

Chapter 1

INTRODUCTION

Introduction

As the name implies, the Leguminosae is characterized by their fruits called "legumes". A legume is basically a dehiscent fruit that develops from a single carpel and splits into two valves that surround the fruit. Although the general structure is uniform in most species, there may be differences in various features of the structure of the pericarpical cells (Fahn, 1967). The relationship between these features, evolution and dehiscence has been described at length by Fahn and Zohary (1955). Not all members of the Leguminosae have fruits that conform to the typological description of the legume; they are not all dehiscent, some being samaras, others drupaceous; some are transversely septate and break up into 1-seeded portions. They may be dry, fleshy, inflated or compressed, greenish or brightly coloured, winged or unwinged.

Legumes are a significant component of nearly all terrestrial biomes in all continents, except Antarctica. It contains plants which are herbs, shrubs, trees, climbers and lianas. Some are fresh water aquatics, but there are no truly marine species. Diversity does exist in all morphological parts to the extent that it needs a thorough and clear understanding. Bentham and Hooker (1876) described 339 genera and 7,453 species in the family Leguminosae, which included Fabaceae, Mimosaceae and Caesalpiniaceae. Estimates of the numbers of genera, and especially species given by Airy Shaw (1966), Hutchinson (1964), Melchior (1964) and others, vary from 590 to 690 genera and 12,000-17,000 species, but all agree that there has been a great increase in the numbers of taxa described since the classic treatments of Bentham (1865) and Taubert (1894). In the present day context, the family Leguminosae (Fabaceae) comprises of more than 700 genera and 20,000 species

and are the largest family of flowering plants after the Orchidaceae and Compositae (Doyle and Luckow, 2003). However, Polhill and Raven (1981) and Humphreys (1996) estimate the number of genera and species under Fabaceae as 750 and 18,000 respectively.

1.1. Economic importance

In terms of economic importance, leguminous plants are the most important family in the dicotyledon (Harbone, 1994). This is because they were among the earliest plants to be domesticated by mankind. The leguminosae are second to cereal crops in agriculture importance, based on area harvested and total production. Most cultivated legumes are found within the Papilionoideae, the subfamily with largest number of species and genera. There are two major groups of cultivated species in the Papilionoideae: the 'tropical' or 'Phaseoloid' legumes (including the genera *Phaseolus*, *Vigna*, *Glycine* and *Cajanus*) and the 'temperate' or 'Galegoid' legumes (including the genera *Melilotus*, *Trifolium*, *Medicago*, *Pisum*, *Vicia*, *Lotus*, *Cicer* and *Lens*). Legume seeds are the second most important protein source of human and animal food (Vietmeyer, 1986).

Common bean (*Phaseolus vulgaris*) is the most important edible food legume and is a major food for urban and rural population in Latin America and East Africa and steadily gaining importance in developed countries. It represents 50% of the grain legumes consumed worldwide. In some countries, such as Brazil and Mexico, it is the primary source of protein in the human diet. Grain legumes provide about a third of all dietary protein nitrogen and a third of processed vegetable oil for human consumption (Graham and Vance, 2003). Seeds of grain legumes contain at least 20-40% protein. In many places of the world, legumes complement cereals or root

crops, the primary source of carbohydrates, in terms of amino acid composition. Today, dry legume seeds are used for food throughout the world. These are referred to as pulse, dry beans, legume seeds or legume grains. Some of the legume seeds like soybeans, groundnuts and winged beans are also sources of oil (Arora *et al.*, 1991). Legumes offer a variety of edible products in addition to seeds. Many immature green pods are edible before the fiber lignify and harden. At this stage, they are still green and succulent and can be used as a green vegetable. Although they have less protein than the mature seed; they are rich in vitamins and soluble carbohydrate. Leaves of leguminous plants are eaten in some parts of the world, particularly in the tropics. As such, common bean is a very important non-processed food crop in third world countries and contributes significantly to the world's protein diet. The importance of this crop as an international protein source is reflected by the fact that the dry bean export market alone has a value of \$1.8 billion to the U.S. economy. Grain legumes such as beans, peas, soybeans, peanuts and lentils, are excellent sources of protein and vitamins in addition to carbohydrate. Although the protein quality is not as good as in meat, generally due to low levels of the essential amino acid methionine, this can be corrected by incorporating grain legumes with cereals in the diet. They are rich in lysine. Especially with soybeans and peanuts, they are good sources of vegetable oils which are used in the preparation of various products such as cooking oil, margarine, mayonnaise, and salad dressings. In Mexico, common bean constitutes the main source of vegetable protein, minerals (Ca, Cu, Fe, Mg, Mn, and Zn) and vitamins (folate). Its balance of essential amino acids in seeds is complementary to that in grains of cereals such as maize (*Zea mays*). It has been estimated that maize and beans should be consumed in a 2:1 ratio to achieve an optimum amino acid balance in the diet (Bressani, 1983).

