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Research Article

# Selection efficiency of molecular markers associated to Yellow Mosaic Virus (YMV) tolerance and identification of donors for earliness, yield and YMV tolerance in blackgram (*Vigna mungo* (L.) Hepper) using GT Biplot analysis

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**Abstract**
The present study was aimed to identify potential Yellow Mosaic Virus (YMV) resistant donors and YMV linked markers for easy and efficient screening of germplasm. Forty - six blackgram genotypes were screened for Yellow Mosaic Virus (YMV) tolerance and yield traits under natural field conditions during summer, 2020 at Dryland farm, S.V.Agricultural College, Tirupati along with molecular validation with 15 SSR (Simple Sequence Repeats) markers associated with YMV tolerance. The genotypes *viz.,* LBG-884, LBG-946, TBG-141, TBG-129, TBG-138, TBG-139, TBG-130 and GBG-1 displayed zero YMV incidence. TBG-104 and VBN-6 were identified as ideal cultivars for yield, earliness and YMV tolerance. The markers *viz.,* CEDG 097, CEDG 180, CEDG 044, and CEDG 139 were adjudged as informative markers as evident indicated by their high PIC scores and Nei's diversity index values. On scrutiny of the selection efficiency of all the alleles, CEDG 180 was found to be more efficient in differentiating resistant and susceptible genotypes. Visual comparison of trait profiles using genotype by trait (GT) biplot analysis identified the genotypes *viz.,* TBG-104 and VBN-6 as ideal cultivars that serve as most desirable parents in breeding programs aimed at developing short duration, high-yielding varieties with YMV tolerance.

**Keywords:** Blackgram, YMV, Molecular validation, SSR markers, GT biplot analysis.

## INTRODUCTION

Blackgram [*Vigna mungo* (L.) Hepper] (2n=22) is the third widely grown pulse crop of India after chickpea and pigeonpea. It contains approximately 22-25% protein, 3.5% - 4.5% fiber, 4.5-5.5% ash and 60-65% carbohydrates on dry weight basis. It is grown as a sole crop, mixed crop, green manure crop, catch crop, relay crop, intercrop and cover crop under varied soils and climatic conditions. During the last few years, domestic production is lagging consumption requirements and imports are found to be inadequate to bridge the supply and demand gap.

In India, among pulses, blackgram accounts for 13 per cent of total pulse area and 10 per cent of total pulse

production with an area of about 5.60 million hectares, production of 3.06 million tonnes and productivity of 546 kg/ha (Anonymous, 2018-19). Andhra Pradesh is one of the leading blackgram growing states of India with an area of 3.81 lakh hectares, production of 3.13 lakh tonnes and productivity of 821.5 kg/ ha(Anonymous, 2018-19).

The productivity of blackgram is also hampered by several biotic factors among which, Yellow Mosaic Virus (YMV), of late, is a wide spread biotic stress causing profound yield losses ranging from 85% to 100% (Rajarathinum *et al.,* 1990). In Andhra Pradesh, the crop area reduced from 6.95 lakh hectares during 2002-03 to 3.81 lakh hectares during 2018-19, majorly due to YMV effect. Management of this deadly disease became the biggest challenge to the farming community that made them switch over to other easily manageable crops like sorghum, maize etc., leading to not only stagnation of black gram yields, but also became a threat to sustainability. Marker assisted selection assists in obviating the need of the laborious and inconsistent phenotypic screening and also allows easy identification of donor sources. Undoubtedly, YMD management in the long-run will rely on the improvements in genomics-assisted breeding. Hence, attention should be placed on the development of linked markers to YMV resistance, for which screening of available markers is inevitable. Marker validation is a process of studying differential patterns of linked markers in different genetic backgrounds.

Yield enhancement is the ultimate objective for most of the breeding programs in many crop species, which often relies on other component traits. Hence, the study of interrelationships among traits is necessary for understanding the association of simple traits with complex traits that ease the process of selection. The genotype by trait (GT) biplot technique evaluates the varieties based on multiple traits and identifies the superior types.

## MATERIALS AND METHODS

Phenotypic screening

Plant material: In the present study, 46 diverse blackgram genotypes (including highly susceptible YMV check LBG-623) were screened for YMV tolerance under natural field conditions during Summer, 2020 (March to May). The Summer season was chosen for field screening as the YMV disease severity is usually high during this season due to presence of congenial conditions such as hot and dry conditions for vector dispersion.

Experimental layout: All the blackgram genotypes were sown in Randomized Block Design with two replications at Dry Land Farm, S.V. Agricultural College, Tirupati. All the entries were sown in single rows of 3 m length, with a spacing of 30 x 10 cm. The infector row method (Nene, 1972) was followed to ensure even distribution of disease pressure throughout the experimental field. A highly susceptible check (LBG-623) was used as an infector line, that is sown alternately after every two genotypes. No plant protection measures were followed to encourage build up of the white fly population for the spread of disease. The crop was frequently monitored for the presence of whiteflies and the development of YMV symptoms.

Disease screening methodology: When 80% of the infector rows were YMV infected, disease scoring was recorded at weekly intervals as per the disease scale (Ahlawat *et al.,* 2016) (**Table 1**). Apart from YMV scoring, twelve yield and yield attributing traits were also recorded randomly from the selected five plants of each genotype except days to 50% flowering and days to maturity which were recorded on plot basis.

