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***Research article***

# GENETIC DIVESITY AND MOLECULAR CHARACTERIZATION OF MUNGBEAN GENOTYPES (*Vigna radiata* (L.) Wilczek)

B. Laxmi Prasanna[1], P.J.M. Rao[2], K.G.K.Murthy[3], K.Kiran Prakash[4], K.N.Yamini[5] and A. Srividhya[6].

[1,3]Department of Genetics and Plant Breeding, Agricultural College, Aswaraopet, Pin- 507301, [2]RARS, Warangal, 4. Agricultural college, Rajamandri, 5,6IBT, Hyderabad.

**ABSTRACT**: The present investigation was undertaken to examine the genetic divergence in 50 mungbean germplasm lines for 13 characters using Mahalanobis $D^2$ statistics. The genotypes grouped into eight clusters. Cluster VII had maximum intra-cluster distance while inter-cluster distance was highest between clusters V and VII. Cluster means indicated that none of the clusters was superior for all the characters studied. Therefore, hybridization between genotypes belonging to different clusters is suggested for development of superior genotypes. 10 SSR primers were used for molecular study of which only one gave slight difference among 19 mungbean genotypes. The quality and quantity of DNA used for amplification by PCR is the key to reproducible results and success of genotyping. Especially, DNA purity is extremely crucial for obtaining clear and discriminate patterns. DNA extraction from mungbean is difficult due to presence of contaminants such as phenols. Therefore, the present study was under taken to obtain high quality and pure DNA in mungbean. With few modifications four different DNA extraction protocols were tried in the present study to obtain high quality and pure DNA *viz.*, (I) Doyle and Doyle (1987), (ii) Method of Murray and Thompson (1980), (iii) Porebski *et al.*(1997), and (iv) Lin *et al.* (2001). Out of the four methods tried for DNA extraction, the method of Lin *et al.* (2001) was found most efficient, as the DNA obtained through this protocol was relatively pure which gave amplyfying products in the PCR. The genotype used for the standardization was MGG -361. Molecular characterization of 19 randomly chosen mungbean genotypes was attempted with the eight standardized primers. None of the primers showed scorable polymorphism. The primers VR4, VR5 and VR9, exhibited non specific bands, in addition to the monomorphic bands.

**Key words:** Mungbean, $D^2$ statistics, Genetic diversity, DNA extraction, Standardization, SSR primers.

## INTRODUCTION

Pulses compliment the daily diet of Indians along with cereals. These are rich in proteins with satisfactory proportion of carbohydrates. Green gram (*Vigna radiata* (L.) Wilczek) or mungbean is a diploid (2n=22), self pollinated widely cultivated pulse crop in India. High protein, easy digestibility and low flatulence production made the crop more acceptable by the people world over. In India, mungbean occupies an area of 34.4 lakh hactares with a production of 14 lakh tonnes and with a productivity of 407 $kg^{-1}$ha (2010-2011). In Andhra Pradesh it is cultivated in 2.84 lakh hactares with a production of 1.34 lakh tonnes and with a productivity of 471 $kg^{-1}$ha. (2011-2012). Genetic diversity is an important factor and also a prerequisite in any hybridization programme. Multivariate analysis by means of $D^2$ statistic is a powerful tool in quantifying the degree of divergence at genotypic level. Success of the hybridization followed by selection depends largely on the selection of parents with high genetic diversity for traits of interest (Murthy and Arunachalam 1966). Therefore the present investigation was planned to assess the genetic divergence among 50 genotypes of mungbean. The quality and quantity of DNA used for amplification by PCR is the key to reproducible results and success of genotyping. Especially, DNA purity is extremely crucial for obtaining clear and discriminate patterns. DNA extraction from mungbean was difficult due to presence of contaminants such as polyphenols. These compounds have also been reported to cause difficulty in DNA purification in other plant species and inhibit enzyme action. Presence of phenols and other contaminants offer difficulty in pipetting DNA and make DNA unamplifiable in PCR reaction by inhibiting *Taq* DNA polymerase. Therefore, the present study was taken to obtain high quality and pure DNA in mungbean. Molecular markers provide an opportunity to characterize genotypes and to measure genetic relationships more precisely than morphological and biochemical markers (Botstein *et al*., 1980).

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Molecular markers being easily reproducible have become favourite tools with breeders and biotechnologists to discern the traits as well as to study diversity among cultivars (Tara Satyavathi *et al*., 2005). Among various molecular markers, because of their abundance, high polymorphism between individuals within populations or closely related genotypes, their multi-allelic and co-dominant nature allowing the genotype establishment of individuals, SSRs or microsatellite markers have been used for individual identification, diversity analysis (Powell *et al.,* 1996).

## MATERIALS AND METHODS

The experiment material consisted of 50 genotypes of mungbean obtained from various pulse coordinating centres of India. The experiment was carried out in a in randomized block design with three replications at Regional Agricultural Research Station, Warangal during *Rabi* 2011-12. Each genotype was sown in three rows of 4m length/genotype/replication was a spacing of 30x10 cm. recommended package of practices were followed to raise the crop. Observations were recorded on randomly chosen five competitive plants on 13 metric characters *viz*., days to 50% flowering, days to maturity, plant height, number of primary branches per plant, number of clusters per plant, number of pods per plant, pod length, number of seeds per pod, 100-seed weight, seed yield per plant physiological traits like harvest index, leaf area and quality trait protein content. The replicated data were subjected to genetic divergence analysis using Mahalanobis $D^2$ statistic (Mahalanobis 1936) as suggested by Rao (1952). All the genotypes were grouped into respective clusters on the basis of $D^2$ values following Tocher's method. A set of ten SSR primers reported by Sreethireddy et *al*. (2008) were used for the present study to characterize 19 randomly selected mungbean genotypes. List of 19 mungbean genotypes used for molecular analysis are furnished in (Table 1). Molecular characterization of 19 mungbean genotypes was attempted using 10 SSR primers at the biotechnology laboratory, RARS, Warangal. The list of genotypes and primers used for the molecular study are presented in table 1 and 2 respectively.

