary lineages of many of the plant groups are poorly understood. Cucurbitaceae, Eriocaulaceae, Leguminosae, and Piperaceae are, however, worked out to some extent. Some of the largest families of the country, such as Orchidaceae, Poaceae, Rubiaceae need serious attention of the molecular taxonomists for a better evolutionary understanding of the country's rich biodiversity. The review provides a consensus from previous studies that nrDNA ITS has been the most extensively used standard marker followed by cpDNA *trnL-F*. On the other hand, the biogeographic and phylogenomic studies have only been carried out for a handful of the angiosperms.

Keywords: Phylogenetic systematics, Evolutionary Relationships, Molecular markers

ABBREVIATIONS USED

DNA- Deoxyribonucleic acid; PCR- polymerase chain reaction; nrDNA- nuclear Deoxyribonucleic acid; cpDNA- chloroplast Deoxyribonucleic acid; AFLP-amplified fragment length polymorphism; RAPD- random amplified polymorphic DNA; ISSR- Inter simple sequence repeats; SSR-Simple sequence repeats; PCR-RFLP- Polymerase Chain Reaction- Restriction fragment length polymorphism; IRAP- inter retrotransposon amplified polymorphism; SRAP- sequence related amplified polymorphism; ITS- internal transcribed spacers; ETS- external transcribed spacers; ORF- open reading frame; NJ- neighbour joining; UPGMA- Unweighted pair group method with arithmetic mean

India is amongst the most biologically diverse countries of the world and hosts as many as four biodiversity hotspots. The richness of biodiversity pertains to complex geological history and tropical climate (Chaitale *et al.* 2014, Singh *et al.* 2020). It represents *ca.* 11% of the world's flora in just about 2.4% of the global landmass. In terms of species diversity, approximately 45,000 plant species are found in India. The angiosperms are represented by approximately 17,500 species out of which 5725 species are endemic to India (Singh *et al.* 2015, Pandey and Shekhar 2018, Singh 2020).

The use of molecular data in phylogenetic systematics and its implications for plant classification and evolution has been well established since the late 1980s (Wiley and Liberman 2011, Stuessy *et al.* 2014). The development of polymerase chain reaction (PCR), cloning, automated sequencing technologies, New Generation Sequencing (NGS), and sophisticated instrumentation, as well as diverse computational evolutionary approaches, have provided the exciting

possibility of establishing inter-relationships of all plant groups. Molecular systematics encompasses a series of approaches in which phylogenetic relationships are inferred using information from macromolecules of the organisms under study. Sequences of the chloroplast, nuclear and mitochondrial genomes are analysed independently and/or in combination for inferring phylogenetic relationships at different hierarchical levels of angiosperm groups.

The past half-century has witnessed impressive advances in floristics, incorporation of new comparative data, and methods of phylogeny reconstruction and classification. Innovations in the use of molecular data, tree-building algorithms, and statistical evaluations have changed the field of systematics (Stuessy *et al.* 2014). Rapidly accumulating DNA sequences from chloroplast, nuclear and mitochondrial genomes have provided new informative sets of data. The most significant developments of the last two decades have been the introduction

of a truly evolutionary approach through the use of cladistic methods, determination of new relationships based on molecular data, and the application of systematics to the problems of biodiversity conservation. In addition to the employment of new DNA data, there has been an integration of data from morphology, anatomy, embryology, palynology, reproductive biology, cytology, and phytochemistry (Pandey 2015). DNA barcodes hold the promise to facilitate rapid assessments of species richness in particular geographic regions or taxonomic groups, aid species delimitation, and speed up the identification of cryptic species. But there is a continued need for carefully curated DNA databases from specimens correctly identified by specialist taxonomists.

A new classification criterion was then unfolded which grouped the plants according to evolutionary relationships (Angiosperm Phylogeny Group, IV Chase *et al.* 2016). An essential part of this idea is that groups of organisms change over time due to many factors like genetic or environmental. Yet it took until the middle of the 20th century for biologists to realize that it is the changed form of a character in time, the “derived state”, that gives us the best clue to phylogenetic relationships and that can be used to group organisms together because it signifies that they share a common ancestry. This realization is the key component of the methodology known as cladistics, which is our current systematic paradigm.

The method uses these derived characters (apomorphies or synapomorphies), to produce explicit, testable patterns of phylogenetic relationships among organisms. In recent years, researchers have continued to refine the methodology, seeking the best ways by which to analyse character data to produce these patterns, as well as devising methods for evaluating the strength of these hypotheses, developing new sources of character information, and realizing the power of the resulting patterns when applied to any questions that deal with the evolution of

organisms.

In India, molecular systematic studies have been carried out only on some members of the flowering plants. In the present communication, a family-wise brief overview of the work done is presented.

