

## Molecular Characterization of Green Gram [*Vigna radiata* (L.) Wilczek] for Future Breeding Programmes

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### ABSTRACT

Molecular characterization is helpful in understanding the phylogenetic relationship among various germplasm to reveal the genetic diversity among the plant genotypes. Morphological and biochemical markers used for discriminating cultivars / varieties are not adequate as they are subject to environmental influences, whereas the molecular markers especially DNA based, have proven better. The latter may or may not correlate with phenotypic expression of a genomic trait. Varietal profiling methods that directly utilize DNA have been found to potentially address all the limitations associated with morphological and biochemical data. They offer numerous advantages over conventional, phenotype-based characters as they are stable and detectable in all situations regardless of growth, differentiation, development or defense status of the cell. Additionally, they are not confounded by environmental, pleotropic and epistatic effects. The DNA markers become the marker of choice for the study of crop genetic diversity, especially those based on DNA sequence variations which are increasingly being utilized in crops for construction of genetic maps and marker-assisted selection studies. Application of molecular markers to plant breeding has established the need for information on variation in DNA sequence even in those crops in which little classical genetic and cytogenetic information is available. Among several efficient methods for revealing genetic variability within and among plant populations, one of the most widely applied methods is molecular marker analysis. Markers are commonly used because they are quick, simple and environment non-sensitive enabling genetic diversity analysis in several types of plant material like natural populations, population in breeding programmes. Evaluation of genetic diversity would promote the efficient use of genetic variations, effective conservation and purity of the genotype to be determined as well as utilization of germplasm in crop improvement.

#### Keywords

Mungbean improvement, Genetic resource, Genetic diversity, Breeding and molecular markers.

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### Introduction

Green gram [*Vigna radiata* (L.) Wilczek] is the most important legume (Pulse) crop in India after chickpea and pigeonpea. India is the primary green gram producer and contributes to about 75 per cent of the world pulses production. It contributes to about 14%

of total pulses cultivation area and 7% of total pulses production in India. Maharashtra, Rajasthan, Madhya Pradesh, Bihar, Punjab and Andhra Pradesh are the leading producers of green gram. For any successful breeding programme to improve grain yield and

component characters, it is essential to know precisely the genetic architecture of these characters under prevailing conditions. Keeping in view the above facts, this review paper was prepared to increase the breeding efficiency, linkage map, genetic diversity in mungbean, detection of quantitative trait loci and to explore the current progress in structural genomic studies in green gram.

Morphological markers with their complex and undeciphered genetic control were used for the individual identification and diversity studies; they may be affected by environmental effects and cultivation practices. In contrast to the morphological markers, molecular markers, are now available (Khan *et al.*, 2000) in plant system involves improvement in the efficiency of conventional plant breeding by carrying out indirect selection through QTL, RAPD and ISSR techniques that provide a new alternative for cultivar identification (Gunter *et al.*, 1996; Lashermes *et al.*, 1996, Bouchired, 1997 and Colombo *et al.*, 2000). Ever since thermostable DNA polymerase was introduced in 1988 (Saiki *et al.*, 1988), the use of PCR (Mullis *et al.*, 1986; Mullis and Faloona, 1987) in research has increased tremendously.

Molecular characterization is helpful in understanding the phylogenetic relationship between plant species and to reveal the genetic diversity within a given taxonomic group. Evaluation of genetic diversity would promote the efficient use of genetic variations (Paterson *et al.*, 1991), effective conservation and purity of the genotype to be determined as well as utilization of germplasm in crop improvement (Samarajeewa *et al.*, 2002). Apart from morphological and biochemical markers, molecular markers have been found to be useful and superior in analyzing the genetic differences in plant populations at the DNA level (Yoon *et al.*, 2000). The molecular

markers are a powerful tool the yield significant information on genetic variation thereby enhancing their scope (Das *et al.*, 2014). Data from even duplicate accessions can be distinguished using molecular markers.

