Chapter 15

Molecular Characterization of *Garcinia* species in the Western Ghats

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Abstract

The genus *Garcinia* L. (Family: Clusiaceae) is an important component of the forest flora of the Western Ghats with 9 species, of which 7 are endemic to the region. Systematics of the genus *Garcinia* is primarily based on morphological data, especially reproductive morphology and the genus is considered as a taxonomically difficult one due to the complexity and diversity in floral characteristics. Molecular tools are getting more acceptances as a convenient tool in the phylogenic studies of such taxonomically difficult groups. Molecular markers are potential in portraying the genetic relationship between plant groups and DNA based molecular taxonomic approaches give an exact and rapid method of distinguishing specimens based on their interspecies variation. In the present study, the genetic profile of 9 *Garcinia* species, *G. gummi-gutta, G. rubro-echinata, G. imberti, G. indica, G. morella, G. talbotii, G. pushpangadaniana, G. travancorica* and *G. wightii* distributed naturally in the Western Ghats of south India, were analyzed for better understanding of interspecific genetic diversity. Molecular profiling using the chloroplast coding region *mat*K could successfully demark different species of the genus *Garcinia*.

Keywords: *Garcinia* species, Western Ghats, Molecular taxonomy, *mat*K

Introduction

Systematics of the genus *Garcinia* is primarily based on reproductive morphology. However, the field identification of *Garcinias* is challenging due to the presence of unisexual flowers and strict seasonality in flowering and fruiting. The morphological assessment and variability studies of *Garcinia* species demonstrated that the morphological variants are enormous within the species with characters always overlapped within and between populations and the genus is often treated as a taxonomically difficult group (Nimanthika and Kaththriarachchi, 2010). Combined approaches based on morphological, molecular and chemical analyses are getting more acceptances in the phylogenic studies of such taxonomically difficult groups (Labra *et al*., 2004). While classical phylogenetic approach relies on morphological characteristics of an organism, in molecular phylogeny, the relationships among organisms were studied by comparing nucleotide sequences of RNA and DNA and sequences of amino acids of a protein. Dissimilarities among the sequences indicate genetic divergence as a result of molecular evolution during the course of time. Molecular markers are a direct assay of hereditary material and unlike morphological markers, molecular markers are not prone to

environmental influences and can complement data from descriptors such as morphological characters (Patwardhan, 2014; Mba and Tohme, 2005). Further, by comparing homologous molecules from different organisms it is possible to establish their degree of similarity, thereby establishing or revealing a hierarchy of relationship through a phylogenetic tree.

Many plant phylogenetic studies are based on chloroplast DNA (cpDNA). In plants, cpDNA is smallest as compared to mitochondria or nuclear genome. It is assumed to be conserved in its evolution in terms of nucleotide substitution with very little rearrangements which permits the molecule to be used in resolving phylogenetic relationships especially at deep levels of evolution. Selection of a gene of sufficient length and appropriate substitution rate is a crucial step and currently used cpDNA genes include rbcL, ndhF, rpl16, matK, atpB and many more.

In *Garcinia*, preliminary molecular phylogenetic work has been started by Rismita-Sari (2000) to test Jones (1980) classifications of *Garcinia* into 14 sections based mainly on male flower characters. Gustafsson *et al*. discussed the phylogenetic status of the Clusiaceae members in detail using chloroplast gene Rbcl and the study supported morphological based classifications (Gustafsson *et al*., 2002). The phylogenetic relationship among mangosteen and several wild relative species were analyzed by comparing sequences of the ITS region of nuclear ribosomal DNA. Both parsimonious and NJ analysis revealed that mangosteen is closely related to *G. malaccensis* (Chinawat and Subhadrabandu, 2004). Results from phylogenetic analyses utilizing chloroplast and nuclear DNA markers agree with morphology in support of the unification of all of *Rheedia* L. and part of *Ochrocarpos* Thouars with *Garcinia* (Sweeney, 2008). Genetic diversity based on morphological and Inter Simple Sequence Repeats (ISSR) of 19 accessions of mangosteen and their close relatives revealed that *G. malaccensis* and *G. celebia* were the ancestors for mangosteen (Sulassih *et al*. 2013).