Acanthaceae

Based on cpDNA *matK* and *rbcL* sequence data, the systematic position of the new species, *Barleria lavaniana* has been ascertained (Patil *et al.*, 2019). The Bayesian inference tree reveals that *B. lavaniana* is placed in subgenus *Barleria* and shows a phylogenetic affinity towards *B. longiflora* and *B. acuminata*.

Anacardiaceae

Ravikanth *et al.* (2004) described a new species of *Semecarpus* based on the evidence from RAPD and ISSR markers. The newly described species *Semecarpus kathlekanensis* shared some features with two other species, *S. travancorica*, and *S. auriculata*, both of which occur in southern Kerala.

Apocynaceae

Surveswaran *et al.* (2009) carried out a molecular systematic study of the genera *Ceropegia* and *Brachystelma* (Ceropegiae, Asclepiadoideae, Apocynaceae) using five molecular markers (two nuclear and three plastid regions). It has been concluded that the Indian members of tribe Ceropegiae were derived from Africa through at least four independent dispersal events. All dispersal events occurred in late Miocene after the establishment of a monsoon climate. One of these early dispersing lineages underwent rapid radiation in peninsular India giving rise to around 50 species. Thus, both dispersal and diversification events coincided with the intensification of monsoons and concomitant aridification (Surveswaran *et al.*, 2020). Apart from the phylogenetic studies and biogeography studies on the group, two new species of *Ceropegia* viz. *C. mizoramensis* and *C. murlensis* from Murlen National Park,

Mizoram have been discovered and published based on information from nrDNA ITS and morphology (Kumar *et al.* 2018).

Araceae

Gholave *et al.* (2017) studied the phylogeny of closely related *Amorphophallus* species of India using plastid DNA markers (*rbcL*, *matK*, *trnH-psbA*, *trnLC-trnLD*) and fingerprinting techniques (RAPD and ISSR markers). These markers were tested for their efficacy to resolve the sectional treatments. Arunkumar *et al.* (2018) employed *trnL-F* and *rpl20-rps12* markers to understand the phylogenetic placement of several Indian *Arisaema* species. The molecular studies helped to ascertain the identity of a dubious species *Arisaema pangii*. Gusman and Gusman (2006) considered *Arisaema pangii* conspecific with *Arisaema nepenthoides* whereas, Li *et al.* (2010) and Govaerts *et al.* (2016) had placed it under *A. wattii*.

Araliaceae

Pandey *et al.* (2002, 2004 and 2009) carried out molecular systematic studies based on ITS sequences of nrDNA on the Indian Araliaceae taxa. The study revealed that *Aralia-Panax* forms a monophyletic group. Three groups were recognised within the clade of Indian *Panax*: *Panax assamicus* group, *P. bipinnatifidus* group, and *P. variabilis-P. pseudoginseng* group.

Arecaceae

Senthilkumar *et al.* (2015) carried out studies on the species delimitation in congeners of the genus *Daemonorops* from India. *Daemonorops* is one of the largest genera of palms and shares a close phylogenetic relationship with the genus *Calamus*. Most of the *Daemonorops* species are morphologically variable and homoplasious. The study mainly focused on the species of Indo-Myanmar (Andaman Islands) because they have shown uncertainty in their stand as distinct species. The study using ITS2 and RPB2 indicated *D. jenkinsiana*, *D. manii*, and *D. kurziana* in

mainland India, and another three species of *D. wrightmyoensis*, *D. aurea*, and *D. rarispinosa* from Andaman group of islands could be the variabilities of *D. manii* that is colonized throughout the archipelago.

Asparagaceae

Patil *et al.* (2016) studied the phylogenetic relationships among 19 species of genus *Chlorophytum* using *matK* and *rbcL* sequences which resolved the species relationships in two major clades. The study used eight plastid (*matK*, *rbcL*, *trnH-psbA*, *rpoC1*, *ycf5*, *rpoB*, *atp* and *psbK-psbI*) and six nuclear (ITS) markers. The *matK* and *rbcL* were found to be ideal markers for the identification and discrimination of *Chlorophytum* species.

Asteraceae

Based on morphological and molecular data (ITS and *trnL-trnF* sequences), Kasana *et al.* (2020) have concluded that the genus *Lipschitziella*, split from *Saussurea*, is monophyletic after the inclusion of *Himalaiella*. Another closely allied genus *Dolomiaea* was recovered monophyletic after the inclusion of *Saussurea costus* and *Frolovia frolovii* (Kasana *et al.* 2018, 2020).

Balsaminaceae

Many of the Indian *Impatiens* taxa are known to have disjunctive distributions in north-eastern India and the Western Ghats, raising several systematic and biogeographic questions. Puri *et al.* (2016) employed ITS and *atpB-rbcL* regions from nine species of *Impatiens*. The study suggested that there were not only dispersals from China and SE Asia into India, but also dispersals out of India into Africa and back into China and SE Asia.

