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# Estimation of genetic diversity of Mungbean (*Vigna Radiata* L.) Cultivar Using SSR and ISSR Marker Analysis

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**Abstract**
DNA Markers provide an opportunity to characterize genotypes and to measure genetic relationships precisely than other markers. In order to obtain an overview of genetic diversity present in mungbean, nineteen genotypes were analyzed at DNA level by simple sequence repeat (SSR) and inter simple sequence repeat (ISSR) markers. Total number of twenty SSR and ISSR primers were used out of which ten SSR primers and seven out of ten ISSR primers showed amplification. The SSR primers amplified 1(VR062, VR188, Phi057) to 2(VR0155, VR0222, VR0223, VR0304, VR225, Phi112 and VR040) alleles of 50-600bp and ISSR primers amplified 1(ISSR GA5), 2(ISSR 842, ISSR 856, ISSR 857, ISSR CA1) and 4(ISSR GA1) alleles of 180-900bp size. SSR primers and ISSR primers showed 85% and 67.85% polymorphism with highest PIC value marked by VR062 is 0.95 and ISSR 842 is 0.60 respectively. The cluster analysis based on unweighted paired group method of arithmetic means (UPGMA) with 20 SSR and ISSR primers grouped nineteen genotypes into four major clusters. The findings of the study allowed discrimination of cultivars which will be useful in backcross breeding programs.

**Keywords:** genetic diversity, mungbean, SSR, ISSR

## Introduction
Mungbean [*Vigna radiata* (L.) Wilczek], also known as green gram or moong is a self pollinated crop that belongs to the subgenus *Ceratotropis* (2n=2x=22)with a genome size of 579 Mbp. It is one of the important pulse crops of India and it ranks third important legume crop after chickpea and pigeonpea. It is grown on an area of 3.7 million hectares in India with a production of 1.57 million tonnes and productivity of 406.98 Kg/ha which is insufficient for internal consumption and highlights the need for new varieties with higher yields. The crosses between the parents with maximum genetic divergence are generally the most responsive for genetic improvement (Arunachalam 1981). The success of any crop improvement programme depends on available genetic diverse cultivars for understanding the progress made in any breeding programme. The methods of detection and assessment of genetic diversity have extended from analysis of discrete morphological traits to molecular traits. The power of discrimination of DNA based markers is so high that very closely related varieties can be differentiated. Several molecular markers have been used in mungbean including AFLP (Bhat *et al.*, 2005), RAPD(Santalla *et al.*,1998; Lakhanpaul *et al*., 2000), SSR (Gwag *et al*., 2010) and ISSR (Reddy *et al.*, 2008). Among several classes of available DNA markers, Microsatellites or Simple Sequence Repeats (SSR) have become major molecular marker after its emergence as a Polymerase Chain Reaction (PCR)-based genetic marker (Chen *et al*., 2002) because of their reproducibility, multiallelic nature, codominant inheritance and good genetic coverage. SSRs are very popular because of their abundant distribution and hypervariable nature. ISSR analysis involves the PCR amplification of the regions between adjacent, inversely oriented microsatellites using a single simple sequence repeat motif (Reddy *et al*., 2008). Although, ISSR is based on microsatellite sequences but it does not need flanking sequence information, therefore easy to develop. It produces more information in terms of number of loci, polymorphic bands and are highly reproducible in nature.

## Materials and Methods
### Plant Material
The experimental materials used in the present investigation consisted of 19 mungbean genotypes. The source of accession of different *Vigna* species is given in Table - 1. The present investigation was carried out at Dept. of Molecular Biology and Genetic Engineering,

College of Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (SVPUA&T).