Rao (2003) studied both intra and inter species relationship among six *Garcinia* species namely *G. indica*, *G. cambogia (G. gummi-gutta)*, *G. cowa*, *G. mangostana*, *G. xanthochymus* and *G. hombroniana*, using RAPD polymorphism. RAPD markers could successfully distinguish different species of the genus *Garcinia*. The study indicated high molecular diversity within *G. cambogia* (Rao, 2003). Parthasarathy *et al*. studied RAPD polymorphism in 33 accessions of *Garcinia* species collected from different areas of Western Ghats (Parthasarathy *et al*., 2013). The dendrogram clearly separated the collections of the 3 main species studied, *G. gummi-gutta*, *G. indica* and *G. xanthochymus,* and suggested high amount of diversity within the collections of the same species. Similar study was also conducted on *Garcinia* collections from North East India using RAPD. High molecular diversity was observed with the heterogeneity index within species ranging from 0.81 to 0.82 in four species, namely *G. gummi-gutta*, *G. indica*, *G. cowa* and *G. xanthochymus* (Parthasarathy *et al*., 2013).

Though Western Ghats is a centre of diversity of *Garcinia* species, a comprehensive study on the molecular profiles of *Garcinia* species of the region including the rare and endemic species has rarely been attempted. Present chapter discusses the molecular characterization of *Garcinia* species naturally occurring in the Western Ghats region, using chloroplast coding region *mat*K.

1. Genomic DNA isolation and sequencing

Genomic DNA was isolated from young leaves using DNeasy plant DNA isolation kit (Qiagen). PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X PCR buffer (150mM Tris HCl, pH-8; 500mM KCl), 0.2mM each dNTPs, 2.5mM $MgCl_2$, 20ng DNA, 1 unit of Ampli Taq Gold DNA polymerase enzyme, 0.1 mg/ml BSA and 4% DMSO, 5pM of forward and reverse primers (Table:01). The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) with an initial denaturation of 95° C for 5.00 min. followed by 40 cycles of 48° C for 0.40 min, 72° C for 1.00 min and 72° C for 5.00 min., followed by 4°C. PCR amplification (**Figure 1**) was followed by sequencing using the BigDye Terminator v 3.1. The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v 5.6.

Table1. Primers used for the molecular study of *Garcinia* species

Target	Primer Name	Direction	Sequence (5' → 3')	Reference
*mat*K	matK-390F	Forward	CGATCTATTCATTCAATATTTC	CBOL Plant Working Group (http://www.barcoding.si.edu /pdf/informationonbarcodeloci.pdf
	matK-1326R	Reverse	TCTAGCACACGAAAGTCGAAGT	

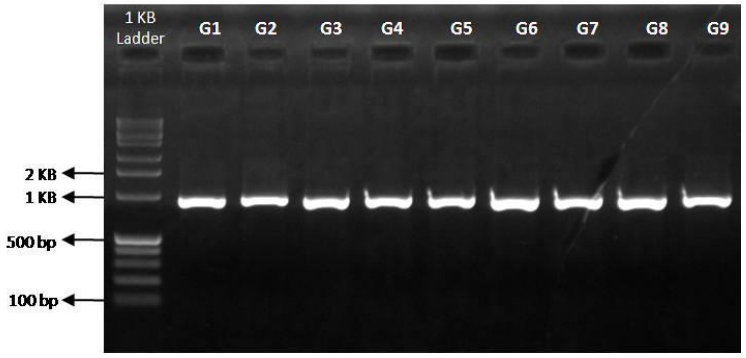

Figure 1. PCR products- *mat*K region of nine *Garcinia* species

2. Sequence analysis

The phylogenetic analyses of 9 *Garcinia* species distributed naturally in the Western Ghats were done using *MatK* with *Clusia criuva* of Clusiaceae family as the out group member (ncbi-TNS:SK08071206). The evolutionary history was inferred using Neighbor-Joining method as elaborated by Saitou and Nei (1987). The optimal tree with the sum of branch length 0.093 is shown in **Figure 2**. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used, to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site. All positions containing gaps and

missing data were eliminated. There were a total of 802 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura *et al.,* 2011).