Molecular data analysis: The genomic DNA was isolated from 46 blackgram genotypes by CTAB (Cetyl Trimethyl Ammonium Bromide) method (Doyle and Doyle, 1990). Several research workers (Anjum *et al.,* 2010; Apraku *et al.,* 2010; Basamma, 2011; Gupta *et al.,* 2013, Gupta *et al.,* 2015 & Behera *et al.,* 2020) have attempted to characterize the untapped germplasm sources with SSR markers for YMV tolerance. A total of 15 SSR (Simple Sequence Repeats) markers (CEDG-149, 264, 271, 139, 008, 180, 176, 097, 044, 056, 198, 228, DMB-SSR 125, BM 146 and BM 170) reported to be associated with YMV were used for molecular validation among 46 blackgram genotypes.

Polymorphism Information Content (PIC) score of each marker was calculated as per the formula proposed by Anderson *et al.* (1993).

$$PIC_i = 1 - \sum_{i=1} pi^2$$

Where, i is the total number of alleles for each locus and p is the proportion of the genotypes with the allele. Nei's genetic diversity was estimated using POPGENE 1.32 (Yeh *et al.,* 1999) software.

Selection efficiency of SSR markers for YMV tolerance: Validation of molecular markers was done by estimating selection efficiency. Selection efficiency of each allele of SSR marker is calculated by dividing the genotypes carrying the respective allele size to its total alleles in resistant and susceptible genotypes.

Genotype by Trait (GT) biplot analysis: The Genotype by Trait (GT) biplot approach (Yan and Rajcan, 2002) was used to display the genotype by trait data in a biplot using "R" packages (version 3.1.1) by plotting PC1 scores against PC2 scores for each genotype and each trait.

$$\frac{\alpha_{ij} - \beta_j}{\sigma_j} = \sum_{n=1}^{2} \lambda_n \xi_{in} \eta_{jn} + \varepsilon_{ij} = \sum_{n=1}^{2} \xi^*_{in} \eta^*_{jn} + \varepsilon_{ij}$$

Because n=2 in a biplot, only PC1 and PC2 are retained in the model and such a model tends to be best for extracting patterns and rejecting noise from the data.

**Table 1. Rating scale for scoring yellow mosaic virus disease (1-9 scale):**

| Disease Scale | Percent Infection | Reaction |
|---|---|---|
| 1 | No symptoms or very minute yellow spots on leaves. | Free (F) |
| 2 | Small yellow specks covering 0.1-5 % leaf area. | Highly Resistant (HR) |
| 3 | Leaf Mottling covering 5.1-10 % leaf area. | Resistant (R) |
| 4 | Yellow mosaic patches on leaves covering 10.1-15 % area. | Moderately Resistant (MR) |
| 5 | Yellow mottling (15.1-20 % leaf area) and sometimes complete leaf discoloration | Moderately Susceptible (MS) |
| 6 | Yellowing of leaves (20.1-30 %) and pods. | Susceptible (S) |
| 7 | Yellowing of leaves (30.1- 50 % leaf area) and pods, reduced leaf area & stunted plant growth | Susceptible (S) |
| 8 | Severe yellow coloration of leaves (50.1-75 % leaf area), stunted plant growth and reduced pod size. | Highly Susceptible (HS) |
| 9 | Complete yellowing of foliage, stunted plant growth with no pods | Highly Susceptible (HS) |

## RESULTS AND DISCUSSION

Phenotypic Screening

Yield attributing traits: The genotypes *viz.,* TBG-104, VBN-6, IPU-2-43, MBG-1058 and MBG-1051 were the desirable genotypes for most of the yield attributing traits (**Table 2**).The early flowering genotypes were VBN-6, TU-40, LBG-787, LBG-946, TBG-129, GBG-79 and IPU-2-43. The genotypes VBN-5, TU-40, VBG-11-301, PU-31 and VBN-6 took minimum days to reach maturity. The superior performers for number of clusters per plant were MBG-1058, TBG-136, VVG-09-005, VBN-6 and MBG-223.The genotypes with more number of pods per cluster were IPU-2-43, TBG-104,VBN-5, LBG-22, P-728 and MBG-1050. The top five performers for number of pods per plant were IPU-2-43, MBG-1058, TBG-104, TBG-136 and VBN-6. The genotype with the longest pod was VBN-6 followed by LBG-752, TBG-104, LBG-884 and GBG-108. Nineteen genotypes had higher number of seeds per pod than general mean (5.81).The bold seeded genotypes were TBG-129, TBG-139, LBG-946, TBG-140 and IPU-2-43. For harvest index, twenty genotypes surpassed the general mean with the lines *viz.,* LBG-946, LBG-787, MBG-1058, MBG-1051 and MBG-1050 occupying the top five positions.

YMV reaction of genotypes: Out of 46 blackgram genotypes screened for YMV reaction, eight genotypes (LBG-884, LBG-946, TBG-141, TBG-129, TBG-138, TBG-139, TBG-130 and GBG-1) were disease free with zero disease incidence (**Table 2 and Fig. 1**).

The number of genotypes that showed Highly Resistant (HR), Resistant (R) and Moderately Resistant (MR) were five (TBG-104, GBG-92, TBG-140, PU-31, TU-67), four (VBN-6, TBG-136, VBN-7, VVG-09-005) and four (GBG-108, TBG-135, TU-40, IPU-2-43), respectively. LBG-904 and LBG-918 exhibited moderately susceptible (MS) disease reaction. The genotypes *viz.,* LBG-922, LBG-933, GBG-79, MBG-1050, MBG-1051, MBG-1058, P-728, P-1032, VBG-11-301, VBN-5, LBG-932, LBG-709, LBG-787, MBG-1061, VBN- 4 showed susceptible reaction (S). Eight genotypes *viz.,* LBG-22, LBG-645, MBG-207, MBG-1037, LBG-752, LBG-685, MBG-223 and LBG-623 came under the category of highly susceptible (HS) group.