Brassicaceae

Pradhan *et al.* (1992) inferred phylogeny of *Brassica* and allied genera based on variations in cpDNA and mtDNA utilizing the PCR-RFLP technique for 60 accessions of 50 species. The cpDNA based phylogeny was found to be congruent with morphology-based

classification. However, mtDNA based phylogeny was not congruent with cpDNA phylogeny and morphology-based classification.

Capparaceae

Tamboli *et al.* (2017) studied *Capparis* species using plastid data and concluded that the Indian *Capparis* are more closely related to Old World taxa and have connections with African, Australian, and Eastern Asian species. Maurya *et al.* (2020) selected plastome representatives from four taxa of the genus *Capparis* and compared them to understand the gene composition and arrangements within the genus. In addition, *rpl32-ccsA*, *atpF-atpH*, *petA-psbJ*, *trnL-trnF*, *trnH-psbA* spacer region and one gene *ycf1* were identified as potential barcodes for the genus *Capparis* based on inter and intraspecific variations.

Cucurbitaceae

The genus *Zehneria* is represented by five species (*Z. bodinieri*, *Z. hookeriana*, *Z. maysorensis*, *Z. odorata*, and *Z. thwaitesii*). Maximum diversity is confined to Peninsular and North Eastern regions. Due to the presence of phenotypic plasticity and unresolved phylogenies, there have been several suggestions for splitting (based on morphological characters) and merging (based on molecular and palynological studies) within the genus. Dwivedi *et al.* (2018) confirmed that the genus is monophyletic after the inclusion of *Anangia*, *Pilogyne*, and *Neoachmandra*. Also, the data generated was found useful in clearing the typification problem related to *Z. thwaitesii* (Bräuchler *et al.* 2016). The work utilized the sequence data from nrDNA ITS and cpDNA (*trnL-F*, *matK*, *rpl20-rps12* and *trnH-psbA*).

Another interesting study on the biogeography of the genus *Dactyliandra* was made by Lindner *et al.* (2017). *D. welwitschii*, considered to be distributed in the African Namib desert and also in western parts of India. It was found that the species has reached the western coast of India as a result of anthropogenic activity. A phylogenetic

analysis of plastid and nuclear ribosomal ITS DNA sequences including all *Dactyliandra* species and *D. welwitschii* from the Thar Desert revealed that this striking disjunction is of very recent origin.

Phylogenetic and biogeographic studies on the *Cucumis melo* have revealed that modern melon cultivars go back to two lineages, which diverged *ca.* 2 million years ago. The study showed that *C. trigonus*, an overlooked perennial and drought-tolerant species from India is among the closest living relatives of *C. melo* (Endl *et al.* 2018).

Shubhanand *et al.* (2015) carried out phylogenetic analysis using two coding regions, *rbcL* and *rpoC1* and two non-coding intergenic spacers *psbA-trnH* and *trnL-F* of plastid DNA for inferring relationships among *Cucumis* species. Maximum evolutionary divergence was shown by *trnL-F* followed by *psbA-trnH* region. The non-coding spacers evolved 1.62 to 9 times faster than coding regions of *rbcL* and *rpoC1*. In the phylogenetic analyses, all species of Asian origin showed clear divergence from *Cucumis dipsaceus* and *Cucumis prophetarum*, which were African in origin. *Cucumis maderaspatanas* (= *Mukia maderaspatana*) and *C. ritchiei* (= *Dicaelospermum ritchiei*) showed very low evolutionary divergence and are reported close to each other. *C. setosus* had been confirmed as distinct species and *C. callosus* was found to be a wild progenitor of *C. melo*.

Ali *et al.* (2009) used internal transcribed spacer (ITS) region of nuclear ribosomal DNA to analyze 18 species of the subfamily Cucurbitoideae (family Cucurbitaceae) to assess their phylogenetic relatedness. The ITS sequence data resolves three major clades. *Benincasa*, *Coccinia*, *Cucumis*, *Diplocyclos*, *Lagenaria* and *Solena*, belong to the tribe Benincaseae, are grouped in a well-supported clade (BS 78). This group is further distinguishable into two subgroups. *Benincasa* forms a clade (80% bootstrap support) with *Cucumis* species. *Lagenaria* is closely related to *Diplocyclos-Coccinia-Solena* subgroup (BP 78). *Trichosanthes* and

Luffa are grouped in a single clade (BP 93). *Edgaria darjeelingensis* (tribe Herpetospermeae), though nested within *Trichosanthes-Luffa* clade (BP 50) is distinct from members of tribes Benincaseae and Joliffeae included in the study. *Momordica cymbalaria* (= *Luffa tuberosa*) forms a clade with *Momordica dioica* (BP 80).