Legumes produce many useful phytochemicals which are utilized in agriculture and industry. Examples of commercially useful phytochemicals are rotenone, tephrosin and deguelin, which are used in limited quantities as pesticides (Beckstrom-Sternberg and Duke, 1994; Gaskins *et al.*, 1972; Minton and Adamson, 1979; Tyler *et al.*, 1976). Some legumes are potential sources of glycosides, biologics, antibiotics and alkaloids which are used in drug manufacturing by the pharmaceutical industry (Tyler *et al.*, 1976). The glycosides include aloemodin, chrysophanol, emodin, and rhein. Winged bean (*Psophocarpus tetragonolobus*) produce useful phytochemicals such as poly-unsaturated fatty acids used as an anti-polyneuritic (Beckstrom-Sternberg and Duke, 1994), it also produces edible leaves, shoots, flowers, pods and tubers as well as seeds whose composition duplicates that of soyabeans. The most interesting feature of winged bean tubers is their protein content. Winged bean tubers average 20% protein as compared to 1% for cassava (*Manihot esculenta*) and 3–7% for potato (*Solanum tuberosum*) (NAS/NRC, 1979). To date, winged bean has not yet met expectations due in part to its intolerance of cold temperatures during the winter. Lablab bean (*Lablab purpureus*) has a myriad of uses. The young pods, dried seeds, leaves and flowers can be eaten.

Cowpea is one of the most important pulse crops globally. FAO estimates that cowpea is now cultivated on at least 12.5 million hectares and an annual production of over 3.3 million tonnes of dry grains were produced worldwide in 2000. Cowpea is mainly used for human and livestock consumption. When fresh, the young leaves, immature pods and peas are used as vegetables, while snacks and main meal dishes are prepared from the dried grain. Cowpea grain matures earlier than cereals, hence becomes a quick source of cash before the maize, millets and cassava are harvested. Cowpea residue after harvest is used for feeding cattle, goats, pigs,

sheep and other farm animals (IITA, 2002). *Vigna unguiculata* is an important crop which provides essential nutrients and high quality protein for human and livestock feed in many areas of the world and is also recognized as a potential source of useful genes for legume breeding (Matos *et al.*, 2011).

In India *Vigna unguiculata* and *Lablab purpureus* are used as food, fodder and as medicine. Although the lablab bean now finds its greatest use in small scale agriculture, its potential for large mechanized production of protein seems impressive. In the monsoon tropics, where other large scale grain legumes (such as soybean) grow poorly, the lablab bean thrives. Green gram (*Vigna radiate*) and Black gram (*Vigna mungo*) are the common and most important pulse crops occupying unique position in Indian agriculture. Among the pulses, they stand fourth in production and acreage (Deepalakshmi and Anand Kumar, 2004; Mandal *et al.*, 2009). They are the staple crop in central and South East Asia but extensively used in India and now grown in the southern United states, the West Indies, Japan and other tropical and sub-tropical countries. They are used for human food, green manure, cover crop, forage, silage, etc. Among legume, they are more useful because they are the main sources of amino acid as well as proteins (Imrie, 2005; Kulsum *et al.*, 2007). Black gram is a rich protein food. It contains about 26 percent protein, which is almost three times that of cereals. Black gram supplies a major share of protein requirement of vegetarian population of the country. It is consumed in the form of split pulse as well as whole pulse, which is an essential supplement of cereal based diet. The combination of dal-chawal (pulse-rice) or dal-roti (pulse-wheat bread) is an important ingredient in the average Indian diet. The biological value improves greatly, when wheat or rice is combined with black gram because of the complementary relationship of the essential amino acids such as arginine, leucine,

lysine, iso-leucine, valine and phenyl-alanine etc. In addition, being an important source of human food and animal feed, it also plays an important role in sustaining soil fertility by improving soil physical properties and fixing atmospheric nitrogen. Being a drought resistant crop, it is suitable for dryland farming and predominantly used as an intercrop with other crops.