**Table 1. List of 19 mungbean genotypes used for molecular analysis**

| S.No | Genotype | Source |
|---|---|---|
| 1 | GIVT -203 | IIPR, Kanpur |
| 2 | KM – 8-657 | IIPR, Kanpur |
| 3 | EC – 19515 | NBPGR, New Delhi |
| 4 | PDM – 11 | IIPR, Kanpur |
| 5 | RM – 9-126 | IIPR, Kanpur |
| 6 | RM -9 -128 | IIPR, Kanpur |
| 7 | LGG – 538 | Lam, ANGRAU |
| 8 | Km – 8 -651 | IIPR, Kanpur |
| 9 | MGG – 335 | Madhira, ANGRAU |
| 10 | KM – 8-666 | IIPR, Kanpur |
| 11 | NM – 1 | NDUAT, Faizabad |
| 12 | MGG-361 | Madhira, ANGRAU |
| 13 | MGG – 349 | Madhira, ANGRAU |
| 14 | Pusa – 9531 | IARI, New Delhi |
| 15 | MGG – 353 | Madhira, ANGRAU |
| 16 | MGG – 351 | Madhira, ANGRAU |
| 17 | KM-8-605 | IIPR, Kanpur |
| 18 | KM-8-664 | IIPR, Kanpur |
| 19 | UPM -84-178 | IIPR, Kanpur |

### Chemicals used: (as per the protocol)

1) Extraction buffer, which is made by mixing of 1.25 mM NaCl, 100 mM Tris cl, 25 mM EDTA, 2% CTAB, 0.2% β-mercapto-ethanol, 3% Poly Vinyl Pyrolidine and required amount of distilled water.
2) Tris EDTA (TE) buffer : 10 mM Tris Cl, 1 mM EDTA
3) 95% cold ethanol, 70% Ethanol (70ml ethanol+30ml Milliq water) and absolute ethanol.
4) Ice cold Isopropanol.
5) C.I: Chloroform: Iso amyl alcohol, 24: 1v/v).
6) P.C.I (Phenol: Chloroform: Iso amyl alcohol, 25: 24: 1).
7) 5 M NaCl
8) RNase A (10 mg/ml).
9) 2 M Sodium acetate.

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**Table2. List of primers used and their Tm as standardized in the present study.**

| S.No | Primer Code | Primer sequence | Tested temperatures ($^oC$) | Best worked at ($^oC$) | Also worked at ($^oC$) |
|---|---|---|---|---|---|
| 1 | VR-1F<br>VR-1R | **F:** GCCCTTCGTGCTAGGAAAT<br>**R:** CCCTACCGGTTGGTTGGT | 53, 55, 56, 58 and 62 | 53 | 55 |
| 2 | VR-2F<br>VR-2R | **F:** CGCCCCTCTAGGTTGGTTGG<br>**R:** GGAAAGACGAAGGGTAGAA | 53, 55 , 56, 58, and 62 | NA | - |
| 3 | VR-3F<br>VR-3R | **F:** GCCCCCCTTAGGTTGGTTGG<br>**R:**CCTTGTATTTGGATTCACAAGA | 53, 55 , 56 , 58 and 62 | NA | - |
| 4 | VR-4F<br>VR-4R | **F:**TGGTTGGTTGGTTCACAAGA<br>**R:**CACGGGTTCTGTCTCCAATA | 58 | 58 | - |
| 5 | VR-5F<br>VR-5R | **F:**TCACAAAGGGAGGGAAGAGA<br>**R:**CCCCAGGTTTGGTTGGTTGGA | 53, 55 , 56 , 58 and 62 | 53 | 55 |
| 6 | MB-7F<br>MB-7R | **F:**CTTGCTTGCGAGGATGAC<br>**R:**TCCAGTGCAGCAGATTGA | 53 , 55 , 56, 58 and 62 | 53 | 55 |
| 7 | VR-9F<br>VR-9R | **F:**TGACGGAGAGAGAGAGAGAGAG<br>**R:**TGCTTCCTTTTGTCTGAGTTAGAA | 58 | 58 | - |
| 8 | MB-3F<br>MB-3R | **F:**GCAGCAACAACAGCAACA<br>**R:**GCAGGTTTTGTGGCTCAG | 58 | 58 | - |
| 9 | MB-4F<br>MB-4R | **F:**TGGAATTTGGAAGGAAGGA<br>**R:**GATGCAGGTGTTTGGGAG | 53, 55, 56, 58 and 62 | 53 | 55 |
| 10 | MB-7F<br>MB-7R | **F:**GGAGAGGAAGGAACAGGG<br>**R:**GGCAGAGCATAACATGGC | 58 | 58 | - |

Four protocols (enlisted below) were followed for standardization of DNA isolation from mungbean. For this purpose radical tips of 0.3 mm length and young leaves of one week old seedlings of mungbean were used. 4 protocols were used for DNA extraction to give good quality DNA enable to PCR analysis.

The details of four DNA extraction protocols tried in the present study are given below:

1. CTAB method of DNA extraction by (Doyle and Doyle 1987)
2. DNA extraction by Murray and Thompson (1980) with few modification
3. DNA extraction by Porebski *et al.* (1997) with few modifications
4. DNA extraction by Lin *et al.* (2001) with few modifications

## CTAB method of DNA extraction (Doyle and Doyle 1987)

Young leaf samples from 1 week old seedlings, were collected directly in eppendorf tubes were ground into fine powder with the help of micro pestle by freezing in liquid Nitrogen which facilitates easy grinding**.** To the powder 400 µl CTAB extraction buffer with 0.2% β- mercapto ethanol was added and the sample tubes were kept in water bath at 60 $^0$C for 30-60 min with occasional and proper mixing after every 5-10 minutes, such that to see not to form any clumps at the bottom. Tubes were removed from water bath and then contents were allowed to cool to room temperature. Equal volume of chloroform: isoamyl alcohol (24:1 v/v) was added and mixed thoroughly by gentle inversion for 15 minutes by keeping in rotator @ 20 rpm (Rotospin, Tarsons) until clear separation of three layers is attained. After that contents were centrifuged at 12000 rpm for 12 minutes at 24$^0$C temperature. The clear aqueous phase (supernatant) was carefully pipetted out into new tubes. The chloroform: isoamyl alcohol (24:1 v/v) step was repeated twice to remove the organic contaminants in the supernatant. To the supernatant, cold isopropanol of about 0.5 to 0.6 volumes (2/3$^{rd}$ of pipette volume) was added. The contents were mixed gently by inversion. Subsequently the tubes were centrifuged at 12000 rpm for 12 min at 24$^0$C temperature to pellet out DNA. The supernatant was discarded gently and the DNA pellet was washed with 70% ethanol and centrifuged at 10,000 rpm for 4-5 min. This step was repeated twice. The tubes were allowed for air drying until the pellet gets completely dried and then the pellet was dissolved in 40 µl TE buffer. DNA was stored in -20$^0$C for further use.

