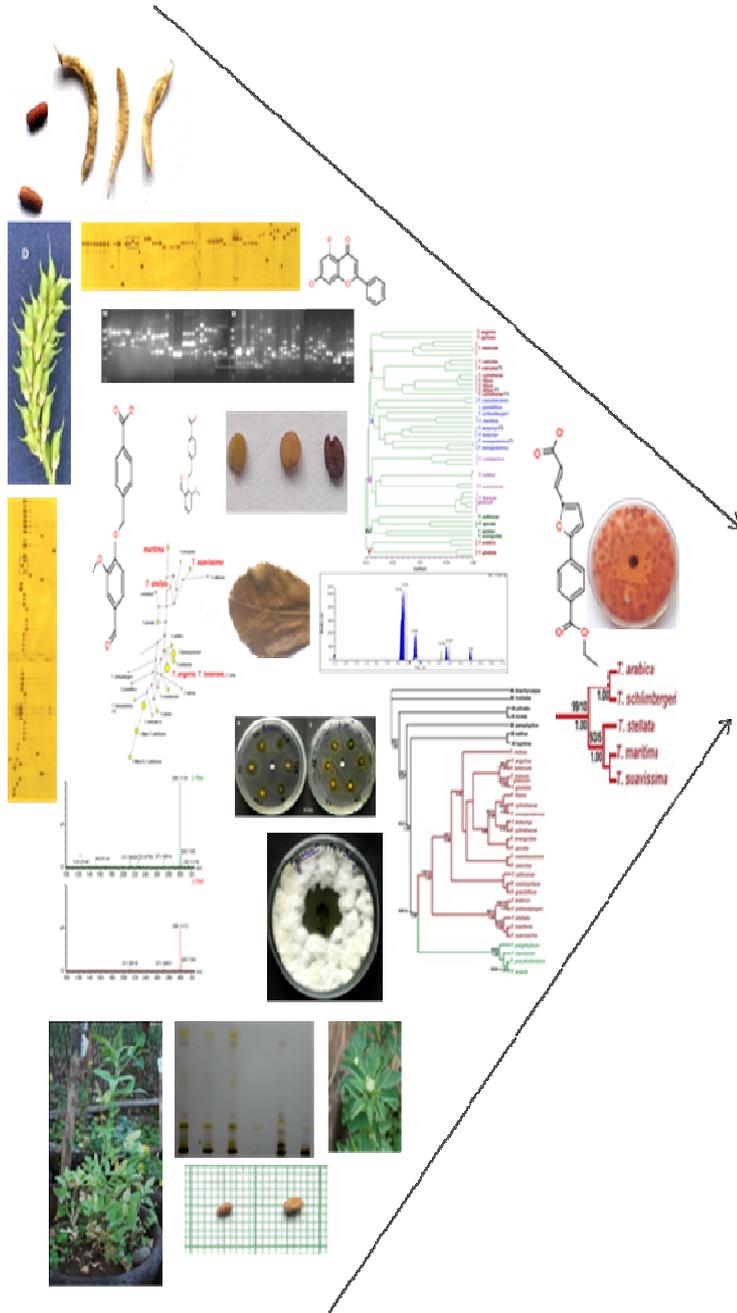


CHAPTER 8



**Summary
and
Future Directions**

8.1 Important research findings

The work presented in the thesis represents an initial step towards molecular and chemical characterization of *Trigonella* species. Important research findings of this work are as follows:

8.1.1 Molecular diversity in *Trigonella*

The present work is the first attempt in molecular characterization of 22 *Trigonella* species using molecular tools. Morpho-taxonomic and molecular markers has offered new insights into the origin of *Trigonella* species, short term evolutionary processes, phylogenetic relationships and ancestry of cultivated fenugreek

Turkey as a Centre of Origin and/or Diversity for *Trigonella*

With ISSR, similarity index values were lower for the accession collected from Turkey in *T. caerulea*, *T. mesopotamica*, *T. kotschy* and *T. filipes*. Intraspecific variability in *T. caerulea*, *T. mesopotamica*, *T. kotschy* and *T. filipes* revealed by Cp SSR was associated with the accessions collected from Turkey. In *T. caerulea* and *T. filipes*, for ITS sequences, intra-individual nucleotide polymorphism was also observed in the accession collected from Turkey with 0% polymorphism in the accessions collected from other countries. In *T. kotschy*, the two accessions from Turkey showed an identical ITS sequence which was 1.03% different from the ITS sequence of the accession collected from Jordan. In *T. mesopotamica* also, the two accessions from Turkey (although they showed different chloroplast haplotype) showed an identical ITS sequence which was 1.4% different from the ITS sequence of the accession collected from Iran and Iraq. With *trnL-F*, insertions/deletions were responsible for the length variation observed in accessions collected from Turkey. Thus, in addition to ISSR and Cp SSR, sequence divergence data also confirmed that these accessions were more divergent as compared to those collected from other countries. In addition to genetic diversity, morphological diversity was also observed in the accessions

collected from Turkey. These results support Vavilov's hypothesis that Turkey is the primary Centre of Origin and/or Diversity for *Trigonella*.