Recently, some bean seed properties have been shown to contribute positively to major health issues, such as the control of type II diabetes. Positive characteristics of bean seeds include the presence of essential minerals, such as Fe and Zn, and high fiber and polyphenolic content. Typically, consumption of bean seeds leads to lower glycemic and cholesterolemic indices and lower incidence of certain types of cancer (Andersen *et al.*, 1984; Hangen and Bennink, 2003). Medicinal applications of members of the genus *Vigna* include the use of *V. gracilis* var. *multiflora* roots as a vermifuge, smoking of leaves of *V. ambacensis* as a cough remedy in the Democratic Republic of the Congo, use of leaves of *V. racemosa* in the treatment of cataract in Nigeria, and the use of the leaf sap and roots in the treatment of diarrhoea in Tanzania. In the Central African Republic, a decoction prepared from the roots of *V. multinervis* is taken on an empty stomach to treat roundworm infections (Burkill, 1995). Tyrosinase found in lablab bean has potential use for antihypertensive treatment (Beckstrom–Sternberg and Duke, 1994).

1.2. Classification

The recognition of one family or three families within the leguminales may still be regarded as a matter of opinion (Brenan, 1967), depending on the emphasis given to the relatively few genera that are transitional between the three major groups, as against the relatively numerous genera about whose position there is no possible difference of opinion. As yet the fossil records provide little evidence about the early

evolution of the family. The three subfamilies were certainly well established and widely dispersed by the Eocene, but earlier records are sparse. Among contemporary system makers, Takhtajan (1966, 1969 & 1973) and Dahlgren (1975) regarded legumes as worthy of ordinal designation. Leguminous genetic resources have hardly been explored and sampled for their offerings. Genetic variation in legume species and their wild relatives is of prime importance to the successful breeding of improved crop cultivars with added value and durable resistance to pests. For proper utilization and conservation, it is important to know the taxonomic relationship among the different crop species and their wild relatives. Traditional taxonomists have less often considered the degree of genetic differentiation among species. Such studies tend to be restricted to well-known crop plants and their allies, where the genetic relationships among the taxa have been extensively studied and the make-up of the genepool is relatively well understood.

The Leguminosae traditionally has been divided into three major groups of very unequal size on the basis of major morphological characters, particularly floral ones. There is still no general agreement regarding the treatment of the major divisions which are sometimes recognized as separate families—Caesalpiniaceae, Mimosaceae and Fabaceae (Papilionaceae) or as subfamilies of a single family, Caesalpinioideae, Mimosoideae and Lotoideae (Papilionoideae/ Faboideae/ Papilionatae). Actually, at a very early stage of taxonomy the concept of a group of papilionaceous legumes with trifoliate leaves and twining stems had developed. The seed characteristics also support the concept of Leguminosae as one family and De Candolle (1825) advocated it. Hutchinson (1926 and 1964) treated these three subfamilies as three distinct families. Taubert (1894) retained the tripartite division of Leguminosae family into three sub-families; however, he removed the Swartzieae

from the subfamily Papilionoideae. This tripartite division of the family Leguminosae has remained stable and is more acceptable by modern taxonomists.

According to Bentham and Hooker (1883), the three sub-families are Caesalpinioideae (152 genera, 2800 species), Mimosoideae (62 genera, 2800 species) and Papilionoideae (440 genera, 12000 species). The sub-family Papilionoideae is the largest of the three sub-families with about two-third of all the genera and species of the family. The species are widely distributed from rain forest to the edges of dry and cold deserts. Hutchinson and Dalziel (1958) divided the sub-family into nine tribes but Giller and Wilson (1991) divided it into 32 tribes. Some important tribes are Dalbergieae, Tephrosieae, Indigoferae, Desmodieae, **Phaseoleae**, Aeschynomeneae, Cicerieae, Crotalarieae and Genisteae.

