CHAPTER V

DISCUSSION

The cereals mainly furnish the carbohydrates while, the grain legumes comprise an important source of proteins in human diet. The pulses are the major sources of proteins in many of the developing countries like India, Pakistan, Bangladesh, Nepal and Srilanka. The lower production of grain legumes/pulses has led to problems of protein malnutrition in many countries.

World agriculture has been successful in the past century in meeting the demand for cereals that are more researched as compared to legumes. To increase future agricultural productivity, it will be necessary to use a broader range of the plant genetic diversity, particularly of legumes and minor crops genetic resources.

Genetic diversity signifies the diversity of the sets of genes carried by different genotypes of a species. In interaction with environment they dictate the patterns of variation and they are the raw materials of evolution. The scope of plant genetic improvement through the manipulation of available genetic variability equally believed by all plant scientists. The importance of genetic diversity in plant breeding is obvious from the results obtained in different crops (Smart, 1990; Rabbani et al., 1998; Ghafoor et al., 2001; Upadhyaya et al., 2002; Upadhyaya, 2003).

In the present study, efforts have been made to determine the genetic diversity and phylogenetic relationship among the selected species of the genus *Vigna savi* which may prove
important in improving the economically important pulse crops by exploiting their wild relatives.

The systematic biochemical analysis has been considered worth utilizing in the present study for the comparative evaluation of the nutritional and antinutritional components as well as the genetic diversity by SDS-PAGE among the selected *Vigna* species which may ultimately prove helpful to increase future agricultural productivity of legumes.

**BIOCHEMICAL STUDIES:**

Now days seed storage proteins have been the subject of extensive investigations due to their economic importance as major protein source for humans and their biochemical utility as a model system in study of genetic variability, characterization and expression by molecular techniques (Khandelwal, 1996). Special emphasis is given to seed proteins because a part of deficiency leading to malnutrition and under nourishment is related to insufficient protein supply.

Bhagya *et al.*, (2006) reported biochemical and protein quality evaluation of tender pods of wild legume *Canavalia cathartica* of coastal sand dunes. They noticed that TI activity was absent in *Canavalia cathartica* and cooking decreased the total phenolics, orthohydric phenols, tannins and phytohaemagglutination activity.

McCue and Shetty, (2002) reported the biochemical analysis of mung bean in which they found that the treatment of microbial polysaccharide stimulates phenolic content and enzyme activity. Salunkhe et al., (1982) reported the biochemical and biological significance of polyphenols in cereals and legumes.

Although legumes constitute one of the richest sources of protein, their utilization is limited due to the presence of antinutritional compounds such as phenolics, tannins, trypsin inhibitors, saponins and lectins (Liener, 1994).

Hence in view of the above, the present biochemical study has been carried out to determine protein profile which included genetic variability by Native and SDS PAGE as well as the indepth studies of antinutritional factors such as trypsin inhibitors, lectin content and polyphenol content among the selected *Vigna* species.

**Protein content:**

As grain legumes provide variety to the diet and contribute proteins, carbohydrates and other valuable proteins, they are regarded as the “health food” in the developing countries. Proteins are the major seed components in all grain legumes. Legumes occupy the second place after cereals as source of calories and protein in human diet (Vadivel and Janardhanan, 2000).

Many nutritionists have suggested partial replacement of animal food with legumes so as to improve the over all nutritional dietary status (Guillon and Champ, 1996).
Several attempts have been made to utilize and induce genetic variations for improvement in protein quality and quantity (Varughese and Swaminathan, 1966; Rao et al., 1975 and Satpute, 1994).

Gottschalk and Muller (1970) proposed that improvement of protein content and composition can be achieved by utilizing genetic variability. The amount and composition of seed proteins are widely influenced both by environmental and endogenous factors.

The present study showed variation in the protein content of selected *Vigna* species. There was an increase as well as decrease of protein content. The soluble seed protein content values have ranged from 42.63 mg/gm to 55.27 mg/gm among the selected *Vigna* species. The highest amount of soluble seed protein content (57.53 mg/gm) could be noticed in *Vigna unguiculata* (L.) Walp. The lowest soluble seed protein content (36 mg/gm) was shown by variety *Vigna mungo* (L.) Hepper-BDU 1.

