Plant Archives Volume 20 No. 1, 2020 pp. 1153-1163 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ASSESSMENT OF GENETIC DIVERSITY FOR YELLOW MOSAIC VIRUS RESISTANCE IN BLACKGRAM CULTIVARS BASED ON MOLECULAR AND MORPHOLOGICAL STUDIES

Malarkodi, A., S. Murugan*, R. Anandan, R. Thirumalai and J. Padmanaban

Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Annamalainagar-608 002 (Tamil Nadu) India.

Abstract

The investigation were conducted to screening for Forty genotypes of blackgram were of different origin were tested for the agronomic performance and YMV resistance. Field screening for YMV disease was carried out during summer, 2017 using infector row technique. Genetic diversity of the population was determined using molecular markers. A set of nineteen SSR markers were validated. The markers CEDG 004, CEDG008, CEDG011, CEDG013, CEDG020, CEDG056, CEDG092, CEDG139, CEDG180, CEDG198, CEDG044, CEDG014, CYR-1, YR-4 and YMV1 got amplified. The disease screening studies resulted in identification of genotypes with high resistance (AUBG 1, AUBG 6, VBN 4, VBN 8) and high susceptibility (AUBG 5, AUBG 11, AUBG 13, AUBG 15, AUBG 17, AUBG 19).

Key word: Blackgram, YMV, SSR Marker, Genetic Divergence.

Introduction

Blackgram (*Vigna mungo* L. Hepper) also called urdbean is a member of the Asian *Vigna* crop group. It is a staple crop in the central and South East Asia; however it is extensively used only in India and now grown in the Southern United States, West Indies, Japan and other tropic as and subtropics (Delic *et al*., 2009). India is the largest producer and consumer of black gram which is cultivated in an area about 3.26 million hectares with a production of 1.76 million tones. Blackgram is a short duration, self-pollinated, diploid grain legume (2n = 22) with a small genome size estimated to be 0.56pg/1C (574 Mbp) (Gupta *et al*., 2008). The productivity of pulses in India is less than half of the productivity levels in the USA and Canada, as the pulses are mainly grown under rainfed condition in India in the areas with high rainfall variability. To fulfil the growing requirement, the country has to produce enough pulses as well as remain competitive to protect the domestic production. It is imperative to develop and adopt more efficient crop-production technologies along with favorable policies and market support to encourage farmers to bring more area under pulses. The lack of an assured market is one of the major issues in the poor performance of pulses.

***Author for correspondence :** E-mail: agriman2001@yahoo.com

Screening may be either on natural field conditions or through forced inoculation employing virulent vectors. In both the situations, vector population remains specific for the disease spread. Practical difficulties include climatic conditions that greatly affect the building up of the vector population. If optimal conditions occur throughout the year, vector population will be present continuously. There is lack of uniform screening procedures and in many cases resistance is governed by recessive genes and hence significant delay in introgression of YMV resistance gene in elite blackgram lines. The efficacy of transmission and behaviour of the whiteflies varies with the host genotypes, vector biotypes and growth conditions. Screening based on natural occurrence in the hot spot areas has not given consistent results. Hence, the plant breeders and pathologists are in need of biotechnological and molecular tools that can lead to the identification of MYMV resistant and susceptible genotypes.

Among various DNA marker systems, SSR markers are considered the most ideal marker for genetic studies because they are multi-allelic, abundant, randomly and widely distributed throughout the genome, co-dominant

that could differentiate plants with homozygous or heterozygous alleles, simple to assay, highly reliable, reproducible and could be applied across laboratories and amenable for automation. Microsatellites or Simple Sequence Repeats (SSR) are DNA sequences with repeat lengths of a few base pairs. Variation in the number of repeats can be detected with PCR by designing primers for the conserved DNA sequence flanking the SSR. As molecular markers, SSR combine many desirable marker properties including high levels of polymorphism and information content, unambiguous designation of alleles, even dispersal, selective neutrality, high reproducibility, co-dominance, rapid and simple genotyping assays. Microsatellites have become the molecular markers of choice for a wide range of applications in genetic mapping and genome analysis (Li *et al*., 2000), paternity determination and pedigree analysis (Ayres *et al*., 1997), gene and quantitative trait locus analysis (Blair and Mc Couch, 1997) and marker-assisted breeding (Ayres *et al*., 1997; Weising *et al*., 1998). Thus the present study was undertaken to validate available SSR markers linked with YMV resistance in blackgram.

Materials and Methods

Plant material

Forty genotypes of blackgram were used for the present study. It comprised of lines whose resistance or susceptible response for YMV disease were not known, while some of the varieties disease reaction was known based on earlier literature.

Molecular markers

A total of fifteen SSR (Simple Sequence Repeats) markers were used for polymorphic studies.

Seed multiplication and sampling

The seeds of 40 genotypes obtained from various sources were raised during October 2016 for seed multiplication. The DNA of 40 blackgram genotypes were isolated from leaf samples collected from the 25 days old seedlings of respective genotypes using CTAB mini-prep method.

Field screening for YMV tolerance / phenotypic variability

Field screening for YMV disease was carried out during summer, 2017 using infector row technique. Forty genotypes were sown in single row with a spacing of 30 × 10 cm in 3 replications. One row of infector line SML 1082 was raised after every five test entries. All the recommended agronomic practices were followed. No insecticidal spray was given in order to allow the whitefly population to spread the disease.

Disease incidence was recorded periodically and the percentage disease incidence was worked out using the formula:

$$\text{Percent disease incidence} = \frac{\text{Number of plants infected in a row}}{\text{Total number of plants in a row)}} \times 100$$

The genotypes were categorised using 0-9 arbitary scale as Immune (HR), Resistant (R), Moderately resistant (MR), Moderately susceptible (MS), Susceptible (S) and highly susceptible (HS) based on disease severity.

In order to assess the genetic diversity among 40 genotypes, D^2 statistic was used following the procedure given by Rao, (1952).

The Principal cpmponents with Eigen value greater than one were selected as proposed by Jeffers, (1967).

