Executive Summary

Minor Research Project

DNA BARCODING OF MURDANNIA (COMMELINACEAE) IN WESTERN GHATS

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By

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Introduction

The Commelinaceae family is well delineated with 41 genera and about 650 species. It is evidenced by several characteristics admitting a distinct closed leaf sheath, a succulent leaf blade, and tri-merous flowers with trenchant petals and sepals (Cronquist 1981; Faden 1985; Faden and Hunt 1991). The distribution is largely tropical and subtropical, but several species extend into temperate areas. The grooviest diversity is noticed in Africa, where, along with Madagascar, nearly half of the genera and around 40% of the species are observed (Faden 1983a).

Murdannia with approximately 50 species is pantropical and warm temperate in distribution and displays richest diversity in tropical Asia (Faden, 2000). It depicts 31% of endemism in India. After Hooker (1892) and Clarke (18181) no revisionary studies has been accomplished on the genus in India.

The present work has implication in the classical taxonomy of the genus *Murdannia* because it is expected to sort out species delimitation which is not possible with the range of morphological variations. The present work also has the significance in applied molecular taxonomy since the data generated through this study will serve as the basic ground data for all other research related to phylogeny of angiosperms (APG-III classification).

(iii) Objectives

1. To collect the germplasm of different species of *Murdannia* in Western Ghats
2. To conserve the collected germplasm through *ex-situ* methods
3. To map the distribution of different species of *Murdannia* in Western Ghats
4. To sequence the DNA of different species of *Murdannia* in Western Ghats
5. To produce DNA barcodes the different species of *Murdannia* in Western Ghats

Materials and Methods

Materials: The present study is focused on the collection and sequencing of different species of *Murdannia*. The locations of the collection were mapped using Geographic Positioning System (GPS).Twenty two species of *Murdannia* were collected during the course of the study.

Study area

The study area includes the South Indian States *viz*. Andhra Pradesh, Karnataka, Kerala, Goa, Maharashtra, Tamil Nadu and union territories of Mahe and Pondicherry. The area includes the major geographic formations such as Deccan plateau, Eastern Ghats and Western Ghats. A wide range of habitats from sea shore to high elevation montane grasslands including wetlands, marshes, mangrove forests, sacred groves, laterite plateau, different forest types such as deciduous, semi evergreen, evergreen, scrub, shola and grasslands are found in the study area.

Field methods:

1. Collection and Herbarium preparation of *Murdannia* species from different localities of Western Ghats
2. Germplasm conservation of *Murdannia* species by *ex-situ* methods.
3. Mapping of different species of *Murdannia* by using Geographical Information System: (GIS) tools- ArcGIS, GPS and Toposheets.

Experimental Method

1. Genomic DNA isolation.
2. PCR amplification.
3. Purification of PCR amplicon.
4. DNA sequencing of PCR amplicon.

Major results

The present study was undertaken to establish a phylogenetic relationship among selected members of genus *Murdannia* of Commelinaceae family based on rbcL gene. The study also provided a distributional record for the genus *Murdannia* Royle.

The genus *Murdannia* favours high altitude habitats (700 – 1000 mts MSL) with high moisture content. The laterite substratum and grassland habitat is the most desirable medium for species endurance and distribution. However *M. juncoides*, *M. esculenta* and *M. japonica* has made adaptations to survive in the rocky crevices where water and moisture availability is low. *Murdannia* prefers exposed habitats for their survival, because most of the species prefers anemophily and seed dispersal by wind. It requires open, exposed high elevation grasslands or laterites mounds. The population assessment also indicated that most of the species are under threat due to anthropogenic activities. So the conservation of *Murdannia* in their natural laterite and grassland habitat is an urgent need.

rbcL gene sequencing studies included BLAST search, Multiple sequence aligment and phylogram construction. BLAST search provided an initial platform for similarity searches within allied sequences. The consensus sequence obtained after sequencing revealed that all the plastid genomes are A+T rich i.e. *Murdannia pauciflora* (301), *Murdannia nudiflora* (303), *Murdannia fadeniana* (318), *Murdannia dimorpha* (310), *Murdannia japonica* (290), *Murdannia satheeshiana* (312) and *Murdannia* species (312). Multiple sequence alignment (BioEdit software) was also performed. MEGA 5 software was used for the construction of phylogram which depicted the phylogenetic relationships among the selected members. The plastid genomes of *Murdannia japonica* (GenBank Accession No.KT067728), *Murdannia dimorpha* (GenBank Accession No.KT067729) *Murdannia satheeshiana* (GenBank Accession No.JQ067697), *Murdannia nudiflora* (GenBank Accession No.KT067730), *Murdannia pauciflora* (GenBank Accession No.KT067731), *Murdannia fadeniana* (GenBank Accession No.KT962113) and *Murdannia sp.nov.* (GenBank Accession No.KT067732) were deposited in GenBank of NCBI.

Two major clusters are identified in the phylogenetic study of members of *Murdannia. M. japonica, M.dimorpha, M.satheeshiana* and *Murdannia sp.nov.* are positioned in the major cluster and the second cluster includes *M. nudiflora*, *M. pauciflora* and *M. fadeniana.* All the species were grouped in major or minor cluster grounded on their high similarity index. *Murdannia sp.nov.* is placed so close to *M. japonica* highlighting their major similarity. Both figures two clades of the same group. *M. dimorpha* and *M. satheeshiana* are pointed in the very

same major cluster, but somewhat away from *Murdannia sp.nov. M. nudiflora*, *M. pauciflora* and *M. fadeniana* were included in the second minor cluster, showing their genetic similarity.

In a comparison of different clades of *Murdannia* species, *M. japonica* shows more similarity with *Murdannia sp.nov*. Besides having a common ancestor, all the seven species of *Murdannia* fleshes distinct clades in two clusters. It is absolved that more genetic connectedness is pictured amongst all the species of *Murdannia* and they present two clusters founded on variance.

Scope and significance of the study

Since there is no recent revision or monograph or molecular phylogenetic studies of *Murdannia* either in South India or in Western Ghats, the present work has its relevance. The study enables to create a preliminary molecular database of selected species of *Murdannia* from the Western Ghats region. This data may resolve the ambiguity of cryptic species in *Murdannia* and helps to establish the phylogeny among the genus. Moreover, DNA barcoding of each species will become a standard for the identity of each species of *Murdannia*.

Major outcome of the study

Molecular systematic studies of *Murdannia* and Commelinaceae are in pioneer stage in Indian subcontinent and it is evident from very limited DNA sequence submissions in GenBank. The present study offer rbcL gene sequences of selected species of *Murdannia* for future molecular phylogenenetic works. The submitted sequences are publicly available in GenBank database so that it can be used for further comparative analysis. Two research papers were published in peer reviewed journals with impact factor. The papers are entitled “Distribution and ecology of the genus *Murdannia* Royle (Commelinaceae) in South India” (Oiirj, ISSN2249-9598, Volume-III, Issue-VI, Nov-Dec2013) and “Notes on the recent species bursts in *Murdannnia* (Commelinaceae) from India” (IJAR, ISSN 2249-555, Volume 5 Issue 7,2015). Based on the samples collected for the study, a new species of *Murdannia* from the Southern Western Ghats region is also about to be published (*Phytotaxa* with impact factor 1.7) after *Murdannia satheeshiana.*