An inspection into the mean performances of all genotypes for different yield traits and YMV reaction revealed that the genotypes TBG-104 and VBN-6 were high yielding lines with moderate levels of YMV tolerance. The only genotype with disease free reaction and good yielding ability was LBG-946.

Molecular validation for YMV tolerance: Of the 15 SSR markers screened, eight showed polymorphism (53.33 %), while remaining seven markers were monomorphic (46.67 %) (**Table. 3, Fig. 2 and 3**). Molecular marker analysis revealed that the number of alleles varied between 2 and 3 with an average value of 2.37. The polymorphism information content (PIC) values ranged from a minimum of 0.08 (CEDG 149) to a maximum of 0.42 (CEDG 097) with an average of 0.28 suggesting existence of less variability for these markers among the tested cultivars. Higher the PIC value, the more informative is the SSR marker. Hence, the primer CEDG 097 (0.42) has the highest PIC value followed by CEDG 180 (0.41), CEDG 044 (0.36), CEDG 139 (0.33) and CEDG 198 (0.31). The values of Nei's genetic diversity index ranged between 0.18 (CEDG 149) and 0.82 (CEDG 097), with an average of 0.53. The appearance of maximum PIC scores and the high Nei's genetic diversity index values for the primers CEDG 097, CEDG 180, CEDG 044, and CEDG 139 indicates that these SSR primers can be utilized to study the genetic diversity.

Selection efficiency of markers: Among the tested SSR markers, 136 bp allele of CEDG180 was able to distinguish 13 out of 21 resistant genotypes (61.91%) and completely absent in susceptible genotypes (**Table 4**). The 163 bp allele of CEDG180 was present in seven out of 21 resistant genotypes (33.33 %) and out of

