

Chapter 6
SUMMARY

Summary

The legume tribe Phaseoleae (*sensu* Polhill and Raven, 1981) is the largest Papilionoideae tribe, with about 84 genera and 1500 species, and is economically the most important, containing the genera *Phaseolus*, *Vigna*, *Glycine*, *Cajanus*, *Clitoria*, *Macrotyloma*, *Lablab*, *Pachyrhizus*, *Pueraria* and *Psophocarpus*. The group is generally recognized by its twining habit and trifoliolate leaves with asymmetrical lateral leaflet margins, although not all taxa have all three characters. Some members of Phaseoleae share a number of derived features with other tribes and for this reasons it has been difficult to delimit the Phaseoleae clearly from other tribes of the Old World tropical lineage (e.g. Millettieae, Desmodieae, Indigofereae and Psoraleeae). Baudet split the traditional Phaseolinae genera into two supergenera: Phaseolastrae centred on the *Phaseolus-Vigna* complex and the Dolichastrae centred on *Dolichos* and its close allies based on the presence or absence of beard below the stigma. Though the use of supergenera has not been widely adopted, the existence of two distinct groups of genera with Phaseolinae is accepted.

Taxonomic confusion still exists with regard to (i) generic delimitation between *Phaseolus* and *Vigna*, *Dolichos* and *Lablab*, *Macrotyloma* and *Dolichos*; (2) conspecificity of *Vigna radiata* and *V. mungo*; (3) status of *Vigna radiata* ssp. *sublobata* and finally, (4) infra-generic classification of the genus *Vigna*. With a view to gather additional evidence to solve the problem relating to the taxonomy and phylogeny of the leguminous plant group described above, molecular characterization of representative members of Phaseolinae were undertaken.

In the present investigation, two PCR-based molecular markers *i.e.* Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) were employed to assess the genetic diversity and establish phylogenetic relationship among 49 species and accessions of *Vigna*, *Phaseolus*, *Dolichos*, *Lablab*, *Macrotyloma*, *Macroptilium*, *Centrosema* and *Psophocarpus* belonging to the sub-tribe Phaseolinae and tribe Phaseoleae of Papilionoid legumes. Besides, molecular characterization of 25 accessions and landraces of rice bean (*Vigna umbellata*) were also done separately. The genomic relationships among the accessions of *Vigna mungo* and *Vigna radiata*, among 9 species of genus *Vigna*, between the genera *Vigna* and *Phaseolus*, *Dolichos* and *Lablab* and among all the 17 genera of the sub-tribe Phaseolinae (tribe Phaseoleae) of Papilionoid legumes have been analysed, discussed, interpreted and compared with previous published literature on the subject.

Genetic diversity and relatedness of 25 landraces of rice bean (*Vigna umbellata*) collected from tribal dominated districts of Odisha (India) were assessed employing 17 ISSR primers. A total number of 1676 fragments were amplified, of which 921 (55%) were polymorphic. The dendrogram showed by and large clustering of landraces according to their geographical location of domestication or cultivation. The landraces collected from Semiliguda and Koraput, both from a single geographical region, shared a similarity of 85%. Similarly, two landraces from Tikabali area of Phulbani district out-grouped from all other accessions at a similarity level of about 75%. However, some accessions got intermingled with genotypes from a different geographical region which may be attributed to a common source of genetic stock and exchange of seed materials among tribal groups inhabiting different districts. The findings are in agreement with Songok *et al.* (2010) and

Muthusamy *et al.* (2008). The genetic diversity parameters and partitioning of gene diversity among 5 populations of rice bean collected from Koraput, Phulbani, Semiliguda, Tikabali and Bhanjanagar (Odisha) were analyzed and the latter two populations were found to possess higher genetic diversity with higher Percent Polymorphic Bands and Shannon Information Index. Among these populations, 45.67% of the total genetic diversity resided within the populations and 54.33% among populations.

The taxonomic treatment of black gram (*Vigna mungo*) and mungbean (*V. radiata*) has been a matter of confusion. While some authorities consider the mung bean and the urd bean to be variants of the same species, others conclude that there is ample evidence in keeping the both as distinct but closely related biological species. In the present study, genetic diversity and relationship among genotypes of *V. mungo* and *V. radiata* were assessed using 11 RAPD and 17 ISSR primers. A total of 944 bands were generated with both ISSR and RAPD markers systems, of which 613 (64.94%) were polymorphic in nature. The extent of polymorphism detected is moderate to low and comparable to what has been previously reported in mungbean (Lakhanpaul *et al.*, 2000) and blackgram varieties from India (Ajibade *et al.*, 2000; Souframanien and Gopalakrishna, 2004). In the current study, the cluster analysis clearly separated the two species *V. radiata* and *V. mungo* although these two self-pollinating species share the same chromosome number ($2n = 20$) and are partially cross-compatible (Seehalak *et al.*, 2006 & Tantasawat *et al.*, 2010). The closer relationship between *V. radiata* and *V. mungo* and distinct species status to both has been strongly supported by molecular studies (Goel *et al.*, 2002; Vir *et al.*, 2010; Ajibade *et al.*, 2000; Fatokun *et al.*, 1993 and Kaga *et al.*, 1996).