Ancestry of cultivated *T. foenum-graecum*

On the basis of morphological similarity, many authors maintain that the ancestor of cultivated fenugreek is the wild *T. gladiata* (Sinskaya 1961). The close genetic relationship between *T. foenum-graecum* and *T. gladiata* revealed by Cp SSR analysis lends support to the hypothesis that *T. gladiata* and *T. foenum-graecum* are sister species sharing a common maternal ancestral linkage. Moreover, at least on the basis of maternal evolutionary relationships displayed by chloroplast microsatellite, along with *T. gladiata*, *T. coelesyriaca*, *T. balansae*, *T. anguina* and *T. cretica* also shared a common maternal ancestral linkage with *T. foenum-graecum*. In agreement with results based on chloroplast microsatellite data, the phylogeny inferred from this study also strongly supports this view.

Phylogenetic relationships in *Trigonella*

Phylogenetic analysis based on nucleotide sequence data revealed the incongruences between morphological classification and molecular phylogenetic relationships. Some interesting results are summarized below:

- Morphological validation of *Trigonella* germplasm revealed three cases of misidentification. The identity of these accessions was established on the basis of previous taxonomic studies in the tribe Trifolieae. In all phylogenetic analysis (separate ITS, ITS+trnL-intron and ITS-trnL-F) the misidentified accessions clustered with a strong BP support with the "medicagoid" clade confirming their identification as *M. pamphylica*, *M. rostrata* and *M. lunata* on the basis of morphology.
- The phylogeny derived from the combined data sets provides strong support for the monophyly of the genus *Trigonella* as delimited by Small

(1987) and indicates that the taxonomic status of some section within the genus *Trigonella* may need revision.

- Molecular results (ISSR, Cp SSR and phylogeny) suggest that changes in the current circumscription of section *Falcatulae* should be considered. The sectional classification of section *Cylindricae* as proposed by Small (1987-b) could not be validated by the phylogenetic analysis due to the position of *T. strangulata* in the phylogram.
- In agreement with morphology and Cp SSR data the phylogeny presented confirms the sister taxon relationship of *T. spicata* with species of section *Cylindricae* and supports the sectional delimitation of section *Verae* and *Callicerates* but with a weak support.
- A well supported change of taxonomic significance based on molecular studies is the possible placement of both *T. arabica* and *T. schlumbergeri* in one section.
- Morphology, ISSR, Cp SSR (shared chloroplast haplotypes) as well as phylogeny indicated that the most closely related species to *T. balansae* is *T. anguina* indicating the use of the latter species in breeding programs in crosses with *T. balansae*.
- Although pod characters have been useful to assess infra generic relationships in *Trigonella* these characters alone are not sufficient to define the sections within genus.

8.1.2 Chemodiversity in *Trigonella*

Bioactive compounds from plants are considered to be safer without side effects. Though antimicrobial compounds have been purified and characterized from a number of medicinal plants no such work has been reported in *Trigonella*. The present work represents the most extensive survey of antimicrobial activity in *Trigonella* done to date. Analysis of chemodiversity in *Trigonella* has also provides valuable information regarding the quantification of diosgenin from aerial parts of *Trigonella* species. Some important results of analysis of chemo diversity in *Trigonella* are summarized below:

- Broad spectrum antibacterial activity was shown by *T. coerulescens*, *T. stellata*, *T. strangulata*, *T. suavissima* and *M. plicata* which could inhibit all the bacteria used. *M. plicata* and *T. suavissima* were the best candidates for further pharmacological investigations.
- *T. coerulescens*, *T. caerulea*, *T. gladiata* and *T. spicata* showed antifungal activity against both *A. niger* and *F. solani*
- *T. coerulescens* was the only *Trigonella* species which showed both antibacterial and antifungal activity.
- A combination of 7'4' dihydroxy flavone and (2E)-3-{5-[4-(ethoxycarbonyl) phenyl] furan-2-yl}prop-2-enoate characterized from *T. suavissima* can be used to prepare potent pharmaceutical formulations along with other antibiotics to treat infections caused by multidrug resistant bacteria.
- The polyhydroxylated alkaloid isolated from *T. spicata* with a novel selective antifungal activity was not reported earlier. Such antifungal compounds identified from plants can be used to develop new strategies to control fungal infections.
- Among the species screened, *T. spicata* showed the highest content of diosgenin in aerial parts as compared to *T. foenum-graecum*.

8.2 Future directions

Legumes like *Trigonella* have an inherent adaptation to challenging environments. However, *Trigonella* species have received little assessment for their agricultural and medicinal potential. These legumes have potential as forage species and the seeds show a high protein content. Hence, they could be used for the dual purpose of grain and grazing. For resources in *Trigonella* to remain available for exploitation, there is a further need for an extensive molecular and chemical based characterization of the germplasm.