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**DNA extraction method of Murray and Thompson (1980) with few modifications**

15-20 radical tips were ground to fine powder in liquid nitrogen using a pestle and mortar. To the powder 500 µl of extraction buffer was added. Suspended the clumps with a spatula then transferred into eppendorf tubes and incubated for 1 hr with frequent mixing in a water bath maintained at 65°C. 500 µl of chloroform: iso-amyl alcohol (24:1) was added and mixed gently @ 20 rpm speed for 10 min. The tubes were centrifuged at 13000 rpm for 15 min and the supernatant was collected into new eppendorf tubes. This step was repeated twice. DNA was precipitated by adding 2/3$^{rd}$ volume of ice-cold Isopropanol followed by gentle mixing and incubated for 1 hr at -20$^{\circ}$C followed by centrifugation at 13000 rpm for 15 min at 4°C. The resultant supernatant was discarded and DNA pellet was washed with 1 ml of 70% ethanol then allowed for air drying and was dissolved in 50µl of TE buffer.

After that the DNA was transferred to new eppendorf tube using half cut tip (to avoid DNA shearing), and 3 µl RNase (10 mg/ml) (pre-boiled) was added to the DNA solution and incubate at 37$^{0}$ C for 1 hr. Re-precipitated the DNA by adding 0.1 volume of 5 M ammonium acetate and two volumes of 100% ethanol followed by gentle mixing and stored at -20$^{0}$ C for 1 hr. The samples were centrifuged at 13000 rpm for 10 min and the supernatant was drained out. 500 µl of 70% ethanol was added to the DNA pellet and centrifuge at 13000 rpm for 10 min. The supernatant was drained carefully and the DNA pellet was air dried over night. The DNA pellet was dissolved in 50 µl of TE buffer.

**DNA extraction method of Porebski *et al.* (1997) with few modifications**

0.5 gm of cotylidonary tips were grounded using mortar and pestle in liquid nitrogen until finely powdered. Frozen ground tissue was transferred to eppendorf tubes. 500 µl of pre heated (60°C) extraction buffer was added. The contents were mixed by gentle inversion and incubated in a water bath maintained at 60 $^{0}$C (with intermittent gentle shaking) for 25-60 min. The tubes were removed from water bath and cooled to room temperature for 4-6 min. 500 µl of chloroform: isoamyl alcohol (24:1) was added and mixed by inversion to form an emulsion. After mixing thoroughly they were centrifuged at 3000 rpm for 20 min in a centrifuge at room temperature. The top aqueous phase was transferred carefully to new eppendorf tubes using wide-bore pipette tip. Chloroform-isoamyl alcohol extraction step was repeated again to remove cloudiness in aqueous phase. 1/2 volume of 5 M NaCl was added to the final aqueous solution recovered and mixed well. Two volumes of cold (-20°C) 95% ethanol was added and mixed by gentle inversion and placed in -20$^{\circ}$C for 10 min to accentuate precipitation. The solution was left at 4-6°C to precipitate overnight. Centrifugation was done at 3000 rpm for 6 min. Supernatant was discarded and pellet was washed with cold (0-4°C) 70% v/v ethanol. The pellet was dried and dissolved in 40 µl of TE buffer and left overnight at 4-6°C.

3 µl RNAse A (10 mg/ml) was added and incubated at 37 $^{0}$C in water bath for approximately 1 hr, the tubes were removed and 3 µl proteinase K (1 mg/ml) was added and incubated at 37$^{0}$ C for 15-30 min. 150 µl of phenol and 150 µl of chloroform was added to each eppendorf tube. They were rotated for a brief period and centrifuged at 14,000 rpm for 10-15 min. Upper layer was collected in new eppendorf tube.

1/10$^{th}$ volume 2 M Sodium acetate and 2 volumes of absolute ethanol were added and mixed gently. The samples were left overnight in -20$^{0}$ C. Samples were centrifuged at 14,000 rpm for 10-20 min. Later the samples were drained and washed with 70% v/v ethanol and the tubes were air dried. 40 µl TE buffer was added and allowed time for complete re-suspension.

**DNA extraction by the method of Lin *et al.* (2001) with few modifications**

Collected leaf samples /root tips of 0.3 mm length from 15-20 germinated seeds of greengram were grounded into fine powder with the help of micro pestle in liquid nitrogen**.** 400 µl CTAB extraction buffer with 0.2% β-mercaptoethanol was added and the sample tubes were kept in water bath at 65$^{0}$C for 30 min with occasional and gentle but proper mixing after every 5-10 minutes. Tubes were removed from water bath and then contents were allowed to cool to room temperature. Equal volume of phenol: chloroform: isoamyl alcohol (25:24:1 v/v) was added and mixed thoroughly by gentle inversion for 15 minutes by keeping in rotator @ 20 rpm until clear separation of three layers is attained. After that contents were centrifuged at 12000 rpm for 12 minutes at 24$^{\circ}$C temperature. The clear aqueous phase (supernatant) was carefully pipetted out into new tubes. To the supernatant cold isopropanol of about 0.5 to 0.6 volume (2/3 rd of pipetted volume) was added. The contents were mixed gently by inversion. Subsequently the tubes were centrifuged at 12000 rpm for 12 min at 24$^{\circ}$C temperature to pellet out DNA. The supernatant was discarded gently and the DNA pellet was washed with 70% ethanol and centrifuged at 10,000 rpm for 4-5 min. This step was repeated twice. The tubes were air dried and then the pellet was dissolved in 40 µl TE buffer and stored at -20$^{\circ}$C over night. 3 ul RNase A (10 mg/ml) was added and the sample tubes were kept in water bath at 37$^{0}$C for 1 hr. Tubes were removed from water bath and then contents were allowed to cool to room temperature. 400 ul of phenol:chloroform:isoamyl alcohol (25:24:1 v/v) was added and mixed thoroughly by gentle inversion for 15 minutes by keeping in rotator @20 rpm until clear separation of three layers is attained. After that contents were centrifuged at 12000 rpm for 12 minutes at 24$^{\circ}$C temperature.