**Table 2. Mean performance of 46 blackgram genotypes for maturity, yield components and Disease reaction**

| S. No. | Genotypes | Days to 50% flowering | Days to maturity | Plant height (cm) | Primary branches per plant | Clusters per plant | Pods per cluster | Pods per plant | Pod length (cm) | Seeds per pod | Seed yield per plant(g) | 100 seed weight (g) | Harvest index (%) | Disease reaction |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | LBG-884 | 36.50 | 76.50 | 31.30 | 2.50 | 11.90 | 2.20 | 26.20 | 4.67 | 6.30 | 5.52 | 4.94 | 40.13 | Free (F) |
| 2 | LBG-946 | 34.50 | 78.00 | 28.50 | 2.50 | 9.10 | 2.45 | 25.20 | 4.49 | 6.50 | 5.68 | 5.28 | 43.59 | Free (F) |
| 3 | TBG-141 | 36.00 | 77.50 | 28.10 | 3.50 | 11.00 | 2.30 | 25.80 | 4.37 | 6.90 | 3.81 | 5.06 | 23.11 | Free (F) |
| 4 | TBG-129 | 34.50 | 76.00 | 29.20 | 2.70 | 10.00 | 2.20 | 19.40 | 3.75 | 5.50 | 4.13 | 5.62 | 25.88 | Free (F) |
| 5 | TBG-138 | 38.00 | 77.50 | 24.30 | 2.30 | 9.70 | 2.10 | 19.90 | 4.32 | 5.60 | 4.07 | 4.75 | 26.93 | Free (F) |
| 6 | TBG-139 | 39.50 | 78.50 | 30.30 | 3.40 | 10.30 | 2.20 | 20.30 | 4.21 | 6.00 | 4.76 | 5.29 | 30.23 | Free (F) |
| 7 | TBG-130 | 35.00 | 75.00 | 23.10 | 2.50 | 10.40 | 2.20 | 21.40 | 4.27 | 5.90 | 3.58 | 4.81 | 22.51 | Free (F) |
| 8 | GBG-1 | 35.50 | 73.50 | 24.30 | 2.70 | 10.00 | 2.40 | 23.60 | 4.31 | 6.10 | 4.87 | 4.95 | 29.22 | Free (F) |
| 9 | LBG-752 | 36.50 | 74.50 | 23.90 | 3.50 | 6.90 | 2.10 | 13.50 | 5.37 | 5.60 | 3.39 | 4.46 | 30.97 | Highly Susceptible (HS) |
| 10 | LBG-22 | 39.50 | 77.00 | 29.50 | 2.60 | 7.00 | 2.60 | 16.40 | 4.01 | 6.40 | 3.84 | 4.17 | 25.10 | Highly Susceptible (HS) |
| 11 | LBG-685 | 43.50 | 76.50 | 30.80 | 2.40 | 8.90 | 2.20 | 17.80 | 4.19 | 5.50 | 3.30 | 4.40 | 19.86 | Highly Susceptible (HS) |
| 12 | LBG-645 | 39.00 | 76.50 | 21.40 | 2.10 | 6.40 | 2.10 | 12.40 | 4.11 | 5.00 | 3.37 | 3.71 | 19.22 | Highly Susceptible (HS) |
| 13 | MBG-207 | 41.50 | 76.50 | 25.90 | 2.10 | 11.00 | 2.10 | 23.70 | 4.22 | 6.10 | 4.41 | 4.15 | 28.51 | Highly Susceptible (HS) |
| 14 | MBG-223 | 40.00 | 74.50 | 25.90 | 2.10 | 13.00 | 2.10 | 25.70 | 4.22 | 5.20 | 4.77 | 4.58 | 28.51 | Highly Susceptible (HS) |
| 15 | MBG-1037 | 44.50 | 78.50 | 32.60 | 2.50 | 9.90 | 2.20 | 18.40 | 4.31 | 5.70 | 3.85 | 4.20 | 19.71 | Highly Susceptible (HS) |
| 16 | LBG-623 | 44.00 | 81.50 | 30.50 | 3.10 | 5.20 | 2.20 | 11.40 | 4.36 | 4.90 | 2.83 | 3.50 | 31.03 | Highly Susceptible (HS) |
| 17 | LBG-904 | 37.50 | 75.50 | 28.90 | 2.30 | 9.40 | 2.40 | 21.50 | 4.44 | 6.20 | 4.33 | 5.01 | 32.41 | Moderately Susceptible (MS) |
| 18 | LBG-932 | 35.50 | 76.50 | 23.50 | 2.40 | 7.30 | 2.00 | 14.24 | 4.29 | 5.40 | 3.42 | 4.34 | 26.90 | Susceptible (S) |
| 19 | LBG-918 | 39.00 | 76.00 | 22.60 | 2.50 | 10.50 | 2.10 | 20.20 | 4.41 | 5.50 | 3.97 | 4.72 | 27.68 | Moderately Susceptible (MS) |
| 20 | LBG-922 | 37.50 | 77.50 | 23.00 | 2.70 | 9.40 | 2.10 | 19.00 | 4.20 | 6.30 | 4.19 | 5.14 | 29.93 | Susceptible (S) |
| 21 | LBG-933 | 36.50 | 78.50 | 24.50 | 3.10 | 11.30 | 2.10 | 21.50 | 4.30 | 6.00 | 4.17 | 5.04 | 28.64 | Susceptible (S) |
| 22 | GBG-79 | 34.50 | 73.50 | 20.80 | 2.30 | 9.10 | 2.20 | 19.60 | 4.32 | 5.80 | 3.88 | 4.62 | 22.14 | Susceptible (S) |
| 23 | GBG-92 | 36.50 | 74.50 | 24.00 | 2.40 | 8.40 | 2.30 | 18.40 | 4.23 | 5.70 | 3.52 | 4.66 | 19.25 | Highly Resistant (HR) |
| 24 | GBG-108 | 37.00 | 76.50 | 28.80 | 2.60 | 9.50 | 2.00 | 16.00 | 4.63 | 5.70 | 3.65 | 4.98 | 21.03 | Moderately Resistant (MR) |
| 25 | TBG-140 | 37.50 | 78.00 | 23.60 | 2.60 | 12.20 | 2.20 | 28.70 | 4.31 | 6.30 | 3.31 | 5.15 | 21.91 | Highly Resistant (HR) |
| 26 | TBG-135 | 35.50 | 74.50 | 17.40 | 2.20 | 7.00 | 2.00 | 15.40 | 3.79 | 5.40 | 3.99 | 4.41 | 22.00 | Moderately Resistant (MR) |
| 27 | TBG-136 | 35.50 | 75.50 | 27.70 | 3.50 | 14.70 | 2.30 | 29.10 | 4.18 | 6.40 | 4.93 | 4.73 | 26.33 | Resistant (R) |
| 28 | TU-40 | 32.50 | 70.50 | 21.50 | 3.10 | 12.00 | 2.20 | 25.80 | 3.92 | 6.40 | 4.61 | 4.75 | 27.46 | Moderately Resistant (MR) |
| 29 | PU-31 | 36.50 | 73.00 | 21.80 | 2.70 | 10.20 | 2.10 | 21.40 | 4.08 | 5.70 | 3.77 | 4.28 | 21.04 | Highly Resistant (HR) |
| 30 | IPU-2-43 | 34.50 | 75.00 | 23.20 | 3.55 | 10.37 | 3.45 | 33.35 | 3.80 | 5.20 | 6.61 | 5.15 | 37.87 | Moderately Resistant (MR) |
| 31 | TBG-104 | 36.00 | 74.50 | 25.35 | 3.70 | 9.80 | 3.20 | 30.45 | 5.01 | 6.70 | 7.83 | 4.38 | 38.68 | Highly Resistant (HR) |
| 32 | LBG-709 | 41.50 | 79.50 | 22.30 | 2.40 | 8.30 | 2.10 | 17.20 | 4.30 | 5.50 | 4.63 | 4.39 | 23.80 | Susceptible (S) |
| 33 | LBG-787 | 32.50 | 73.50 | 23.20 | 2.40 | 11.00 | 2.20 | 24.40 | 4.03 | 6.40 | 5.21 | 4.28 | 43.54 | Susceptible (S) |
| 34 | MBG-1050 | 44.00 | 77.50 | 22.30 | 2.50 | 7.50 | 2.50 | 18.70 | 4.42 | 5.60 | 4.76 | 4.42 | 41.07 | Susceptible (S) |
| 35 | MBG-1051 | 41.00 | 75.50 | 21.80 | 2.30 | 10.70 | 2.40 | 28.00 | 4.26 | 5.70 | 6.01 | 4.48 | 42.04 | Susceptible (S) |
| 36 | MBG-1058 | 39.00 | 79.50 | 33.00 | 2.40 | 15.80 | 2.30 | 32.20 | 4.13 | 5.70 | 6.43 | 4.44 | 42.92 | Susceptible (S) |
| 37 | MBG-1061 | 36.50 | 73.50 | 22.40 | 2.60 | 12.80 | 2.30 | 27.30 | 4.37 | 5.60 | 5.61 | 4.76 | 36.35 | Susceptible (S) |
| 38 | P-728 | 35.00 | 74.00 | 21.90 | 2.50 | 10.80 | 2.50 | 26.05 | 4.33 | 5.60 | 5.09 | 4.40 | 29.15 | Susceptible (S) |
| 39 | P-1032 | 37.50 | 76.50 | 15.90 | 2.10 | 10.00 | 2.10 | 20.90 | 3.64 | 5.20 | 3.81 | 5.10 | 23.74 | Susceptible (S) |
| 40 | TU-67 | 36.00 | 73.50 | 22.90 | 3.25 | 11.50 | 2.20 | 24.20 | 4.04 | 6.20 | 5.34 | 4.61 | 35.01 | Highly Resistant (HR) |
| 41 | VBG-11-301 | 35.50 | 71.50 | 23.10 | 2.70 | 10.50 | 2.00 | 20.80 | 4.59 | 6.10 | 5.23 | 4.91 | 32.09 | Susceptible (S) |
| 42 | VBN-5 | 35.50 | 70.50 | 22.40 | 3.15 | 10.20 | 2.60 | 25.30 | 3.90 | 5.60 | 5.13 | 4.57 | 35.37 | Susceptible (S) |
| 43 | VBN-6 | 32.00 | 73.00 | 24.30 | 2.65 | 13.50 | 2.30 | 28.80 | 5.87 | 5.90 | 6.98 | 5.01 | 37.72 | Resistant (R) |
| 44 | VBN-7 | 37.00 | 73.50 | 15.80 | 2.65 | 9.10 | 2.50 | 24.25 | 4.57 | 5.35 | 5.24 | 4.84 | 36.86 | Resistant (R) |
| 45 | VVG-09-005 | 36.50 | 74.50 | 22.40 | 3.15 | 13.60 | 2.00 | 26.20 | 4.00 | 5.40 | 5.44 | 4.67 | 27.19 | Resistant (R) |
| 46 | VBN- 4 | 41.50 | 78.50 | 19.20 | 2.50 | 11.20 | 2.20 | 26.10 | 4.51 | 5.70 | 5.94 | 4.72 | 31.43 | Susceptible (S) |
| | Max. | 44.50 | 81.50 | 33.00 | 3.70 | 15.80 | 3.45 | 33.35 | 5.87 | 6.90 | 7.83 | 5.62 | 43.59 | |
| | Min. | 32.00 | 70.50 | 15.80 | 2.10 | 5.20 | 2.00 | 11.40 | 3.64 | 4.90 | 2.83 | 3.50 | 19.22 | |
| | Mean | 37.41 | 75.70 | 24.55 | 2.68 | 10.18 | 2.27 | 22.31 | 4.31 | 5.81 | 4.63 | 4.67 | 29.70 | |
| | SE(m) | 0.76 | 0.84 | 0.75 | 0.09 | 0.57 | 0.10 | 1.03 | 0.08 | 0.18 | 0.2 | 0.19 | 1.94 | |
| | SE(d) | 1.08 | 1.19 | 1.06 | 0.12 | 0.81 | 0.14 | 1.46 | 0.12 | 0.26 | 0.28 | 0.27 | 2.74 | |
| | C.D. (5%) | 2.19 | 2.40 | 2.16 | 0.25 | 1.64 | 0.29 | 2.94 | 0.24 | 0.51 | 0.57 | 0.54 | 5.55 | |
| | C.V.(%) | 2.89 | 1.57 | 4.35 | 4.56 | 7.81 | 6.33 | 6.37 | 2.75 | 4.39 | 6.023 | 5.71 | 9.17 | |

