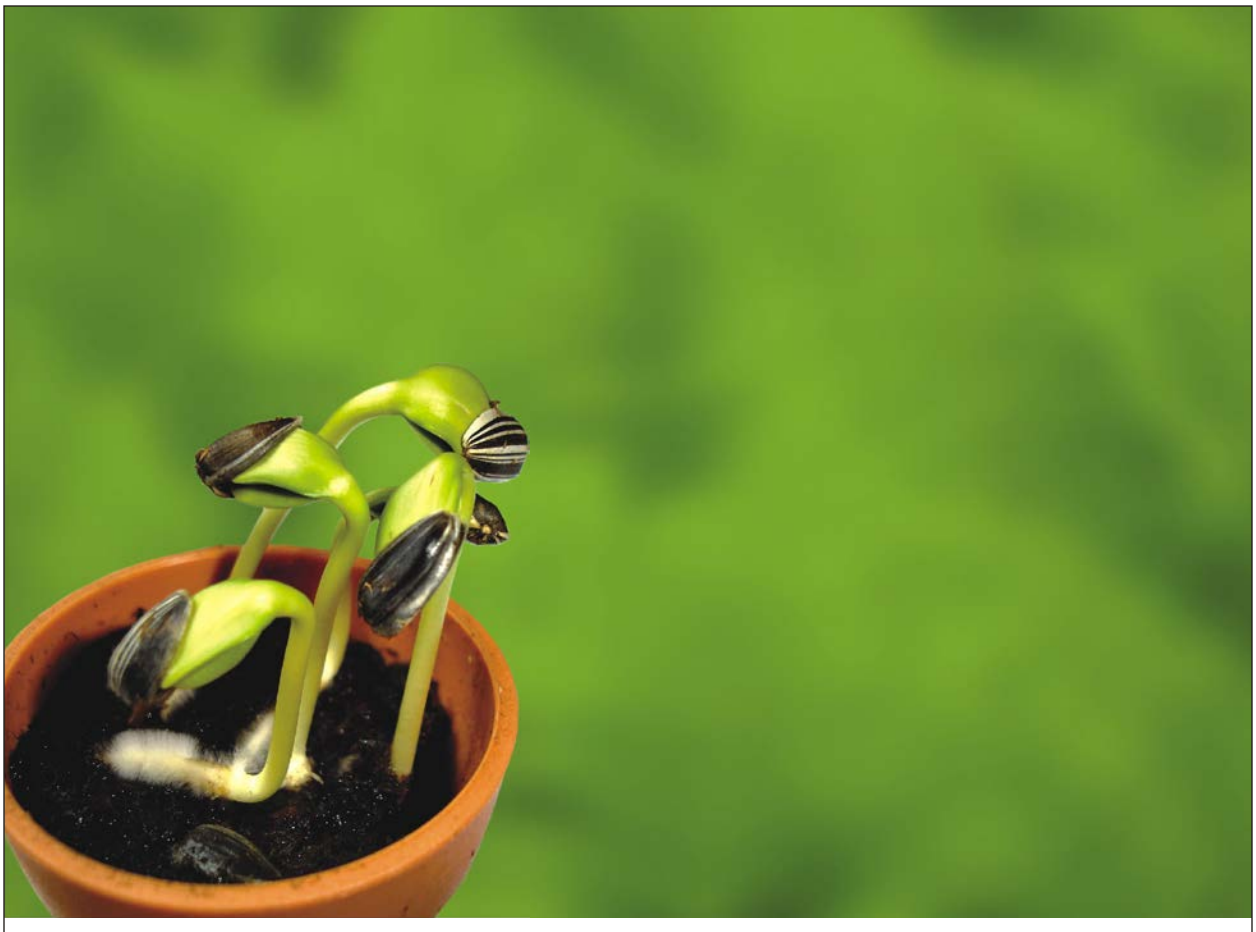

International Journal of Plant Breeding and Genetics

ISSN 1819-3595

Academic Journals Inc.

www.academicjournals.com

International Journal of Plant Breeding and Genetics 9 (4): 238-246, 2015
ISSN 1819-3595 / DOI: 10.3923/ijpbg.2015.238.246

Evaluation of Genetic Diversity in *Vigna radiata* (L.) Using Protein Profiling and Molecular Marker (RFLP)