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The clear aqueous phase (supernatant) was carefully pipetted out into new tubes. To the supernatant cold isopropanol of about 0.5 to 0.6 volume (2/3 rd of pipetted volume) was added. The contents were mixed gently by inversion. Subsequently the tubes were centrifuged at 12000 rpm for 12min at 24°C temperature to pellet out DNA. The supernatant was discarded gently and the DNA pellet was washed with 70% ethanol and centrifuged at 10,000 rpm for 4-5 min. This step was repeated twice. The tubes were allowed for air drying until the pellet gets completely dried and then the pellet was dissolved in 40 µl TE buffer. DNA was stored in -20°C for further use.

**DNA quantification**

0.8 gm of agarose was weighed and added to 100 ml of 1X TAE buffer to get final concentration of 0.8%. Agarose was dissolved properly by boiling in microwave oven and then cooled to room temperature. Just before complete cooling 3 µ1 of ethidium bromide (10 mg/ml) was added to it and mixed thoroughly. Gel casting tray was prepared by fitting the comb properly into the gel casting tray in such a way that 2mm gap was maintained between the bottom of the tray and comb tip. The melted cool agarose (with ethidium bromide) was poured into the casting tray carefully without formation of any air bubbles and left for solidification for 20-30 min. After proper solidification of the gel, the casting tray along with gel was placed horizontally in electrophoresis apparatus and the comb from casting tray was removed carefully. Required amount of 1X TAE solution was added to the apparatus such that the gel was completely immersed. DNA samples for loading were prepared by placing of 2 µ1 of 6X loading buffer on parafilm, to which further 2 µl of DNA was added. The DNA samples from parafilm were gently loaded in to the wells of the gel.

λ DNA of known quantity having concentration of *viz.*, 50 ng, 100 ng and 200 ng was loaded in order to quantify the DNA samples. The gel was run at 70V, till the tracking dye migrated to the bottom of the gel. The electrophoresed DNA samples were visualized using a UV gel documentation system (Syngene G BOX HR, UK) and the same was photographed and documented.

**Polymerase Chain Reaction (PCR)**

Polymerase chain reaction (PCR) is used to selectively amplify *in vitro* a specific segment of the DNA to a billion fold (Mullis et al., 1986). In the present study, amplification was carried out in 10 µl reaction mixture by using thermo cycler PCR. Reaction mixture used for SSR PCR containing 2 µl (15 ng/ul) of DNA, 1 µl (10X) of Taq buffer (10 mM Tris Cl, pH 8.3, 50 mM KCl, 0.001% gelatin), 1 µl (1mM) dNTPs, 0.3 µl (2units/ µl) of Taq polymerase, 1µl each of forward and reverse primer (5pmols/ µl), made upto 10 µl with milliq water.

**Standardization with primers**

Ten primers were standardized (table 2) for their Tm using one genotype *i.e* MGG-361**.** The PCR conditions used for standardization was: Initial denaturation of 94°C for 4 minutes; 35 cycles of denaturation at 94°C for 1 minute, annealing temperatures (as given in table 2) for 1 min, extension at 72°C for 1 min; final extention at 72°C for 7 min; storage at 4°C. The PCR amplified products were resolved on 3% agarose gel. The agarose gel was prepared by adding 3 gm of agarose to 100 ml 1X TAE buffer and boiled carefully till the agarose was completely melted. Just before complete cooling 3µ1 ethidium bromide (10 mg/ml) was added and the gel was poured in the tray containing the comb carefully avoiding air bubbles to form. The solidified gel was transferred to horizontal electrophoresis apparatus and 1X TAE was added to immerse the gel. PCR product was mixed with 3 µl of 6X loading dye and the sample was loaded in the well carefully. 50 bp ladder was loaded as reference marker. The gel was run at constant voltage of 70V for about 4 hours, until the ladder got properly resolved. Gel was photographed using Gel Documentation system.

Genomic DNA was extracted from young leaves by CTAB method (Lin *et al.* (2001) with few modifications. SSR amplification reactions were carried out in volume of 10 µl containing 30 ng/ µl of genomic DNA, 0.6 Units of *Taq* DNA polymerase, 0.1 mM dNTP, 0.15 µM of primer (each forward and reverse). PCR conditions were programmed for initial denaturation at 94 $^0$C for 3 min, 30 cycles of 30 sec denaturation at 94$^0$ C, 30 sec annealing at different temperatures of 53 $^0$C, 55 $^0$C, 58 $^0$C and 1min extension at 72$^0$ C ,followed by final extension for 7 min at 72$^0$ C. Amplified products were electrophoresed on 4% agarose gel.

## RESULTS AND DISCUSSION

Analysis of variance revealed that a wide range of variability existed for all the traits studied indicating the presence of significant variation among the genotypes. Based on the $D^2$ analysis, all the genotypes were grouped into eight different clusters. (Table-3) Cluster one was largest with 41 genotypes, cluster VII with three genotypes and other clusters II, III, IV, V, VI and VIII possessing one genotype each. Clustering pattern indicated that the genotypes originating from different geographical regions grouped together into different clusters showing no parallelism between genetic diversity and geographical distribution. Our results are on par with the findings of Raje and Rao *et al*. (2001), Dasgupta *et al*. (2005) and Majumder et al. (2007).

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This implies that the seletion of parents for hybridization based on geographical origin would be arbitrary. The grouping of genotypes from same source into different clusters as observed in present study may be because of free exchange of breeding material among different regions, there by the character constellation associated with a particular region, in nature loose their individuality under human interference. This may be attributed to the fact that these promising lines of mungbean were received from different breeding centres of the country (Birari and Ghanekar 1992).