In case of *Vigna mungo* varieties, the higher soluble seed protein content (38.20 mg/gm) was noticed in *Vigna mungo* (L.) Hepper - Tau 1 as compared to *Vigna mungo* (L.) Hepper - BDU 1 which revealed comparatively a lower soluble seed protein value (36.23 mg/gm).

The pertinent observations have conclusively indicated the scope for increasing the seed protein content through utilization of genetic variation among the selected *Vigna* species.
ANTINUTRITIONAL FACTORS:

The nutritional value and protein digestibility of legume plant parts would always be very poor unless subjected to cooking (Liener, 1962). This happens due to the presence of protease inhibitors which are the major antinutritional factors of edible legumes (Liener and Kakade, 1969 and George, 2006). In addition to this the legumes also comprise lectins, amylase inhibitors, tannins and polyphenolic compounds (Gaikwad, 2002; Khadke, 2005 and Bhalerao, 2009).

Like soybean, several antinutritional factors have been found in winged bean as well. Such factors include the trypsin inhibitors (Kortt, 1979), the chymotrypsin inhibitors (Sohonie and Bhandarkar, 1954), the lectins (Renkonen, 1948).

Trypsin inhibitor content:

Protease inhibitors are widely distributed among many species in several plant families, particularly legumes (Liener and Kakade, 1980). Proteinase inhibitor proteins are present in almost all life forms as component of cytoplasm, secretions and intercellular fluids (Laskowski and Kato, 1980). These inhibitors have been proposed to function as storage proteins, regulators of endogenous protease and as factors that protect plants from insects and pathogen attack (Liener and Kakade, 1980; Ryan, 1990).

Since early 1940’s, the plant inhibitors have been extensively studied as antinutritional factors due to the potential adverse effects they produce on human and animal intestinal tract

The legumes have provided large amount of proteinase in human diet, however, their use as sole source of proteins has been subdued due to the presence of antinutritional factors, in particular, the proteinase inhibitors. Nevertheless, there has been a remarkable improvement in the nutritional quality of legume food due to various treatment methods (Friedman and Guibman, 1986 and Rackis et al., 1986). The thermolabile nature of legume protease inhibitors has been known (Liener, 1969).


Very few reports are available in regard to trypsin and chymotrypsin inhibitor genes in plants. In legumes trypsin and chymotrypsin inhibitors have been studied from different plants like soybean (George, 2006), urd bean (Sagade, 2008), moth bean (Khadke, 2005) and winged bean (Dadke, 1999). The adverse dietary effects of protease inhibitors have been studied by many workers (Liener and Kakade, 1969; Gunn et al., 1980 and Higuchi et al., 1983). Presence of protease inhibitors in plants was
recognized by Read and Hass (1938). The systematic study of plant protease inhibitors was undertaken by Borchers and Ackerson (1947).

Tibe et al., (2007) reported the trypsin inhibitor (TI) activity and condensed tannin content in Bambara groundnut (*Vigna subterranea* (L.) Verdc) grown in Southern Africa. They found that the trypsin inhibitor activity of each selected landrace differed from country to country with no simple pattern revealed but the landraces from Namibia have shown the highest TI activity and those grown in Botswana had the least. The trypsin inhibitor activity reported in Bambara groundnut was found higher than in soybean and pigeon pea.

In the present study, the selected *Vigna* species have shown considerable variation regarding the trypsin inhibitor (TI) level. The trypsin inhibitor (TI) content values ranged from 51.40 to 455.80 TIU/ml/gm meal in selected *Vigna* species.

The highest TI content (455.80 TIU/ml/gm meal) was recorded in *Vigna aconitifolia* (Jacq.) Marechal, while the *Vigna mungo* (L.) Hepper - BDU 1 revealed the lowest TI content (51.40 TIU/ml/gm meal).

Among the selected *Vigna radiata* varieties, the highest TI content value (297.80 TIU/ml/gm meal) was observed in *Vigna radiata* (L.) Wilczek- BM 2002-01 whereas the lowest TI content value (94.73 TIU/ml/gm meal) was shown by *Vigna radiata* (L.) Wilczek- NVL 1.