Ali *et al.* (2010) confirmed the systematic position of *Luffa tuberosa* using nrDNA ITS sequences from 16 accessions which include five accessions of *Luffa* (under four species i.e. *Luffa acutangula*, *L. cylindrica*, *L. aegyptiaca* and *L. tuberosa*), nine accessions of *Momordica* (under eight species i.e. *M. angustisepala*, *M. balsamina*, *M. cabraei*, *M. charantia*, *M. charantia* subsp. *macroloba*, *M. cissoides*, *M. cochinchinensis*, *M. dioica* and *M. foetida*) and two accessions of *Trichosanthes* under two species (i.e. *T. lepiniana* and *T. tricuspidata*). The results reveal that *Luffa tuberosa* should be transferred to genus *Momordica* and the species to be referred as *M. tuberosa*.

Dioscoreaceae

Mukherjee and Bhat (2013) analysed the relationship between wild and cultivated yams. For the study, 29 accessions of 17 *Dioscorea* species belonging to seven different sections were analysed. The PCR-RFLP data, chloroplast DNA (cpDNA) loci *psbA-trnH*, and *trnL-F* regions reveal the relationship between wild and cultivated yams.

The molecular phylogenetic analyses (*matK*, *rbcL*, *atpB-rbcL*, and *ndhF*) supported the tuberous and rhizomatous species belonging to independent clades with strong support. The analyses formed the basis for the sampling of species for comparing the secondary metabolites (phenolics and flavonoid) in Indian *Dioscorea* (Barman *et al.* 2017).

Sheikh *et al.* (2019) in their communication analysed the morphologically distinct species of *Dioscorea* belonging to section *Enantiophyllum*, *Botryosicyos*, and *Opsophyton* using three markers (18S rDNA,

matK and *rbcL*). The result revealed 18S rRNA gene to be highly conserved as compared to cpDNA regions like *matK* and *rbcL* loci. They proposed 18S rRNA gene as a better candidate for delimitation of specific boundaries in *Dioscorea*. The phylogenetic analysis of the combined loci showed that the species belonging to section *Enantiophyllum* were monophyletic.

Ericaceae

Berry *et al.* (2018) analysed 98 species of *Rhododendrons* based on a published molecular dataset of sequences of RNA Polymerase II subunit (RPB2-I). The evolution of the floral traits was studied using phylogenetic correlation tests and ancestral state reconstructions (maximum parsimony, MP, and ML methods). Significant correlations were found between corolla pigment pattern and type of floral symmetry at the level of corolla form, stamen flexion or arrangement, and pistil flexion.

Eriocaulaceae

The study of Darshetkar *et al.* (2017, 2019a, 2019b, 2020) focussed on morphological characterization, phylogeny, and DNA Barcoding of *Eriocaulon* species, especially from the Western Ghats. Two new species were identified based on morphological and molecular evidence (Darshetkar *et al.* 2017, 2019a). For phylogenetic study, one nuclear (ITS) and one chloroplast marker (*trnL-F*) were selected. DNA Barcoding was carried out using loci based and plastome based (Superbarcoding) approaches. The loci *rbcL*, *rbcL+ITS*, *trnL-F+rbcL*, *rbcL+psbA-trnH* were identified as probable barcodes using loci based approach while *trnS-GGA-trnG-GCC* and *atpH-atpI* intergenic spacers and *ycf1*, *ndhD*, *ndhA* genes were identified as probable barcodes using plastome based approach. Phylogenomic analysis based on 81 protein-coding genes placed Eriocaulaceae sister to Mayacaceae. This study enhanced our understanding of the evolution of Poales by analysing the plastome data from the order.

Euphorbiaceae

Jagtap *et al.* (2018) carried out phylogenetic and population studies of geophytic *Euphorbia* species (subgenus *Euphorbia*) from some deciduous forests and hilltop plateaus in India. Phylogenetic studies using nuclear ribosomal internal transcribed spacers (nrITS) and *matK* sequences showed that the Indian geophytic *Euphorbia* species lie within the section *Euphorbia* clade that includes African and Indian non-geophytic species. The study also suggested that local adaptation and phenotypic plasticity are responsible for taxonomic ambiguities in the classification of Indian geophytic *Euphorbia* species.

Hydatellaceae

Sampling of all the species and analysing cpDNA (*atpB*, *matK*, *ndhF*, *rbcL*) and nrDNA (ITS) data reveal that Indian *Trithuria konkanensis* nests with Australian *T. polybracteata* and *T. lanterna*. (Iles *et al.* 2012)

Iridaceae

Singh *et al.* (2017) made a comparative phylogenetic analysis of ornamental *Gladiolus* cultivars using cpDNA *psbA-trnH* and *trnL-F*. The relationships among the cultivars could prove useful for generating cultivars with phenotypically stable *Gladiolus* crop. From this study, it was also revealed that *psbA-trnH* is highly variable for the group and hence could be a choice of marker for further phylogenetic studies.

