

Exploring Phylogenetic Relationships in Malvaceae Using Sequence Data of *MatK* Gene

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ABSTRACT

Malvaceae, flowering plant family belongs to order Malvales. It consists of around 244 genera and over 4225 accepted species all over the world. The members of this family used as a source of fiber, food, beverages, medicines, timber etc. this shows its great economic importance. Several phylogenetic studies have been conducted using different single and multiple molecular signatures and different phylogenetic approaches. However, the subfamilial relationships in Malvaceae is still unclear. The focus of the current study is to infer the subfamilial relationships in Malvaceae using sequence data of *matK* gene through Bayesian Inference (BI) analysis. The phylogenetic analysis carried out using 35 species as representation of subfamilies of Malvaceae and one species of closely related family as outgroup. The *matK* sequence data identified well supported topology of the phylogenetic tree that confirmed the division of the Malvaceae *s.l.*, family into further subfamilies; Malvoideae, Bombacoideae, Tilioideae, Sterculioideae, Helicteroideae, Dombeyoideae, Grewioideae, Byttnerioideae. These subfamilies grouped into Byttneriina and Malvadendrina clade. The result suggested that first Byttneriina clade was originated and then Malvadendrina clade. In Byttneriina clade, two subfamilies; Grewioideae and Byttnerioideae were found in sister association. Within Malvadendrina clade, Dombeyoideae was emerged as most primitive subfamily followed by Sterculioideae, Tilioideae. These subfamilies found sister to Malvatheca clade that comprised two subfamilies Malvoideae and Bombacoideae. The current study presented a phylogenetic framework for Malvaceae may help in understanding the evolutionary relationships of the subfamilies. Increased number of sample size and other regions of chloroplast and nuclear genome could further help in evolutionary and taxonomic study in Malvaceae.

Keywords: Bayesian analysis, Evolutionary relationships Malvaceae, *matK* gene, Phylogenetic, Subfamilial association

INTRODUCTION

Malvaceae is a flowering plant family belongs to order Malvales. This family consists of over 2400 genera and over 4225 species all over the world. Nineteen (19) genera and 94 specific and interspecific taxa reported from Pakistan. Globally, three other families (Sterculiaceae, Tiliaceae, and Bombacaceae) of same order Malvales merged into Malvaceae based on molecular data. With inclusion of other three families in Malvaceae, the total representation of extended Malvaceae from Pakistan is 36 genera and 141 species. Due to this merger, subfamilial relationship of extended Malvaceae is unclear at global and regional level. In Pakistan, this circumscription not implemented yet and no study conducted to infer merging of four families into one.

OBJECTIVE

To infer subfamilial relationships in extended Malvaceae using sequence data of *matK* gene.

METHODOLOGY

Field survey conducted to collect fresh samples from different areas of Pakistan. Dried plant samples also collected from Center for plant conservation Herbarium, university of Karachi. Collected plant species identified with the help of flora of Pakistan and expert taxonomist and then deposited in Center for plant conservation to get the accession numbers. Some DNA sequences also retrieved from GenBank as well. Total 35 species (fresh and dried) collected that were representing the extended Malvaceae. Total genomic DNA was isolated from fresh leaf and dried leaf samples as well. The chloroplast coding region *matK* amplified using forward and reverse primers, amplified products purified and then sequenced from commercial laboratory. DNA sequences were analyzed using bioinformatics tool and deposited in GenBank to get the accession numbers. Using *matK* sequence dataphylogenetic tree reconstructed through Bayesian Inference (BI) analysis.

RESULTS

The phylogenetic analysis carried out using 35 species as representation of subfamilies of Malvaceae and one species of closely related family as outgroup. The *matK* sequence data identified well supported topology of the phylogenetic tree that confirmed the division of the Malvaceae *s.l.*, family into further subfamilies; Malvoideae, Bombacoideae, Tilioideae, Sterculioideae, Helicteroideae, Dombeyoideae, Grewioideae, Byttnerioideae. These subfamilies grouped into Byttneriina and Malvadendrina clade. The result suggested that first Byttneriina clade was originated and then Malvadendrina clade. In Byttneriina clade, two subfamilies; Grewioideae and Byttnerioideae were found in sister association. Within Malvadendrina clade, Dombeyoideae was emerged as most primitive subfamily followed by Sterculioideae, Tilioideae. These subfamilies found sister to Malvatheca clade that comprised two subfamilies Malvoideae and Bombacoideae.

