CHAPTER 1
INTRODUCTION

The genus *Vigna* Savi belongs to family Fabaceae, which comprises of all the leguminous taxa. Fabaceae is one of the most morphologically diverse families of angiosperms, ranging from trees to aquatics to xerophytes, with the majority being herbaceous perennials. Fabaceae is normally divided by taxonomists into three subfamilies, Mimosoideae, Caesalpinioideae and Faboideae. It is the third largest family of the flowering plants consisting of approximately 650 genera and 18,000 described species and ranks only after the Compositae and Orchidaceae (Polhill and Raven, 1981) and second most important, with respect to economic importance after Gramineae (Heywood, 1978; Heywood and Chant, 1982). *Vigna* is a large genus comprises of 104 described species (Lewis *et al.*, 2005) and is pantropical in distribution and reported around the globe. The history of the taxonomic treatments of the Asian *Vigna*, at the genus and subgenus level is summarized. The Asian *Vigna* were initially classified into the genus *Phaseolus* by De Candolle (1825). Ohwi (1953) proposed a new genus *Azukia* for this group. Subsequently, Maekawa (1955) further divided this group into two genera, *Azukia* and *Rudua*, mainly based on seedling characteristics. However, these treatments, that consider the Asian *Vigna* constitute a distinct genus or genera, have not gained general acceptance. Verdcourt (1970) proposed a restricted concept for *Phaseolus* limiting it to those American species with a tightly coiled style and pollen grains lacking coarse reticulation. The concept of the genus *Vigna* was revised to include the Asian *Vigna* as subgenus *Ceratotropis* by Verdcourt (1970) and listed 17 species in the subgenus *Ceratotropis*.
Marechal et al. (1978) in their monograph on the Phaseolus- Vigna complex, which has become the standard taxonomic system for this group, recognized seven subgenera in the genus Vigna, namely Ceratotropis, Haydonia, Lasiospron, Macrorhycha, Plectotropis, Sigmoidotropis and Vigna. The relationships and geographical distributions of these subgenera are summarized in Fig.1. Marechal et al. (1978) also recognized 17 species in the subgenus Ceratotropis. Tateishi (1985) proposed a revision of the Asian Vigna based both on examining specimens in European and Asian herbaria and intensive field studies in many Asian countries. Thulin et al. (2004) later transferred the subgenus Macrorhynchus, which was previously placed in the genus Vigna to genus Wajira.

Among the subgenera in the genus Vigna, only Ceratotropis has its centre of species diversity distributed across Asia (Verdcourt, 1970; Marechal et al., 1978; Tateishi, 1996). Tomooka et al. (2002a) recognized 21 species in the subgenus Ceratotropis, out of which six species have been domesticated, which includes V. angularis (azuki bean), V. radiata (mung bean), V. mungo (black gram), V. umbellata (rice bean), V. aconitifolia (moth bean) and V. reflexo-pilosa var. glabra (creole bean). Among them, mung bean, azuki bean and black gram are the most important in terms of economic importance (Tomooka et al., 2002a). Mung bean and urd bean provide a significant portion of the dietary protein in many societies. Several others species, including adzuki bean, moth bean, rice bean and bambara groundnuts are important in the diets of many societies. Mung bean, in particular, is especially important as the major food crop under the subgenus Ceratotropis in developing countries in South and South-east Asia where 80% of the
Fig.1: Scheme of classification and main areas of distribution of the genus *Vigna*

after Tomooka *et al.* (2002a)
Genus: *Vigna*

**ASIA**
- Subgenus: Ceratotropis
  - Section: Angulares
    - *V. angularis*  
    - *V. dalzelliana*  
    - *V. exilis*  
    - *V. minima*  
    - *V. reflexo-pilosa*  
    - *V. riukiuensis*
  - Aconitifoliae
    - *V. nakashimae*  
    - *V. nepalensis*  
    - *V. tenancialis*  
    - *V. trinervia var. bournea*  
    - *V. umbellata*
- Subgenus: Haydonia
  - Section: Glossostylus
    - *Haydonia*
  - Section: Pseudolibrechtsia
    - *Microspermae*
- Subgenus: Macrorhyncha
  - Section: Plectotropis