Bioprospecting of *Trigonella* germplasm for biologically active compounds

Trigonella species are aromatic and medical herbs and therefore there is an immense scope to isolate and purify new bioactive molecules with novel

activities. Screening of *Trigonella* species for antibacterial activity opens the possibility to isolate natural antibacterial compound(s) from *T. coerulescens*, *T. stellata* and *T. strangulata*. Besides these *Trigonella* species, *M. plicata* is also a good candidate for further purification and characterization of antibacterial compound(s) against MDR bacteria including *S. aureus* (VRSA). Further work on these lines should also focus on the characterization of the unknown compounds in the ethyl acetate fraction of *T. suavissima* as well as on the isolation and characterization of other new antimicrobial compounds from this species. A novel cytotoxic combination of *Trigonella* derived phytochemicals active against multidrug resistant bacteria can be prepared to reduce the emergence of antibiotic resistant variants.

Laboratory screening of *Trigonella* species for antifungal activity could identify plants with antifungal activity against filamentous fungi indicating that a more extensive chemical prospecting of *Trigonella* genus (besides *T. spicata*) is likely to identify compounds with potential use in medicine and agriculture. Of particular interest is *T. coerulescens* which has shown both antibacterial and antifungal activity.

Establishment of plant cell culture systems obtained from *T. spicata*, could represent a potential renewable source of valuable medicinal compounds like diosgenin. For this, it would be necessary to examine whether the ability of *T. spicata* to accumulate diosgenin under field conditions would persist under the conditions of tissue culture as well. It will also be interesting to examine if the type and dose of auxins and cytokins and the addition of precursors can have a marked effect on the production diosgenin. If successful, design and development of bioreactors for this valuable secondary metabolite may be a possibility in future.

Need for taxonomic reassessment of genus *Trigonella*

The present data has clearly shown incongruence between the classification based on morphological characters and genetic relationships resolved by molecular data. Molecular data has allowed a greater resolution of relationships

in *Trigonella* on the basis of some well supported clades obtained in the combined nr DNA and the *trnL-F* analysis. However, a majority of clades were weakly supported indicating that a number of questions regarding relationships among *Trigonella* species remain to be resolved. Additional molecular data from different gene regions would be required to substantiate these relationships. This is especially true for species belonging to sections *Uncinatae*, *Capitatae*, *Samaroideae* and *Spinosaes*. Additional data will allow greater understanding of the evolution of morphological, molecular, and biochemical characters of taxonomic relevance in this genus. Data from different gene regions along with intensive sampling, mostly in the poorly understood section *Ellipticae* will be required for taxonomic reassessment and possibly taxonomic revision of the genus.

Potential for DNA bar coding of *Trigonella* species

“DNA barcoding” in angiosperms using nr DNA and non-coding Cp DNA sequences has been reported for many plants. In *Trigonella*, growing some of the wild germplasm to maturity is time consuming, and many of the species are difficult to bring to flower and fruit outside of nature. A “DNA barcoding” approach would be a useful supplement to existing identification methods which are mainly based on morphology. In our study, 97% of the sampled *Trigonella* species had unique combinations of nr DNA ITS and *trnL-F* sequences indicating that a DNA based identification of *Trigonella* species is quite feasible. It will also be of interest to characterize *Trigonella* germplasm for other proposed bar coding regions like *trn H-psb A*, *matk* etc.

Genomics assisted improvement

India is one of the major producer and exporter of fenugreek. This spice occupies third place in area and fourth in production among all the minor spices grown in this country. Although improved varieties have been developed through conventional breeding (pure line selection and mutational breeding), fenugreek germplasm has not been subjected to intensive selection through advanced plant

biotechnological approaches. Owing to little success in hybridization, the genes from related *Trigonella* species have not been exploited in fenugreek. However, double haploid breeding can now offer better scope for improvement in productivity and medicinal quality (Shiva and Malhotra 2008). Wild *Trigonella* species grow in dry, stony and marginal habitats suggesting that they may carry genes for resistance to heat, drought, and cold stress (Muehlbauer, 1993). In a time of rapid climate changes, such species may have a role in agriculture of the future. The knowledge of interspecific relationships in *Trigonella* can be used for further exploring these species for genetic improvement of this crop.

Using molecular markers, accelerated progress has been made in recent years towards a better understanding of the genetics of chickpea, lentil and pea. Genome sequences of legumes like *Lotus*, *Medicago*, soybean and pigeon pea are also available. Most genes in a papilionoid legume species are likely to be found within a relatively large (several hundred kb to several Mb) syntenic region with respect to any other given papilionoid species. The information from gene-rich regions of the *Lotus* and *Medicago* can be used to optimize the limited genetic information available for many other orphan legumes like *Trigonella*. The genome sequences of model legumes combined with other new genomics technologies will be important tools for basic research in these species. Possible understanding of genes involved in nodulation and synthesis of compounds with nutraceutical values such as galactomannans, steroidal sapogenin (diosgenin and tigogenin), isoleucine and trigonelline content for which there is a demand in pharmaceutical industry will be of particular interest.