Shahidul Islam, Javeed Iqbal Mir and Rajdeep Kudesia
Bundelkhand University, Jhansi, Uttar Pradesh, India

Corresponding Author: Javeed Iqbal Mir, Bundelkhand University, Jhansi, Uttar Pradesh, India

ABSTRACT

Seed storage protein profiling through SDS-PAGE and DNA Restriction Fragment Length Polymorphism (RFLPs) were surveyed in 5 accessions of mungbean (*Vigna radiata* L.) sampled from NBPGR Thrissur, Kerala, India, to evaluate genetic diversity. The protein profile showed a low level of polymorphism (26.66%). The similarity index calculated ranged from (42.30-50%). The dendrogram constructed revealed two clusters. Cluster 1st comprises two accessions IC 251432 and IC 251433. Cluster 2nd comprises 3 cultivars viz., IC 251431, IC 251430 and IC 251434. Cluster 2nd also revealed a 100% homology between IC 251431 and IC 251430 accessions. The RFLP analysis was carried out by single restriction enzyme *Eco*RI. The RFLP profile revealed a high level of polymorphism (90.90%). A total of 11 clear bands were scored. The only band with fragment size 630 kb was monomorphic while, the rest were polymorphic. Similarity index ranged from (42.82-11.11%). The UPGMA analysis divided the accessions into 2 clusters viz., cluster 1st comprising 4 accessions (IC 251431, IC 251432, IC 251433 and IC 251430). Cluster 2nd includes only one accession (IC 251434) occupying a distinct position in constructed dendrogram.

Key words: Genetic diversity, SDS-PAGE, restriction fragment length polymorphism, UPGMA dendrogram

INTRODUCTION

The genus *Vigna*, family Fabaceae (formerly Leguminosae), is a tropical plant and comprises of more than 150 species that are native to the warm regions of both old world and the new world (Faris, 1965). Mungbean has been considered to have been domesticated in India (Vavilov, 1926). The annual world production area of mungbean is about 5.5 million ha of which about 90% is in Asia (Lambrides and Godwin, 2007). India is the biggest producer of mung bean having about 2.99 million ha cultivated land (Singh and Ahlawat, 2005). Mungbean is a substantive source of dietary protein (24-28%) and carbohydrates (59-65%) on a dry weight basis and provides about 3400 kcal energy kg^{-1} of grain. The remainder is comprised of fat (1-1.5%), fibre (3.5-4.5%) and ash (4.5-5.5%) (Srivastava and Ali, 2004). To meet global mungbean demand, it is imperative to improve the current average global productivity (~400 kg ha^{-1}) as well as to expand the crop into new regions (Nair *et al*., 2012). Mungbean (*Vigna radiata* L. Wilczek) is diploid in nature with {2n = 2x = 22}. Mungbean and blackgram have small genome sizes estimated to be 0.60 pg $1C^{-1}$ (579 Mbp) and 0.59 pg $1C^{-1}$ (574 Mbp), respectively, which are similar to those of the other *Vigna* species (Arumuganathan and Earle, 1991).

Genetic diversity refers to any variation in nucleotides, genes, chromosomes or genomes of the organism. One practical application of knowledge of genetic diversity is in the design of populations

for genome mapping experiments (Kaga *et al*., 1996). Genotyping of different species is necessary for characterization of different accessions of crop germplasm, testing varietal purity and registration of newly developed cultivars (Chowdhury *et al*., 2002).