Leguminosae

The molecular systematics of six genera of Leguminosae (*Alysicarpus*, *Crotalaria*, *Indigofera*, *Trigonella*, *Vicia* and *Vigna*) has been carried out in India. The genus *Alysicarpus* is represented by *ca.* 18 species, of which seven are endemic. Gholami *et al.* (2017) studied the molecular systematics of *Alysicarpus* species using ITS sequences. The ITS sequence data strongly supported the monophyly of the genus *Alysicarpus*. Analyses revealed four major well-supported clades within the genus. Ancestral state

reconstructions were done for two morphological characters, namely calyx length in relation to the pod (macrocalyx and microcalyx) and pod surface ornamentation (transversely rugose and non-rugose). Analyses revealed macrocalyx and non-rugose pods are plesiomorphies for the genus, microcalyx and transversely rugose pods are apomorphies, and evolution of macrocalyx and non-rugose pods are nonhomologous and microcalyx and rugose pods evolved from a single common ancestor.

Subramaniam *et al.* (2013) studied the molecular systematics of *Crotalaria* using ITS sequences representing all the six recognized sections of Indian *Crotalaria*. Ancestral state reconstructions were also done for two morphological characters, corolla keel beak, and leaf form. Analyses revealed five major well-supported clades within a monophyletic *Crotalaria*. Apart from the revisionary work on the genus, recently three new species have been published. Danda *et al.* (2016) described *C. meghalayensis* using morphological and molecular markers. Rather *et al.* (2018) described *C. suffruticosa* and *C. multibracteata* based on data from nrDNA ITS, *matK* and morphometric analysis, and PCoA.

Based on ITS and cpDNA (*matK* and *trnL-F*) sequence data, phylogenetic relationships in 40 species of Indian *Indigofera* were assessed (Chauhan *et al.* 2013a, b). The individual markers as well as the combined data set supported the monophyly of the genus. Moreover, the evolution of leaf forms (unifoliolate, trifoliolate, and multifoliolate) was also examined by ancestral state reconstruction. Their findings revealed that the immediate last common ancestor for the ingroup was either trifoliolate or multifoliolate. Unifoliolate feature within the group appeared as synapomorphy which evolved at least four times (Meena *et al.* 2020).

Phylogenetic relationships in 22 species representing 11 of the 12 sections recognized within the genus *Trigonella* were analysed using nuclear ITS and plastid *trnL-F* DNA sequences (Dangi *et al.* 2016). Trees

resulting from maximum parsimony as well as Bayesian inference of combined data provided evidence for *Trigonella* being monophyletic with high support but did not agree with the traditional subgeneric division. Phylogenetic reconstructions indicated three major lineages supported by apomorphies in inflorescence and stipule. The study supported the delimitation of sections *Cylindricae*, *Verae*, *Samaroideae*, *Pectinatae*, *Erosae*, and *Callicerates*. Strong support was obtained to combine monotypic sections *Pectinatae* and *Erosae* into one. However, species belonging to section *Foenum-graecum* and *Falcatulae* clustered in different subclades, contrary to their current classification.

Saini and Jawali (2009) studied the evolution of 5S rDNA in 26 accessions of cultivated *Vigna* spp. belonging to different geographical regions of the world. The study was conducted to compare the efficacy of 5S rDNA with markers like nrDNA ITS, and cpDNA *atpB-rbcL* in estimating the phylogenetic relationships. The study revealed that the 5S IGS is highly informative, nearly as informative as nrDNA ITS region, and much better than *atpB-rbcL* and can easily be used to infer species relationships among *Vigna* species.

Shiran *et al.* (2014) made an attempt to resolve the complex taxonomic history in the genus *Vicia* using nrDNA ITS sequences of 49 *Vicia* spp. The phylogenies obtained using MP estimation revealed that *Vicia faba* and *V. bithynica* are distant from the Narbonensis species complex (NSC) in the traditional section *Faba*. *V. faba* is more closely aligned with section *Peregrinae* while *V. bithynica* is more closely aligned with section *Vicia*. Also, separate sectional status is needed for the complex based on the synapomorphies present within the section.

Malvaceae

The Indian species of family Malvaceae have been poorly worked out. However, some efforts have been made recently to resolve the taxonomic intricacies of the genera *Abutilon*

and *Sida* using molecular data. The molecular studies helped to describe a new species *Sida sivarajanii* from India (Tambde *et al.*, 2020). Whereas, a revision in the circumscription of *Abutilon* was suggested by the ITS dataset (Nimbalkar, 2019).

Menispermaceae

In a view to delimit species boundary in *Tinospora*, Ahmed *et al.* (2006) conducted phylogenetic analyses using RAPD, PCR-RFLP techniques, and ITS sequence data. The result reveals that the phylogeny of *Tinospora* species deduced from the ITS and 5.8S sequences were congruent with the phylogenetic results obtained by RAPD analysis. Both markers have helped to sort out the phylogenetic placement of three *Tinospora* species.