**Table.3: Clustering pattern of mungbean (Vigna radiata (L). Wilczek) genotypes by Tocher's method.**

| Cluster No | No. of genotypes | Names of the Genotypes |
|---|---|---|
| I | 41 | EC-19515, KM-8-651, KM-8-664, NM-1, WGG-2, MGG-351, C0GG-975,PDM-99-3, MGG-347, CO-7,PUSA-105, MGG-351, KM-203, SML-1023, MGG-361,LGG-521, MGG-335, LGG-538, COGG-936, RM-9-126, PDM-54, PUSA- 9531, KM-8-657, RM-9-136, GIVT-213, CO-6, UPM-84-178, MGG-353, MGG-349, KM-8-666, GIVT-203, LGG-410, KM-11-542, KM-11-567, WGG-37, KM-8-662, LGG-528, ML-326, KM-8-605, KM-11-546, RM-9-122, ASHA. |
| II | 1 | LGG-547 |
| III | 1 | RM-9-128 |
| IV | 1 | KM-11-351 |
| V | 1 | PDM-11 |
| VI | 1 | KM-11-570 |
| VII | 3 | VG-6197A, WGG-42, EC-396117 |

Seed yield per plant contributed maximum towards genetic divergence followed by test weight , primary branches per plant, number pods per plant, harvest index (%), seeds per pod, plant height, leaf area and days to maturity, protein (%), days to 50% flowering, clusters per plant, pod length respectively to the genetic divergence in decreasing order, (Table-4). Seed yield contributed maximum (29.55%) to the total divergence. Our findings are confirmed with the resulta of Backiyarani *et al*. (2000), Indradeo Pandey (2007) and Haritha and Reddy Sekhar (2003).

The average intra and inter cluster $D^2$ values are presented in Table 5. Intra cluster $D^2$ values ranged from 0.00 (cluster II, III, IV, V, VI, VIII) to 7.09 (cluster VII). Higher intra cluster distance recorded for cluster VII followed by cluster I. Maximum intercluster distance was observed between cluster V and cluster VII (11.65) followed by cluster III and cluster VIII (11.42), cluster III and cluster VII (11.25), cluster II and cluster VII (11.14) and cluster VI and cluster VII (11.05), while the minimum inter cluster distance was noticed between cluster II and cluster IV (4.70) followed by cluster IV and cluster VI (5.58) and cluster II and cluster V (5.79) suggesting that the genotypes of these four clusters were not genetically much diverse. The highest intra-cluster distance in cluster VII indicates the presence of wide genetic diversity among the 3 genotypes (VG-6197A, WGG-42 and EC-396117) within the cluster. The maximum inter cluster distance (11.65) was observed between cluster V and VII indicates the presence of wide diversity between two clusters that expected to give higher frequency of better segregants or desirable combinations for development of useful genetic stocks or varieties. The cluster means of different characters for each of 13 characters are presented in Table 6. From the data we can conclude that considerable differences exist for all the traits studied. It indicated that the cluster mean for days to 50 per cent flowering was highest in cluster II and cluster III (39.67) and the lowest in cluster VI (31.33). Days to maturity was highest in cluster II (72.33) and the lowest in cluster VI (58.33). Cluster V recorded highest cluster means per plant height (63.30 cm) and lowest in cluster VII (41.98 cm).

Cluster III recorded the highest number of primary branches per plant (3.00) and the lowest number of primary branches per plant was in cluster IV (0.93). Cluster II (12.13) and Cluster VI (5.33) recorded the highest number of clusters per plant respectively. The number of pods per plant was highest in cluster V (46.80) and the lowest in cluster cluster VII (22.73).

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**Table 4: Relative contribution of each character to the diversity in mungbean (*Vigna radiata* (L.) Wilczek).**

| S. No. | Characters | Times ranked first | Contribution (%) |
|---|---|---|---|
| 1 | Days to 50% flowering | 10 | 0.82% |
| 2 | Days to maturity | 58 | 4.08% |
| 3 | Plant height (cm) | 53 | 4.41% |
| 4 | Number of primary branches per plant | 149 | 12.16% |
| 5 | Number of clusters per plant | 16 | 1.31% |
| 6 | Number of pods per plant | 102 | 8.33% |
| 7 | Pod length (cm) | 11 | 0.90% |
| 8 | Number of seeds per pod | 91 | 7.43% |
| 9 | Test weight (gm) | 168 | 13.71% |
| 10 | Seed yield per plant (gm) | 362 | 29.55% |

The maximum cluster mean for pod length was recorded in cluster VII (9.31) and the minimum in cluster IV (6.90). The number of seeds per pod was highest in cluster IV (13.33) and the lowest in cluster VII (10.42). For test weight highest value was recorded in cluster VII (6.52gm) and the lowest in cluster II (3.27gm). Cluster VIII recorded the highest seed yield per plant (20.67g) while, in cluster I it was low (7.65g). High protein (%) was recorded in cluster III (23.81 %) and low in cluster VI (19.23%). Cluster IV recorded the while it was lowest in cluster II (31.83). Cluster IV (40.83%) recorded the highest harvest index in the cluster IV while it was lowest in cluster II (31.83). High cluster mean for leaf area was recorded in cluster VI (471.67) and low in cluster VIII (257.00). Cluster VIII showed the highest mean value for seed yield per plant. Cluster V recorded highest mean value for number of pods per plant. Cluster VII showed maximum mean value for test weight. Cluster IV recorded the highest mean value for number of seeds per pod. Cluster II recorded highest mean value for number of clusters per plant. Crosses between genotypes VG-7890A (VIII) and PDM-11 (V) and VG-6197A, WGG-42, EC-396117 (VII), KM-11-351 (IV) and LGG-547 (II) are expected to exhibit high heterosis and might result in high yielding segregants with desired traits. Cluster III has the highest mean value for protein content, cluster IV for high harvest index, cluster VII showed highest mean value for leaf area and good harvest index with lowest mean value for days to 50% flowering and days to maturity. Hence, the genotype KM-11-570 can be considered for earliness and VG-7890 A might result in high yielding early duration greengram varieties. Crosses between genotypes VG-7890A (VIII) and PDM-11 (V) and VG-6197A, WGG-42, EC-396117 (VII), KM-11-351 (IV) and LGG-547 (II) are expected to exhibit high heterosis and might result in high yielding segregants with desired traits. The promising genotypes having outstanding mean values for yield and yield component traits are VG-7890A (Cluster-VIII) for Seed yield per plant, PDM-11 (Cluster V) for number of pods per plant, VG-6197A, WGG-42 and EC-396117 (Cluster V) for test weight, KM-11-351 (Cluster IV) for number of seeds per pod, LGG-547 (Cluster II) for number of Clusters per plant, KM-11-570 (Cluster VII) for days to 50% flowering, days to maturity, leaf area and harvest index.