Among numerous techniques available for assessing the genetic variability and relatedness, seed storage protein analysis represents a valid alternative to varietal identification (Manella *et al.,* 1999). The SDS-PAGE have provided a promising tool to distinguish cultivars of a particular crop species (Jha and Ohri, 1996). It has been used successfully to resolve taxonomic and evolutionary problems of several plants (Rabbani *et al*., 2001). Analysis of SDS-PAGE are simple and inexpensive, which are added advantages for use in practical plant breeding (Sadia *et al*., 2009). Measurement and characterization of genetic diversity have always been a primary concern in population and evolutionary genetic studies, because genetic variability provides the material basis for evolutionary change (Zhang *et al*., 1993). Presently improved DNA markers are available to assess the genetic diversity e.g., RFLP, AFLP, SSR, which provide much better information as compared to protein profiling to assess the genetic diversity. The variation in the length of DNA fragments produced by a specific restriction endonuclease from genomic DNA is termed Restriction Fragment Length Polymorphism (RFLP) (Beckmann and Soller, 1986). The RFLPs are attractive markers because they are generally numerous, co-dominant, phenotypically neutral and insensitive to plant growth environment. The RFLP techniques have been applied to a wide range of theoretical and applied genetic studies such as mapping genes of economic value (Diers *et al*., 1992), identifying useful germplasm (Zehr *et al*., 1992), resolving phylogenetic relation-ships among taxa (Song *et al*., 1990) and diallel analysis of quantitative traits (Lee *et al*., 1989; Smith *et al*., 1990). The RFLPs provide an opportunity to more precisely measure genetic differences among individuals compared to morphological or biochemical markers because these are generally abundant in populations of interest, have no apparent effect on plant fitness per se and are not affected by the environment (Soller and Beckmann, 1983). Seed storage protein profiling through SDS-PAGE and DNA Restriction Fragment Length Polymorphism (RFLPs) were surveyed in 5 accessions of mungbean (*Vigna radiate* L.) sampled from NBPGR Thrissur, Kerala, India, to evaluate genetic diversity.

MATERIALS AND METHODS

Plant material: Five mungbean varieties viz. IC 251431, IC 251432, IC 251433, IC 251430 and IC 251434 given in Table 1 with their sample names collected from NBPGR, Regional station, Thrissur, Kerala, India. Plants were raised in pots and leaf samples pooled from plants of each variety were collected into labelled bags. The study was done in june 2014 and all the samples collected were stored in deep freezer prior to genomic DNA Isolation.

SDS-PAGE: The electrophoretic procedure was carried out using slab type SDS-PAGE with 12% polyacrylamide gel. A 12% resolving gel (3.0 M Tris HCL, pH 8, 0.4% SDS and 4.5 stacking gel) was

Table 1: Seed accessions and their source

Accession name	Sample name	Source
IC 251431	SI	NBPGR Thrissur
IC 251432	S2	NBPGR Thrissur
IC 251433	S3	NBPGR Thrissur
IC 251430	S4	NBPGR Thrissur
IC 251434	S5	NBPGR Thrissur

prepared and polymerized chemically by addition of 17 mL of (N,N,N,N) tetramethylenediamine and 10% ammonium per sulphate. Electrode buffer solution was poured into the bottom pool of the apparatus. Gel plates were placed in the apparatus carefully so as to prevent bubble formation at the bottom of the gel plates. The electrode buffer (0.025 M tris, 1.29 M glycine, 0.125% SDS) was added to the top pool of the apparatus. The 200 µL of the extracted protein was loaded with the help of micropipette into each well of the gel. The apparatus was connected with constant electric supply (75 V) till the tracking dye bromophenol blue (BPB) reached the bottom of the gel. Gels were then stained with staining solution comprising 0.2% (W/V) Coomassie Brilliant Blue (CBB) R 250 dissolved in 10% (V/V) acetic acid and 40% (V/V) methanol for about an hour at room temperature. Gels were destained in a solution containing 5% (V/V) acetic acid and 20% (V/V) methanol. Gels were shaked using Double Shaker Mixer Model DH-10 gently until the background of the gel became clear and proteins bands were clearly visible. After destaining, the gels were photographed using gel documentation system.

Genomic DNA isolation and RFLP: One gram of fresh young leaves from each accession was taken for DNA isolation. The sample was ground to fine powder in pre chilled pestle and mortar with 2 mL of CTAB (2X) buffer. The resultant homogenates were transferred into centrifuge tubes and 3 mL of CTAB buffer was added to each tube. The centrifuge tubes were incubated at 65°C for one hour in water bath. Equal volume of chloroform: Isoamyl alcohol was added to each sample and was mixed gently by inverting them for 15-20 min. The whole samples were spinned at 10,000 rpm for 15 min. Then, the supernatants were taken out into 1.5 mL centrifuge tubes. Twice the volume, chilled alcohol was added to precipitate DNA. After this the samples were incubated overnight at -20°C. The next day, washing of the samples (2 times) with alcohol was done and then after, the DNA was dissolved in 40 µL double distilled water. DNA was extracted by cetyltrimethyl-ammonium bromide (CTAB) method (Doyle, 1990).