Among several efficient methods for revealing genetic variability within and among plant populations, some of the most widely applied methods are RAPD (Wolff 1993; Wachira *et al.*, 1995; Brummer *et al.*, 1995; Swoboda and Bhalla, 1997) and ISSR (Joshi and Dhawan, 2007; Heikal *et al.*, 2008a). Further, RAPD and ISSR, markers are commonly used because they are quick, simple and environment non-sensitive enabling genetic diversity analysis in several types of plant material like natural populations, population in breeding programmes and germplasm collections (Williams *et al.*, 1990). To achieve breakthrough in the yield and quality characters of *V. radiata* L., genetic divergence analysis has been attempted so that the highly diverse genotypes could be selected for molecular breeding programmes. Molecular markers have been utilized for genetic diversity, phylogenetic analysis, germplasm evaluation, genome mapping, genetic linkage map construction and marker-assisted selection to investigate structural genomics in crop plants (Muthamilarasan and Prasad, 2015).

### **Randomly Amplified Polymorphic DNA (RAPD)**

Among the DNA markers, development of RAPD-PCR based DNA finger printing has proved to be versatile (Gherardi *et al.*, 1998). RAPD markers have been used for the identification and assessing the genetic diversity among cultivars of several crops like green gram (Saini *et al.*, 2010). Moreover, RAPD-derived genetic information helps to compare each germplasm and to choose

competent parents for hybridization. Among the various molecular markers, PCR based RAPD markers have become popular since their application does not need any prior information about the target sequences on the genome and is simple and fast. At first the RAPD study of mungbean was conducted to determine the genetic variability among 23 accessions of five species of wild and cultivated plants in the subgenus *Ceratotropis*, including *V. umbellata*, *V. angularis*, *V. radiata*, *V. aconitifolia*, and *V. mungo* (Kaga *et al.*, 1996). Earlier RAPD analyses in Greengram mainly done for examining genotypic diversity and genetic similarity among different genotypes for crop improvement (Santalla *et al.*, 1998 and Bisht *et al.*, 1998). 32 Indian cultivars of green gram were randomly amplified polymorphic DNA (RAPD) using 21 decamer primers. A total of 267 amplification products were formed at an average of 12.71 per primer with an overall polymorphism of 64 per cent. The extent of polymorphism was moderate to low. Jaccard's similarity coefficient values ranged from 0.65 to 0.92. Mainly three clusters revealing greater homology between cultivars (Lakhanpaul *et al.*, 2000). A linkage map was developed from the F<sub>2</sub> mapping populations of *V. radiata* ssp. *radiata* and *V. radiata* ssp. *sublobata*, with integration of 52 RFLP and 56 RAPD markers localized to 12 linkage groups to detect genetic polymorphism among greengram genotypes (Lambrides *et al.*, 2000). 75 random RAPD primers to differentiate between 12 black gram varieties. Consistent results were obtained with about 40 primers. Use of these 40 primers resulted in the amplification of 74 polymorphic bands. Putative variety specific markers were amplified in four cases. The black gram varieties could be discriminated by analysing the amplification patterns generated by four primers (Ranade and Gopalakrishna (2001). 13 germplasm collection of *Vigna* species characterized by using RAPD markers. Total

ten RAPD primers were used, out of which five were found to produce clear banding pattern. A total of 134 bands were produced, out of which 104 were found to be polymorphic. SAS program (version 8.0) was used to construct dendrogram which divided 13 accessions into two clusters comprising of six accessions in one and the other included rest of the accessions (Samarajeewa *et al.*, 2002). 21 green gram cultivars were subjected to RAPD analysis using 34 decamer primers. All the primers produced polymorphic amplification products with some extent of variation. Green gram cultivars exhibited 75.0 per cent polymorphism. Jaccard's similarity coefficient ranged from 0.54 to 0.85 and concentrated mostly between 0.61 to 0.74. This indicated a rather narrow genetic base of tested cultivars. Genetic similarity obtained in this study may be used for selecting parents for breeding purposes. Clustering of cultivars into four groups showed reasonable variability that may be exploited for yield improvement (Afzal *et al.*, 2004). Eight long primers used for AP-PCR analysis of 46 green gram genotypes. A total of 173 fragments were amplified of which 39.08 per cent were polymorphic. AP-PCR profiles from only three primers were sufficient to differentiate all the genotypes. A high degree of genetic variation was observed among different genotypes, whereas, those originating from the same source were highly related (Saini *et al.*, 2004). Markers like RAPD and ISSR employed to assess the genetic diversity among selected germplasm comprising varieties, landraces and wild accessions. Though polymorphism among the varieties was moderate, it was high (83%) when the whole set of germplasm was considered. One green gram variety, PS-16 with determinate growth habit and a wild accession, Sub-14 was found most diverse as revealed from the lowest Jaccard's similarity coefficient value (0.34) (Chattopadhyay *et al.*, (2005). RAPD and SSR techniques used to assess genetic