**Table 1:** List of mungbean genotypes used in this study with characteristics

| S. No | Name of genotype | Origin | Characteristics |
|---|---|---|---|
| 1. | SML-134 | PAU | For Summer/Spring |
| 2. | NUV-585 | IIPR, Kanpur | Moderately resistant to MYMV |
| 3. | TARM-18 | BARCK/PKV | Resistant to PM, suitable for Rabi, small seed |
| 4. | PDM-139 | IIPR, Kanpur | Summer season, medium height, moderately resistant to YMV |
| 5. | PUSA-4891 | IARI | Moderately resistant to MYMV |
| 6. | IPM-409 | IIPR, Kanpur | Early duration, short height, short pod length, medium size oval seed, black pod colour at maturity |
| 7. | SMM-15-48 | IIPR, Kanpur | Resistant to MYMV |
| 8. | MH-427 | HAU, Hisar | Resistant to MYMV |
| 9. | SMM-15-76 | IIPR, Kanpur | Resistant to MYMV |
| 10. | IPM-02-03 | IIPR, Kanpur | Erect, synchronous, medium-large size seed, resistant to MYMV |
| 11. | COGG-912 | TNAU | Resistant to YMV and CLS |
| 12. | MH-521 | HAU, Hisar | Multiple resistance against MYMV, leaf curl virus and leaf crinkle virus. |
| 13. | MH-318 | HAU, Hisar | Early maturing, it matures in 60 days.Its optimum yield is 23 q/ha within an average yield of 10-12 q/ha in summer and 16-18 q/ha in Kharif. It is resistant to shattering and MYMV. |
| 14. | PUSA-0891 | IARI, New Delhi | Moderately resistant to MYMV |
| 15. | PUSA-0672 | IARI, New Delhi | Resistant to MYMV released by IARI for NHZ during 2009 for Kharif season |
| 16. | AKM-984 | PDKV, Akola | Moderately susceptible to MYMV |
| 17. | PUSA-9531 | IARI, New Delhi | Resistant to YMV, Tolerant to Jassids and whitefly, suitable for summer |
| 18. | BDRY-1 | Bidnapur | Large-seeded, Resistant to MYMV |
| 19. | SMM-15-70 | IIPR, Kanpur | Moderately resistant to MYMV |

**Isolation of genomic DNA**

Genomic DNA was isolated from the seeds of nineteen genotypes grown by using germinator which provides suitable temperature and environment by using Cetyltrimethyl Ammonium Bromide (CTAB) method. The DNA pellet was dissolved in TE buffer (pH 8.0).

**Quality and quantity of the purified DNA**

For purification of DNA, RNase treatment was given. The amount of DNA was determined through BIO-RAD Smart SpecTM Plus Spectrophotometer. The absorbance at 260nm wavelength gave the quantity of the total DNA, and the ratio of the absorbance at 260nm and 280nm indicated the quality of the purified DNA. The quantification was done in comparison with the known standard. The DNA was also loaded in 0.8% agarose gel alongside diluted uncut lambda DNA as standard to recheck the quality and quantity of DNA.

**ISSR amplification**

ISSR amplifications were carried in a 20µl volume containing 2.5µl 10X PCR Buffer (with $MgCl_2$), 4.0µl of sterile distilled water, 4.0 µl of 1mM each dNTPs Mix, 100ng/µl of 1.0µl primer, 8.0µl of genomic DNA and 1U/µl of 2.0 µl Red Taq Polymerase (Genei, Banglore). Amplification was performed in Eppendorf Master cycler gradient. DNA amplification was performed in the BIO-RAD CyclerTM Thermal cycler, programmed for 4min at 94°C, 35 cycles of 1min at 94°C, 1min at 35°C and 1min at 72°C and final extension for 7min at 72°C followed by storing at 4°C till loading to the agarose gel. The amplified products were loaded on 2.4% agarose gel containing 1.5µg/ml Ethidium Bromide and electrophorised in a constant voltage at 75V. The amplifications were checked for their reproducibility.