The RFLP analysis was carried out individually with one restriction base enzyme *Eco*RI. The RFLP reaction started with the incubated DNA where its final volume was usually kept at 20 µL. This was the amount typically run on the gel (Fig. 1). Following this 5 µL of *Eco*RI restriction

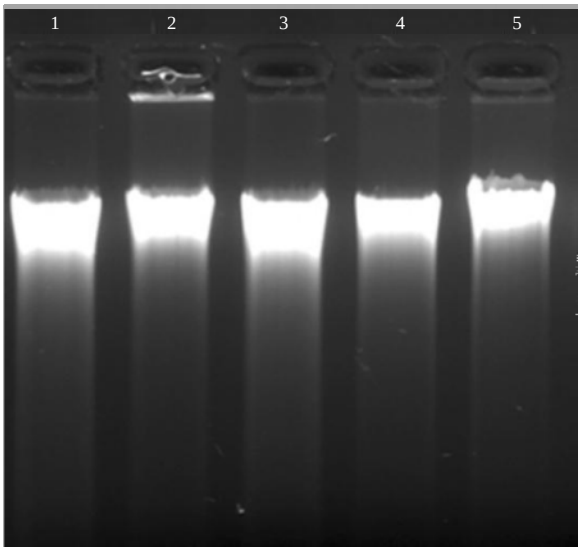

Fig. 1: Genomic DNA of five accessions of *Vigna radiata*

enzyme was also added along with sterile distilled water to make the final volume up to 20 µL. The tubes were tapped several times in order to ensure complete mixing. The tubes were centrifuged briefly, so that the liquid was made to concentrate at the bottom following which they were being placed in water bath for 30-60 min usually kept at 37°C. Finally, 5 µL of tracking dye was also added to the sample on an agarose gel in order to locate the exact position of DNA fragments in the gel and also to track down the distance of migration. The gel was viewed under ultraviolent (UV) transilluminator and photographed using gel documentation. All the selected four primers produced recognizable bands. Scorable bands for a primer in each genotype were compared and allotted 0 (absence) or 1 (presence) values. Two microliter of 10X restriction buffer was added to the reaction mixture in order to make the final concentration up to 1X.

RESULTS AND DISCUSSION

The SDS-PAGE is a promising tool for distinguishing cultivars of a particular crop species (Cooke, 1984; Gardiner and Forde, 1988). The present study was therefore undertaken in order to distinguish among the five given cultivars of mungbean through the use of SDS-PAGE. The SDS-PAGE analysis led to the detection of a total number of 15 bands among the five given accessions. Out of the 15 polypeptide bands calculated, only four bands were polymorphic and the rest (11) were monomorphic. The average polymorphism calculated was 26.66%. The accession IC 251433 (S3) showed the maximum number of bands (14) while, as the minimum number of bands (11) were present in the accessions IC 251431 (S1) and IC 251430 (S4). Thirteen bands were observed in IC 251432 (S2) and twelve in IC 251434 (S5). The protein band with highest molecular weight i.e., 97 kDa and the protein band with lowest molecular weight i.e., 24 kDa were generated in all the five accessions (Fig. 2).

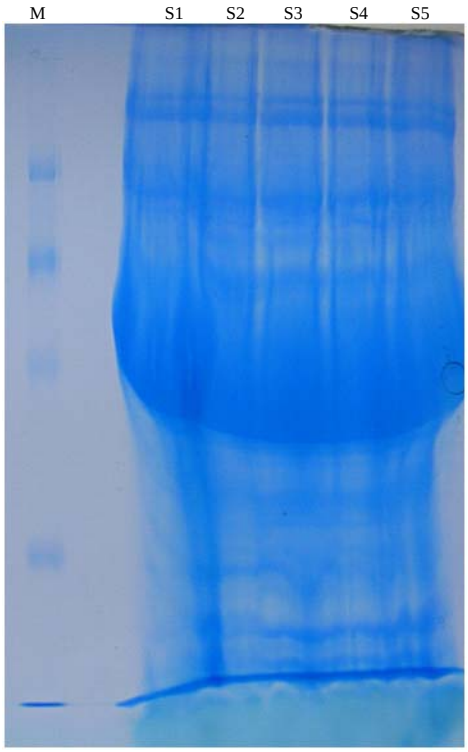

Fig. 2: Electrophoretic pattern of five mungbean accessions as revealed by SDS-PAGE