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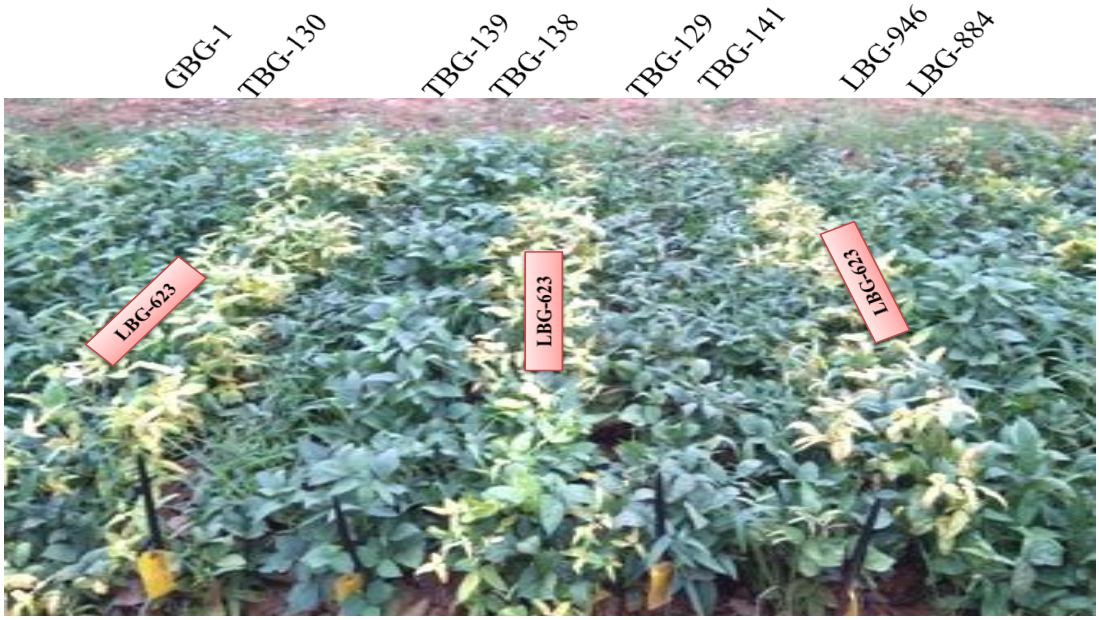