DISCUSSION

In the current study, we used the sequence data of *matK* gene for the inference of phylogenetic relationships in core Malvales. The focus of the presented study was to infer subfamilial relationships in extended Malvaceae *s.l.*, using single locus of chloroplast genome. The analysis of *matK* sequence data was mainly consistent with the results obtained in previous studies that uses the limited number of loci or plastid genome of 48 accessions (Cvetković, Tijana, 2021). The *matK* sequence data resolved the subfamilial relationships in Malvaceae that remained unresolved in previous study that were based on few plastid loci or plastid genome (Cvetković, Tijana, 2021).

The *matK* based phylogenetic analysis provided support for the monophyly of the three previously recognized major clades within Malvaceae *s.l.* namely Byttneriina, Malvadendrina, and Malvatheca. The inferred *matK* based phylogenetic tree with major clades presented in Figure 1.

Byttneriina clade comprising the subfamilies, Grewioideae and Byttnerioideae and placed at the base of Malvadendrina clade that comprising all remaining subfamilies of extended Malvaceae *s.l.*, The *matK* sequence analysis confirmed the placement of Byttneriina as sister to Malvadendrina (Cvetković, Tijana, 2021). Our *matK* analysis strongly supports the monophyly of Byttneriina. This monophyly also supported by plastome data set (Cvetković, Tijana, 2021) but not supported by some morphology based traditional classification in which Grewioideae included in Tiliaceae.

The *matK* analysis weakly supported the monophyly of Malvatheca (Bombacoideae and Malvoideae). However, the other previous studies (Bayer et al. 1999; Cvetković, Tijana, 2021) strongly supported this monophyly. Within Malvatheca, the monophyly of Bombacoideae and Malvoideae strongly supported.

The *matK* analysis strongly supported the monophyly of Malvadendrina clade. The phylogenetic relationships of subfamilies; Dombeyoideae, Helicteroideae, Sterculioideae and Tilioideae is uncertain in different

morphological and molecular based classifications (Bayer et al. 1999; Nyffeler et al. 2005; Le Péchon and Gigord 2014) and require molecular based revision to clear their association. In the current study, Dombeyoideae retrieved as sister to all remaining Malvadendrina subfamilies and Malvatheca clad. This sister association is not corroborate the previous study in which Helicteroideae was observed as sister to remaining subfamilies (Cvetković, Tijana, 2021). Helicteroideae with moderate support retrieved as sister to Sterculioideae, Tilioideae and Malvatheca clade. In the current study, the association of Sterculioideae and Tilioideae supported poorly. The Malvatheca clade containing Malvoideae and Bombacoideae found at the base of Malvadendrina. The placement of Malvatheca clade at the base poorly supported. However, the monophyly of Malvoideae and Bombacoideae strongly supported.

CONCLUSION

The current study presented a phylogenetic framework for Malvaceae may help in understanding the evolutionary relationships of the subfamilies. Increased number of sample size and other regions of chloroplast and nuclear genome could further help in evolutionary and taxonomic study in Malvaceae.

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REFERENCES

1. Bayer, Clemens, et al. "Support for an expanded family concept of Malvaceae within a recircumscribed order Malvales: A combined analysis of plastid atpB and rbcL DNA sequences." *Botanical Journal of the Linnean Society* 129.4 (1999): 267-303.
2. Cvetković, Tijana, Damien Daniel Hinsinger, and Joeri Sergej Strijk. "The first complete chloroplast sequence of a major tropical timber tree in the Meranti family: *Vatica odorata* (Dipterocarpaceae)." *Mitochondrial DNA Part B* 2.1 (2017): 52-53.
3. Cvetković, Tijana, et al. "Phylogenomics resolves deep subfamilial relationships in Malvaceae sl." *G3* 11.7 (2021): 136.
4. Le Péchon, Timothée, and Luc DB Gigord. "On the relevance of molecular tools for taxonomic revision in Malvales, Malvaceae sl, and Dombeyoideae." *Molecular Plant Taxonomy*. Humana Press, Totowa, NJ, 2014. 337-363.
5. Nyffeler, R., and D. A. Baum. "Phylogenetic relationships of the durians (Bombacaceae-Durioneae or Malvaceae/Helicteroideae/Durioneae) based on chloroplast and nuclear ribosomal DNA sequences." *Plant Systematics and Evolution* 224.1 (2000): 55-82.