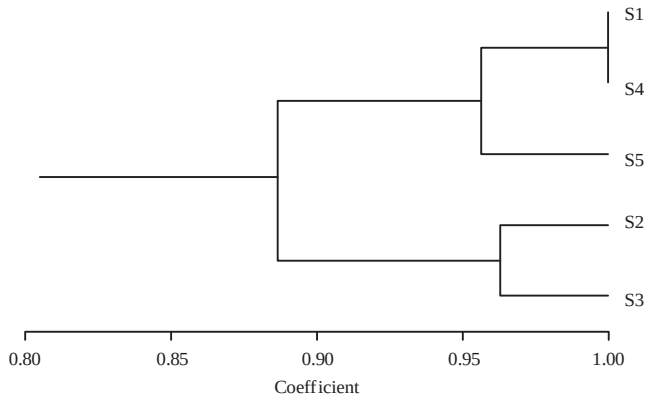

Fig. 3: UPGMA dendrogram showing genetic relationship among five accessions of *Vigna radiata* based on SDS-PAGE

The similarity index calculated among the five accessions ranged from 42.30-50%. Among the five given accessions, the accessions (IC 251431 and IC 251430) showed the highest similarity index i.e., 50%, while as the lowest similarity index was found between (IC 251433 and IC 251434). The accessions (IC 251432 and IC 251433) showed 48.14% followed by (IC 251430 and IC 251434), (IC 251431 and IC 251434) showing 47.82%, (IC 251431 and IC 251432), (IC 251432 and IC 251430) showing 45.83% similarity index.

The data obtained from SDS-PAGE analysis was used for construction of a dendrogram using UPGMA shown in Fig. 3. The five given accessions were grouped into 2 clusters. Cluster 1st comprises IC 251432 and IC 251433 cultivars. Cluster 2nd comprises IC 251431, IC 251430 and IC 251434 cultivars. The cluster analysis revealed that IC 251431 and IC 251430 are closely related with 100% homology while as IC 251431 and IC 251434 and IC 251430 and IC 251434 are distinctly related to each other.

The study depicted a low amount of variation in the five genotypes based on electrophoresis of seed storage proteins which was found in concordance with Hameed *et al*. (2012), who also documented low amount of genetic diversity within 13 mungbean genotypes. The average polymorphism calculated was 26.66% and is more or less in harmony with the findings of Ghallab *et al*. (2007), who recorded an average polymorphism of 44.4% within nine newly bred mungbean genotypes. The results obtained also strengthened the previous findings of Shafique *et al*. (2011) and Ghafoor *et al*. (2005), who also enumerated a low magnitude of genetic diversity within *Vigna mungo* genotypes. However, Win *et al*. (2011) observed distinct polymorphism in cowpea accessions.

The RFLPs are simply inherited naturally occurring Mendelian characters. During the present scenario, *Eco*RI restriction enzyme supplied by Fermentas company was used to restrict the genomic DNA of five accessions of *Vigna radiata*. Only major bands were calculated. Minor bands not clearly scorable were not included in band calculation. A total of 11 clear bands were scored in all the five accessions (Fig. 4). The accessions IC 251432 (S2) and IC 251434 (S5) showed the maximum number of five bands while, as the minimum number of three bands were present in the accession IC 251431 (S1). Four bands were observed in IC 251430 (S4) and IC 251433 (S3). The largest fragment i.e., 630 kb of size was generated in the accession IC 251430 (S4) while as the

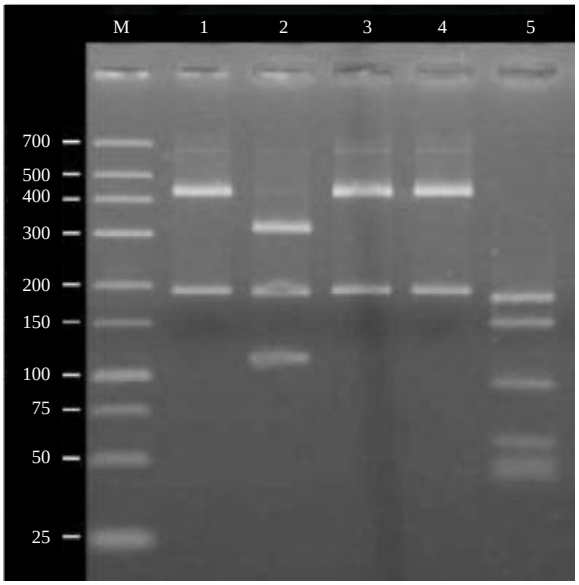

Fig. 4: RFLP profile of five accessions of *Vigna radiata* produced by using *Eco*RI