**AFRICA**
- Subgenus: Vigna
  - Section: Catiang
    - *Comosae*  
    - *Liebrechtsia*  
    - *Macrodontae*  
    - *Reticulatae*
- Subgenus: Lasiospron
  - Section: Caracallae
    - *Condylostylis*  
    - *Leptospron*  
    - *Peduncilares*  
    - *Sigmoidotropis*
- Subgenus: Sigmoidotropis
  - Section: Aconitifoliae
    - *V. aconitifolia*  
    - *V. aridicola*  
    - *V. khandalensis*  
    - *V. stipulacea*  
    - *V. trilobata*

**AMERICA**
- Subgenus: Sigmoidotropis
  - Section: Ceratotropis
    - *V. grandiflora*  
    - *V. hiriella*  
    - *V. mungo*  
    - *V. radiata*  
    - *V. subramaniana*
world's mung bean is grown. It is rich in protein (17-24%) and is easily digestible, thus providing an alternative and inexpensive source of vegetable dietary protein (Fernandez and Shanmugasundaram, 1988) and constitute one of the most economically important groups in the genus *Vigna* for subsistence agriculture (Santalla *et al*., 1998).

Some of the *Vigna* species are cultivated worldwide and are adapted to a wide range of extreme environmental conditions. They grow in poor soils without supplementary nitrogen and some of them are also cultivated as cover crop, green manure and for the control of soil erosion etc. (Chandel *et al*., 1982). Many of the species produce multiple edible products, and these products provide subsistence to farmers with a food supply throughout the growing season as well as dry seeds in off season since the seeds are easy to store and transport. Tender shoot tips and leaves of cowpeas can be consumed as soon as the plants reach the seeding stage and immature pods and immature seeds can be consumed during the fruiting stage. Harvested dry seeds of all of the *Vigna* crops can be consumed directly, and are also produce sprouts and/or commonly used to make flour. Plant residues can be used as fodder for farm animals. *Vigna* food products exhibit many excellent nutritional attributes and these products provide much needed complements in diets comprised mainly of roots, tubers or cereals.

The *Vigna* constitute an important group of cultivated and wild species for which rich diversity occurs in India. Taxonomically, cultigens and conspecific wild forms are recognised in all cultivated Asiatic pulses, *V. radiata*, *V. mungo*, *V. umbellata* and *V. angularis* except for *V. aconitifolia* which have retained wild
type morphology (Marechal et al., 1978; Lukoki et al., 1980). The cultivated species, *V. radiata* and *V. mungo* are of Indian origin, as it can trace back its remains in archaeological sites in the sub-continent (Arora et al., 1973; Chandel et al., 1984). *V. radiata* assumes additional importance due to the fact that it has been reported to cross with a number of *Vigna* species when it is used as a female parent. It is also proposed to be one of the first species of the genus *Vigna*, to have undergone domestication on the basis of information obtained through interspecific crosses (Dana, 1966). The domesticated *V. acontifolia* is confined only to the tropical region of India, while that of *V. angularis* and *V. umbellata* is widely domesticated across the South-east Asia. The origin of *V. umbellata* is considered to be in Indo-China region and also partly from South-east Asia (Marechal et al., 1978; Baudoin and Marechal, 1988). The tribal dominated belts of Mizoram, Manipur, Meghalaya, Tripura, Sikkim, North Bengal and parts of Nagaland and Arunachal Pradesh, are rich in local variability in *V. umbellata*, *V. angularis*, *V. mungo* and *V. vexillata* etc. *V. umbellata* occurs sporadically in the North-eastern region with profuse branching types reported from Mizoram and Manipur. Higher number of seeds per pod from Mizoram; higher number of pods per peduncle, bold seeds and high grain yield from Manipur; higher polymorphism has also been recorded in local landraces for seed colour. Landraces with a rare uniform light green colour occur in the Mao hills (Senjam et al., 2012), while seed colour is commonly yellow, brown or red. The red type is commonly known as red small bean and considered as herb in traditional Chinese medicine.
Twenty four species of Vigna under the subgenus Ceratotropis are reported to occur in India (Sanjappa, 1992), which partly represents centre of species diversity for all the three sections of subgenus Ceratotropis, are also known as Asian Vigna. The cultivated species in the mungo-radiata complex with conspecific wild forms are V. mungo and V. radiata. The conspecific wild species in V. mungo is V. mungo var. silvestris. In V. radiata, more diversity in wild types occurs and two conspecific species V. radiata var. setulosa and V. radiata var. sublobata are reported. Two more wild species, V. hainiana and V. khandalensis, endemic to India, have also been accommodated in the mungo-radiata complex owing to their similarity to both cultivated V. mungo and V. radiata. Other cultivated species with conspecific wild forms include V. umbellata (wild type occurring, conspecific with cultigen types V. umbellata var. gracilis), V. aconitifolia (wild occurring variability conspecific with cultigen types) and the semi-domesticated V. trilobata (wild occurring and variability being conspecific with cultigens type). No wild types are reported to occur in India for V. angularis. V. dalzelliana, V. minima, V. bourneae and V. glabrescens, which are other important species in the subgenus Ceratotropis reported to occur in India. The use of wild relatives as sources of new germplasm is well established in breeding programmes for crop improvement on a worldwide level. However, the efficiency of introduction of useful traits from wild germplasm such as disease resistance and other agronomic characters into elite cultivars varies greatly. Wild Vigna species have great potential for use in crop improvement programmes. Systematic
survey and collection of wild *Vigna* species occurring in India was undertaken during 1999-2001.