**Table 5: Mean intra (diagonal) and inter- cluster distance among 8 clusters formed by Tocher's method in mungbean (*Vigna radiata* L.) genotypes during *rabi*, 2011-2012.**

| Clusters | Cluster I | Cluster II | Cluster III | Cluster IV | Cluster V | Cluster VI | Cluster VII | Cluster VIII |
|---|---|---|---|---|---|---|---|---|
| **Cluster I** | **5.21** | 7.99 | 7.66 | 7.15 | 8.98 | 7.44 | 9.93 | 10.71 |
| **Cluster II** | | **0.00** | 10.70 | 4.70 | 5.79 | 7.23 | 11.14 | 6.67 |
| **Cluster III** | | | **0.00** | 9.91 | 7.96 | 7.99 | 11.25 | 11.42 |
| **Cluster IV** | | | | **0.00** | 7.23 | 5.58 | 10.10 | 7.54 |
| **Cluster VI** | | | | | **0.00** | 6.46 | 11.65 | 6.81 |
| **Cluster VII** | | | | | | **0.00** | 11.05 | 8.59 |
| **Cluster VIII** | | | | | | | **7.09** | 10.93 |
| | | | | | | | | **0.00** |

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Four different DNA extraction protocols were tried with few modifications to obtain high quality and pure DNA. Out of the four methods tried for DNA extraction, the method of Lin *et al.* (2001) (Fig.1) was found most efficient, as the DNA obtained through this protocol was relatively pure which gave amplifying products in the PCR. Standardization was done by using one genotype *i.e* MGG-361. Of the 10 primers used, 8 could produce amplicons at different Tms (Table 2; Fig. 2). Four of the primers *i.e.,* VR-4, VR-9, MB-13 and MB-77 produced best amplicons at 58°C and four other primers *i.e.,* VR-1, VR-5, MB-7 and MB-14 amplified best at 53°C. Two primers VR-2 and VR-3 did not produce any amplicons at tested temperatures (Table 2). Hence the eight amplified primers were used for further study.

**Table: 6: Mean values of 8 clusters obtained by Tocher's method estimated from genotypes of Mungbean (*Vigna radiata* L.*)* during *rabi,* 2011-12.**

| Cluster No. | Days to 50% flowering | Days to maturity | Plant height | Number of primary branches per plant | Number of Clusters per plant | Number of pods per plant | Pod length | Number of Seeds per pod | Test weight | Seed yield per plant | Protein content (%) | Harvest index (%) | Leaf area |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cluster I | 38.93 | 67.94 | 54.20 | 1.13 | 7.40 | 25.44 | 7.24 | 10.67 | 3.51 | 7.65 | 22.02 | 32.87 | 414.6 |
| Cluster II | 39.67 | 72.33 | 53.53 | 1.20 | 12.13 | 40.40 | 7.03 | 11.60 | 3.21 | 16.50 | 20.84 | 31.83 | 413.13 |
| Cluster III | 39.67 | 64.67 | 53.27 | 3.00 | 6.33 | 31.53 | 7.37 | 12.47 | 3.85 | 8.07 | 23.81 | 37.23 | 322.07 |
| Cluster IV | 34.67 | 62.67 | 57.67 | 0.93 | 8.70 | 24.67 | 6.90 | 13.33 | 3.37 | 14.34 | 21.43 | 40.83 | 450.00 |
| Cluster V | 39.00 | 70.67 | 63.30 | 2.60 | 8.67 | 46.80 | 7.10 | 10.47 | 3.49 | 18.17 | 23.25 | 36.07 | 382.43 |
| Cluster VI | 31.33 | 58.33 | 60.00 | 1.87 | 5.33 | 27.67 | 7.97 | 10.67 | 3.23 | 13.45 | 19.23 | 40.30 | 471.67 |
| Cluster VII | 36.11 | 67.11 | 41.98 | 1.29 | 6.60 | 22.73 | 9.31 | 10.42 | 6.52 | 9.99 | 22.43 | 38.68 | 386.98 |
| Cluster VIII | 37.00 | 64.33 | 36.33 | 1.80 | 6.60 | 29.47 | 7.30 | 10.47 | 4.32 | 20.67 | 23.21 | 35.97 | 257.00 |