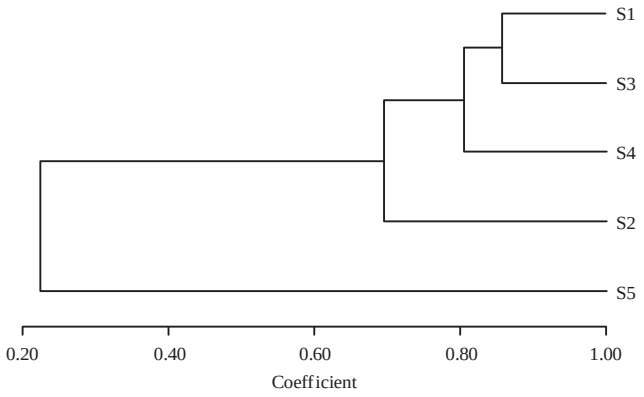

Fig. 5: UPGMA dendrogram showing genetic relationship among five accessions of *Vigna radiata* based on RFLP

smallest fragment 150 kb of size was generated in IC 251434 (S5). Among the 11 scorable bands calculated, only one band at position 6 with 380 kb was monomorphic and the rest were polymorphic (Fig. 4).

Similarity index calculated was found to be highest i.e., 42.82% between the cluster of IC 251431 and IC 251433 and another cluster of IC 251431 and IC 251430 while, lowest similarity index of 11.11% was found between the cluster with IC 251433 and IC 251434 and the cluster with IC 251430 and IC 251434. Similarity index equal to 37.5% was found in between (IC 251431 and IC 251432) and (IC 251433 and IC 251430) accessions and 33.33% in between (IC 251432 and IC 251433) and (IC 251432 and IC 251430) accessions.

The dendrogram obtained from UPGMA method is shown in Fig. 5. The cluster analysis divided the five accessions into two main clusters. Cluster 1 comprising four (IC 251431, IC 251432,IC 251433 and IC 251430) accessions. Accession IC 251434 occupies a distinct position in the

dendrogram. Therefore, it becomes evident from the dendrogram that IC 251431 and IC 251433 are closely similar to each other and IC 251431 and IC 251434 are genetically dissimilar. Since, the cultivar IC 251431 and IC 251434 occupy a distinct place in the dendrogram.

The level of polymorphism observed in the entire data set was found to be 90.90%, which were in more or less harmony with those of Velasquez and Gepts (1994), who observed 70% polymorphism in *Phaseolus vulgaris*. The results obtained further strengthened the previous findings of Nodari *et al*. (1992) and Silberstein *et al*. (1999).

Gentzbittel *et al*. (1992) also recorded a high level of polymorphism in *Helianthus*. However, a low magnitude of polymorphism 38% was observed by Singh *et al*. (2008) by using a single restriction enzyme in *Elaeis guineensis*. Similar results were published by Keim *et al*. (1990) in wheat.

Comparison: The electrophoretic SDS-PAGE and RFLPs analysis, in general were performed to establish the fingerprints in five different mungbean cultivars and to elucidate their genetic relationships. The resulted protein banding pattern showed low magnitude (26.66%) of polymorphism among the five given cultivars. On the contrary, RFLP profile showed a relatively high level of polymorphism i.e., 90.90%. This variation might be due to the use of single restriction enzyme (*Eco*RI). If more than one restriction enzyme would have been used, the results might have been different.