Baker's (1876-78) classification is used commonly for the subtribal classification of the tribe Phaseolae, though there have been many other systems, both before and after his work. The sub-tribal classification of the large tribe Phaseolae has been the subject of debate. It was not until the work of De Candolle (1825) that a rational classification of the tribe was presented. A more mature synthesis quickly followed with the work of Bentham (1837), in which most of the present subtribes were outlined, and in the *Genera Plantarum* (1865), which is his final view of the tribe. Later work by Taubert (1894), Harms (1915, 1921), Piper (1925, 1926) and Hutchinson (1964) added new genera and modified minor intra-tribal concepts. The Phaseoleae contains the largest number of genera and comprises the most economically important legumes. Aside from the most widely cultivated legumes which the common man refers to as beans, viz. *Phaseolus* spp., *Vigna* spp. and *Glycine max*, a substantial portion of the remaining genera in the tribe contain

members of considerable importance as food, fodder and ornamental plants. Lackey (1981) divided this tribe into eight sub-tribes. (1) *Cajaninae* (2) ***Phaseolinae*** (3) *Clitoriinae* (4) *Ophrestiinae* (5) *Kennediinae* (6) *Erythrinae* (7) *Diocleinae* and (8) *Glycininae*.

The bulk of the Phaseoleae, including the *Diocleinae*, *Phaseolinae*, more remotely the *Glycininae*, and probably the *Kennediinae*, form one large natural group. The *Phaseolinae* seem to be specialised derivatives from *Diocleinae*-like ancestors. Linnaeus (1753) described 23 species, some of which are important crop plants, like *Dolichos* and *Phaseolus*. His genera *Phaseolus*, *Dolichos* and *Glycine* are more heterogeneous than might have been expected, even at that era.

1.3. Origin and evolution

It is used to be thought that legumes evolved, probably in the humid tropics, in the late Cretaceous. The exact time was difficult to pinpoint because of the paucity and uncertain affinities of many fossils. Recent molecular studies have helped to improve this situation. Lavin *et al.* (2005) carried out an extensive molecular analysis and correlated them with fossil characters and produced an over all chronogram for the family and related this to phylogeny. The general conclusion was that legumes probably evolved approx. 60 million years ago (Mya), early in the Tertiary period. The fossil record of the legumes is not particularly rich until about 35 to 54 Mya (Eocene or mid-tertiary), when papilionoid and mimosoid legumes become abundant and diverse in both North American and European fossil floras (Taylor, 1990; Herendeen *et al.*, 1992). They were called a "Western Gondwanan" family, and often were cited as an example of the biotic connection that existed between Africa and South America during the Cretaceous (65-145 mya), when these continents were in

close proximity. Advances in our understanding of continental drift and the availability of more precise phylogenies for legumes have not supported the Gondwanan hypothesis, and most recent biogeographic studies have concentrated on Eocene (35-55 mya) or later events to explain legume distributions. Thus, an alternative general explanation for the distribution of legumes hypothesizes that continental breakup and progressively cooler climates since the Eocene have led to the patterns of distribution we see today. Under this model, any archaic African taxa are interpreted as relicts of the once widespread boreotropical forest that have managed to survive the increased aridity and cooler temperatures that led to their extinction in the northern hemisphere (Lavin *et al.*, 2000).

1.4. Diversity, distribution and phylogeny of Phaseolinae

A large number of species of *Vigna*, *Phaseolus*, *Psophocarpus*, etc. are found in the wild in India or are cultivated. The Asiatic *Vigna* species are adapted to a diverse range of agroclimatic conditions. *Vigna aconitifolia* is undoubtedly the most drought resistant, and is particularly common in semiarid to arid regions, especially in the north-western desert region of the Indo-Pakistan subcontinent. It is said to be cultivated sporadically in Sri Lanka, Burma, southern China and Malaysia, but it seems that even in these countries it is mostly grown in relatively dry habitats. It is most common in drylands with poor sandy soils which hold little water. In contrast, *Vigna umbellata* is adapted to humid, subtropical to temperate regions. In India, *Vigna umbellata* is cultivated largely in the humid north-eastern parts and it occurs wild from Kerala to the Himalayas, ascending to 1500 m in the Khasi hills, and onwards to central China through Malaysia. Wild *Vigna trilobata* is found from the Himalayas to Sri Lanka, and also in Burma, Malaysia, China, Pakistan, Afghanistan

and Ethiopia, but it is cultivated only in India, as a cover crop and for fodder. The tribal people of India eat seeds gathered from wild plants.