diversity among cowpea genotypes. A total of 61 RAPD primers used and twelve shows polymorphism. Fifteen of the 30 microsatellite primer pairs were polymorphic, detecting one to nine alleles per locus in cowpea landraces (Diouf *et al.*, (2005). Genetic diversity of greengram genotypes at DNA level analyzed by RAPD analysis using 20 decamer primers which generates 200 bands with an average of 10 per primer and exhibited 83.0 per cent polymorphism. Jaccard's similarity coefficient ranged from 0.64 to 0.93 and concentrated mostly between 0.76 and 0.93. This indicated a rather narrow genetic base of tested green gram landraces. Clustering of green gram landraces into two groups showed reasonable variability that may be exploited for selecting parents for breeding purposes (Karuppanapandian *et al.*, 2006), the isolation and characterization of new polymorphic microsatellites in green gram (*Vigna radiata* L.) was done and out of 93 designed primer pairs, 7 were found to amplify polymorphic microsatellite loci, which were then characterized using 34 green gram accessions (Gyun Gwag *et al.*, 2006). Genetic variability among 32 Indian green gram cultivars were studied using RAPD marker. Total 21 decamer primers were used that produced 267 bands with an average of 12.71 per primer. Polymorphism percentage was 64. Jaccard's similarity coefficient values ranged from 0.65 to 0.92. The cluster analysis divided all the cultivars into three clusters revealing greater homology between cultivars, as the genotypes were released from the same source and showing commonness with their pedigree (Lakshanpaul *et al.*, 2006). 54 accessions of green gram for RAPD profiling to identify the extent of diversity, the RAPD profiles were analysed for Jaccard's similarity coefficients that was found to be in the range from 0 to 0.48, indicating the presence of wide range of genetic diversity at molecular level. The dendrogram resolved all the accessions into two major clusters, I (with 11

accessions) and II (with 43 accessions). The distribution of the accessions in different clusters and sub clusters appears to be related to their performance in field conditions for 10 morphological traits (Lavanya *et al.*, 2008). From 80 diverse origin accessions of green gram one green gram genotype (B1) was differentiated from wild and cultivated accessions belonging to twelve other indigenous *Vigna* species and subspecies by employing one each of microsatellite, ISSR and RAPD primers. The highly informative primers could be utilized in generating useful molecular descriptors for fingerprinting green gram genotypes (Chattopadhyay *et al.*, 2008). Evaluation of genetic variation through RAPD markers in four black gram mutants (high seed protein, tall, bushy and dwarf mutants) along with parent cultivar (control) by taking 20 random primers which had generated 202 fragments that scored 58 polymorphic DNA bands. The average DNA bands were 10.1 per locus that ranged from 1 to 9. The average polymorphic rates were 38.37 per cent. Primer OPK-06 and OPK-11 revealed 62.5 per cent DNA polymorphism. Jaccard's similarity coefficient ranged between 0.621 and 0.785 (Arulbalachandran *et al.*, 2009). Evaluation of genetic variations in seven *Vigna* species using RAPD and ISSR markers were done, total five random primers generated 64 fragments, of which 31 were polymorphic with an average of 12.8 bands per primer. The amplified products varied in size from 2556 to 255 bp; eleven selected ISSR primers produced 128 bands across seven genotypes of which 89 were polymorphic with an average of 11.64 per primer. The size of amplified bands ranged from 2838 to 264 bp. The results indicated that both the marker systems RAPD and ISSR, individually or combined can be effectively used in determination of genetic relationship among the *Vigna* species (Abd El-Hady *et al.*, 2010). RAPD primers used for differentiate a set of 39 green gram



