To ascertain the degree of intrageneric similarities in plants, studies on physicochemical characterization of DNA have been carried out in species of *Lathyrus* (1-3), *Vicia* (4), *Lolium* (1), *Triticum* (5), *Anemone* (6) Cichoreae of compositae (7) and *Allium* (8) while the seed proteins have been compared in case of genera like *Phaseolus* (9-12), *Cicer* (13), *Pisum* (14) and *Vigna* (15-18). In the present thesis, I have used an integrated approach of studying DNA characteristics as well as seed storage globulins in four species of *Vigna* and pigeonpea. To our knowledge, such a combined approach involving simultaneous comparison of DNA as well as proteins has not been used before at least in plants.

Although results in individual chapters have already been discussed, I wish to state here some general trends that emerge when all the results are considered together.

The four *Vigna* species show enough homology/relatedness to be considered as species of the same genus *Vigna*

The DNAs of the four *Vigna* species show similar base composition with G + C contents in the range of 30-35%. This similarity in base composition is further evident when HPLC and restriction endonuclease cleavage patterns of their DNAs are compared. The four species show similar HPLC elution
profiles with two unidentifiable peaks, at least one of which may correspond to methylated adenine. The presence of the latter modified base has been inferred from a comparison of the digestion patterns of all the Vigna DNAs with MboI and Sau3AI. Sau3AI digests all Vigna DNAs to a much greater extent than MboI and as discussed in chapter V, these data indicate the presence of methylated adenine.

A comparison of DNA reassociation kinetics further reveals additional similarities in the four Vigna plants. The DNA reassociation data for all the four plants can be modelled into a two-component system only, where in one component is of repetitive DNA sequences and the other of unique/single copy DNA sequences. The proportion, rate constant and reiteration frequencies of the repetitive DNAs of three of the four Vigna plants are similar with only cowpea showing a very high frequency of repeats. Furthermore, the DNA-DNA hybridization studies have revealed that the total Vigna DNAs show greater than 50% homology to cowpea total DNA (upto Cot 1.0 x 10\(^{-3}\) M.s.) and that the repetitive DNAs (upto Cot 1.0 x 10\(^{-1}\) M.s.) are even more homologous to cowpea repetitive DNA (homology as high as 83%).

A comparison of the total seed globulins by SDS-PAGE reveals that the abundant subunits of molecular weights ranging from 43-68 kilodaltons as well as 18-20 kilodaltons are present in all of
these *Vigna* plants. Finally, when reactivity of these globulins to the antibodies raised against cowpea globulins is considered, it is found that all of the other three *Vigna* globulins react substantially with the former (anti-cowpea globulin antibodies). All the above data clearly suggest the overall relatedness of seed globulins in these plants.

Earlier, it was shown that the Asiatic *Vigna* species (previously classified as Asiatic *Phaseolus* species) could be distinguished as separate from true *Phaseolus* species like *Phaseolus vulgaris* (19). These studies were, however, rather isolated. Beridze (20,21) for example, showed that the DNAs of Asiatic *Phaseolus* species (now *Vigna* species) exhibited either no distinct satellite or only a very small proportion of satellite (5-15%) while a true *Phaseolus* species (*P. vulgaris*) had as much as 30% satellite DNA. Similarly Kloz, Klozova and co-workers (9-12) have shown initially using antibodies against the seed protein of *P. vulgaris* that cowpea, mothbean, mungbean and urdbean are less closely related to *P. vulgaris*. In a subsequent report (18) they have shown that the plants like mothbean, mungbean and urdbean are more closely related to cowpea while Frenchbean (*P. vulgaris*) exhibits a poor relationship to cowpea. In this latter experiment, they had used antibodies raised against seed proteins of cowpea (18).

The molecular data obtained in the present studies (especially in case of Asiatic *Vigna* species, mothbean, mungbean
and urdbean) thus substantiate their similarities to cowpea and reaffirm their taxonomic affinities to the genus *Vigna*.

Despite overall similarities, cowpea can be distinguished from the other three *Vigna* species by virtue of some striking features of its DNA.