Genotypic diversity by molecular studies

Genetic diversity of the population was determined using molecular markers. A set of nineteen SSR markers were validated using 40 genotypes. PCR reaction was carried out in a volume of 15 µl containing 50 ng of genomic DNA, 1X PCR buffer, 10 mm dntp's, 25 mm $mgcl_2$, 5 µm of (forward and reverse) primer and 1 unit of Taq DNA polymerase. Amplification was performed in master cycler gradient PCR (eppendorf). Amplification conditions were, initial denaturation at 94°C for 3 minutes followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 45-58°C for 1 minute and extension 72°C for 1 minute and a final extension at 72°C for 10 minutes. The PCR amplified products were subjected to gel electrophoresis in 3% agarose gel in 1X TBE at 100 V for 5 hours using gel electrophoresis unit. The ethidium bromide stained gels were documented using BIO-RAD gel documentation system. The markers CEDG 004, CEDG008, CEDG011, CEDG013, CEDG020, CEDG056, CEDG092, CEDG139, CEDG180, CEDG198, CEDG044, CEDG014, CYR-1, YR-4 and YMV1 got amplified at the same annealing temperature as mentioned in the original publications (Basak *et al*., 2004; Maiti *et al*., 2011). The annealing temperature for the markers were fixed through gradient PCR and the amplification was performed. The details of the markers used in this study are mentioned in table 1. The results obtained for the fifteen markers were verified through independent PCR.

The reaction mixture was given a short spin for thorough mixing of the cocktail components. PCR samples were stored at 4°C for short periods and at 20°C for long duration. The amplified products were loaded on ethidium bromide stained agarose gels (1.2%) and polymorphic primers were noted. The PCR amplified products were resolved on 3.0% agarose gel. A 100 bp ladder was loaded as a reference marker. The gel was run at constant

Table 1: SSR primers used for molecular analysis of MYMV disease in blackgram.

S. No	Marker name	Primer sequence	Repeat Motif	Product size (bp)	Annealing temperature (°C)
1	CEDG 133	F-CAAACTTCCGATCGAAAGCGCTTG R-GTTTCTCCTCAATCTCAAGCTCCG	(AG) 19	190	58
2	CEDG 008	F – AGGCGAGGTTTCGTTTCAAG R – GCCCATATTTTTACGCCCAC	(AG)26	110-140	55
3	CEDG 011	F – GTCCGACTTTATGTGTGGAG R – TTTCTAGTTCCAGCCCCGAC	(AC)9(AT)8 (AT)7(AC)4	118	59
4	CEDG 013	F – CGTTCGAGTTTCTTCGATCG R – ACCATCCATCCATTCGCATC	(AG)22	92-82	54
5	CEDG 020	F-TATCCATACCCAGCTCAAGG R-GCCATACCAAGAAAGAGG	(AT)18 (AG)20	143-149	56
6	CEDG 056	F- TTCCATCTATAGGGGAAGGGAG R- GCTATGATGGAAGAGGGCATGG	(AG) 14	172-220	61
7	CEDG 092	F-TCTTTTGGTTGTAGCAGGATGAAC R-TACAAGTGATATGCAACGGTTAGG	(AG) 17	150-170	55
8	CEDG 139	F-CAAACTTCCGATCGAAAGCGCTTG R-GTTTCTCCTCAATCTCAAGCTCCG	(AG) 19	190	58
9	CEDG 180	F-GGTATGGAGCAAAACAATC R-GTGCGTGAAGTTGTCTTATC	(AG) 11	136-163	55
10	CEDG 198	F-CAAGGAAGATGGAGAGAATC R-CCTTCTAAGAACAGTGACATG	(AG) 30	227-209	50
11	CEDG 044	F-TCAGCAACCTTGCATTGCAG R-TTTCCCGTCACTCTTCTAGG	(GT)10AT (AG)18	172-210	58
12	CEDG 014	F – GCTTGCATCACCCATGATTC R- AAGTGATACGGTCTGGTTCC	(AT)12 (AG)14	176-116	58
13	CYR1	F-GGGTGGTTTGGGTAAGACCAC R-TTCGCGGTGTGTGAAAAGTCT	-	1236	58
14	YR4	F-GGTAAGACGACACTCGCTTTA R-GACGTCCTTGTAACTTTGATCA	-	456	58
15	YMV1	F-GAGAGAGAGAGAGAGACAAAG R-GAGAGAGAGAGAGAGACAGGA	-	1357	58

voltage of 80V for about 1-3 hours, until the ladder got properly resolved. Gel was photographed using the Gel Documentation system. (Vilber lourmat + Imaging system).

The presence of the band was scored as 1 and absence of the band was score 0. In case of absence of any band the particular genotype was re-run using the same marker to conform the absence of particular band.

Marker statistics

The frequency of particular band produced by a particular marker was estimated based on its presence in the entire germplasm. This was repeated for all the bands produced by a particular marker and polymorphism information content was estimated the formula, PIC = 1- Σ $(Pi)^2$. The markers which showed no polymorphic bands and those with no amplification at all were dropped from further analysis.

Genetic diversity of the forty genotypes was determined from the polymorphic molecular marker pattern by estimating the genetic distance using DICE dissimilarity co-efficient estimated using the following formula. The clustering was done by using the dissimilarity coefficient matrix for the forty genotypes by employing the unweighted neighbour joining method bootsraped over 5000 times. The dendogram was constructed by using the joining pattern of the genotypes. The clads showing more than 70% of the bootstrap value where considered arbitrarily as a strong cluster.

Cluster analysis

Cluster analysis was performed using the neighbor-joining (NJ) method with the Darwin v. 5.0.157 software (Perried and Jacquemound-Collet, 2006). In addition, genetic distance dendrograms were drawn using Darwin 5 software program which was based on DICE matrix and UPGMA in Hierarchical Clustering module and also based on Unweighted Neighour-Joining in Neighbour-Joining module.

Table 2: Components of PCR reaction.