The intra-generic classification of *Vigna*, as suggested by Verdcourt (1970) and modified by Maréchal *et al.* (1978), has a series of subgenera and sections. It is comprised of 82 species distributed among 7 subgenera namely, *Vigna*, *Haydonia*, *Plectotropis*, *Ceratotropis*, *Lasiospron*, *Sigmoidotropis* and *Macrorhyncha*. The genus *Vigna* subgenus *Ceratotropis* consists of 16 to 17 recognised species (Verdcourt, 1970; Marechal *et al.*, 1978), which are distributed across Asia. In the present work, genetic diversity and phylogenetic relationship among 9 species of *Vigna* with 29 accessions were studied using 11 RAPD and 16 ISSR primers. A total of 2426 fragments were amplified with both the primer sets, of which 2368 bands (97.6%) were of polymorphic nature. The phylogenetic tree generated using RAPD data clearly segregated *Vigna pilosa* from all other species justifying its placement in a separate genus *Dysolobium* (Sanjappa, 1992). However, the data obtained from ISSR and combined RAPD and ISSR markers could not establish the above fact.

Of the *Vigna* species selected for the present work, clustering of species in the dendrograms constructed using RAPD, ISSR and combined data was not in complete agreement with the intra-generic classification stated above but certain species did. Based on ISSR data, two subspecies of *Vigna unguiculata* (*V. unguiculata* ssp. *unguiculata* and *V. unguiculata* ssp. *cylindrica*) belonging to the subgenus *Vigna* got separated from members of two other subgenera viz. *Ceratotropis* and *Sigmoidotropis*. The combined RAPD and ISSR markers data revealed grouping of taxa into two distinct clusters, one with *Vigna radiata*-*Vigna mungo* belonging to the sect. *Ceratotropis* of the subgenus *Ceratotropis* along with *V. unguiculata* of the sect. *Vigna* of subgenus *Vigna* and the other with members of sect. *Leptospron* of subgenus *Sigmoidotropis*; sect. *Angulares* and sect. *Aconitifoliae* of the subgenus *Ceratotropis*. Though both *V. aconitifolia* and *V. trilobata* were

members of the sect. *Aconitifoliae* of the subgenus *Ceratotropis*, they were placed remotely.

Vigna sublobata, which is considered as a variety of *V. radiata* (Verdcourt, 1970; Saini & Jawali, 2009; Bisht *et al.*, 2005; Dikshit *et al.*, 2005), was found to be quite distinct from *V. radiata* and did not come even in the same clade. This authenticates the view of Bairiganjan *et al.* (1985) in treating this widely accepted variety as a true species. Morphological distinction, analysis of seed protein content, amino acid composition and crossability experiments by Babu *et al.* (1985) established *V. sublobata* as a putative progenitor of *V. mungo*, which is remotely related to *V. radiata*. The above view has been supported and authenticated by Vir *et al.* (2009) and Undal *et al.* (2011).

Recently, the subgenus *Ceratotropis* has been divided into three sections *Aconitifoliae*, *Angulares* and *Ceratotropis* (Tomooka *et al.*, 2002). However, the sectional classification of the subgenus *Ceratotropis* could not be resolved using either RAPD or ISSR markers in the present study. Though *V. radiata* and *V. mungo* belonging to the section *Ceratotropis* came in one cluster but *V. sublobata* (*V. radiata* var. *sublobata*) of the same section formed a different clade with members of other sections. Similarly, *V. aconitifolia* and *V. trilobata* of the section *Aconitifoliae* were grouped in a larger cluster but away from each other and intermingled with other taxa of the section *Ceratotropis*, *Angulares* and even unrelated species of subgenus *Sigmoidotropis*. Saini and Jawali (2009), Doi *et al.* (2002) and Tomooka *et al.* (2002) also observed that the species relationship in *Vigna* is not in agreement with sectional classification stated above. Similar result obtained in the present study necessitates reinvestigation of the sectional classification of the sub-genus *Ceratotropis*.

One of the major problems surrounding the taxonomy of *Vigna* has been the generic delimitation between it and closely related genus *Phaseolus*. In the present study, employing RAPD markers, the generic distinction between the two genera *Phaseolus* and *Vigna* could not be established. However, on the basis of ISSR data, most of the accessions and with RAPD+ISSR markers all genotypes of *Phaseolus vulgaris* got separated from *Vigna*. This authenticates the fact that these two are distinct genera distinguishable by clear-cut morphological and molecular characteristics. This has been supported by the work of Verdcourt (1970), Vir *et al.* (2010) and many others.