**SSR amplification**

SSR amplifications were carried out in 20µl volume containing 2.0µl 10X PCR Buffer(with $MgCl_2$), 3.0µl sterile distilled water, 4.0 µl of 1mM each dNTPs Mix, 100ng/µl of 1.0 µl Forward and Reverse Primer each, 8.0µl of genomic DNA and 1U/µl of 1.0 µl Red Taq Polymerase (Genei, Banglore). Amplification conditions were 1 cycle for 4min at 94°C, 35 cycles of 1min at 94°C, 1min at 35°C and 1min at 72°C and final extension for 7min at 72°C. Amplified products were electrophorised on 2.4% agarose gel and separated in 1X TAE buffer at 75V. The gels were visualized under UV after staining with Ethidium Bromide and were documented using a Gel documentation and image analysis system.

**Data Analysis**

The genotypes used in the study were scored for the presence and absence of ISSR and SSR bands, data entered into binary matrix as discrete variables, 1 for presence and 0 for absence of the character. The binary data was imported into NT Edit of NTSYS-pc version 2.1. The SIMQUAL programme was used to calculate the Jaccard's coefficient. Similarity matrices were utilized to construct the UPGMA( unweighted pair group method with arithematic average) dendograms. To measure informativeness of the markers, the polymorphism information content (PIC) for each ISSR and SSR locus was calculated.

**Results and Discussion**

**ISSR band pattern**

Ten ISSR primers were used for analyzing the polymorphism in mungbean, of which seven primers resulted in amplification. The total number of alleles, number of polymorphic and monomorphic alleles, PIC value and Resolving power values are shown in Table - 2 as described

below.

| S. No | Primer code | No of alleles | Polymorphic alleles | Monomorphic alleles | PIC value | Resolving Power |
|---|---|---|---|---|---|---|
| 1. | ISSR 842 | 2 | 2 | 0 | 0.60 | 3.12 |
| 2. | ISSR 856 | 2 | 2 | 0 | 0.12 | 3.76 |
| 3. | ISSR 857 | 2 | 2 | 0 | 0.29 | 3.36 |
| 4. | ISSR CA1 | 2 | 2 | 0 | 0.12 | 3.76 |
| 5. | ISSR 836 | 1 | 0 | 1 | 0.12 | 3.76 |
| 6. | ISSR GA5 | 1 | 1 | 0 | 0.30 | 1.68 |
| 7. | ISSR GA1 | 4 | 3 | 1 | 0.48 | 5.76 |
| Total | | 14 | 12 | 2 | 2.03 | 23.44 |
| Average | | 2.0 | 1.5 | 0.2 | 0.29 | 3.34 |

Amplification of genomic DNA of nineteen genotypes, using ten ISSR primers produced 14 fragments that could be scored with an average of 2.0 bands per primer (Table 2). The number of amplified fragments ranged from 1-4 which varied in size from 180-900 bp. Of 14 amplified bands, 12 were polymorphic with an average of 1.5 polymorphic fragments per primer. The average rate of polymorphism 67.85% indicating the presence of moderate variability in mungbean genotypes. Amplification profile of genotypes using ISSR GA1 primer with highest number of allele as given below:

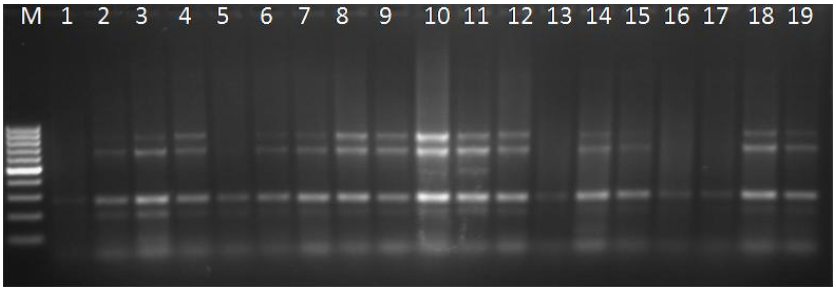


**Fig 1.1:** ISSR profiling pattern of nineteen mungbean varieties with ISSRGA1.

**SSR band pattern**

Total number of ten forward and reverse primers were used to analyze polymorphism in mungbean, of which each primer showed amplification, by producing 17 fragments that could be scored with an average of 1.7 bands per primer(Table 3). The list of SSR primers showing amplification is as follows.