The data on chromosome numbers and comparative karyotype is fundamental to overall understanding of genome in different species or in morphologically diverse populations within a species (Stace, 2000). Karyomorphological analysis is a useful method for characterizing plant chromosomes and genome organization. The structure and morphology of the chromosome is of vital importance when studying the origin, evolution and classification of taxa (Yang *et al*., 2005). The difference and similarities in the karyotype are regarded as basis of genetic variation, as well as distance or relatedness among diverse genomes (Kumar and Rao, 2002a). Since karyomorphological study alone does not supply sufficient data for authentic characterization of the species, it is necessary to investigate the differential fluorophore banding pattern for providing more data to support the species differentiation or affinities. Fluorochrome banding has the advantage of being a simpler, more reproducible and less destructive method, as compared with C-banding (Guerra, 1993). Some of the commonly used fluorochromes in plants are CMA, which preferentially stains GC-rich DNA and DAPI, which preferentially stains AT-rich regions. Using these two fluorochromes, heterochromatin blocks in plant can be characterized as GC-rich or AT-rich region (Guerra, 2000). Heterochromatin is one of the chromosomal components that have attracted most attention from cytogeneticists for the fact that it stains differently to the rest of the chromosome, of its unknown function, its apparent lack of genes refer to as a “genomic wasteland” or a
repository of “junk” DNA (John, 1998). Nowadays this idea is obsolete; in fact, in the past two decades molecular genetic studies have implicated that it play an important cellular function in structural rearrangement of the chromosome (Dimitri et al., 2004, 2005; Corradini et al., 2007).

Most of the cytogentical studies such as chromosome counts, associations/behaviour during meiosis, ploidy level etc. in the genus Vigna have been focussed on cultivated species viz. V. mungo, V. radiata, V. umbellata and V. aconitifolia. Such basic knowledge of chromosomes are still lacking for some wild Indian species. Therefore, being one of the centres of origin and diversity, there is an urgent need to evaluate the cytogenetical mechanism underlying genome organization and speciation in the genus Vigna.