CONCLUSION

The knowledge of genetic diversity is a useful tool in gene-bank management and planning experiments because it facilitates efficient sampling and utilization of germplasm either by identifying or by eliminating duplicates in the gene stock ultimately resulting in the development of core collection philosophy. In the present scenario, it was observed that a low magnitude of genetic diversity at seed storage protein level was present in *Vigna* genotypes. Despite of this low magnitude of genetic diversity at SDS-PAGE level, this technique can be used in breeding programs in resolving genetic diversity disputes and can be aimed for the efficient development of new improved genotypes of *Vigna radiata*. On the contrary, RFLPs analysis was efficient in predicting the usable level of polymorphism among the given mungbean cultivars. More so, RFLP analysis in the present investigation has been successful in depicting the similarity and variance among the cultivars. Since, variance was found between IC 251431 and IC 251434 accessions, thus it provides an insight for future breeding programmes. Hence, RFLP is quite convenient to apply. The only option left over is to validate the RFLP based assessment of genetic diversity by using maximum number of restriction base enzymes for the samples provided.

REFERENCES

Arumuganathan, K. and E.D. Earle, 1991. Nuclear DNA content of some important plant species. Plant Mol. Biol. Rep., 9: 208-218.

Beckmann, J.S. and M. Soller, 1986. Restriction fragment length polymorphisms and genetic improvement of agricultural species. Euphytica, 35: 111-124.

Chowdhury, M.A., B. Vanderberg and T. Warkentin, 2002. Cultivar identification and genetic relationship among selected breeding lines and cultivars in chickpea (*Cicer arietinum* L.). Euphytica, 127: 317-325.

Cooke, R.J., 1984. The characterization and identification of crop cultivars by electrophoresis. Electrophoresis, 5: 59-72.

Diers, B.W., P. Keim, W.R. Fehr and R.C. Shoemaker, 1992. RFLP analysis of soybean seed protein and oil content. Theo. Applied Gen., 83: 608-612.

Doyle, J.J., 1990. Isolation of plant DNA from fresh tissue. Focus, 12: 13-15.

Faris, D.G., 1965. The origin and evolution of the cultivated forms of *Vigna sinensis*. Canadian J. Gen. Cytol., 7: 433-452.

Gardiner, S.E. and M.B. Forde, 1988. Identification of cultivars and species of pasture legumes by SDS-PAGE of seed proteins. Plant Var. Seeds, 1: 13-26.

Gentzbittel, L., A. Perrault and P. Nicolas, 1992. Molecular phylogeny of the *Helianthus genus*, based on nuclear restriction fragment length polymorphism (RFLP). Mol. Biol. Evol., 9: 872-892.

Ghafoor, A., Z. Ahmad and M. Afzal, 2005. Use of SDS-PAGE markers for determining quantative traits loci in black gram [*Vignamungo* (L.) Hepper] Germplasm. Pak. J. Bot., 37: 263-269.

Ghallab, K.H., A.M. Ekram, S.A. Afiah and S.M. Ahmed, 2007. Characterization of some superior mungbean genotypes on the agronomic and biochemical genetic levels. Egypt. J. Desert Res., 57: 1-12.

Hameed, A., M. Qureshi, M. Nawaz and N. Iqbal, 2012. Comparative seed storage protein profiling of mung bean genotypes. Pak. J. Bot., 44: 1993-1999.

Jha, S.S. and D. Ohri, 1996. Phylogenetic relationships of *Cajanus cajan* (L.) Millsp. (pigeonpea) and its wild relatives based on seed protein profiles. Genet. Resources Crop Evol., 43: 275-281.

Kaga, A., N. Tomooka, Y. Egawa, K. Hosaka and O. Kamijima, 1996. Species relationship in the subgenus Ceratropis (*Genus vigna*) as revealed by RAPD analysis. Euphytica, 88: 17-24.

Keim, P., B.W. Dier, T.C. Olson and R.C. Shoemaker, 1990. RFLP mapping in soybean: Association between marker loci and variation in quantitative traits. Genetics, 126: 735-742.

Lambrides, C.J. and I. Godwin, 2007. Mungbean. In: Genome Mapping and Molecular Breeding in Plants 3: Pulses, Sugar and Tuber Crops, Kole, C. (Ed.). Springer, Berlin, pp: 69-90.

Lee, M., E.B. Godshalk, K.R. Lamkey and W.W. Woodman, 1989. Association of restriction fragment length polymorphisms among maize inbreds with agronomic performance of their crosses. Crop Sci., 29: 1067-1071.