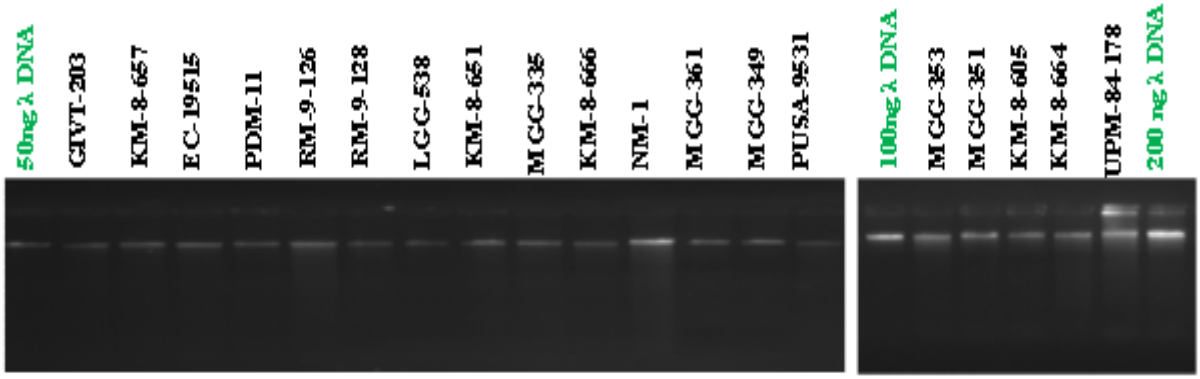


**Figure-1: Genomic DNA isolation by Lin et al (2001) method with few modifications.**

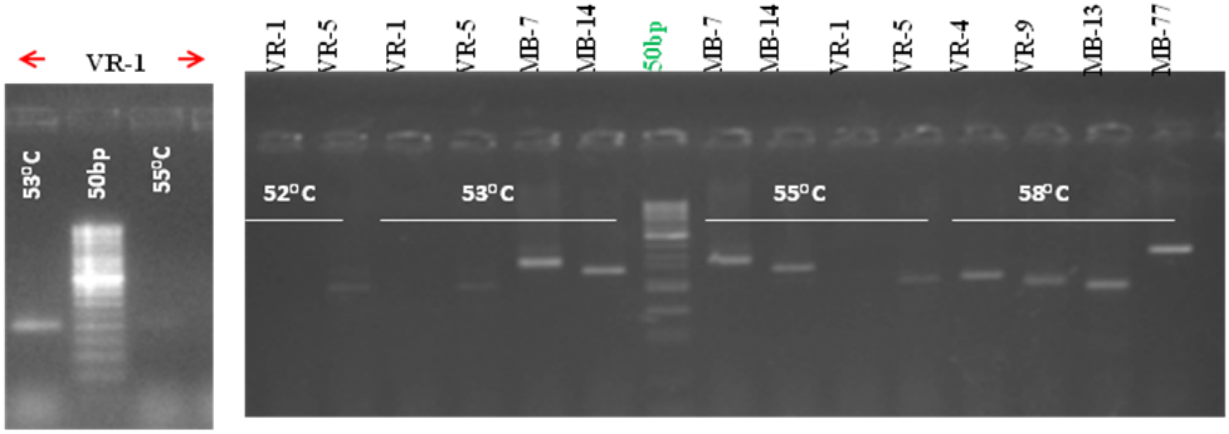


**Figure-2: Standardization of primers with green gram genotype MGG-361**

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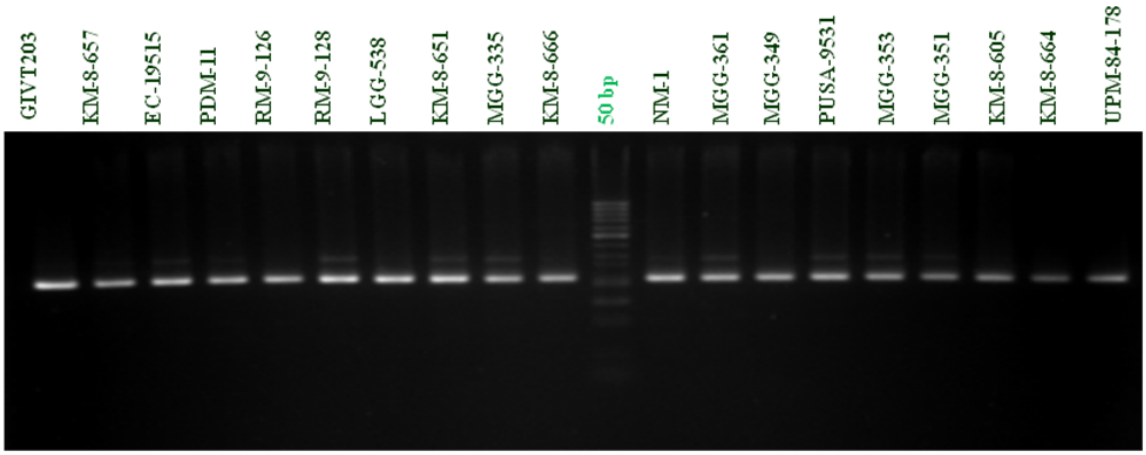


**Figure-3: Amplication profile of 19 green gram genotypes with VR-4 primer**

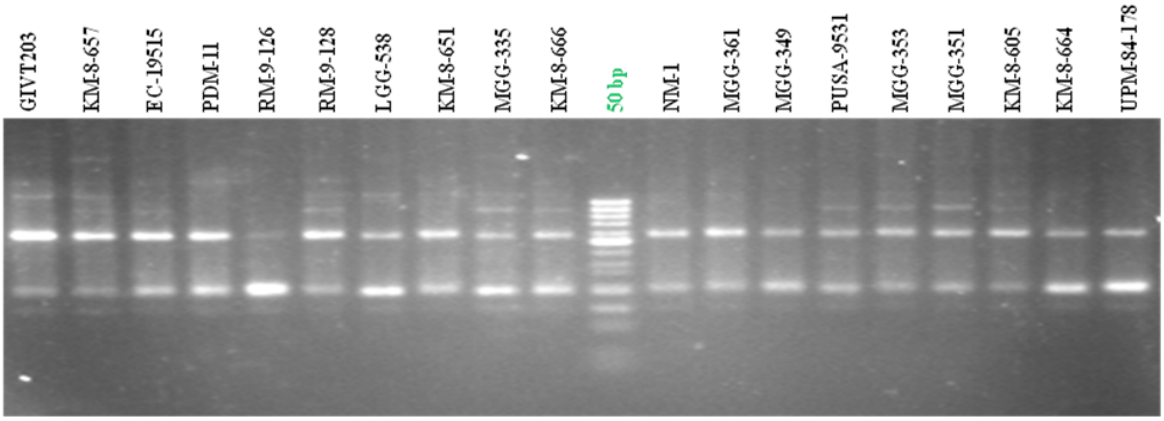


**Figure-4: Amplication profile of 19 green gram genotypes with VR-9 primer**

Molecular characterization of 19 randomly chosen mungbean genotypes was attempted with the eight standardized primers. None of the primers showed scorable polymorphism. The primers VR4, VR5 and VR9, exhibited non specific bands, in addition to the monomorphic bands. Amplification profiles of primers VR-4 and VR-9 are shown in Figs. 3 and 4 respectively. In the present study, as all genotypes produced similar sized amplicons with all the eight primers, it was not possible use them for diversity analysis. Though, same primers were used by Sreethi Reddy *et.al.* (2008), they reported seven primers to be polymorphic, with an average of 1.6 bands per primer. This might be due to the inclusion of genotypes from different geographical locations, including exotic collections, in their study which could result in considerable diversity. Gwag (2006) observed that of the 93 SSR primer pairs used, seven were polymorphic. This necessitates the utilization of more number of microsatellites to detect the polymorphism for genetic diversity studies especially while genotyping local varieties. The potentiality of SSRs in mungbean diversity analysis has also been reported by many earlier workers (Sangiri *et al.* 2007, Dikshit *et al*. 2007). Ranade, 2009 revealed that using of more sensitive techniques for DNA fragment size analysis like PAGE or capillary electrophoresis (Sutapa Dutta *et al*. 2011) may give better results. In the present study too, if more number of SSR primers are used or if more sensitive resolving systems are used, it would be possible to obtain better results and detect the diversity among the mungbean genotypes used.