Musaceae

Lamare *et al.* (2016) presented phylogenetic implications of the internal transcribed spacers of nrDNA and chloroplast DNA fragments of *Musa* in deciphering the ambiguities related to the sectional classification of the genus. Results reveal that there exist homoplasy and sectional classification based on morphology that does not corroborate with the clades recovered using phylogenetic analyses.

Singh *et al.* (2020) assessed the systematic position of the species belonging to the genus *Musa* sampled from the Andaman and Nicobar Islands. In this study, both morphological and molecular data (individual and combined) were analyzed and phylogenetic analysis was done by the Maximum Likelihood and Bayesian methods using cpDNA (*trnL-F*) and nrDNA (ITS1-5.8S-ITS2) markers and a matrix comprising of 43 taxa of *Musa*. The results revealed that the genus is now represented by 4 wild and two cultivated species in contrary to 6 wild (5 species and one variety) and 2 cultivated species. Based on the individual as well as a combined analysis, *M. balbisiana* var. *andamanica* and *M. sabuana* are synonymized with *M. balbisiana*.

Myricaceae

Yanthan *et al.* assessed the genus *Myrica* based on morphometric (2011) and nrDNA ITS sequence data and phylogenetically differentiated *Myrica nagi* from *M. esculenta*. The sampling for the analyses covered three morphotypes and a total of 111 individuals from different localities in Meghalaya with basic variation in mean leaf length, fruit length, and ripe fruit colour. Work utilized nuclear rRNA genes segment of DNA of these variants utilising the amplicon restriction patterns, nucleotide sequences, and secondary folding of the 5.8S rRNA. Authors propose that *M. nagi* and *M. esculenta* should be treated as two distinct species.

Nymphaeaceae

Dkhar *et al.* (2012) estimated the phylogenetic implications of nrDNA ITS in the genus *Nymphaea* in India. The phylogenetic estimate revealed accessions of *N. rubra*, *N. pubescens* are in a polytomy. *Nymphaea lotus*, *N. petersiana* are sister taxa. *Nymphaea lotus*, *N. petersiana* clade is sister to *N. rubra*, *N. pubescens* clade and placed within *N.* subg. *Lotus*. *Nymphaea caerulea* accessions are in a polytomy with *N. nouchali*. Other accessions belonging to species *N. tetragona* and *Nymphaea × marliaceae* have been placed in *Nymphaea*. subg. *Nymphaea*.

Oleaceae

Jeyarani *et al.* (2018) studied the phylogenetic relationships in 22 taxa of *Jasminum* in India based on the nuclear (internal-transcribed spacer region of nrDNA and chloroplast markers (*matK*, *trnL-F*, and *trnH-psbA*). Maximum likelihood and Bayesian analyses from individual markers, as well as from the combined dataset, revealed that the group is monophyletic if *Menodora* spp. are excluded from the analyses. The analyses recovered three strongly supported clades. Ancestral character state reconstruction of taxonomically useful characters (leaf forms, leaf arrangement, and flower colour) which were used to demarcate sections within the genus revealed

homoplasy. The study also suggested at least four reversals to unifoliolate condition that after split from the last common ancestor. The study also suggested that yellow-flowered condition evolved from the white-flowered ancestor. It was the pioneering work to estimate the evolutionary history of Indian species of *Jasminum*.

Orchidaceae

Sharma *et al.* (2012) used nrITS region to assess phylogenetic inter-relationships and infra-generic classification of ten *Cymbidium* species collected from north-east India. The study validated the utility of ITS rDNA region for understanding the phylogenetic relationships among *Cymbidium* spp. The study also suggested ITS2 as probable DNA barcode for the genus.

Pandanaceae

Phylogenetic relationships among Indian *Pandanus* (28 spp.) and *Bemstonea* (17 spp.) deciphered using three cpDNA sequence regions (*trnL* UAA 5'exon – *trnL* UAA 3' exon, *trnL* UAA 3' exon – *trnF* GAA and *atpB* – *rbcL* chloroplast intergenic spacers) have revealed that both genera form a monophyletic group (Nadaf and Zanan 2012).

Piperaceae

Divergence dating analysis (Sen *et al.* 2019) has revealed that the genus *Piper* originated during lower Cretaceous around 110 Ma and colonized Peninsular India five times independently, from Southeast Asia starting from the Oligocene. The study provided evidence for the Miocene overland dispersal of *Piper* species to Africa from South Asia.

Pittosporaceae

Systematic position of *Pittosporum eriocarpum* has been ascertained. The study utilized the potential nrDNA ITS sequences from four accessions collected from Uttarakhand and rest were retrieved from GenBank. The phylogeny revealed that *P. eriocarpum* share sister relationship with *P.*

balfouri from Mauritius and *P. manii* from Kenya (Dwivedi *et al.* 2020).