Component	Quantity	Reaction volume
Taq buffer (10X) with Mg Cl_2	1X	1.5 µl
Dntp mix	2.5 Mm	1.5 µl
Taq DNA polymerase	3U/µl	2.0 µl
Forward primer	0.2 µm	2.0 µl
Reverse primer	0.2 µm	2.0µl
Genomic DNA	50 ng/µl	2.0 µl
Sterile distilled water		4.0 µl

Results

Yellow mosaic virus is a serious disease which affects black gram in India. Many varieties of blackgram are susceptible to the disease facilitating transfer of resistant genes into the popular cultivars using marker assisted breeding. In the present study forty genotypes of different origin were tested for the agronomic performance and YMV resistance. Further, they were used for assessing genetic diversity and testing the validity of SSR markers identified as linked to YMV 15 resistance in blackgram. The disease screening studies resulted in identification of genotypes with high resistance (AUBG 1, AUBG 6, VBN 4, VBN 8) and high susceptibility (AUBG 5, AUBG 11, AUBG 13, AUBG 15, AUBG 17, AUBG 19).

Phenotypic variability

Percentage disease infection ranged from 1.29 to 90.55. The genotype G38 showed minimum percentage disease infection and the genotype G22 showed maximum percentage disease infection. Out of forty genotypes studied, twenty two genotypes had significantly higher mean value than the general mean (31.86) (Table 6).

The trait, seeding fresh weight exhibited a range of 0.07 to 1.80. The genotype G33 showed minimum seeding fresh weight and the genotype G17 showed maximum seeding fresh weight. Two genotypes, out of forty genotypes studied had significantly higher mean value than the general mean (1.21) (Table 6).

The trait, seeding dry weight exhibited a range of 0.06 to 0.80. The genotype G3 showed minimum seeding dry weight and the genotype G31 showed maximum seeding dry weight. No genotypes, out of forty genotypes studied had significantly higher mean value than the general mean (1.59) (Table 6).

The trait, single plant seed yield exhibited a range of 7.50 to 20.44. The genotype G38 showed minimum single plant seed yield and the genotype G11 showed maximum single plant seed yield. Thirty five genotypes, out of forty genotypes studied had significantly higher mean value than the general mean (11.97) (Table 6).

Table 3: PCR temperature regime.

S. No	Step	Temperature	Time	Cycles
1.	Initial denaturation	94°C	3 min.	1
2.	Denaturation	94°C	1 min.	35
3.	Annealing	49-62°C	1 min.	
4.	Extension	72°C	2 min.	
5.	Final extension	72°C	7 min.	1
6.	4°C		∞	

Variability studies

The phenotypic co-efficient of variation, genotypic co-efficient of variation, heritability and genetic advance as percent of mean were estimated for all the nine characters and presented in table 7.

Phenotypic co- efficient of variation (PCV)

The phenotypic co-efficient of variation ranged from 7.13 percent (days to 50 percent flowering) to 114.39 percent (number of seeds per pod). High PCV was observed for number of seeds per pod (114.39 percent), percentage of disease infection (86.47), seedling dry weight (76.12), single plant seed yield (60.41 percent) and number of clusters per plant (58.99 percent). Moderate PCV was observed for number of pods per plant (33.20 percent), seedling fresh weight (49.77) and plant height (21.10). Low PCV was observed for number of primary branches per plant (18.57 percent), pod length 16.91 percent) and days to 50 percent of flowering (7.13 percent) (Table 7).

Genotypic co-efficient of variation (GCV)

The GCV ranged from 7.10 percent to 114.32 percent for days to 50 percent flowering and number of seeds per pod respectively. High GCV was observed for number of seeds per pod (114.32), percentage disease infection (86.42 percent), seedling dry weight (76.12) single plant seed yield (60.37 percent) and number of clusters per plant (58.97 percent). Moderate GCV was observed for number of pods per plant (33.10 percent), seedling fresh weight (44.03) and plant height (20.20). Low GCV was observed for number of primary branches per plant (18.29 percent), pod length (15.82 percent) and days to 50 percent of flowering (7.10 percent) (Table 7).

Heritability (h^2)

High heritability was observed for the traits *viz.*, percentage disease infection (99 percent), number of clusters per plant (99 percent), days to 50 percent flowering (99 percent), single plant seed yield (99 percent), number of primary branches per plant (96 percent), number of pods per plant (99 percent), pod length (87 percent), plant height (91 percent), number of seeds per pod (99 percent) seedling fresh weight (78 percent),

Table 4: Disease scoring scale (0-9) for MYMV based on percentage disease incidence (Bashir *et al*., 2005) and Diseases scoring for MYMV in Annamalainagar.

Scale	Percentage infection	Category	Reaction group	Diseases scoring for MYMV in Annamalai nagar (Genotypes)
0	All plants free of virus symptoms	Highly Resistant	HR	
1	1-10% infection	Resistant	RR	G1, G6, G22
2	11-20% infection	Moderately resistant	MR	G2, G4, G7, G10, G14, G24, G25
3	21-30% infection	Moderately Susceptible	MS	G18, G20
4	30-50% infection	Susceptible	S	G3, G8, G9, G12, G16, G21, G23, G26, G27, G28
5	More than 50%	Highly susceptible	HS	G5, G11, G13, G15, G17, G19, G29

seedling dry weight (76.20 percent). The heritability values ranged from 78 percent for seedling fresh weight to 99 percent percentage disease infection (Table 7).

Genetic advance as percent of mean

A perusal of genetic advance for all the quantitative characters under study ranged from 14.59 percent for days to 50 percent flowering to 49.27 percent for single plant seed yield respectively. The estimate of high genetic advance was observed for the traits *viz*., single plant seed yield (49.27 percent), plant height (39.82 percent), number of primary branches per plant (37.08 percent) percentage of disease infection (35.52 percent), number of pods per plant (33.17 percent) and pod length (30.79 percent). Moderate genetic advance was observed for seedling fresh weight (27.31), number of seeds per pod (24.47 percent) and seedling dry weight (22.43). Lower GCV value observed for number of clusters per plant (20.21 percent) and days to 50 percent flowering (14.59 percent) (Table 7).

Phenotypic divergence

The quantitative assessment of genetic divergence was made by adopting Mahalanobis D^2 statistic for various traits. Genetic divergence was estimated for 40 genotypes and the results are presented here under.