## REFERENCES

Backiyarani, S., Nadarajan, N., Rajendran, C and Shanthi, S. 2000. Genetic divergence for physiological traits in cowpea (*Vigna Unguiculata* (L).Walp). Legume research. 23(2) : 114-117.

Botstein, D., White, R. L., Skolnick, M and Davis, R. W. 1980. Constructio of genetic linkage map in man using restriction fragment length polymorphism. Am. J. Hum. Genet. 32: 314-331.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Birari, S.P., Ghanekar, S.L. 1992. Genetic diversity in Lablab bean. Journal of Maharastra Agricultural University. 17(2): 257-260.

Dasgupta, T., Mukherjee, K., Roychoudhury, B., Nath, D. 2005. Genetic diversity of horsegram germplasms. Legume Res., 28 (3): 166-171.

Dikshit, H. K., Jhang, T., Singh, N. K, Koundal K. R., Bansal, K. C., Chandra N, Tickoo J. L and Sharma, T. R 2007. Genetic differentiation of *Vigna* species by RAPD, URP and SSR markers. *Biologia Plantarum***,** 1(3): 451-457.

Doyle, J.J and Doyle, J.L. 1987. A rapid DNA isolation procedure for small amount of fresh leaf tissue. Phytochem. Bull. 19: 11-15.

Gwag, J. G, Chung, J. W. Chung, H. K. Lee, J. H. Ma, K. H. Dixit, A. Park, Y. J., Cho, F.G. Kim, T.S. and. Lee, S. H. 2006. Characterization of new microsatellite markers in mungbean, *Vigna radiata* (L.). Molecular Ecology Notes**.,** 1132-1134.

Haritha, S and Reddy Sekhar, M. 2003. Comparision of cluster formation by Mahalanobis's $D^2$ and Metroglyph analysis in mungbean (*Vigna radiata* (L.) Wilczek). Legume Research. 26 (2): 100 – 104.

Indradeo Pandey. 2007. Genetic diversity in grain cowpea (*Vigna unguiculata* (L.) Walp) *Legume Research*. 30 (2): 92-97.

Lin, R.C., Ding, Z.S., Lil, B and Kuang, T.Y. 2001. A rapid and efficient DNA minipreparation suitable for screening transgenic plants. Plant molecular biology reporter. 19: 379a–379e.

*Mahalanobis, P.C. 1936. On the generalised distance in statistics. *Proceedings of National Institute of Sciences*, India 2: 49-55.

Majumder, N.D., Mandal, A.B., Ram, T., Kar, C.S. (2011). Assessment of genetic diversity and other genetic parameters in blackgram. Crop Improvement**.** 38(1): 35-37.

Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, G and Erlich, H. 1986. Specific enzymatic amplification of DNA *in vitro*: the polymerase chain reaction. *Cold Spring Harb.* Symp. Quant. Biol. 51:263-273.

Murray, M.G. and Thompson, W.F. 1980. Rapid isolation of high molecular weight plant DNA. Nucl. Acid Res. Vol 8, 19: 4321-4325.

Murthy, B. R. and Arunahalam, V. 1966. The nature of genetic divergence in relation to breeding system in some crop plants. Indian Journal of Genetics and Plant Breeding. 26: 188-198.

Powell, W.M., Morgante, J.J., Doyle. J, W McNicol, S.V. Tingey and Rafalski, A.J. 1996. Genepool variation in genus *Glycine* sub genus *soja* revealed by polymorphic nuclei and chloroplast microsatellites. Genetics. 144: 793-803.

Raje, R.S and Rao, S.K. 2001. Genetic diversity in a germplasm collection of mungbean (*Vigna radiata* (L.) Wilczek). Indian Journal of genetics and plant Breeding**.** 61 (1): 50-52.

Rao, C.R. 1952. Advanced statistical methods in Biometric research. John Willey and Sons Inc., New York: 390.

Ranade,R and Gopalakrishna,T. 2009. Genetic diversity in blackgram (*Vigna mungo* L. Hepper) using amplified fragment length polymprphism (AFLP). Journal of Food Legumes**.** 22(3): 153-157.

Sangiri, C., Kaga, A., Tomooka, N., Vaughan, D and Srinives, P. 2007. Genetic diversity of the mungbean (*Vigna radiata*, Leguminosae) genepool on the basis of microsatellite analysis. Australian Journal of Botany, 55: 837–847.

Sreethi Reddy, K., Souframanien, J., Reddy, K.S and Dhanasekar, P. 2008. Genetic diversity in mungbean as revealed by SSR and ISSR markers. Journal of Food Legumes**.** 21(1): 15-21.

Sutapa Dutta, Giriraj Kumawat, Bikram P Singh, Deepak K Gupta, Sangeeta Singh, Vivek Dogra, Kishor Gaikwad, Tilak R Sharma, Ranjeet S Raje, Tapas K Bandhopadhya, Subhojit Datta, Mahendra N Singh, Fakrudin Bashasab, Pawan Kulwal, Wanjari, K.B., Rajeev K Varshney, Douglas R Cook and Nagendra K Singh. 2011. Development of genic –SSR markers by deep transcriptome sequencing in Pigeonpea (*Cajanus cajan* (L.) Millspaugh). BMC Plant Biology 11:17

Porebski S L, Bailey L G and Baum B R 1997 Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant molecular biology reporter 15(1): 8-15.

Tara Satyavathi, C. Bhat, K. V, Bharadwaj, C. Tiwari, S.P and Chaudhary, V. 2005. AFLP based DNA profiling and genetic diversity assessment of Indian soyabean (*Glycine max* (L.) Merrill) varieties. Genetic Resources and crop evolution 53: 1069-1079.