In case of two *Vigna mungo* varieties, the lower TI content value (51.40 TIU/ml/gm meal) could be seen in *Vigna mungo* (L.)
Hepper - BDU 1, as compared to Vigna mungo (L.) Hepper -Tau 1 which has shown the higher TI content value (264.63 TIU/ml/gm meal).

Cheung et al., (2009) reported the inhibitory activity of the trypsin inhibitors getting attenuated in the presence of reducing agent dithiothreitol in Vigna mungo.

The Vigna species carrying lowered levels of trypsin inhibitors are likely to assume significant importance and immense economic value especially in regard to their nutritional potential. The detailed understanding of the genetics of inhibitors and other antinutritional components present in Vigna species would be immensely helpful to the breeders in planning their breeding programmes directed towards qualitative improvement.

**Lectin content:**

Lectins are the carbohydrate binding, cell-agglutinating proteins of non immune origin (Lis and Sharon, 1981). The presence of lectins may have an adverse effect on the nutritional quality of raw legume seeds (Liener, 1976). Bender, (1983) reported the lectins in bean and their side effects on human being.

According to some researchers like Janzen et al., (1976) and Gatehouse et al., (1995), the seed lectins have a protective role against various predators. Similar types of results have been reported earlier by Alexander and Caldwell (1987) in tubers of Winged bean.

Besides seeds, the lectins have been found in all kinds of vegetative tissues of plants (Lis and Sharon, 2004). The toxic
effect of lectins on animals and human beings was proved by using purified lectins from various sources like Ricin, the toxic lectin from castor bean seeds and root lectins from Adenan-
volkensii roots (Goldstein and hayes, 1978). Two acidic lectins viz., WBAI and WBAII were reported from Winged bean seeds (Mitra et al., 2002 and Manoj et al., 1999).

Studies revealed that the lectin account for about 25% growth inhibition in both animals and man (Liener, 1986 and 1994). Lajolo and Genovese, (2002) reported nutritional significance of lectins and enzyme inhibitors from legumes.

Ayyagari et al., (2003) reported the lectins, trypsin inhibitors and tannins in some legumes and cereals and the effects of processing and they revealed that lectin content of different cultivars of Lathyrus sativus showed significant variation whereas tannin, trypsin inhibitor and total protein content did not vary much.

In the present investigation, the specific activity values were ranging from 23.04 to 1085.44 HAU/mg protein. It was observed that Vigna mungo (L.) Hepper - BDU 1 revealed lowest (23.04 HAU/mg protein) where as Vigna aconitifolia (Jacq.) Marechal demonstrated the highest (1085.44 HAU/mg protein) specific activity.

From the above results it is concluded that reduction in lectin level is possible in Vigna species by utilizing genetic variability and genetic manipulation. Further investigations and comprehensive studies are required for testing seeds of low lectin
content for animal feeding and their subsequent industrial/food utility aspect.

**Polyphenol content:**

The polyphenols inhibit the digestive enzymes and interfere with the biological value of grains, but the phenolic compounds are beneficial to plants against bird depredation, insect attack and diseases caused by fungi, bacteria and viruses.

Tannin precipitate protein and reduce the food protein quality (Tan *et al*., 1983 and Cabrera and Martin, 1986), hence low tannin plant type is required for the nutritional improvement of crop plants.

Polyphenols in cereals and legumes have been receiving considerable attention largely because of their adverse influence on color, flavor, and nutritional quality. These compounds belong to the flavonoid and tannin groups and are mostly located in the seed coat or pericarp of the grains and the treatment of alkaline reagents and ammonia can remove 90% of polyphenols (Salunkhe *et al*., 1982).

The adverse dietary effects of polyphenol have been studied by many workers (Klu *et al*., 1997; Singh, 1984 and Azra *et al*., 2008).

Tannins are the polyphenolic compounds which lead to decreased nutritional quality of food hence low tannin varieties are supposed to improve the nutritional quality of the pertinent plant product. The heat-stability inhibits the lowering of phenolics
hence genetic alteration for reduction in phenolics is considered necessary.