Mahalanobis's generalized distance (D^2)

In order to assess the genetic diversity among 40 genotypes, D^2 statistic was used following the procedure given by Rao, (1952). Since the entire seven yield component characters were correlated, they were transformed into uncorrelated linear combination through pivotal condensation method. The statistical distance **(**Mahalanobis's D^2**)** between a pair of genotypes was obtained as the sum of squares of the difference between the pairs of corresponding uncorrelated values of any two genotypes considered at a time.

Cluster analysis

The quantum of genetic divergence was also assessed by cluster analysis using Mahalanobis's euclidean squares distances which grouped the entire material into more precise clusters and estimates the average distance between them. Genetic divergence was estimated on the basis of D^2 value and 40 genotypes under study were grouped into three clusters by Tocher's method as presented in table 8. Cluster I was the largest with 37 genotypes, G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13, G14, G15, G16, G17, G18, G19, G20, G21, G22, G23, G24, G25, G26, G27, G28, G29, G30, G31, G32, G33, G34, G35, G36, G37, Cluster II with 2 genotypes of G38 and G40 and Cluster III with only one genotype G40 (Table 8).

Table 5: Analysis of variance for different characters in blackgram.

Source of variation	Replication df=2	Genotype df=39	Error df=78
Days to 50% flowering	0.0484	20.9882**	0.0474
Plant height (cm)	0.2125	106.573	5.5328
No. of primary branches per plant	0.0991	1.1050**	0.0354
No. of clusters per plant	0.0570	6.3624**	0.0299
No. of pods per plant	2.1343	114.09**	0.8149
Pod length (cm)	0.0435	0.7361**	0.0830
No. of seeds per pod	0.0606	0.8793**	0.0462
Percentage of disease infection (%)	2.8297	1897.70	0.9742
Seedling fresh weight (g)	0.0835	0.1384*	0.0937
Seedling dry weight (g)	0.0135	0.0899**	10.9627
Seed yield per single plant(g)	0.0605	10.9627**	0.0396
*, ** Significant at 5% and 1% respectively.			

Intra and Inter cluster distances

The highest divergence occurred between cluster I and cluster III (44067.70) followed by cluster I and cluster II (41157.70) in that order. This indicates greater divergence between these clusters. The crosses involving parents from these divergent clusters are expected to yield good amount of heterosis in F_1 and high frequency of transgressive segregants leading to genetic variability in subsequent generations (Table 8).

The cluster means for each of the seven characters are presented in

Table 6: Mean performance of blackgram genotypes for Eleven traits.

Geno type	Days to 50 % flowering	Plant height (cm)	No. of primary branches per plant	No. of cluster per plant	No. of pods per plant	Pod length (cm)	No. of seeds per pod	Perce-ntage of disease infection	Seed-ling fresh weight (g)	Seed-ling dry weight (g)	Single plant seed yield (g)
G1	35.303	43.500	5.68	9.050	39.567**	5.610*	6.80	40.683	0.950	0.077	13.180**
G2	37.820**	39.500	6.233	10.480	40.167**	4.380	7.400	20.893**	1.500	0.317	15.620**
G3	33.650	41.433	5.920	10.630	26.60	5.337	6.633	20.703**	1.507	0.063	15.690**
G4	36.720	37.133	5.590	13.620**	34.600	4.563	7.100	10.713**	1.477	0.364	12.853**
G5	35.233	37.500	4.850	10.880	32.033	4.657	8.233	35.540	1.650	0.078	14.110**
G6	38.180**	28.567**	5.810	13.257**	42.600**	4.973	7.100	60.433	1.267	0.294	18.720**
G7	37.913**	36.733	4.710	11.637	37.600**	5.037	6.800	21.373**	0.950	0.081	15.350**
G8	40.243**	35.800	6.033	10.690	37.10**	4.737	6.0	65.130	1.500	0.098	13.690**
G9	41.630**	42.63	6.230*	13.273**	43.60**	4.527	6.900	35.600	1.507	0.111	14.523**
G10	33.823	34.700	7.610**	13.813**	27.800	5.507	7.200	53.310	1.477	0.288	16.063**
G11	35.963	37.66	6.430**	11.513	36.33*	5.823**	6.200	20.607**	1.650	0.611	20.44**
G12	36.723	41.867	5.693	11.60	44.30**	4.500	6.233	30.657	1.507	0.559	17.180**
G13	35.210	31.133**	5.270	11.250	36.20*	4.440	6.833	33.740	1.493	0.344	13.513**
G14	39.633**	35.80	5.490	11.040	36.733**	5.660*	6.667	72.573	1.700*	0.547	14.040**
G15	34.520	42.50	5.410	12.260	40.500**	4.743	7.500	7.520**	1.160	0.645	14.290**
G16	36.280	42.100	6.120	10.230	41.33**	5.117	6.633	21.213**	1.163	0.176	16.673**
G17	39.033**	51.73	5.960	10.113	31.600	5.787**	7.800	7.583**	1.800*	0.620	15.540**
G18	36.820	40.400	4.880	12.420	45.267**	4.477	7.400	13.297**	1.377	0.405	12.213
G19	33.240	44.500	6.210*	8.850	38.300**	4.537	7.067	75.390	1.603	0.167	12.623**
G20	42.617**	47.800	6.233*	10.630	39.200**	4.510	6.80	65.440	1.613	0.122	13.213**
G21	38.323**	59.033	5.910	9.413	42.533**	4.467	6.267	40.550	1.417	0.274	12.420**
G22	40.120**	41.767	4.820	12.230	48.267**	5.240	6.33	90.553	1.587	0.384	15.160**
G23	33.203	31.033**	5.250	9.127	51.600**	4.543	6.433	86.270	1.527	0.179	14.270**
G24	34.033	34.100	5.430	9.240	45.33**	4.473	6.600	88.837	1.300	0.154	12.073
G25	36.320	38.367*	6.430**	10.610	38.300**	4.490	7.467	76.017	1.297	0.088	16.610**
G26	40.120**	44.277	5.290	9.430	42.600**	4.620	6.567	9.433**	1.650	0.137	14.020**
G27	42.030**	41.833	6.250*	11.130	45.633**	4.427	6.633	23.963**	1.670	0.154	15.120**
G28	39.210**	42.367	5.430	10.030	36.200*	4.633	6.100	30.457	1.603	0.073	12.990**
G29	36.060	35.867	4.873	11.630	41.567**	5.180	5.600	33.510	1.613	0.076	15.610**
G30	33.950	32.100**	5.470	11.853	34.867*	5.320	6.467	6.643**	1.417	0.100	13.210**
G31	35.370	37.00	4.890	9.490	36.100*	4.307	5.80	29.387**	1.583	0.144	11.50
G32	37.540*	41.50	6.610	10.230	28.567	5.160	6.427	9.503**	1.527	0.589	12.61**
G33	39.230**	44.433	5.230	10.630	27.133	4.587	6.833	26.433**	0.073	0.052	13.5**
G34	36.813	38.100	6.270*	10.257	29.60	4.383	7.267	1.867**	0.076	0.612	14.50**
G35	34.090	31.867**	5.640	10.010	28.30	4.533	7.59	1.733**	0.100	0.072	12.71**
G36	34.360	30.500**	5.890	10.230	36.500*	6.533**	7.79	1.527**	0.144	0.503	12.89**
G37	36.020	34.500	5.613	6.630	5.950	7.233**	6.25	1.300**	0.121	0.129	13.49**
G38	37.530*	33.633*	5.323	38.033**	4.810	7.433**	7.833	1.297**	0.302	0.310	7.51
G39	39.720**	38.100	10.64**	34.167**	5.160	6.500**	8.23	1.733**	0.390	0.029	19.51**
G40	42.160**	27.50**	8.84**	43.267**	5.563	6.867**	31.437**	1.33**	0.182	0.652	11.51
MEAN	37.169	38.253	5.912	12.872	34.610	5.096	11.03	31.869	1.211	1.520	11.97
C.V	0.2857	6.034	3.281	1.607	2.396	5.88	3.16	2.659	1.47	1.59	1.36
S.E	0.1257	1.358	0.108	0.099	0.521	0.166	0.124	0.569	0.176	0.114	0.114
C.D.5%	0.352	3.802	0.304	0.279	1.459	0.465	0.347	1.595	0.494	0.320	0.321
C.D.1%	0.467	5.051	0.404	0.371	1.938	0.618	0.461	2.119	0.657	0.425	0.427