The concept of genetic markers is not a new one; Gregor Mendel used phenotype-based genetic markers in his experiment in the nineteenth century. Later, phenotype based genetic markers for Drosophila led to the establishment of the theory of genetic linkage. The limitations of phenotype based genetic markers led to the development of more general and useful direct DNA based markers that became known as molecular markers. A molecular marker may be defined as a particular segment of DNA that is representative of the differences at the genome level. Molecular markers may or may not correlate with phenotypic expression of a trait. Molecular markers offer numerous advantages over conventional phenotype based alternatives as they are stable and detectable in all tissues regardless of growth, differentiation, development, or defence status of the cell are not confounded by the environment, pleiotropic and epistatic effects. Molecular
analysis of genetic diversity in *Vigna* species have been performed by using DNA markers like (RAPD) randomly amplified polymorphic DNA (Samec *et al*., 1998; Santalla *et al*., 1998; Banerjee *et al*., 1999; Lakhanpaul *et al*., 2000; Lambridges and Lawn, 2000; Betal *et al*., 2004), (ISSR) inter simple sequence repeats (Souframanien and Gopalkrishna, 2004), (RFLP) restriction fragment length polymorphism (Lambridges and Lawn, 2000), (AFLP) amplified fragment length polymorphism (Bhat *et al*., 2005) and (SSR) simple sequence repeat (Wang *et al*., 2004) markers. RAPD is a valuable tool for identifying genetic variation because it is inexpensive, quick, and simple (Williams *et al*., 1990). It permits the identification of DNA polymorphisms and can be used to amplify particular fragments of genomic DNA (Bielawski *et al*., 1996). Inter-simple sequence repeats (ISSR) is a type of molecular marker, proposed by (Zietkiewicz *et al*., 1994) for fingerprinting. ISSR primers can confirm specific amplified DNA polymorphic fragments within the variety (Leian *et al*., 2005). Three methods involving single primer for amplification reactions are commonly used in diversity analysis of higher plants which include (a) direct amplification of minisatellite DNA regions (DAMD) (Heath *et al*., 1993); (b) inter simple sequence repeat (ISSR) (Gupta *et al*., 1994) and (c) random amplified polymorphic DNA (RAPD) (Williams *et al*., 1990; Welsh and McClelland, 1990). In the recent years, the PCR based single primer amplification reaction (SPAR) methods are gaining prominence as effective tools for genetic diversity studies in plants and they collectively provide a comprehensive description of the nature and extent of the diversity (Bhattacharya *et al*., 2005; Ranade *et al*., 2009). The
efficiencies of this cumulative markers approach has been tested and trusted by several researchers engaged in genetic diversity analysis study in a wide range of plants including Neem (Ranade and Farooqui, 2002); Mulberry (Bhattacharya et al., 2005); Pomegranate (Ranade et al., 2009) Papaya (Saxena et al., 2005); Prosopis (Sharma et al., 2010b); Cymbidium (Sharma et al., 2011); Jatropha (Kumar et al., 2011) and reveals quite promising and reliable results.

From the beginning of molecular systematics to the present day, the most popular phylogenetic markers in plants are various regions in the chloroplast genome. They are used both at the high taxonomic levels (divisions and classes) and at low levels (genera and species). In most cases protein-coding genes (and corresponding amino acid sequences) are used for molecular phylogenetic analysis at the high taxonomic level, although examples of successful usage in such cases of non-coding plastome sites are known (Borsch et al., 2003; Lohne and Borsch, 2005). The set of chloroplast genes for phylogenetic analysis at the high taxonomic level was subsequently supplemented with genes atpB (Wolf, 1997; Soltis et al., 1999), atpF-atpH (Drager and Hallick, 1993), psbK-psbl (Meng et al., 1991) and accD (Yuji et al., 2005) and some others. Moreover the atpB gene has been used successfully in phylogenetic studies at family and higher levels (Hoot et al., 1997, 1999; Hoot and Douglas, 1998; Bayer et al., 1999; Chase et al., 1999). Hence, these regions are expected to be useful in studying phylogenetic relationship at lower taxonomic levels.

The genetic diversity in cultivated and wild forms in the genus Vigna subsection Ceratotropis occurring in the Indian gene centre is extremely rich and
interesting. Despite the presence of rich diversity for these cultivated species and wild relatives in India, these wild-relative complexes have not been the subject of intense studies, which is essential for identification, classification and management of the genetic resources. The information related to chromosome number and karyotypes besides meiotic studies on chromosome associations, recombination frequency, anaphase distribution etc. is the basic requirement for understanding of genome organization. A number of earlier workers did such studies in genus *Vigna* but incidentally they are all confined to cultivated species/varieties/genotypes (Rao and Chandel, 1991). Most earlier studies demonstrated species relationships based on a limited number of accessions of individual species and information on intraspecific diversity is lacking. Information on intraspecific diversity is essential for effective use of wild species germplasm in crop improvement programmes. Wild species/accession did not receive the due attention from geneticists and cytogeneticists. Hence, an effective strategy to better understand the cytogenetics and evolution of this genus, there is a need to undertake comprehensive cytogenetical and DNA marker approaches to define the existing natural variation at inter- and intra specific levels of genetic relationships and diversity analysis that would be useful for breeding programs through selection of diverse parents, and molecular phylogeny based on chloroplast gene sequences, to trace, observe and define the evolutionary rates of sequence divergence within the genus for phylogenetics and evolutionary relationship of the *Vigna* species.
Thus the present investigation has been carried out with the following major objective:

- To study inter- and intraspecific chromosomal variation in Vigna species.

Studies related to above objective form different chapters of the thesis, and result of each aspect has been discussed in individual chapters.