When many animals consumed diets rich in tannins, decreased growth could be invariably recorded (Mole et al., 1993 and Zucker, 1983). The polyphenols also lower protein as well as starch digestibility and hinder mineral adsorption from the diet in different systems (Deshpande and Salunkhe, 1982 and Thompson and Yoon, 1984).

Barroga et al., (1985) reported polyphenols in mung bean (Vigna radiata (L.) Wilczek) and they found that polyphenols in mung bean had low protein precipitating capacity.

In the present study, the selected Vigna species were evaluated for polyphenol content according to Folin-Denis procedure (Swain and Hills, 1959). The polyphenol content values were ranging from 405.83 to 915.20 mg /100 gm seed meal. It was observed that Vigna aconitifolia (Jacq.) Marechal carried lowest (405.83 mg /100 gm seed meal), where as Vigna radiata (L.) Wilczek- BM 4 has shown the highest (915.20 mg /100 gm seed meal) polyphenol content.

The other selected Vigna species such as Vigna umbellata (Thunb.) Ohwi and Ohashi and Vigna unguiculata (L.) Walp. also demonstrated significant variability regarding polyphenol content.
ELECTROPHORESIS (Native and SDS-PAGE):

The seeds are the storehouse of different biomolecules, especially the proteins which are of much importance. The high protein content of seeds especially in legumes, has offered scope to the geneticists for undertaking detailed biochemical characterization of that material and understanding the genetic control involved in their synthesis.

Now-a-days the highly versatile polyacrylamide gel electrophoresis technique has become the choice of priority for the biochemical researchers for making its use in characterizing the proteins (Khadke, 2005).

Seed storage proteins are the products of gene expression with genetic stability and are not affected by environment. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) technology is widely used to detect the seed polypeptides and to study plant taxonomy, affinities and genetic diversity.

Seed protein patterns obtained by electrophoresis have been successfully used to resolve the taxonomic and evolutionary problems of crop species (Khan 1992; Rao et al., 1992).

Genetic variants showing differences in composition of seed proteins have been reported in many species and genera of higher plants (Colloda et al., 1991 and Ignacimuthu and Arockiadass, 1993).

Siriamornpun et al., (2010) reported protein fractionation of cowpea (Vigna unguiculat (L.)Walp.) by electrophoresis. They
found that seed tissues were most suitable for varietal identification by electrophoresis.

Ghafoor and Ahmad (2005) reported the variation in storage protein banding pattern by SDS-PAGE, that exists the selected black gram species, but the magnitude found was low, because out of 105 genotypes tested for SDS-PAGE, only 46 exhibited variation which was about 44 % of the total genotypes under investigation.

The similarity in the seed protein profiles of wild and cultivar species has been recorded in wheat (Johanson, 1967), *Glycine max* (Miles and Hymowitz, 1973) and chickpea (Ladizinisky and Adler, 1975).

The SDS-PAGE polypeptide profiles have been studied in different legumes like *Vigna* and its cultivars (Chanyou *et al.*, 2006), winged bean (Dadke, 1990 and Bhalerao, 2010), soybean (George, 2006) and different other grain legumes (Valizaden, 2001).

Duke and Glassman (1968), while working on *Drosophila* proposed that electrophoretic mobility of xanthine dehydrogenase isozymes tended to reduce as the species became advanced. This relationship was further confirmed in wheat by Siddiq (1972).

Sharma and Maloo (2006) observed all types of protein bands i.e. low, medium and high intensity in various Soybean varieties. The polymorphism in protein banding pattern has been obtained by Nayeem *et al.*, (1999) in wheat mutant ‘sharbati’ and Kumar *et al.*, (2003) in *Trifolium*. 
Seed protein patterns obtained by electrophoresis have been successfully used as a promising tool for distinguishing cultivars of particular crop species (Ferguson and Grabe, 1986; Moller and Spoor, 1993 and Jha and Ohri, 1996).

Ahmad and Slinkard (1992) reported phylogenetic relationship among *Cicer* species based on SDS-PAGE data and suggested *Cicer reticulatum* as the wild progenitor of cultivated chickpea.