| S.No | Primer code | No of alleles | Polymorphic alleles | Monomorphic alleles | PIC value | Resolving Power |
|---|---|---|---|---|---|---|
| 1. | VR062 | 1 | 1 | 0 | 0.95 | 10.42 |
| 2. | VR0155 | 2 | 1 | 1 | 0.00 | 2.0 |
| 3. | VR0222 | 2 | 2 | 0 | 0.50 | 2.72 |
| 4. | VR0223 | 2 | 2 | 0 | 0.51 | 2.52 |
| 5. | VR0304 | 2 | 2 | 0 | 0.29 | 0.59 |
| 6. | VR188 | 1 | 1 | 0 | 0.73 | 1.04 |
| 7. | VR225 | 2 | 2 | 0 | 0.75 | 1.95 |
| 8. | Phi057 | 1 | 1 | 0 | 0.00 | 2.0 |
| 9. | Phi112 | 2 | 0 | 2 | 0.00 | 2.0 |
| 10. | VR040 | 2 | 2 | 0 | 0.78 | 1.88 |
| Total | | 17 | 14 | 3 | 4.51 | 27.12 |
| Average | | 1.7 | 1.4 | 0.2 | 0.45 | 2.71 |

The number of amplified fragments ranged from 1-2 which varied in size from 50-600 bp of 17 amplified bands, 14 were polymorphic(85%) with an average of 1.4 polymorphic fragments per primer. According to Li *et al.*, (2001), polymorphism in the SSR could also be due to changes in the SSR region itself, caused by the expansion or contraction of SSRs, or interruption. Amplification profile of genotypes using VR0223 and VR0304 primer with highest number of alleles as given below.

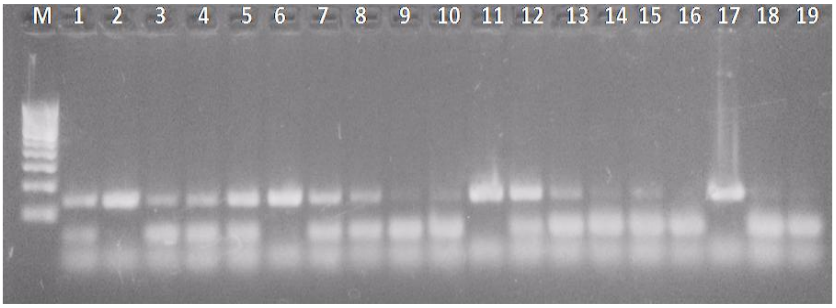


**Fig 1.2:** SSR profiling pattern of nineteen mungbean varieties with VR0223.

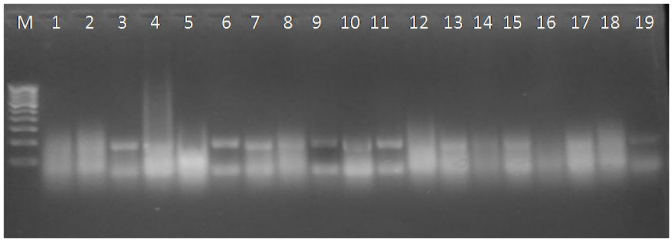


**Fig 1.3:** SSR profiling pattern of nineteen mungbean varieties with VR0304.

**ISSR and SSR Data**

Genetic markers are used to portray diversity within the cultivated germplasm and to identify grouping of cultivars which are adapted to particular regions (Paterson *et al*. 1991). DNA-based markers are ubiquitous, repeatable, stable and highly reliable (Virk *et al*., 2000; Singh *et al*., 2003). They are less affected by age, physiological condition of samples and environmental factors. Polymorphisms at DNA level can be studied by numerous approaches like polymorphism information content etc (Singh *et al*., 2009).