genotypes. The primers S-1 and S-2 were more efficient with iso-frequency distribution of most of their banding patterns and a combination of any one of the primers with S-1 or S-2 could identify all the genotypes (Saini *et al.*, 2010). Assessment of genetic diversity among 15 green gram genotypes of Pakistan was done through Random Amplified Polymorphic DNA (RAPD) analysis. Total 30 random decamer primers were used and showed 91.6 per cent polymorphism. Maximum similarity was observed between NM-51, NM-54 and NM-98 (0.86). The analysis revealed that the inter-varietal genetic relationship of several genotypes is related to their centre of origin (Ullah *et al.*, 2010). RAPD analyses of genomic DNA, which was carried out using 80 RAPD primers in ten different genotypes each of A and R lines of pigeonpea. 76 of the 80 RAPD primers produced clear and unambiguous banding pattern among A and R lines. Out of these, 72 were polymorphic and 4 were monomorphic. A total of 702 bands were amplified, out of which 544 were polymorphic. This evinced on an average 7.55 polymorphic bands per primer; though the average numbers of amplified bands per primer were 8.77 (Sheikh *et al.*, 2011). Screening of forty black gram varieties/lines was done against the virus under field conditions, but none of the lines appeared to be highly resistant. RAPD analyses revealed an extensive amount of variation, which could be used for cultivar identification. Genetic differentiation among black gram genotypes was similar to the field screening data (Binyamin *et al.*, 2011). Genetic diversity and relationships among eight black gram (*V. mungo*) varieties from diverse geographic locations was done using RAPD markers. Forty decamer primers could generate a total of 346 RAPD fragments, of which 338 or 97.68 percent were polymorphic. The similarity coefficient was maximum between PLU-289 and PLU-456(0.76) that indicated

less divergence between them. Lower similarity was observed between LBG-20 and PLU-289 (0.4337) indicating more divergence (Srivastava *et al.*, 2011). Research was done to know genetic diversity and their phylogenetic status among the wild and cultivated species of green gram using 36 random decamer primers. Twenty eight out of 36 primers amplified across all species scoring 537 amplicons of which 498 were polymorphic and 39 were monomorphic. Average percent polymorphism among the seven species was 92.82%, respectively. Out of 28, two primers were reported to generate a maximum of 95 percent polymorphism. The dendrogram obtained categorized the seven species in to two distinct clusters (Undal *et al.*, 2011). Thirty seven black gram genotypes analyzed using RAPD markers. Among 53 primers 36 produced polymorphic fragments in black gram. The RAPD markers were found useful for studying genetic diversity but clustering did not exhibited indication for agronomic performance, whereas quantitative traits contributed more towards agronomic performance. Cluster revealed that only a portion of genetic diversity has been exploited for black gram improvement that should broaden involving diverse parents from various clusters (Ghafoor *et al.*, 2012). Genetic diversity analysis was done among 36 accessions of cultivated and wild *Vigna* species using 12 quantitative morphological cultivars. The 36 *Vigna* accessions formed 8 clusters at 16.77 dissimilarity level. The accessions namely *V. dalzelliana*, *V. unguiculata*, *V. trilobata*, *V. mungo* var. *mungo* and *V. trinervia* var. *bourneae* were clustered in the separate groups. Hence the variations were found to occur at species level (Pandiyani *et al.*, 2012). Genetic diversity of 13 green gram cultivars was done using RAPD markers analysis. 20 arbitrary decamer oligonucleotide primers used, out of those 10 produced total 379 different bands with an average of 37.9 bands per primer. Banding