Poaceae

The taxonomy of family Poaceae has been one of the least worked out at the molecular level in India. However, few efforts have been undertaken recently to resolve the complexities associated with several genera like *Oryza* (Rangan *et al.* 2002), *Glyphochloa* (Gosavi *et al.* 2016), *Ischaemum* (Bokil *et al.* 2020) using molecular tools. Rangan *et al.* (2002) studied twenty-one species belonging to *Oryza*, including wild rice varieties and compared them with a tetraploid halophytic wild rice relative *Porteresia coarctata* (= *Oryza coarctata*) for the genetic relatedness using AFLP and RAPD markers. The study suggested *O. australiensis* a descendent of the same lineage as of *P. coarctata*. Of late, the molecular systematics of an endemic genus *Glyphochloa* was studied by Goasvi *et al.* (2016) based on nuclear ribosomal ITS sequences and plastid intergenic spacer regions (*atpB-rbcL*, *trnT-trnL*, *trnL-trnF*). Two distinct clades of *Glyphochloa* (*sensu lato*) were recovered. However, a sister-group relationship between group I and II taxa was not well supported, although the genus recovered as monophyletic in the shortest trees inferred using ITS or concatenated plastid data. The other genus *Ischaemum* has been challenging to the taxonomists due to the presence of overlapping characters, interspecific variation, and its polymorphic nature. Over the years, species delimitation in *Ischaemum* has been dealt with by taxonomists exclusively based on morphology. Considering polymorphism and heterogeneity in *Ischaemum*, molecular phylogenetic studies helped to understand the morphological variations and addressing their ecological diversification.

Podostemaceae

Khanduri *et al.* (2015) studied the infrageneric relationships of the Indian taxa which were unresolved using morphological and molecular

data. The study suggested that the Indian species of *Podostemum* and *Hydrobryopsis* should not be merged with *Zeylanidium* as suggested earlier. In Tristichoideae, Indian species of *Dalzellia* and *Indotristicha* formed a clade, sister to *Tristicha*. However, the analyses indicated that within the *Indotristicha–Dalzellia* clade, *I. tirunelveliana* is a sister species, with high support to *Dalzellia* instead of its congener *I. ramosissima*. Based on morphological and molecular data, the removal of *I. tirunelveliana* from the genus *Indotristicha* was proposed. The study also provided insights into the evolution of some morphological characters in Podostemaceae.

Polygonaceae

Choudhary *et al.* (2012) made the first attempt to study the *Polygonum (sensu lato)* of the Indian Himalayas using ITS sequences from 44 taxa to investigate relationships among various sections. The analysis suggested eight major groups of the Indian *Polygonum* species. The relationships among different sections were largely congruent with those inferred from morphological characters. The study provided a high-resolution phylogeny of the Himalayan *Polygonum (sensu lato)* and supported the merger of the section *Amblygonon* in section *Persicaria*.

Punicaceae

Narzary *et al.* (2016) analysed two species of genus *Punica* viz. *P. granatum* L. and *P. protopunica* using nrDNA ITS to ascertain the systematic position and their relationship with other members of the order Myrtales. The sequence analyses using Maximum likelihood and Bayesian inference revealed that all the accessions of the species are monophyletic, also family Lythraceae (*sensu lato*) is recovered monophyletic and sister relationship with family Onagraceae is weakly supported. However, it has been suggested that a wider sampling would help understanding the phylogenetic placement of the genus *Punica* under family Lythraceae

Ranunculaceae

Based on morphological and ITS sequence data, Singh *et al.* (2018) described *Thalictrum nainitalense* from Nainital, Uttarakhand.

Rutaceae

Jena *et al.* (2009) incorporated 50 accessions of wild, semi-wild, and domesticated taxa of *Citrus*. Based on sequences from *trnD-trnT*, *rbcL*-ORF, and *trnL-F* sequence data, Indian *Citrus* species have been grouped in 6 distinct clusters. The study further concludes that *C. maxima*, *C. medica*, and *C. reticulata* are the basal taxa of edible *Citrus*.

Hynniewta *et al.* (2014) used ITS region to study *Citrus* species from north eastern India and suggested *C. reticulata* to be a close relative of *C. ichagensis*. On the other hand, the study did not demonstrate a clear differentiation between subgenera *Citrus* and *Papeda* at the rDNA level.

Sapindaceae

Phylogenetic relationships among Indian species of soap nut and other closely related taxa within the family were analysed using nrDNA ITS sequences (Mahar *et al.*, 2017). The maximum likelihood analyses using the newly generated sequences and those downloaded from NCBI revealed substantial genetic differences between the accessions of *Sapindus oligophyllus*, *S. emarginatus*, and *S. trifolius*. Hence, they were vouched to be treated as distinct species. The sister group of *Sapindus* is genus *Lepisanthes* and supports the previous study.