Table 7: Phenotypic co-efficient of variation (PCV), Genotypic co-efficient of variation (GCV), Heritability (h^2), Genetic advance as percent of mean (GA) for various characters in blackgram genotypes.

S. No	Traits	Mean	PCV (%)	GCV (%)	Heritability (%)	GA (%) of mean
1	Days to 50%flowering	35.6	7.132	7.108	99.3	14.59
2	Plant height(cm)	5.5	21.109	20.202	91.5	39.82
3	Number of primary branches	11.4	18.578	18.293	96.9	37.10
4	Number of clusters	28	58.995	58.979	99.9	20.21
5	Number of pods per plant	4.5	33.209	33.106	99.3	33.17
6	Pod length	6.7	16.916	15.820	87.4	30.79
7	Number of seeds per pod	9.6	114.397	114.326	99.8	24.47
8	Percentage of disease infection	1.3	86.479	86.420	99.8	35.52
9	Seedling fresh weight	0.09	49.770	44.033	78.2	27.31
10	Seedling dry weight	14.7	176.245	176.122	99.8	22.43
11	Single plant seed yield(g)	31.8	60.414	60.379	99.8	49.27

table 9. Single plant seed yield was highest in cluster I (11.9) and lowest in cluster II and III (0.00). Cluster II recorded highest mean value for number of clusters per plant, plant height (cm), days to 50% flowering and pod length. Cluster III showed maximum mean value for seeds per pod (Table 9).

The number of times that each of the seven characters appeared first rank and its respective percent contribution towards genetic divergence is presented in table 10. Seed yield per plant (55.12%) followed by percentage of disease infection (22.1795%), seedling fresh weight (10.64%), days to 50% flowering (4.74%), number of seeds per pod (2.82%), number of clusters per plant (1.92%) seedling dry weight (1.92) number of pods per plant (0.641) and plant height, number of primary branches, pod length (0.00) contributed to the genetic divergence in decreasing order.

Principal component analysis

The principal component analysis was done with 40 breeding lines of Urdbean. The data presented in table 11 evidently revealed that the first five components having the Eigen values more than 1 contributed 71 % of the variability among the breeding lines. In this investigation first component was much influenced by the vegetative characters *viz.*, plant height, number of pods per plant and seedling fresh weight.

The second component was contrastingly much influenced by seedling dry weight, pod length. The third component was influenced by number of pods/plant. The fourth component was strongly influenced by days to 50% flowering. The fifth component had influenced with single plant seed yield.

Molecular diversity

Genotypic variability of the germplasm containing forty genotypes were analyzed using fifteen SSR markers. It comprised of lines whose resistance or susceptible response for YMV disease were not known, while some of the varieties disease reaction was known based on earlier literature. The YMV disease reactions of the genotypes were studied in Departmental Farm, Department of Genetics and Plant Breeding Annamalainagar of Chidambaram, Cuddalore District. The test genotypes were initially screened with 15 SSR markers, to find out the markers showing polymorphism

Table 8: Composition of D^2 clusters for blackgram genotypes & Average inter (D^2) and intra (diagonal) cluster distance for blackgram genotypes.

Cluster 1	G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13, G14, G15, G16, G17, G18, G19, G20, G21, G22, G23, G24, G25, G26, G27, G28, G29, G30, G31, G32, G33, G34, G35, G36, G37	5834.15 (**76.38**)	41157.69 (202.87)	44067. 69 (209.92**)**
Cluster2	G 38, G 40		6945.75 (**83.34**)	24863.71 (157.68)
Cluster3	G 39			0.000 (**0.00**)

Table 9: D^2 cluster mean of black gram genotypes.