Vigna radiata is the most widely cultivated as it is relatively drought tolerant and well adapted to a range of soil conditions including light soils and limited irrigation. The black gram (*Vigna radiata*) is considered to have been domesticated in India from its wild ancestral form *Vigna mungo* var. *silvestris*. It is widely cultivated throughout the southern half of Asia, especially India, Pakistan, Bangladesh, Sri Lanka, Thailand, Laos, Cambodia and Vietnam. *Vigna mungo*, on the other hand, requires wetter conditions and thrives in relatively heavier soils. While *Vigna radiata*, *Vigna mungo* and *Vigna aconitifolia* can grow well in the temperature range of 20°C to 45°C, *Vigna mungo* tolerates cooler temperatures, and *Vigna aconitifolia* warmer ones. Natural distribution of *Vigna mungo* var. *silvestris* ranges from India to Myanmar (Tateishi, 1996).

Rice bean (*Vigna umbellata* var. *umbellata*) is considered to have been domesticated in Southeast Asia. Wild forms of rice bean are distributed in Northeastern India, Burma, Thailand, Laos and Vietnam (Ohashi *et al.*, 1988). Moth bean (*Vigna aconitifolia*) is an important underutilized pulse crop having high degree of adaptation towards harsh and hostile soils, atmosphere and poor input situations. Moth bean is considered to have been domesticated in India, Pakistan, Myanmar or Ceylon (Purseglove, 1974., Marechal *et al.*, 1978). Indian sub-continent is the place of origin of this crop. Appreciable amount of variation exists in the traditional growing areas among different varieties and landraces. Cowpeas (*Vigna unguiculata*) are most important as pod vegetables and for fodder.

Common bean (*Phaseolus vulgaris*) is an ancient pulse crop originating in Central and South America and a crop that is adapted to many niches, both in agronomic and consumer preference. They are found from sea level up to 3000 MSL and are cultivated in monoculture, in associations, or in rotations. The centre of origin of *Psophocarpus tetragonolobus* is not yet known but, on the basis of linguistic evidence, Burkill (1995) postulated that Arab traders brought it to the East from the African side of the Indian Ocean. Vavilov (1951), however, included winged bean in his Indian centre of origin, while Hymowitz and Boyd (1977) proposed Papua New Guinea as the major distribution centre on the basis of its long history of cultivation in the highlands. Similarly, *Centrosema* is a well known genus endemic to the American tropics and subtropics, with a few species naturalised elsewhere in tropical climates.

Phaseolinae show palynological and morphological characters that make its taxonomic position uncertain. Due to difference of opinion, the inclusion of particular genus under a tribe or splitting of one genus into several genera/sub-genera and elevation of the rank of a lower taxa to higher category remain controversial. Despite the attention the group has received, consistent and well-resolved relationships among its members have proven difficult. Several phytochemical and morphological studies have attempted to improve the generic delimitation (Marechal *et al.*, 1978; Zalocchi, 1992; Di Stilio, 1994; Drewes, 1995; Palacios and Hoc, 2001). Embryological investigations which are of great importance in addressing taxonomic problems are few (Johri *et al.*, 1992) and are restricted to a few species of *Phaseolus* (Brown, 1917; Weinstein, 1926; Desphande and Bhasin, 1974; George *et al.*, 1979) and *Vigna unguiculata* (Ojega and Samyolu, 1970). It is important to resolve relationships among these taxa to provide a framework for understanding the

evolution of organellar genomes in the group. Thus always there is a great debate on the inclusion or removal of genera within a sub-tribe. The major problem in this subtribe Phaseolinae, as described by number of workers, is the placement of *Vigna* and *Phaseolus*. The majority of *Vigna* species has been described and classified using a combination of morphological characteristics which overlap with the allied species of the genus *Phaseolus*.