Moller and Spoor (1993) used SDS-PAGE for discrimination and identification of *Lolium* species and reported differences in the resulting seed protein banding patterns for identification.

Gantait *et al.*, (2009) reported the evaluation of genetic diversity among the *Vigna mungo* (L.) Hepper genotypes by SDS-PAGE.

In the present investigation, protein profiles and polypeptide profiles of selected *Vigna* species have been analyzed on Native and SDS-PAGE to understand genetic variability in *Vigna* species. The selected *Vigna* species have exhibited significant variability in their protein profiles.

The relative mobility (REM) values of the protein profiles indicated that the last band travelled upto 3/4\(^\text{th}\) of the gel even on 10% PAGE. The better resolution coupled with band sharpness was observable only with 12% PAGE.

The protein polymorphism of selected *Vigna* species revealed a wide variability with respect to the number and mobility of bands. A total of 130 bands were resolved on the
native gel among selected *Vigna* species which were categorized into 42 polypeptide bands. The REM values of these 42 bands ranged from 0.04 to 0.69. The selected germplasm differed with regards to the presence of various protein bands.

The *Vigna* species selected for the analysis by SDS-PAGE exhibited a considerable genetic variance in the analyses of total germplasm and hence the results obtained by this study could be of broader spectrum.

SDS denatured protein gels could resolve a total of 62 bands in selected *Vigna* species, which were grouped as 33 distinct SDS protein bands. These SDS protein bands belonged to different molecular weights ranging from 17 KDa to 97 KDa. The relative mobility of these bands varied from 0.06 to 0.80 among the selected *Vigna* species. Low, medium and high mobility bands were observed in all the cases.

*Vigna mungo* (L.) Hepper - Tau 1 exhibited maximum number of bands i.e. all the 9 bands were visible, followed by *Vigna radiata* (L.) Wilczek- BMR 145 which showed 8 bands. A high molecular weight polypeptide band of medium to high intensity with REM 0.09 (MW 91.2 KDa) was unique among the six selected *Vigna* germplasm. *Vigna aconitifolia* (Jacq.) Marechal could be further differentiated from other germplasm by the presence of a strong protein band of highest molecular weight i.e. 97.7 KDa.

Variability of protein bands was well expressed in the entire gel. The presence or absence type of polymorphism of SDS
proteins was revealed in this study and the germplasm showed both homology and diversity in their banding pattern.

**Zymogram analysis based on Native and SDS-PAGE:**

Kouhsari et al., (2006) reported the zymogram analysis of six isoenzyme systems in some species of *Hyoscyamus*. They found that *Hyoscyamus tenuicaulis* was completely distinct from the other species.

Gantait et al., (2009) reported the zymogram analysis of selected *Vigna mungo* (L.) Hepper genotypes by SDS-PAGE. They revealed that clustering pattern of seed protein not reflected the geographical distribution of genotypes.

Biochemical analyses including seed protein profiling provide consistent results as they are less likely to be altered by the environment (Roy et al., 2001).

The seed proteins of selected *Vigna* species on Native PAGE exhibited the existence of 42 protein bands located in three zones A, B and C. Zone A representing the heaviest molecular weight protein was sub divisible into eighteen sharp and distinct bands. Similarly zone B representing mostly medium dark sharp bands, was sub divisible into twelve bands. The next zone C representing lighter bands with few faint bands was sub divisible into twelve bands. Thus, a total of 42 bands could be resolved in seed protein.

The two bands i.e. A₁ and B₆ were common in nine and eight genotypes under study, respectively. These bands can serve as a source of reference for inter-gel or inter-laboratory
comparison. Therefore, the dissimilar groups were mainly due to the absence or presence of bands.

Lot of variability was observed in polypeptide seed protein profiles of selected *Vigna* species by SDS PAGE. The seed proteins on SDS gels exhibited the existence of 33 protein bands located in four zones A, B, C and D. Zone A representing the heaviest molecular weight protein was sub divisible into six sharp and distinct bands. Similarly, zone B representing mostly sharp and dark bands was sub divisible into eleven bands. The next zone C representing dark to lighter bands with few faint bands was sub divisible into eight bands. The next zone D characterized by lighter to faint bands was sub divisible into eight bands.