Among several classes of available DNA markers, Inter-simple sequence repeat (Reddy *et al*., 2008) and Simple sequence repeats (Guag *et al*., 2010) have become major molecular markers for a wide range of studies in plants and animals after their emergence as a Polymerase Chain Reaction markers (Chen *et al*., 2002) because of their co-dominant segregation and their ability to detect large number of discrete alleles repeatedly, accurately and efficiently (Olufowote *et al*., 1997). The assessment of diversity of mungbean germplasm has been carried out by many researchers in the past (Lakhanpal *et al*., 2000; Chattopadhyay *et al.*, 2008; Nirmal and Bhat, 2016; Datta *et al*., 2012; Molla *et al*., 2016; Kaur *et al*., 2016; Rekha *et al*., 2015; Reddy *et al*., 2015; Sao *et al*., 2015).

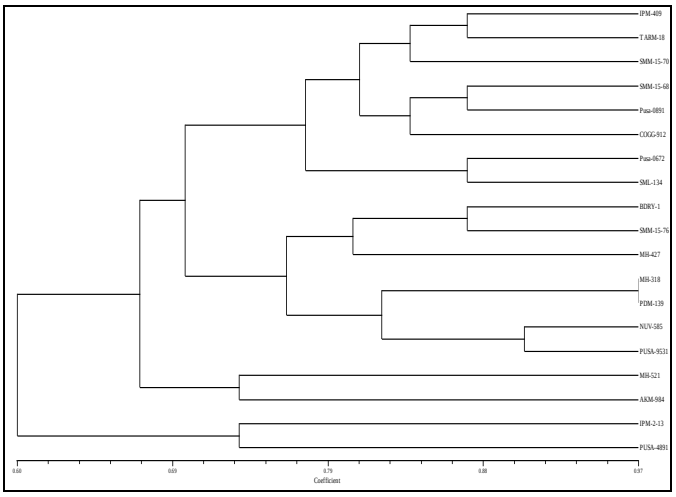


**Fig 2:** Dendogram generated using UPGMA analysis showing relationships among mungbean genotypes obtained by ISSR and SSR data.

The data collected from ISSR and SSR were combined for UPGMA cluster analysis. The combined cluster analysis of nineteen genotypes grouped into three major clusters showing correlation between phylogenetic divergence and geographical distribution. It is difficult to accurately identify varieties by their morphological characteristics; however, ISSR and SSR markers used in the study detected a high level of polymorphism as well as monomorphism and were successful in distinguishing accessions. Group 1 was the most diverse among all consisting of 15 genotypes viz., IPM-409, TARM-18, SMM-15-70, SMM-15-68, PUSA-0891, COGG-912, PUSA-0672, SML-134, BDRY-1, SMM-15-76, MH-427, MH-318, PDM-139, NUV-585, PUSA-9531. The second and third cluster includes two genotypes each i.e., MH-521, AKM-984 and IPM-2-13 and PUSA-4891 respectively. The accessions belonging to the same region or developed by the same organization have shown significant grouping eg., PUSA-0891, PUSA-0672 and PUSA-9531 belongs to the same origin IARI, New Delhi.

It is concluded from the study that assessment level of diversity and species relationship among genotypes of *Vigna* species have a great significance for designing breeding strategies which allows selection of the desired accessions for crossing. Many of the accessions included in the study are morphologically similar but lack the pedigree information. Thus, identification of the genetic distance among accessions will be important to maximize their use in breeding strategies.