No one queried the placing of the Asiatic beans in *Phaseolus* until Kurz (1876), when transferred an Indian species from *Phaseolus* to *Vigna*, for which he offered no explanation. These Asiatic plants, and some South American ones, were very closely related to several species of *Vigna*, and Wilczek transferred *P. radiatus* and *P. campestris* in 1954. One year earlier, Ohwi (1953) had separated many of the Asiatic species as a genus *Azuki*. *Phaseolus* was considered to be restricted to the more upland parts of America except for one pantropical, more or less littoral species, *P. adenanthus*. This clearly made much more phytogeographical sense. *Phaseolus adenanthus* has also been re-examined, it too can be referred to *Vigna* rather than to *Phaseolus*, which removes the last geographical anomaly. *Vigna* is probably a quite ancient pantropical genus, and *Phaseolus*, a more recently evolved genus restricted to the American cultivation. In an attempt to resolve the problem of the boundaries between *Phaseolus* and *Vigna*, Verdcourt (1970) took a monographic approach and considered merging *Vigna* with *Phaseolus*, or subdividing one or both genera into smaller genera to remove the difficult groups. However, he considered the former unsatisfactory as the type species of the two genera are so different, clearly representing distinct natural groups, while the latter answer, on the other hand, would result in too many small new genera. Thus, based on a combination of characters, including floral morphology, pollen grain sculpture and biochemical

evidence, he restricted *Phaseolus* to those American species with a tightly coiled style (as originally suggested by Bentham, 1865) and whose pollen grains lacked wide reticulation. Recently, several deletions and rearrangements of chloroplast sequences have been characterized in phaseoloid legumes which provided characters suggesting relationships among these taxa (Bruneau *et al.*, 1990; Doyle *et al.*, 1995; Bailey *et al.*, 1997).

The genus *Vigna*, together with the closely related genus *Phaseolus*, forms a complex taxonomic group. According to Verdcourt's (1970) proposal, mungbean and its relatives (which are now recognized as the subgenus *Ceratotropis*) were transferred to the genus *Vigna* from *Phaseolus*. Taxonomic treatment of black gram (*Vigna mungo*) and mungbean (*Vigna radiata*) has been confused by many. Verdcourt (1970) proposed that these two species should be treated as a single species. However, Marechal *et al.* (1978) considered these two as distinct species and this was supported by many taxonomists. Two botanical varieties were recognized in *V. mungo*: *V. mungo* var. *mungo* as the cultivated form (black gram) and var. *silvestris* as the wild ancestral form of black gram (Lukoki *et al.*, 1980). Cytoplasmic differentiation seems to be a major factor in speciation among the Asiatic *Vigna* species. However, *V. radiata* when crossed with *V. mungo* or the wild *V. sublobata* produces viable hybrids. Furthermore, recombinant types can be recovered in the backcrosses, F2 and successive generations from both sets of hybrids, which indicate a close relationship between *V. radiata* and the other two species.

While the origin of the different cultivated species is far from clear, the morphologically similar looking wild species and forms can be considered as their

possible progenitors. The relationship between *V. sublobata* (*Phaseolus sublobatus*) and *V. trilobata* is of particular importance. It seems most probable that *V. sublobata* is the ultimate ancestor to both *V. radiata* and *V. mungo*. It is so similar to *V. radiata* that some taxonomists have preferred to describe it as *V. radiata* var. *sublobata*, and the cultigen as *V. radiata* var. *radiata*. Sharma *et al.* (2006), using scanning electron microscopy, recognized two major seed-coat surface patterns in the *V. mungo-radiata-sublobata* complex. They found difference in seed coat surface characteristics and the quantity and form of wax deposition. They concluded that *V. sublobata* is not the likely progenitor of *V. radiata* but could instead be the putative ancestor of *V. mungo*. *Vigna umbellata* is similar in many morphological traits to *V. radiata*, and may be closely related. However, wild forms of *V. umbellata* have also been reported. *Vigna angularis* resembles *V. umbellata* in many ways, but is less similar to *V. radiata*; all three seem to form a single taxonomic complex.

Vigna aconitifolia, on the other hand, differs markedly from *V. radiata* and other species resembling it. So does *V. trilobata*, which appears to be closer to *V. aconitifolia* than any other *Vigna* species. As described above, confusion exists in species delimitation in the genus *Vigna* even today.