Of the 3 bands, $A_2$ was common in six genotypes and $A_6$ and $D_6$ were common in five genotypes under study, respectively. These bands can serve as a source of reference for inter-gel or inter-laboratory comparison.

**Cluster analysis based on SDS-PAGE (Rooted and Unrooted dendrogram):**


Upadhyaya *et al.*, (2002) studied phenotypic diversity for morphological and agronomic characteristics in 1956 accessions
of chickpea core collection, comprising desi, kabuli and intermediate types.

Elizabeth et al., (2001) investigated 19 Sesbania accessions to characterize them on morphological and agronomic data using multivariate methods. Principal component analysis indicated that variance accumulated by the first two components for morphological and agronomic data was 74.4% and 77.0%, respectively.

Manivannam (2002) analyzed 33 mung bean genotypes derived from ten crosses to determine genetic diversity using multivariate analysis. The genotypes were grouped into seven clusters.

Upadhyaya, (2003) studied phenotypic diversity for morphological and agronomic characteristics in 1704 accessions of groundnut. Principal coordinate and principal component analyses showed that 12 morphological descriptors and 15 agronomic traits, respectively, were important in explaining multivariate polymorphism.

An UPGMA dendrogram (Rooted) based on selected Vigna species data was established. Thus, the dendrogram of total seed proteins based on distance coefficient revealed three distinct clusters cluster A, cluster B and cluster C.

The cluster A included 6 germplasms, cluster B included 4 germplasms and cluster C included 1 germplasm, as the most divergent genotype. The cluster A included four Vigna radiata varieties along with Vigna mungo (L.) Hepper–BDU 1 and Vigna aconitifolia (Jacq.) Marechal whereas the other three species of
*Vigna savi* got included in cluster B. The cluster A was divided into 2 sub-clusters, namely AI and AII.

An UPGMA dendrogram (Unrooted) based on selected *Vigna* species seed protein analyses data was constructed. Although each species contained different number of germplasm, it is convenient to do so through adjusting the program. Results showed that six species/varieties of *Vigna savi* had close affinities to form one cluster firstly than they clustered with other *Vigna savi* germplasm.

**Electrophoretic detection of TI:**

Trypsin isoinhibitors have been reported to be present in the seeds of leguminous plants (Belitz and Weder, 1986).

Mulimani and Paramjyothi (1993) reported the detection of protease inhibitors by the gel X-ray film contact print technique in redgram (*Cajanus cajan* L.).

The electrophoretic profiles of the trypsin inhibitors on X-ray film revealed 3 to 8 isoinhibitors in different species of *Vigna savi*. The maximum 8 trypsin isoinhibitor bands were observed in *Vigna umbellata* (Thunb.) Ohwi and Ohashi and minimum 3 isoinhibitor bands in *Vigna radiata* (L.) Wilczek AKM 8802.
MOLECULAR STUDIES:

Plant genomic DNA content:

The genomic DNA was isolated from fresh, young and disease-free leaves by using modified CTAB method (Doyle and Doyle, 1987).

Genome size variations have been confirmed in angiosperm (Bennett and Smith, 1991), especially in *Scilla bithynica* (Greilhuber, 1998) and *Eleusine floccifolia* (Hiremath and Salimath, 1991).

The quantification of extracted DNA was carried out by using Eppendorf make Biophotometer which directly gives the quantity of DNA present in the sample. DNA yield of selected species of *Vigna savi* demonstrated considerable variability ranging from 530-660 µg/gm tissues.

The highest DNA yield (660 µg/gm tissues) could be recorded in *Vigna radiata* (L.) Wilczek- NVL 1. While the lowest value for DNA content (530 µg/gm tissues) could be recorded in *Vigna unguiculata* (L.) Walp.

Inter simple sequence repeats (ISSR) marker studies:

The objectives of the present day plant breeding can be successfully achieved, if the conventional plant breeding is supplemented with molecular breeding approaches including both, the transgenic crops and marker-assisted selection methodology (Gupta and Roy, 2002).

Genetically modified crops like wheat, rice, cotton, maize, soybean and some other legumes are under cultivation for
improved traits. It has also become possible to clone the mutated genes that are responsible for a certain phenotype; this part of genetics is better known as forward genetics (Barr and Emmanuel, 1990).