**Fig. 1. Blackgram genotypes with zero YMV incidence**

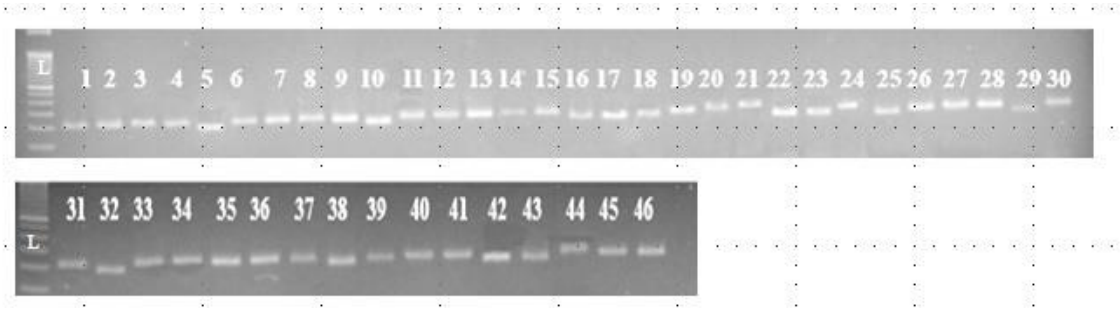


**Fig. 2. Amplification profile of CEDG 180 among 46 blackgram genotypes**

1-LBG-752, 2-LBG-22, 3-LBG-685, 4-LBG-645, 5-MBG-207, 6-MBG-223, 7-MBG-1037, 8-LBG-623, 9-LBG-904, 10-LBG-932, 11-LBG-918, 12-LBG-922, 13-LBG-933, 14-GBG-79, 15-GBG-108, 16-LBG-709, 17-LBG-787, 18-MBG-1050, 19-MBG-1051, 20-MBG-1058, 21-MBG-1061, 22-P-728, 23-P-1032, 24-VBG-11-031, 25-TBG-140, 26-TBG-126, 27-VBN-6, 28-VBN-7, 29-VVG-09-005, 30-TBG-135, 31-VBN-5, 32-LBG-884, 33-LBG-946, 34-TBG-141, 35-TBG-129, 36-TBG-138, 37-TBG-139, 38-TBG-130, 39-GBG-1, 40-TBG-104, 41-TU-40, 42-IPU-2-43, 43-TU-67, 44-PU-31, 45-TBG-136, 46-GBG-92

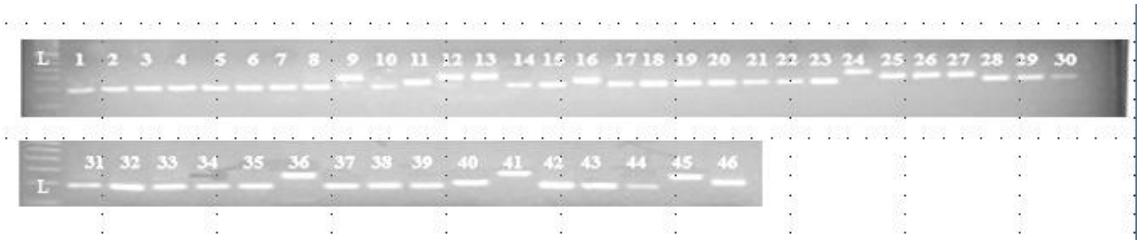


**Fig. 3. Amplification profile of CEDG 097 among 46 blackgram genotypes**

1-LBG-884, 2- LBG-946, 3-TBG-141, 4-TBG-129, 5-TBG-138, 6-TBG-139, 7-TBG-130, 8-GBG-1, 9-LBG-752 , 10-LBG-22, 11-LBG-685, 12- LBG-645, 13-MBG-207, 14-MBG-223, 15-MBG-1037, 16-LBG-623, 17-LBG-904, 18-LBG-932, 19-LBG-918, 20-LBG-922, 21-LBG-933, 22-GBG-79, 23-GBG-92, 24-GBG-108, 25-TBG-140, 26-TBG-135, 27-TBG-136, 28-TU-40, 29-PU-31, 30-IPU-2-43, 31-TBG-104, 32-LBG-709, 33-LBG-787, 34-MBG-1050 , 35-MBG-1051, 36-MBG-1058, 37-MBG-1061, 38-P-728, 39-P-1032, 40-TU-67, 41-VBG-11-301, 42-VBN-5, 43-VBN-6, 44-VBN-7, 45-VVG-09-005, 46-VBN- 4, L-50 bp ladder.

**Table 3. Molecular analysis of SSR markers**

| S. No. | Primer name | Ann. Temp (°C) | Forward Sequence (5'-3') | Reverse Sequence (3'-5') | Allele size (bp) | Na | PIC | Nei's |
|---|---|---|---|---|---|---|---|---|
| 1. | CEDG 149 | 57 | GGCTGAAGGTGATGACA GAAG | GGCACTGGTTTTCTAAGGTTGTTG | 190-210 | 2 | 0.08 | 0.18 |
| 2 | CEDG 139 | 62 | CAAACTTCCGATCGAAAG CGCTTG | GTTTCTCCTCAATCTCAAGCTCCG | 90-180 | 3 | 0.33 | 0.66 |
| 3. | CEDG 008 | 60 | GGAATTAGAGATGATT GGAC | CACCACTTCATTATGTATGG | 110-140 | 2 | 0.18 | 0.34 |
| 4. | CEDG 180 | 57 | GGTATGGAGCAAAAC AATC | GTGCGTGAAGTTGTCTTATC | 100-163 | 3 | 0.41 | 0.66 |
| 5. | CEDG 176 | 57 | GGTAACACGGGTTCA GATGCC | CAAGGTGGAGGACAAGATCGG | 150-180 | 2 | 0.11 | 0.34 |
| 6. | CEDG 097 | 60 | GTAAGCCGCATCCATAA TTCCA | TGCGAAAGAGCCGTTAGTAGAA | 100-150 | 3 | 0.42 | 0.82 |
| 7. | CEDG 044 | 57 | TCAGCAACCTTGCATT GCAG | TTTCCCGTCACTCTTCTAGG | 130-180 | 2 | 0.36 | 0.67 |
| 8. | CEDG 198 | 57 | CAAGGAAGATGGAGA GAATC | CCTTCTAAGAACAGTGACATG | 210-230 | 2 | 0.31 | 0.59 |

Na- Number of alleles per locus, PIC- Polymorphism Information Content, LG – Linkage Group, Nei's- Nei's genetic diversity index.