In *Dolichos*, the confusion arises not so much from intrinsic taxonomic difficulties as from man-made difficulties of nomenclature. *Dolichos* was a completely heterogeneous group from which most of the species described by Linnaeus were soon transferred. *Dolichos lablab* was usually considered an aberrant species, and Adanson (1763) separated it into a separate genus, *Lablab. Macroptilium*, in which the end of the style is curved like the handle of an umbrella and the petals have a characteristic shape, was established as a genus.

1.5: Molecular markers in classification and phylogeny

Earlier, the classification was solely based on morphological characters which exhibit a range of variation in response to environmental variables. Subsequently, biochemical and molecular markers were put to use for species delimitation and phylogenetic studies. Isozyme characterization can provide reliable characteristics to establish distinctness over and above morpho-agronomic characteristics (Mauria *et al.*, 2000). There are several leguminous taxa in the plant kingdom where there is always controversy regarding their rank and systematic position.

Biochemical markers like proteins, isozymes, terpenes, flavonoids, etc may prove useful, but proteins and isozymes have been widely used. The multiple forms, distinct chemical properties and developmental differences in plant enzymes have led the researchers to use it as a tool for plant systematic studies. Detection of genetic diversity in plant population using protein and isozyme markers has revolutionized the field of experimental and evolutionary plant genetics. Isozyme markers might be useful in detection of ecological races, lines, evolutionary relationship among taxonomical categories and other evolutionary problems at populationary level (Turner, 1969). Protein and isozyme analyses provide an efficient approach for directly comparing the magnitude and distribution of genetic diversity between different populations and species. These are also useful for identification of species, cultivars, variety and their purity test, determination of population structure and evolutionary potential of a species and discrimination between species and their inter specific hybrids (Desborough & Peloquin, 1966; 1968; 1971; Weir, 1990; Puecher *et al.*, 1996; Hahn & Schoberlein, 1999; Gao & Hong, 2000; Kumar *et al.*, 2001; etc.).

Molecular markers are more costly than biochemical markers but they provide better accuracy and expanded scope of biosystematics inference and facilitate statistical analysis of phylogenetic trees. DNA based molecular marker techniques have been proven to be powerful in genetic diversity estimations. With the introduction of Polymerase Chain Reaction (PCR) based techniques, a multitude of tools became available. These recently developed multi-loci marker techniques, which include Randomly Amplified Polymorphic DNA (RAPD), Arbitrary Primed (AP) PCR, Amplified Fragment Length Polymorphism (AFLP), Microsatellite (SSR), DNA Amplification Fingerprinting (DAF) and Inter Simple Sequence Repeat (ISSR) is playing increasingly important roles in this type of research. The simplicity and cost effectiveness of RAPD and the number and speed of markers generated by this has encouraged the application of RAPD markers to many types of biodiversity-associated problems and attracted many researchers, particularly those interested in either genetic fingerprinting or the patterns and levels of genetic diversity; since 1990. RAPD has been widely used for the evaluation of taxonomic position of family, genus and species, analysis of genetic diversity and phylogenetic relationships within and among related genera, species and varieties, investigation of hybridization and introgression events, identification of cultivars and varieties, studying the population structure and clonal structure, etc. (Welsh *et al.*, 1991; Ratnaparkhe *et al.*, 1995; Gimenes *et al.*, 2000; Parentoni *et al.*, 2001). RAPD helps to indicate closeness of species and hybrids quickly and efficiently and thus help to predict the outcome of a cross based on genotypic information (Lim *et al.*, 1999). In addition to studies of genetic diversity there has been increasing number of publications concerning population genetics, phylogenetics, parentage analysis, phenotypic and molecular characterization of species (Lewis & Snow, 1992; Sullivan *et al.*, 1995; Nair *et al.*, 1999; Khan *et al.*, 2000; Kafkas & Perl-Treves, 2001; 2002).

Considering the potentials of the DNA marker-based genetic diversity analysis, the present study is aimed at evaluating the usefulness of molecular markers in assessing and analysing the nature and the extent of genetic diversity among the members of the subtribe Phaseolinae collected from different geographical areas of Eastern India and to derive phylogenetic relationship among different constituent taxa of the group.