Dolichos has affinities with *Macrotyloma* and the monotypic genera *Dipogon* and *Lablab*, with which it is often confused. *Lablab* can be distinguished from *Dolichos* and related genera by several characters including a blade-like style; a non-penicillate stigma and verrucose margins on the pods (Verdcourt, 1978). In the present work, the genetic relationships among four accessions of *Dolichos trilobus* and three landraces of *Lablab purpureus* were assessed with the application of combined RAPD and ISSR markers. The phylogenetic tree so generated placed all accessions of *Dolichos trilobus* and *Lablab purpureus*, except one, in separate clades. The above findings justify granting separate generic status to both the taxa as has been done on morphological and molecular basis (Songok *et al.*, 2010). Further, most of the genotypes of *L. purpureus* and *D. trilobus* collected from a particular geographical region had close similarities among them.

The phylogenetic tree constructed using RAPD and ISSR combined markers, segregated all the 17 species of the sub-tribe Phaseolinae belonging to 8 genera

namely, *Vigna*, *Phaseolus*, *Dolichos*, *Lablab*, *Macrotyloma*, *Macroptillium*, *Centrosema* and *Psophocarpus* into two major groups; the smaller cluster containing accessions of *Macroptillium lathyroides*, *Centrosema pubescens* and *Psophocarpus tetragonolobus*. The segregation of *Psophocarpus tetragonolobus* and *Centrosema pubescens* in the dendrogram was supported on the basis of morphological studies by Baker (1878). The genus *Centrosema* is now considered as a member of a different sub-tribe Clitoriinae on the basis of morphological characters. Lackey (1981) kept *Psophocarpus* separate with the remarks that the same is a peripheral member requiring possible realignment with other sub-tribes. In view of the above observation, the remote placement of *Psophocarpus* in a distant clade in the phylogram gets validated on molecular grounds. However, grouping of *Macroptillium*, which has closer affinity with *Phaseolus-Vigna* complex, with *Centrosema* and *Psophocarpus* in a distant cluster could not be explained. *Dolichos* is closely related to *Macrotyloma* (Moteetee & Van Wyk, 2012; Verdcourt, 1970; Baker, 1878) but contrary to this, they were distantly placed in the cladogram constructed on the basis of molecular data in the present study. The close genetic relationship between *Dolichos* and *Lablab* was also validated.

With regard to the intra-generic classification and phylogeny of *Vigna*, the clustering of species in the dendrogram was not in complete agreement with the intra-generic classification proposed by Maréchal *et al.* (1978) who divided the genus into 7 subgenera (*Vigna*, *Haydonia*, *Plectotropis*, *Ceratotropis*, *Lasiospron*, *Sigmoidotropis* and *Macrorhyncha*) but certain taxa followed the accepted pattern of classification. The dendrogram revealed two distinct clusters, one with *Vigna radiata-Vigna mungo* group belonging to the subgenus *Ceratotropis* along with *V. unguiculata* of the subgenus *Vigna* and the other with members of two other subgenera *Sigmoidotropis*

and *Ceratotropis*. Though both *V. aconitifolia* and *V. trilobata* belong to the sect. Aconitifoliae of the subgenus *Ceratotropis*, they were placed remotely. However, similar observations were made by Ajibade *et al.* (2000) using ISSR polymorphism. Tomooka *et al.* (2000) proposed a revised list of taxa in the subgenus *Ceratotropis* and suggested three groups, giving them taxonomic rank as Section Angulares (azuki bean group), Radiatae (mungbean group) and Aconitifoliae (mothbean group). The present study was able to distinguish two genepool groups within the Asiatic *Vigna* (sub-genus *Ceratotropis*) *i.e.* mungbean group with *V. radiata*, *V. mungo* and others and a miscellaneous group with *V. sublobata*, *V. trilobata*, *V. aconitifolia*, *V. umbellata* etc. belonging to various intra-generic taxa.

Molecular phylogenetics has dramatically reshaped our views of organismal relationships and evolution. This impact has been manifested at all taxonomic levels of the hierarchy of life, from the infra-species level to kingdoms. Over the last few decades there has been increased interest in the wild relative of crops in general and of legumes in particular. These wild relatives play a two-fold role in the conservation of genetic resources and their utilization. They constitute a geographic framework of reference against which the diversity of the domesticated gene pool can be tested and are becoming increasingly important as additional source of genetic diversity. Whereas wild relatives have been used primarily as a source of major genes for disease or pest resistance, recent progress in molecular mapping will certainly provide opportunities to identify and transfer genes for quantitative traits leading to crop improvement. Molecular characterization and phylogenetic analyses revealed during the present study of phaseoloid legumes will help to identify the closest relatives of many cultivated crops to be use in plant breeding and 'model organisms' for molecular genetic studies.