Clusters	Character										
	1	2	3	4	5	6	7	8	9	10	11
C1	36.955	39.237	5.721	10.795	36.986	4.947	9.032	34.335	1.28	2.46	11.98
C2	39.84	20.13	7.08	40.65	5.38	7.15	19.63	1.31	1.28	2.46	3.21
C3	39.72	38.1	10.64	34.16	5.16	6.5	66.6	1.7	0.39	14.3	1.59

Table 10: Contribution of different characters to divergence.

Character	Contribution %
Days to 50% flowering	4.7436
Plant height (cm)	0.0000
No. of primary branches per plant	0.0000
No. of clusters per plant	1.9231
No. of pods per plant	0.6410
Pod length (cm)	0.0000
No. of seeds per pod	2.8205
Percentage of disease infection (%)	22.1795
Seedling fresh weight (g)	10.6410
Seedling dry weight (g)	1.9231
Seed yield per single plant(g)	55.1282
Total	**100**

between the parents.

Marker Allele Distribution

Nine out of fifteen markers (63.2%) showed polymorphic among the genotypes tested. Five markers were bi-alleic producing two distinct alleles among the genotypes while one marker were mono-allelic showing a dominant allele pattern. Out off fifteen markers nine markers showed intermediate PIC value ranged from (0.309-0.658) table 12.

Genotypic diversity

The genetic distance announced using DICE dissimilarity co-efficient indicated lowest dissimilarity (0.16) between G1 and G3. The highest divergence of 0.8 was between G35 and G37 and between G26 and G28.

The Dendogram constructed using the DICE dissimilarity co-efficient between genotypes showed seven apparent clusters based on marker allele distribution. The first cluster consisted of five genotypes (G 18, G 17, G 16, G 26 and G 25) and accommodated 10.5 % of the total population based on allelic similarity. The second cluster consisted of six genotypes having a membership density of 6.6%. It consisted of (G 11, G 4, G 10 G 2, G 1 and G 5). The third cluster consisted of eight genotypes accommodating 20.5% of total genotypes. It consisted of genotypes of G 31, G 6, G 9, G 13, G 33, G 12, G 20 and G 19. The clustering of series of genotypes in a same cluster might be due to their origin from the same geographical location. The fourth cluster consisted of six genotypes accommodating 13% of the population consisting of genotypes of G 40, G 7, G 29, G 38, G 27 and G 35. The fifth cluster consisted of four genotypes (G 28, G 32, G 8 and G 30) and population of 15.7%. The sixth cluster consisted of six genotypes accommodating 17.2 of total genotypes and consisted of genotypes G 39, G 36, G 37, G 14, G 22 and G 21. The Seventh cluster had remaining 16.5% of the population consisting of genotypes G 34, G 23, G 24, G 15 and G 3.

Table 11: Principal component analysis of blackgram genotypes.

Statistics	PC1	PC2	PC3	PC4	PC5
Standard deviation	1.4821	1.3988	1.1828	1.0860	1.0439
Proportion of Variance	0.1997	0.1779	0.1272	0.1072	0.0991
Cumulative Proportion	0.1997	0.3776	0.5048	0.6120	0.7110
Eigen Values	2.1967	1.9566	1.3990	1.1793	1.0897

Table 12: Polymorphic information content (PIC) value for the SSR primers.

S. No	Name of the marker	PIC
1	CEDG008	0.499
2	CEDG013	0.563
3	CEDG020	0.309
4	CEDG056	0.658
5	CEDG092	0.471
6	CEDG139	0.483
7	CEDG180	0.580
8	CEDG014	0.382
9	CYR1	0.546

Although the neighbor joining procedure was bootstrapped 5000 times none of the clusters or class showed bootstrap value more than 48%. The highest bootstrap value of 48% was shown by two genotypes G 7 and G 40 followed by their grouping with G 29.

Discussion

The present study was carried in the Department of Genetics and Plant Breeding to validate the usefulness of 15 SSR markers identified as linked to YMV resistance in 40 test genotypes. Field screening for YMV disease was carried out in the Genetics and Plant Breeding department Farm using infector row technique to assess YMV resistance and the phenotypic diversity of the test genotypes. In order to assess the worth of the population for isolating high yielding lines besides looking for resistance to YMV the variability parameters like mean, range and variance were computed for seven quantitative traits *viz.*, days to 50% flowering, plant height, number of branches per plant, number of clusters per plant, number of pods per plant, pod length, number of seeds per pod, percentage disease infection, seedling fresh weight, seedling dry weight, single plant yield were also carried out.

The results of analysis of variance indicated that variance due to genotypes was significant for all characters indicating that the genotypes selected for the present study were genetically different. Among the genotypes G 11 had the highest mean value for seed yield per plant. Percentage disease infection

ranged from 1.29 to 90.55. The genotype G38 showed minimum percentage disease infection and the genotype G22 showed maximum percentage disease infection. Out of forty genotypes studied, twenty two genotypes had significantly higher mean value than the general mean (31.86) (Table 6).

High PCV and GCV estimates were observed for seed yield per plant, number of pods per plant, plant height and seedling dry weight. Lowest PCV and GCV estimates were recorded for days to 50% flowering, number of primary branches, plant height followed by pod length. Similar findings were found in mungbean by Priyanka bhareti *et al.,* (2011) for seed yield.

Varma and Garg, (2003) observed low PCV and GCV values for days to flowering in mung bean. However, in the present investigation, pod length and number of seeds per pod had moderate GCV and PCV values. These results are in accordance with results of Singh *et al*., (2009) and Konda *et al.,* (2009). Low to moderate GCV and PCV values for above two characters indicate the influence of the environment on these traits and also limited scope of selection for improvement of these characters.

The estimates of phenotypic coefficient of variation (PCV) were higher than the estimates of genotypic coefficient of variation (GCV) for all the traits under study indicating the environmental influence over the traits. Similar results were observed by Konda *et al.,* (2009) and Reddy *et al*., (2011) in black gram.

High heritability was observed for plant height followed by number of pods per plant, seed yield per plant and number of clusters per plant. Low heritability was observed for pod length, number of seeds per pod and number of branches per plant. However, heritability does not give a correct measurement of the genotypic variation; hence it should be observed with genetic advance as percent of mean.