**Table 4. Selection efficiency of polymorphic markers in 46 blackgram genotypes**

| S. No. | SSR marker | Allele (bp) | Percent representation in resistant genotypes | Percent representation in susceptible genotypes |
|---|---|---|---|---|
| 1 | CEDG 097 | 100 | 71.43 | 68.00 |
| | | 120 | 19.05 | 12.00 |
| | | 150 | 9.52 | 20.00 |
| 2 | CEDG 044 | 130 | 52.38 | 68.00 |
| | | 180 | 47.62 | 32.00 |
| 3 | CEDG 149 | 190 | 100.00 | 92.00 |
| | | 210 | 0.00 | 8.00 |
| 4 | CEDG 180 | 100 | 4.76 | 4.00 |
| | | 136 | 61.91 | 0.00 |
| | | 163 | 33.33 | 96.00 |
| 5 | CEDG 139 | 90 | 0.00 | 4.00 |
| | | 150 | 71.43 | 76.00 |
| | | 180 | 28.57 | 20.00 |
| 6 | CEDG 008 | 110 | 90.48 | 88.00 |
| | | 140 | 9.52 | 12.00 |
| 7 | CEDG 176 | 150 | 100.00 | 88.00 |
| | | 180 | 0.00 | 12.00 |
| 8 | CEDG 198 | 210 | 66.67 | 80.00 |
| | | 230 | 33.33 | 20.00 |

25 susceptible genotypes it was represented among 24 genotypes (96.00%).

Based on molecular validation results it can be inferred that the SSR markers CEDG 097 followed by CEDG 180, CEDG 044 and CEDG 139 were more efficient. Hence, these markers could be utilized in breeding programs aimed at developing YMV resistant varieties for easy and efficient selection of potential donors.

Inter-relationships among yield, yield traits, earliness and YMV reaction in blackgram using Genotype by Trait (GT) biplot analysis: The GT biplot (**Fig. 4**) captured 47.14 % of the total variation of the standardized data, of which 32.69% was explained by the first principal component (PC1) and 14.45% explained by the second principal component (PC2). This low level of variation conveys the complex inter-relationships among the traits studied (Yan and Rajcan, 2002). From GT biplot results it was evident that the traits *viz.,* number of pods per cluster, harvest index, pod length, number of pods per plant, number of primary branches per plant, number of clusters per plant, number of seeds per pod, 100- seed weight and plant height showed positive association with seed yield per plant. The traits showing significant positive relationship with seed yield would be helpful in the improvement of the seed yield. Seed yield was negatively associated with YMV scores, days to 50% flowering and days to maturity. Similar kind of association among yield and yield attributing traits was also reported by (Aghaee *et al.,* 2010; Singh *et al.*, 2014; Paramesh, 2014; Oladejo *et al.,* 2011; Sharma *et al.,* 2018; Mahmoud *et al.,* 2020).

YMV scores among genotypes was found to be positively associated with days to 50 % flowering, days to maturity and plant height. This trait was negatively associated with 100- seed weight, number of seeds per pod, number of clusters per plant, number of primary branches per plant, number of pods per plant, seed yield per plant, number of pods per cluster, harvest index and pod length. The length of the attribute vector also is a good marker to show ability of traits in discriminating accessions; the traits with longer vector will be more successful in discriminating accessions. In the present study, traits *viz.,* seed yield plant per plant, number of pods per plant, days to 50 % flowering and harvest index had higher discriminating ability. The traits *viz.,* pod length, number of primary branches per plant, plant height and number of seeds per pod had the least discriminating ability.

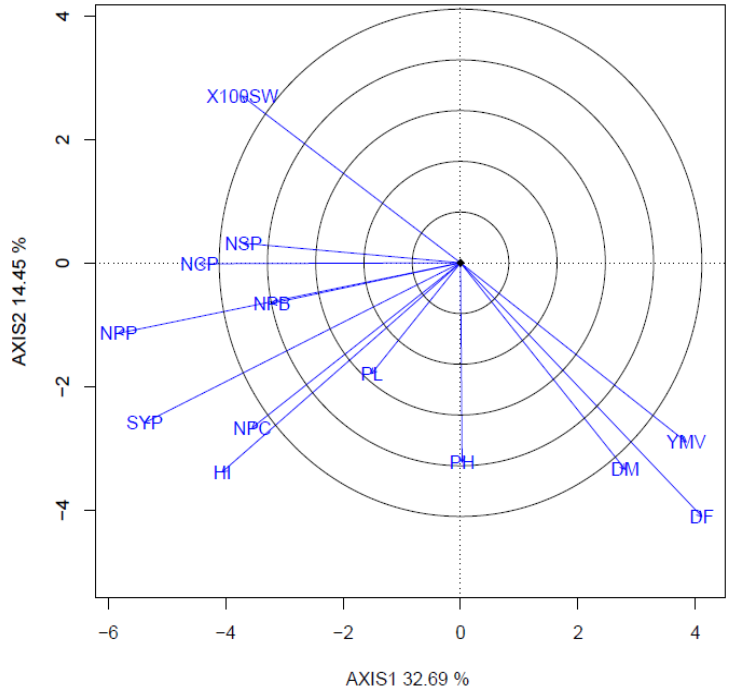


**Fig. 4. Genotype-by-trait biplot showing trait inter- relationships**

DF: Days to 50% flowering, DM: Days to maturity, PH: Plant height (cm), NPB: Number of primary branches plant$^{-1}$, NCP: Number of clusters plant$^{-1}$, NPC: Number of pods cluster$^{-1}$, NPP: Number of pods plant$^{-1}$, PL: Pod length (cm), NSP: Number of seeds pod$^{-1}$, SYP: Seed yield plant$^{-1}$ (g), 100-SW: 100- seed weight (g), HI: Harvest index (%)