By using techniques like high resolution thermal denaturation of DNA, equilibrium centrifugation in CsCl and DNA reassociation kinetics, it has also been possible to locate or identify specific differences among seemingly related species. Of the four *Vigna* species, cowpea DNA exhibits a prominent heavy satellite DNA component in neutral CsCl. The analyses of its DNA reassociation kinetics has revealed that the total repetitive DNA has a frequency of reiteration of 13490 as against 192-826 in the other three *Vigna* repetitive DNAs. Since satellite DNA is generally assumed to consist of highly repeated DNA sequences, the high frequency of repetition of cowpea repetitive DNA may be due to the presence of a heavy satellite DNA. Furthermore, repetitive and total DNA homology studies (by DNA-DNA hybridization) as well as seed globulin comparisons with reference to cowpea reveal that cowpea on one hand and the three *Vigna* species on the other constitute two groups of varying relatedness (especially so in case of seed globulin reactivities to anticowpea globulin antibodies).

The above molecular differences between cowpea (a true *Vigna* species) and the other three *Vigna* species (reclassified)
can be attributed to their gene pools and centres of origin and domestication (19, 22-24). Thus while cowpea has an African gene pool and centre of origin and domestication, the other three *Vigna* species, namely, mothbean, mungbean and urdbean are of Asiatic (particularly Indian subcontinent and South East Asia) origin and domestication with the gene pools also being confined to the same regions. As a result of this, amongst the three Asiatic *Vigna* species, limited gene flow was/is possible as shown by interspecies hybridization, while cowpea could only be crossed with its immediate wild ancestor *Vigna dekindtiana*. Such a distinction of the species suggests that the two groups (African and Asiatic *Vigna* species) have apparently domesticated/evolved at rates and to an extent that are different from each other. Furthermore, since neither natural nor artificial gene flow/mixing was possible between the two, their rate and extent of domestication/evolution becomes even more significantly different. However, given the present day status of these cultivated plants as crops, it is clear that they are not still truly domesticated like the cereals (especially wheat and corn) and this suggests that a good possibility now exists to attempt a genetic exchange between the two groups. This is especially of importance in view of the fact that the gene pool resources in these plants, as of present, are poor enough to prohibit further man-directed evolution of these crop plants. Moreover, since the seeds
of *Vigna* species in general have either no or very low proportions of antinutritional factors like cyanogenic factors, protease inhibitors and hemagglutination factors as compared to the other cultivated legumes like pea, kidneybeans/French bean and soybean, a well established gene flow system for the *Vigna* species gains tremendous importance in pulse/legume breeding and improvement programmes.

**Perspectives**

During the period of last six years, tremendous technological progress has been observed in the field of molecular biology. Specific genes are cloned sequenced and are used as probes for exploring homologies in related organisms. This kind of work, however, could not be undertaken in India due to severe constraints in the availability of restriction enzymes, radioactive isotopes and other fine chemicals. It is only since last two years, that these constraints are being gradually eliminated and a few laboratories have been able to initiate work in recombinant DNA technology.

In the present studies in four *Vigna* species we have been able to identify specific molecular parameters that have been affected by differences in gene pool and centre of origin and domestication. Using cloned DNA sequences, it would be possible to examine the above species in greater depth. Thus, the cloned repetitive DNA sequences from cowpea can be used
to probe the evolutionary fate of similar repeats in other Vigna species so as to identify more precisely the molecular basis/effects of a separate gene pool of cowpea and that of other Vigna species. The extent and rate of 'turnover' in these species can also be estimated from homology studies of cloned repetitive DNAs. Furthermore, use of cloned non-repetitive sequences as well as specific genes can illustrate the extent of nucleotide sequence divergence. Such data can go a long way in establishing the molecular basis/effects of speciation in these Vigna species. Given the fact that some of the cultivated Vigna species are known to mankind since ancient times and also the observation that Africa and Indian subcontinent/S.E.Asia have been the two major centres of origin of the cultivated Vigna species, it is clear that these species have evolved by diverging from each other (24) and thus, do not have any gene flow between them (19). Therefore, an indepth study of cloned sequences in these Vigna groups can establish possible routes of gene flow amongst them. It is this aspect, of application of recombinant DNA technology to Vigna species which can play an important role in identifying the useful germplasm for subsequent plant improvement work.
REFERENCES