Manella, G., S.V. Onofaro, A. Tonidni and V. Mangifico, 1999. Seed storage protein characterization of *Solanum species* and of cultivars and androgenic lines of *S. melongena* L. by SDS-PAGE. Seed Sci. Technol., 27: 23-35.

Nair, R.M., R. Schafleitner, L. Kenyon, R. Srinivasan, W. Easdown, A. Ebert and P. Hanson, 2012. Genetic improvement of mungbean productivity. Proceedings of the 12th SABRAO Congress on Plant Breeding towards 2025: Challenges in a Rapidly Changing World, January 13-18, 2012, Chiang Mai, Thailand, pp: 27-28.

Nodari, R.O., E.M.K. Koinange, J.D. Kelly and P. Gepts, 1992. Towards an integrated linkage map of common bean : 1. Development of genomic DNA probes and levels of restriction fragment length polymorphism. Theo. Applied Genet, 84: 186-192.

Rabbani, M.A., M.A. Qureshi, R. Anwar and S. Komatsu, 2001. Characterization of mustard [*Brassica juncea* (L.) czern. and coss] germplasm by SDS-PAGE of total seed proteins. Pak. J. Bot., 33: 173-179.

Sadia, M., S.A. Malik, M.A. Rabbani and S.R. Pearce, 2009. Electrophoretic characterization and the relationship between some *Brassica species*. Electron. J. Biol., 5: 1-4.

Shafique, S., M.R. Khan, M. Nisar and S. Rehman, 2011. Investigation of genetic diversity in black gram [*Vignamungo* (L.) Hepper]. Pak. J. Bot., 43: 1223-1232.

Silberstein, L., I. Kovalski, R. Huang, K. Kostas-Anagnostou, M.M.K. Jahn and R. Perl-Treves, 1999. Molecular variation in melon (*Cucumis melo* L.) as revealed by RFLP and RAPD markers. Scientia Hortic., 79: 101-111.

Singh, D.P. and I.P.S. Ahlawat, 2005. Green gram (*Vigna radiata*) and Black gram (*Vigna mungo*) improvement in India: Past, present and future prosdpects. Indian J. Agric. Sci., 75: 243-250.

Singh, R., S.G. Tan, J.M. Panandam, R.A. Rahman and S.C. Cheah, 2008. Identification of cDNA-RFLP markers and their use for molecular mapping in oil palm (*Elaeis guineensis*). AsPac J. Mol. Biol. Biotechnol., 16: 53-63.

Smith, O.S., J.S.C. Smith, S.L. Brown, R.A. Tenborg and S.L. Wall, 1990. Similarities among a group of elite maize inbreds as measured by pedigree, F_1 grain yield, grain yield, heterosis and RFLPs. Theor. Applied Genet., 80: 833-840.

Soller, M. and J.S. Beckmann, 1983. Genetic polymorphism in varietal identification and genetic improvement. Theor. Applied Genet., 67: 25-33.

Song, K., T.C. Osborn and P.H. Williams, 1990. Brassica taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). Theo. Applied Gen., 79: 497-506.

Srivastava, R.P. and M. Ali, 2004. Nutritional quality of common pulses. Indian Institute of Pulses Research, Kanpur, India, pp: 65.

Vavilov, N.I., 1926. Studies on the origin of cultivated plants. Institute of Applied Botany and Plant Breeding, Leningrad.

Velasquez, V.L.B. and P. Gepts, 1994. RFLP diversity of common bean (*Phaseolus vulgaris*) in its centres of origin. Genome, 37: 256-263.

Win, K.T., Z.O. Aung, T. Hirasawa, T. Ookawa and H. Yutaka, 2011. Genetic analysis of *Myanmar vigna* species in responses to salt stress at the seedling stage. Afr. J. Biotechnol., 10: 1615-1624.

Zehr, B.E., J.W. Dudley, J. Chojecki, M.A.S. Maroof and R.P. Mowers, 1992. Use of RFLP markers to search for alleles in a maize population for improvement of an elite hybrid. Theo. Applied Gen., 83: 903-911.

Zhang, Q., M.A. Maroof and A. Kleinhofs, 1993. Comparative diversity analysis of RFLPs and isozymes within and among populations of *Hordeum vulgare* ssp. spontaneum. Genetics, 134: 909-916.