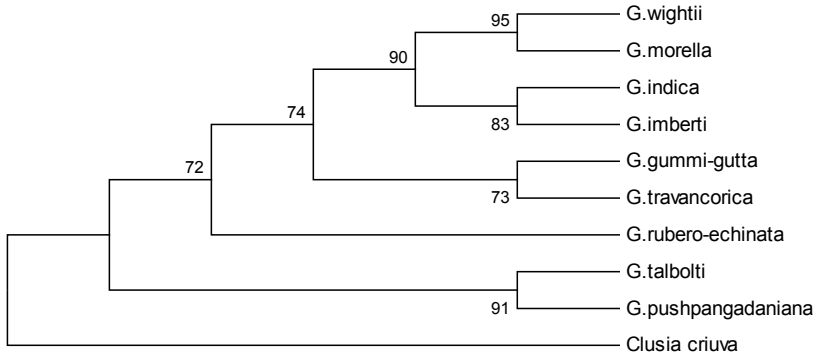

Figure 2. NJ- Phylogram based on *matK* loci of 9 species of *Garcinia* and the out group *Clusia criuva.*

All the accessions of the *Garcinia* species were clustered together in the NJ phylogram based on *matK* loci and the phylogram distinctly delimit all the 9 species and were also clearly differentiated from the out group *Clusia criuva*. In the first clad the accessions of *G. morella* and *G. wightii* were clustered together with a bootstrap value of 95%. The second clad includes *G. indica* and *G. imberti* and showed sister relationship with 83% bootstrap support. The third clad includes two sub clusters with *G. travancorica* in one cluster and *G. gummi-gutta* in the second cluster with bootstrap value of 73%. The fourth cluster is purely monophyletic with *G. rubro-echinata.* The fifth cluster includes *G. talbotii* and a recently published species *G. pushpangadaniana* with bootstrap value of 91%.

Generally, the classical morphology based classification and molecular analysis based classification complement each other since morphology of an organism is the manifestation of its genome, proteome and transcriptome profiles. The results of the current molecular study are in part congruent with the classification based on morphological features (Chapter 1). The species status of *G. pushpangadaniana* is confirmed and also its allied nature to *G. talbotii* (Sabu *et al*., 2013). *G. pushpangadaniana* and *G. talbotii* were morphologically distinct from other species by the characteristic features of stamens in 5 phalanges and 5 numbered sepals and petals. *G. morella* and *G. wightii* that showed as a separate clad in molecular phylogeny were allied and distinct from other species based on sessile fruits and 4 lobed stigma. *G. rubro-echinata* also stands distinct based on morphological features with echinate fruits and supports the monophyletic nature of *G. rubro-echinata* in the molecular phylogram. Combined multidisciplinary analysis of vegetative and reproductive morphology, along with molecular taxonomy yield more robust phylogeny which could be used for studies of phytogeography and evolutionary radiation of the *Garcinia* species.

Conclusions

The genus *Garcinia* is one of the taxa with poorly resolved phylogenetic relationships. Although widely practised even now, traditional morphology based systems of classification can have some limitations while systematics based on molecular markers can complement the traditional morphology based method for phylogenetic studies. Further, the genetic profile of

the *Garcinia* species of the Western Ghats can be used to solve the taxonomic enigmas and for analyzing the phylogeny of the group. The present work shows that the *Garcinia* species can be distinctly identified by the phylogram based on *matK* loci of the *Garcinia* species and molecular profiling has been successfully used to resolve species circumscriptions and identification of *Garcinia* species in the Western Ghats.

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