Solanaceae

Kumar *et al.* (2011) studied the relationship between wild and cultivated germplasm of *Withania somnifera* (Indian ginseng) and taxonomic status of *W. ashwagandha* in India using AFLP and nrDNA ITS1 and ITS 2 sequences. The results obtained using the UPGMA dendrogram revealed relationships among wild, cultivated, and hybrid taxa. The wild, cultivated and hybrid taxa form separate clades, and resurrection of *W. ashwagandha*

from *W. somnifera* was suggested. Rahman & Misra (2015) tapped the potential of 18S rRNA and cpDNA *rbcL* genes to decipher the phylogenetic relationship among various *Solanum* taxa. The sequence data suggested a close evolutionary relationship between - 1. *Solanum nigrum* and *Solanum clavatum*, 2. *Solanum kurzii* and *Solanum gilo* and 3. *Solanum sisymbriifolium* and *Solanum khasianum*.

Rhizophoraceae

Molecular phylogeny of 22 mangrove species was analyzed using RAPD and RFLP. The dendrogram showed grouping of all the major mangroves, except for *Nypa fruticans* (Arecaceae), into one group. All species under the tribe Rhizophorae formed a sub-cluster, to which *Xylocarpus granatum* was found to be the most closely related species. The clustering pattern implied that *Excoecaria agallocha* and *Acanthus ilicifolius* should be considered as true minor mangroves. The present study also provided molecular data favouring the separation of *Avicennia* spp. from the Verbenaceae to create a monotypic family the Avicenniaceae. The separation of *Viscum orientale* into the Viscaceae was also supported. (Parani *et al.* 1998).

Zingiberaceae

Das *et al.* (2011) analysed eight species of economically important *Curcuma* spp. from northeast India viz., *C. amanda*, *C. angustifolia*, *C. caesia*, *C. zedoaria*, *C. aromatica*, *C. longa*, *C. domestica* and one unidentified were analysed using AFLP, RAPD, ISSR markers to understand the genetic relationship among them. The result reveals that ISSR markers are best among AFLP and RAPD in deciphering the phylogenetic relationship. However, the relationships among the species were not constant for different markers yet dendrograms were congruent for major clades. Basak *et al.* (2014) studied the genetic diversity and relationship of *Hedychium* spp. from Northeast India using ISSR and AFLP. The study recommended the

use of these markers for phylogenetic studies, tests of bio-geographic hypotheses, and models of trait evolution.

Lamo (2017) carried out phylogenetic analysis of the genus *Curcuma* using sequence data of nuclear ribosomal DNA (ITS; internal transcribed spacer region) and that of plastid DNA (*matK* gene and *psbA-trnH* intergenic spacer) along with morphological and cytological data. The analysis included a total of 27 taxa belonging to *Curcuma*, *Hitcheniopsis*, *Peirreana*, *Ecomata* and *Petiolata* groups described by earlier workers. Phylogenetic analyses using *matK* and *psbA-trnH* revealed a close genetic relationship of the species between the groups or the uniparentally inherited chloroplast gene but could not reveal the true phylogenetic relationship. However, cladogram of ITS and concatenated sequences could successfully resolve the species into different group viz. *Curcuma*, *Hitcheniopsis*, *Ecomata* and *Pierreana*. The reticulate position of *C. petiolata* and *C. roscoeana* was confirmed and it was speculated that they might have evolved from hybridization of species belonging to the *Curcuma* and *Hitcheniopsis* clade.

CONCLUSION

The floristic wealth of India comprises more than 250 Angiosperm families. However, at the molecular level a handful of them have been worked out to date. Some of the dominant families like Poaceae, Orchidaceae, Euphorbiaceae, Rubiaceae, Rosaceae, etc. are still waiting to be researched at the molecular level. Some well-represented genera like *Impatiens*, *Primula* and *Dendrobium* are to be investigated on priority in view of the high degree of their habitat destruction in the country. More than 80 monotypic genera are endemic to India of which majority belong to the family Poaceae, hitherto poorly studied at the molecular taxonomic level. Considering the high degree of endemism in the country, it is

the need of the hour to understand their speciation, diversification, and other associated processes such as interpretation of gene function and the genomic evolution using advanced phylogenomic data. Target enrichment and sequencing of multiple nuclear loci, though rarely used in Indian taxonomic studies, have proved useful to provide strong support for construction of robust phylogenies. They also direct us to explore the presence of important metabolites in the particular plant groups and prioritize the conservation efforts of the threatened and endemic taxa. Efforts are, however, being made by the selected group of Indian taxonomists and some of the significant works have been carried out in the last two decades. These efforts have resulted in the discovery of several new species and a better understanding of the taxonomy and biogeography of some complex angiosperm groups like Araliaceae, Eriocaulaceae, Fabaceae, Piperaceae etc. However, a lack of funding, infrastructure, and proper training has hindered research in this direction. To ensure a better understanding of the native Indian flora, it is imperative to support the classical taxonomists with more funding and advanced training in the field of molecular systematics.

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