The genetic advance as percent of mean was high for plant height followed by number of pods per plant. Lowest genetic advance as percent of mean was observed for number of branches per plant. Moderate genetic advance as percent of mean was observed in seed yield per plant.

High heritability along with high genetic advance as percent of mean was observed for plant height, number of primary branches, percentage of disease infection, number of pods per plant and pod length. The association of high heritability with high genetic advance for these traits was indicative of additive effects. High heritability with moderate genetic advance was observed for seedling fresh weight, number of seeds per pod, single plant seed yield and seedling dry weight. High heritability with low genetic advance was observed for days to 50% flowering followed by number of clusters per plant which indicates non additive gene action. Low heritability with low genetic advance was observed for remaining traits indicating less scope for selection. Similar results were obtained by Anju Pathania *et al*., (2010). Talukdar and Biswas, (2008) recorded high heritability values for days to 50 percent flowering, 100 seed weight and seed yield per plant. Sharma, (1999), Natarajan and Rathinasamy, (1999) reported similar findings of high heritability and high genetic advance for number of pods per plant, number of seeds per pod and seed yield per plant.

Genetic divergence by D^2 analysis

Genetic divergence was estimated on the basis of D^2 values and 40 genotypes under study were grouped into six clusters by Tocher's method as presented in table 8. Cluster I was the largest with 37 genotypes, G 1, G 2, G 3, G 4, G 5, G 6, G 7, G 8, G 9, G 10, G 11, G 12, G 13, G 14, G 15, G 16, G 17, G 18, G 19, G 20, G 21, G 22, G 23, G 24, G 25, G 26, G 27, G 28, G 29, G 30, G 31, G 32, G 330, G 34, G 35, G 36,G 37, Cluster II with 2 genotypes of G 38 and G 40 and Cluster III had only one genotype G 39.

The highest divergence occurred between cluster I and cluster III (44067.70) followed by cluster I and cluster II (41157.70) in that order. This indicates greater divergence existed between these clusters. The crosses involving parents from these divergent clusters are expected to yield good amount of heterosis in F_1 and high frequency of transgressive segregants and genetic variability in subsequent generations.

The minimum inter cluster distance was noticed between cluster II and cluster III (24863.72) suggesting that the genotypes of these clusters were not genetically much diverse.

The study indicated that the inter-cluster distances were greater than intra-cluster distances, revealing considerable amount of genetic diversity among the accessions. Similar results were also reported by Ali *et al.,* (2008) and Chauhan *et al.,* (2008). Hybridization between genotypes from two divergent clusters could produce desirable recombinants for grain yield**.**

The relative contribution of individual characters towards the expression of genetic diversity was estimated over character wise D^2 value. The three top contributors were single plant seed yield (55.12 %), percentage disease infection (22.18 %) and seedling fresh weight (10.64%). De *et al.,* (1998) observed that the traits contributing

maximum towards the D^2 values needed to be given more emphasis for detecting the clusters to be taken for the purpose of selection of parents for hybridization programme.

Principal component analysis

The principal component analysis was done with 40 breeding lines of blackgram. Evidently revealed that the first five components having the Eigen values more than 1 contributed 71% of the variability among the breeding lines. In this investigation first component was much influenced by the vegetative characters *viz.*, plant height, number of pods per plant and seedling fresh weight. The second component was much influenced by vegetative traits seedling dry weight and pod length.

The third component was influenced by number of pods per plant. The fourth component was strongly influenced by 50% flowering. The fifth component was influenced by single plant seed yield. The sixth component was strongly influenced by number of primary of branches, pod length, while the character, number of clusters per plant influenced this component negatively. The seventh component was influenced by seedling fresh weight and pod length. Singh *et al.,* (2005) studied principal component analysis in black gram and the first 5 PCA together explained 71% of variation present in the material. Thus the most important descriptors were plant height, seedling fresh weight and seed yield per plant in component 1 and number of pods per plant in component 2.

Molecular diversity

In the present study nine out of fifteen markers (63.2%) showed polymorphic bands among the genotypes tested. Out of the remaining six markers, five markers were bi-allelic producing two distinct alleles among the genotypes while one marker was mono-allelic showing a dominant allele pattern. Polymorphic information content of the nine polymorphic markers ranged from 0.30 to 0.65. Four markers namely CEDG013, CEDG056, CEDG180 and CYR1 had PIC values above 0.5 and hence can be considered as informative markers for YMV screening. The genetic distance announced using DICE dissimilarity co-efficient indicated lowest dissimilarity (0.16) between G 1 and G 3. The highest divergence of 0.8 was between G 35 and G 37 and between G 26 and G 28.

The dendogram constructed using the DICE dissimilarity co-efficient between genotypes showed four apparent clusters based on marker allele distribution. The first cluster consisted of five genotypes (G 18, G 17, G 16, G 26 and G 25) and accommodated 10.5 % of the total population based on allelic similarity. The second cluster consisted of six genotypes having a membership density of 6.6%. It consisted of (G 11, G 4, G 10 G 2, G 1 and G 5). The third cluster consisted of eight genotypes accommodating 20.5% of total genotypes. It consisted of genotypes of G 31, G 6, G 9, G 13, G 33, G 12, G 20 and G 19. The clustering of series of genotypes in a same cluster might be due to their origin from the same geographical location. The fourth cluster consisted of six genotypes. It accommodated 13% of the population consisted of genotypes of G 40, G 7, G 29, G 38, G 27 and G 35. The fifth cluster consisted of the four genotypes (G 28, G 32, G 8, G 30) with population of 15.7%. The sixth cluster consisted of six genotypes accommodated 17.2% of total genotypes. It consisted of genotypes G 39, G 36, G 37, G 14, G 22 and G 21. The Seventh cluster had remaining 16.5% of the population consisting of genotypes G 34, G 23, G 24, G 15 and G 3.

Although the neighbor joining procedure was bootstrapped 5000 times none of the clusters showed bootstrap value more than 48%. The highest bootstrap value of 48% was shown by two genotypes G 7 and G 40. While selecting parents for yellow mosaic virus tolerance cluster II (G 38 and G 40) must be given importance because they had high field tolerance. Incidentally the two genotypes were grouped in the same cluster II as described by molecular marker diversity analysis.