pattern showed 100 per cent polymorphism. Dendrogram based on Nei's genetic distance using UPGMA segregated these cultivars into two major clusters. The highest genetic distance (1.0852) was found between cultivars BARI Mung-2 and Mung-6 (Sony *et al.*, 2012). Molecular markers have been used to study the genetic diversity in green gram cultivars. RAPD marker association with YMV resistance gene in green gram has been identified. For validation and confirmation several marker band like VMYR 1, CEDG 180 and several RGA marker are taken into consideration to identify the exact marker which is linked to YMV resistance (Panigrahi *et al.*, 2014). Analysis of genetic diversity and relationship in 7 exotic and 3 advance green gram germplasm using 3 RAPD primers (OPA01, OPB06 and OPB07), on an average, 6 amplified products per primer were formed and per cent polymorphism was found to be 78.33. The similarity coefficient values was highest (0.93) between genotypes AVRDC-3 and AVRDC-4, indicating less divergence amongst those genotypes and was least (0.39) between AVRDC-5 and AVRDC-6, indicating more divergence (Bhuyan *et al.*, 2014).

### **RFLP markers**

Earlier mungbean studies, RAPD markers were commonly utilized using Sanger sequencing approaches, when the availability of mungbean sequence information was insufficient (Chavan and Gacche, 2014). RFLP probes were used because these markers are co-dominant and their use yields reproducible results. The first study of RFLP technology in mungbean was done to analyze the genetics of bruchid resistance. In this study, 153 RFLP markers were mapped to 14 linkage groups covering 1,295 cM, with an average distance of 9.3 cM between markers (Young *et al.*, 1992). Molecular mapping and QTLs study for locating different traits in

mungbean was done based on RFLP markers (Humphry *et al.*, 2005). Comparative mapping among different legumes like, mungbean, cowpea, and common bean was done by using RFLP markers based on a common set of DNA clones (Boutin *et al.*, 1995). RFLP markers were used for development of initial linkage map in mungbean based on clones from mungbean, cowpea, soybean, and common bean. The map consists of 171 markers covering a total of 1,570 cM on 11 linkage groups, with an average interval of 9 cM between loci, as well as several important traits such as resistance to seed bruchids, resistance to powdery mildew and seed size (Menancio *et al.*, 1992). The genetic relationships between mungbean and cowpea were also investigated by establishing the linkage arrangement of RFLPs. As RFLP approach is most laborious and costly and specific probes for target sequences, an alternative method, the PCR-based RAPD technique, has been used to identify and characterize mungbean genotypes (Williams *et al.*, 1990).

### **ISSR markers**

ISSR offers several advantages and this technique is already used in many crop plants. ISSR markers have been successfully utilized for assessing the genetic diversity in the genus *Vigna* by various scientists.

An investigation was done to know the utility of inter simple sequence repeat (ISSR) DNA polymorphisms to distinguish taxa within the genus *Vigna*. Total 19 primers were used out of which 15 primers showed effective polymorphisms for distinguishing taxa at the species level. In contrast, ISSR analysis was not able to clearly differentiate subgeneric divisions within *Vigna*. They attribute this loss of resolution at the sub generic level to the high rate of evolution of the sequences examined (Ajibade *et al.*, 2000). ISSR and

RAPD were employed to assess the genetic diversity among selected mungbean germplasm varieties, landraces and accessions. Through polymorphism among the varieties was moderate, it was high (83%) when the whole set of germplasm was considered. One green gram variety, PS-16 with determinate growth habit and a wild accession, Sub-14 was found most diverse as revealed from the Jaccard's similarity coefficient value. The efficiency of ISSR markers over RAPD markers was well visualized from higher frequency polymorphic bands and polymorphic information content values (Chattopadhyay *et al.*, 2005). Inter simple sequence repeat (ISSR) marker technique has been employed to identify markers linked to the MYMV resistance gene. Of the 100 primers screened, 54 showed amplification of which 36 exhibited polymorphism. The ISSR8111357 marker was sequenced and sequence characterized amplified region (SCAR) primers were designed (YMV1-F and YMV1-R) to amplify the marker (Souframani and Gopalakrishna, 2006). Genetic diversity in green gram 30 genotypes were analysed at the DNA level by simple sequence repeat (SSR) and intersimple sequence repeat (ISSR) markers. A total of 19 SSR and 35 ISSR primers were used, of which 10 and 20 primers, respectively, showed amplification. The 20 ISSR primers, which showed amplification, produced 116 bands of which 72 were polymorphic (62%). The number of amplified bands ranged from 2 to 12 and the size ranged from 200 to 2700 bp (Reddy *et al.*, 2008). Genetic diversity among 23 black gram genotypes involving 16 released varieties, six prerelease cultures and one wild species *V. mungo var. silvestris* were analyzed using twelve ISSR primers. The level of polymorphism was 82.05 per cent. The resulting dendrogram distributed the 23 black gram genotypes into five main clusters. The results of PCA were comparable to that