**References**


1. Arunachalam V. Genetic distance in plant India. Indian Journal of Genetics & Plant Breeding. 1981; 41(2):226-236.
2. Bhat KV, Lakhanpaul S, Chadha S. Amplified Fragment Length Polymorphism (AFLP) analysis of genetic diversity in Indian mungbean (Vigna radiata [L.] Wilczek] cultivars. Indian Journal of Biotechnology. 2005; 4:55-64.
3. Chattopadhyay K, Bhattacharya S, Mandal N, Sarkar HK. PCR-based Characterization of Mung Bean (*Vigna radiata*) Genotypes from Indian subcontinent at Intra- and Inter-Specific Level. Journal of Plant Biochemistry and Biotechnology. 2008; 17(2):141-148.
4. Chen X, Cho YG, McCouch SR. Sequence divergence of rice microsatellite in Oryza and other plant species. Mol. Genet. Genome. 2002; 268:331-343.
5. Datta Subhojit, Gangwar S, Kumar S, Gupta S, Rai R, Kaashyap M *et al*. Genetic diversity in selected Indian mungbean (*Vigna radiata* L. Wilczek) cultivars using RAPD markers. American Journal of Plant Sciences. 2012; 3:1085-1091.
6. Gwag JG, Chung JW, Chung HK, Lee JH, Ma KH, Dixit A. Characterization of new microsatellite markers in mungbean, *Vigna radiata* (L.). Mol. Ecol. Notes. 2006; 6:1132-1134.
7. Gwag JG, Dixit A, Park YJ, Ma KH, Kwon SJ, Cho GT. Assessment of genetic diversity and population structure in mungbean. Genes and Genomics. 2010; 32:299-308.
8. Kaur G, Joshi A, Jain D, Choudhary R, Vyas D. Diversity analysis of greengram (*Vigna radiata* (L) Wilczek) through morphological and molecular markers. *Turkish* Journal of Agriculture and Forestry. 2016; 40:229-240.
9. Lakhanpaul S, Chadha S, Bhat KV. Random amplified polymorphic DNA (RAPD) analysis in Indian mungbean (*Vigna radiata* (L.) Wilczek) cultivars. Genetica. 2000; 109:227-234.
10. Li CD, Fatokun CA, Ubi B, Singh BB, Scoles GJ. Determining genetic similarities and relationships among cowpea breeding lines and cultivars by microsatellites markers. Crop Science. 2001; 41:189-197.
11. Molla R, Ahmed I, Rohman M, Hossain A, Chowdhary AZ. Genetic diversity analysis and DNA fingerprinting of mungbean (*Vigna radiata* L.) genotypes using SSR Markers. Plant Science. 2016; 4(6):153-164.
12. Nirmal Bharathi M, Sumangala Bhat. Molecular diversity

analysis for pod shattering using SSRs markers in Mungbean (*Vigna radiata* L. Wilczek). European Journal of Biotechnology and Bioscience. 2016; 4(12):53-56.
13. Paterson AH, Damon S, Hewitt JD, Zamin D, Rabinowitch HD, Lincoln SE. Mendelian factors underlying quantitative traits in tomato: comparison across species, generation and environment. Genetics. 1991; 127:181-197.
14. Reddy KS, Souframanien J, Reddy KS, Dhanasekar P. Genetic Diversity in mungbean as revealed by SSR and ISSR markers. Journal of Food Legumes. 2008; 21:15-21.
15. Rekha KS, Reddy DM, Reddy BR, Reddy KH, Reddy B. DNA fingerprinting and genetic diversity analysis using RAPD, ISSR and SSR Markers in Mungbean (*Vigna radiata* (L) Wilczek). Journal of Agriculture and Veterinary Science. 2015; 8(7):63-68.
16. Sao Kumar Manoj, Nair SK, Verulkar SB, Saxena RR, Nanda HC. Molecular profiling and genetic diversity of mungbean (*Vigna radiata* L.) genotypes using ISSR and SSR markers. Indian J. Agric. Res. 2015; 48(1):373-376.
17. Singh Akanksha, Dikshit HK, Jain Neelu, Singh D, Yadav RN. Efficiency of SSR, ISSR and RAPD markers in molecular characterization of mungbean and other Vigna species. Indian Journal of Biotechnology. 2013; 13:81-88.
18. Virk PS, Ford-Lloyd BV, Jackson MT, Newbury HJ. Use of RAPD for the study of diversity within plant germplasm collections. Heredity. 1995; 74:170-179.