The number of clusters formed by phenotypic divergence was only three while molecular diversity produced seven clusters. Although the genotypes were subjected to natural epiphytotic condition for YMV, the phenotypic clustering did not correlate with genotypic clustering by SSR markers specific for yellow mosaic virus. Many studies have found less relationship between molecular diversity and genetic distances estimated from morphological data (Gepts, 1993).

References

Ali, M.N., Suparna, Gupta, B.Somnath and H.K. Sarkar (2008). Evaluation of blackgram (*Vigna mungo* (L.) Hepper) germplasm using multivariate analysis, *Environment and Ecology.,* **26(2A):** 943-945.

Ayres, N.M., A.M. McClung, P.D. Larkin, H.F.J. Bligh, C.A. Jones and W.D. Park (1997). Microsatellites and a single-nucleotide polymorphism differentiate apparent amylase classes in an extended pedigree of US rice germplasm. *Theor. Appl. Genet.,* **7:** 773-781.

Bhareti, Priyanka, D.P. Singh and R.K. Khulbe (2011). Genetic variability and association analysis of advanced lines and cultivars following intervarietal and interspeccific crosses in blackgram. *Crop Improv.,* **38(1):** 67-70.

Basak, J., S. Kundagrami, T.K. Ghose and A. Pal (2004). Development Of Yellow Mosaic Virus (YMV) Resistance Linked DNA Marker In *Vigna mungo* From Populations Segregating For YMV-Reaction, *Molecular Breeding*., **14:** 375-383.

Blair, M. and S.R. McCouch (1997). Microsatellite and sequence-tagged site markers diagnostic for the bacterial blight resistance gene, *xa-5*. *Theor. Appl. Genet.*, **95:** 174-184.

Burton,G.W. and E.M. Devane (1952). Estimating heritability in fall fescue (*Festecd cirunclindcede*) from replicated clonal material. *Agronomy J.*, **45:** 478-481.

Chauhan, M.P., A.C. Mishra and K.S. Ashok (2008). Genetic divergence studies in urdbean (*Vigna mungo* (L.) Hepper). *Legume Res*., **31(1):** 63-67.

De, R.N., R. Seetharaman, M.T. Sinha and S.P. Banerjee (1998). Genetic divergence in rice. *Indian Genet. Plant Breed.*, **48:** 189-194.

Delic, D., O. Stajkovic, D. Kuzmanovic, N. Rasulic, S. Knezevic and B. Milicic (2009). The effects of rhizobial inoculation on growth and yield of *Vigna mungo* L. in Serbian soils. *Biotechnology in Animal Husbandry.*, **25(5-6):** 1197-1202.

Gepts, P. (1993). The use of molecular and biochemical markers in crop evolution studies. *Evol. Biol*., **27:** 51-94.

Gupta, S.K. and T. Gopalakrishna (2008). Molecular markers and their application in grain legumes breeding. *J. of Food Legumes*., **21:** 1-14.

Jeffers, J.N.R. (1967). Two case studies in the application principal component analysis. *Applied Statistics.*, **16:** 225-236. *doi:10.2307/2985919*

Konda, C.R., P.H. Salimath and M.N. Mishra (2009). Genetic variability studies for productivity and its components in blackgram (*Vigna mungo* (L.) Hepper) *Legume Res*., **32(1):** 59-61.

Li, D.C., C.A. Fatokun, B. Ubi, B.B. Singh and G.J. Scoles (2001). Determining genetic similarity and relationships among cowpea breeding lines and cultivars by microsatellite markers. *Crop Sci.*, **41:** 189-197.

Maiti, S., J. Basak, S. Kundagrami, A. Kundu and A. Pal (2011). Molecular marker-assisted genotyping of mungbean yellow mosaic India virus resistant germplasms of mungbean and urdbean, *Mol. Biotechnol*., **47:** 95-104.

Natarajan, C. and R. Rathinasamy (1999). Genetic variability, correlation and path analysis in blackgram. *Madras Agricultural J*., **86(4-6):** 228-231.

Pathania, Anju, B.C. Sood and S. Bhateria (2010). Genetic architecture of radiation induced variability for quantitative traits in chickpea (*Cicer arietinum* L.). *Legume Res.*, **34(3):** 155-165.

Perrier, X. and J.P. Jacquemoud-Collet (2006). DARwin software *http://darwin.cirad.fr/*. Accessed 30 Sept 2015.

Rao, C.R. (1952). Advanced Statistical Methods in Biometrical research. John Wiley and Sons, New York. 236-272.

Reddy, D.K.R., O. Venkateswarlu, G.L.S. Jyothi and M.C. Obaiah (2011). Genetic parameters and interrelationship analysis in blackgram [*Vigna mungo* (L.) Hepper]. *Legume Res.*, **34(2):** 149-152.

Sharma, R.N. (1999). Heritability and character association in non segregating populations of mungbean. *J. of Inter-academica*., **3:** 5-10.

Singh, K.P., A. Malik, S. Sinha, K. Vinod and R.C. Murthy (2005). "Estimation of source of heavy metal contamination in sediments of Gomti River (India) using principal components analysis," *Water Air and Soil Pollution.*, **166(1-4):** 321-341.

Singh, S.K., I.P. Singh, B.B. Singh and Onkar Singh (2009). Genetic divergence in mungbean greengram (*Vigna radiata* (L.) Wilczek). *Legume Res.*, **32(2):** 98-102.

Talukdar, D. and A.K. Biswas (2008). Variability, Heritability and Scope of Selection for some Quantitative Traits in Induced Mutant Lines of Grasspea (*Lathyrus sativus* L.). *Inter. J. of Plant Sci.*, **3:** 528-53

Varma, P. and D.K. Garg (2003). Estimation of genetic parameters among a set of mungbean (*Vigna radiate* (L.) Wilczek) genotypes. *Annals of Agricultural Res., New series*, **24(1):** 156-158.

Weising, K., P. Winter, B. Huttel and G. Kahl (1998). Microsatellite markers for molecular breeding, *J. Crop. Prod.*, **1:** 113-143.