of grouping based on UPGMA in that the 23 genotypes could be grouped into four clusters (Kanimozhi *et al.*, 2009). Determination of genetic diversity and relatedness in 17 green gram (*V. radiata* (L.) Wilczek and 5 black gram (*V. mungo* (L.) Hepper) genotypes were done by means of ISSR analysis and morphological characters. In total, 341 fragments were amplified for the two *Vigna* species by ISSR analysis using 18 ISSR primers. The analysis revealed 90.6 per cent polymorphism. UPGMA analysis (based on ISSR) exhibited 2 major clusters; cluster I containing all green gram genotypes and cluster II containing all black gram genotypes (Tantasawat *et al.*, 2010). Evaluation of plant genetic diversity of seven *Vigna* species was done by scientists using RAPD and ISSR markers. They selected 11 ISSR primers that produced 128 bands out of which 89 were polymorphic with an average of 11.64 bands per primer. The size of amplified bands ranged from 264 to 2838 bp. The similarity coefficient values ranged from 0.118-0.822 (ISSR) and 0.115-0.899 (RAPD and ISSR). The results indicated that both marker systems RAPD and ISSR, individually or combined can be effectively used in determination of genetic relationship among the *Vigna* species (Elham *et al.*, 2010). Phylogenetic relationships of ten *Vigna* genotype analyzed by using inter simple sequence repeats (ISSR) markers. Average polymorphism was 54.05 per cent and average of 6.66 polymorphic fragments per primer. ISSR based dendrogram classified ten genotypes into 3 clusters (I, II and III) with 6, 3 and 1 genotypes respectively (Pardhe and Satpute, 2011). 20 ISSR and 22 SSR markers had been used to differentiate 2 major pulse crops (*Cicer arietinum* L. and *Cajanus cajan* L.) possessing different important traits. The average number of amplicon generated was 1.3 (SSR) and 8.1 (ISSR) that showed a polymorphism of 95% (ISSR) and 93% (SSR). UPGMA cluster analysis of eight

genotypes using both the markers showed clear demarcation between the two genera falling in two separate clusters with their respective resistant and susceptible genotypes (Datta and Lal, 2011). DNA polymorphism of black gram cultivars identified by using 10 ISSR markers, amplification pattern yielded 71 bands, of which 65 were polymorphic with an average of 6.5 polymorphic bands per primer. Cluster analysis grouped the genotypes into two main clusters, I and II comprised of 22 and 11 that genotypes, respectively. The Jaccard's similarity coefficient values between different *Vigna* genotypes ranged from 0.47 to 1.00 (Bhareti *et al.*, 2012). Utilization of inter-simple sequence repeat (ISSR) markers was done for identifying putative F1 hybrids from six cross combinations whose morphological characteristics were very similar to those of their respective female parents and could not be visually discriminated from the self-pollinated progeny. Based on 10 ISSR primers, polymorphisms were found between female and male parents of all six cross combinations. F1 hybrids of all six cross combinations could be differentiated from the self-pollinated progeny of their female parents by using only either ISSR 841 or 857 primers, together with the ISSR 835 primer (Khajudparn *et al.*, 2012). Five species of *Pinellia* endemic in China were analyzed using inter-simple sequence repeat (ISSR) marker examined genetic relationship among. Twelve ISSR primers showed 88.58 percent polymorphism, whereas 38 SRAP primer combinations showed 78.37 percent polymorphism. Cluster analysis showed that ISSR, SRAP and ISSR + SRAP dendrograms of the five species of *Pinellia* generally exhibited similar clustering patterns (Liu *et al.*, 2012). ISSR markers were used to study the DNA polymorphism in elite green gram genotypes. A total of nine AFLP primer combination and 22 ISSR primers were used. The 22 ISSR primers used in the study