Genetic markers are useful in screening germplasm in minimum time and labour (Nakajima et al., 1994).

George (2006) used the RAPD and ISSR molecular markers for the genetic diversity analysis of soybean, with reference to lectin content of seeds. She detected 69% DNA polymorphism through ISSR and 53.50% through RAPD.

RAPD markers have been used for assessing the genetic diversity among cultivars of several crops like cowpea (Mighouna et al., 1998), pea (Hoey et al., 1996), mung bean (Lakhanpal, 2000; Chittopadhyay, 2005) and Brassica juncea (Ali et al., 2007).

Ray et al., (2006) evaluated genetic stability of three economically important micropropagated banana (Musa spp.) cultivars as assessed by RAPD and ISSR markers. Among the two marker systems used, the ISSR fingerprinting revealed more polymorphism than the RAPD.

The genetic relationship among Turkish cultivars and breeding lines of Lens culinaris have been analyzed by Yuzbasioglu et al., (2006) They noted highest genetic distance of 58.30% in these lines.

Vural et al., (2007) concluded that ISSR technique is superior over RAPD for fingerprinting of chickpea genotypes and the two marker systems have shown clear differentiation of chickpea genotypes.

The present study, using ISSR was undertaken to characterize genetic variation in selected Vigna species. The selected Vigna species shown considerable polymorphic bands. Such types of genetic diversity were earlier reported by Ohri (1998) and Fana et al., (2004) in cowpea.

For ISSR analysis, nine ISSR primers were tested for DNA amplification initially. Among them, three ISSR primers revealed amplification for the present selected Vigna species. Each primer varied greatly for the ability to resolve variability among selected Vigna species. Mostly the primers generated several markers and were able to show a high level of genetic diversity.

**Cluster analysis based on ISSR markers:**

The data obtained by scoring the ISSR profiles with different primers individually as well as collectively were subjected to the construction of similarity matrix using Neighbour-joining tree construction method of Nei and Li/Dice. The similarity values were used for cluster analysis.

Ghafoor et al., (2001) studied genetic diversity in 484 black gram germplasm accessions. Quantitative traits were analyzed for cluster and principal component analyses.
Maqbool et al., (1997) reported phylogenetic relationship of 15 genotypes of the genus *Lens* and seven of their interspecific hybrids.

... Amplification of genomic DNA of ten genotypes using ISSR marker analysis yielded total amplified 304 fragments. ISSR analysis yielded 37 fragments that could be scored, of which 20 were polymorphic while the remaining were monomorphic in nature. In ISSR analysis number of amplified fragments ranged from 05 (UBC 819) to 17 (UBC 821) which varied in size from 125 bp to 1500 bp. Of the 37 amplified bands, 20 bands (54.05%) were polymorphic with an average of 6.66 polymorphic fragments per primer. The percentage of polymorphism ranged from 20% (primer UBC 819) to 88.23% (primer UBC 821). The primers based on poly (GT) produced maximum number of bands (17 bands) while poly (GT with TA at 3') produced minimum number of bands (5 bands). The PCR amplification using ISSR primers gave rise to reproducible amplification products. The complete data was based on a total of 304 bands.

Cross breeding between genetically different individuals are recommended, rather than involving individuals belonging to related genetic group (Rocha et al., 2002). The evaluation of genetic diversity and construction of linkage maps has been considered desirable for the efficient use of genetic variations in the breeding programme. ISSR analysis reported in the present work could be useful to select parents to be crossed for generating appropriate populations intended for both genome mapping and breeding purposes.
Through the above study, it was observed that *Vigna radiata* (L) Wilczek BM 4 showed most genetic variability compared to other selected *Vigna radiata* species, suggesting utilization of this species over others for breeding programme and in transferring the characters into *Vigna radiata* cultivars. Similarly, *Vigna mungo* (L) *Hepper* - BDU 1 showed genetic variability which is also suitable for breeding programme. The genetic divergence can provide visual idea about variability present in studied *Vigna* species in addition to assuring its continued genetic improvement prospects.