produced 108 bands across 30 genotypes, of which 68 were polymorphic. The number of amplified bands varied from two to ten (Singh *et al.*, 2013). A total of 108 bands obtained using 30 green gram genotypes through 22 ISSR markers of which 68 were polymorphic. The average numbers of bands per primer and polymorphic bands per primer were 4.9 and 3.1, respectively. ISSR primers gave 58.3 per cent polymorphism and the PIC value ranged from 0.09 to 0.71 with an average of 0.46. The UPGMA distributed the 30 genotypes into five main clusters; ISSR markers are formed useful in the assessment of green gram diversity and the selection of core collection to enhance the efficiency of germplasm management for use in green gram breeding and conservation (Singh *et al.*, 2013). An experiment was undertaken to identify ISSR markers associated with powdery mildew resistance in green gram (*Vigna radiata* (L.) Wilczek) cross between Kopargaon (susceptible) and BPMR-48 (resistant), among the 75 ISSR markers, 54 were amplified in DNA extracted from green gram. Four markers were polymorphic for the two parents (Kopargaon and BPMR-48) whereas, only one marker was found polymorphic between the parents as well as resistant and susceptible population (Bainade *et al.*, 2014). A total number of 10 ISSR primers that produced polymorphic and reproducible fragments were selected to amplify genomic DNA of the green gram genotypes. Ten primers amplified a total number of 353 bands under 93 loci across five genotypes. The cultivars exhibited an overall polymorphism of 52.69 per cent. The value of Jaccard's similarity coefficient ranged from 0.724 to 0.793 and these five green gram cultivars were grouped into 3 clusters at 72.4 per cent similarity. Overall a moderate degree of genetic diversity among the green gram cultivars was recorded (Das *et al.*, 2014). The extent of genetic diversity among 35 *Vigna* genotypes analyzed using SSR, ISSR and



RAPD markers. SSR (21), ISSR (17) and RAPD (25) markers produced a total of 319 bands, of which 284 exhibited polymorphism. Higher marker indices were obtained for ISSR markers, which also proved to be the most efficient marker system in terms of average heterozygosity values. The pooled allelic diversity data grouped 35 genotypes into 4 major clusters with most of the genotypes reflecting relationship according to the distribution (Singh *et al.*, 2014).

In conclusion, currently, the genetic diversity of plants has been assessed more efficiently after the introduction of the method that reveals polymorphism directly at the DNA levels. Ever since thermostable DNA polymerase was introduced, the use of PCR (Mullis *et al.*, 1986) in research has increased tremendously. Genotypic selection at the DNA level can be exploited in marker assisted selection to identify desirable genotypes. DNA based markers find widespread application in plant science and plant production. Morphological markers with their complex and undeciphered genetic control were used for the individual identification and diversity studies, they may be affected by environmental effects and cultivation practices. Due to the importance of mungbean, molecular markers involving inter simple sequence repeat (ISSR), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and simple sequence repeat (SSR) markers have long been applied toward improving mungbean, with a focus on yield, nutritional improvement, and disease resistance; these techniques are that could be useful for analyzing germplasm and genetic diversity and for constructing linkage maps. The present review study was conducted with an objective of selecting suitable parents for future use in improvement of green gram, molecular analyses aimed for evaluating genetic diversity in mungbean, linkage map

construction for detecting quantitative trait loci and to explore the current progress in structural genomic studies in green gram. Incorporation of the molecular approaches along with the conventional techniques is genetically more informative.

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