Performance of blackgram genotypes for yield, yield traits, earliness and YMV reaction based on GT biplot analysis: ("Which won what" genotype by trait biplot analysis)

High values for all the studied traits were desirable except for the traits, days to 50% flowering, days to maturity and YMV scores for which low values are desirable. The traits were considered as the tester and the cultivars as entries. The genotype at vertex position in the biplot (**Fig. 5**) is known as vertex genotype. The vertex genotypes are the ones with the highest value within the sector. These vertex genotypes could be utilized as parents in hybridization programs.

The genotypes (**Fig. 5**) *viz.,* TU-40, TBG-135, LBG-645, LBG-623, MBG-1058 and TBG-104 were regarded as vertex genotypes as they were outperformers for the traits within the sector. The genotype MBG-1058 was found to be better performer for plant height. The genotype TU-40 came out as better performer for 100- seed weight. LBG-623 exhibited higher values for the traits within the sector *viz.,* days to 50% flowering, days to maturity and YMV scores indicating that this is a long duration variety highly susceptible to YMV. The genotype TBG-104 was observed to be outstanding genotype for the characters *viz.,* harvest index, number of pods per cluster, seed yield per plant, pod length, number of pods per plant, number of primary branches per plant, number of clusters per plant and number of seeds per pod.

Ranking of genotypes using GT biplot analysis: The best genotype is the one with the longest projection onto ATC abscissa and positioned closest to the ideal entry (innermost concentric circle with an arrow in **Fig. 6**). Based on the ranking pattern, TBG-104 was identified as the ideal genotype, while IPU-2-43, MBG-1058, VBN-6 were ranked close to the ideal genotype.

Based on their performance, the genotypes were ranked as follows,TBG-104 > IPU-2-43 > MBG-1058 > VBN-6 ≈ LBG-946 ≈ LBG-884 ≈ MBG-1051 > TBG-136 ≈ MBG-1061 ≈ VBN-4 > LBG-787 ≈ TBG-139 > VBN-5 > TU-67 ≈ P-728 ≈ LBG-904 ≈ MBG-1050 > TBG-141 ≈ VBN-7 > VVG-09-005 ≈ LBG-933 ≈ MBG-223 ≈ MBG-207 > GBG-1 ≈ VBG-11-031 > TBG-140 ≈ LBG-922 ≈ LBG-22 > TBG-138 ≈ LBG-918 ≈ LBG-752 > LBG-709 ≈ MBG-1037 >

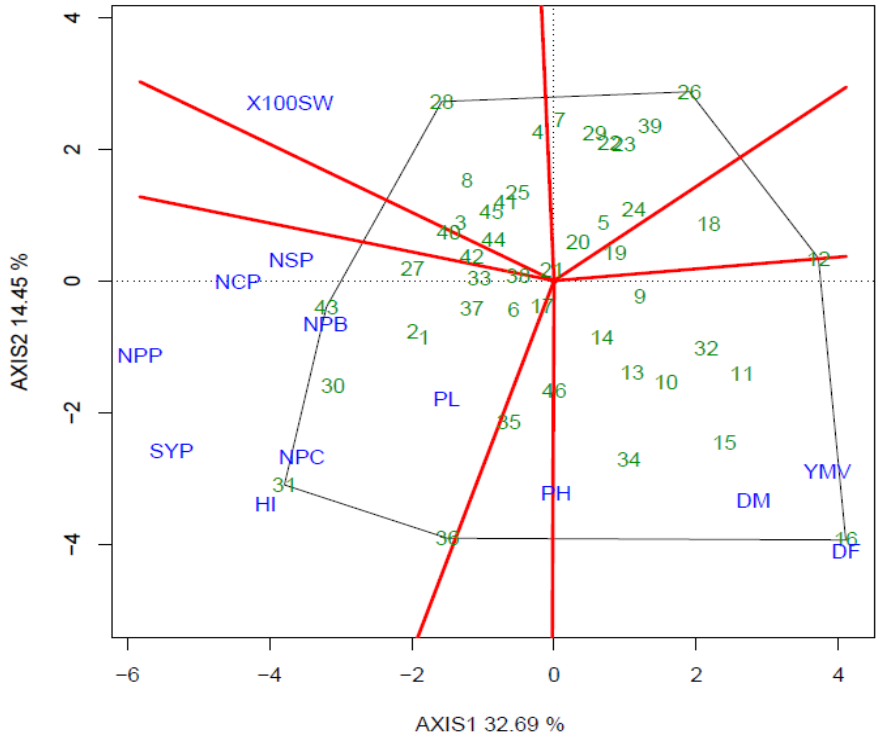


**Fig. 5. A "Which won what" genotype-by-trait biplot of yield, yield traits, earliness and YMV reaction among 46 blackgram genotypes**

DF: Days to 50% flowering, DM: Days to maturity, PH: Plant height (cm), NPB: Number of primary branches plant$^{-1}$, NCP: Number of clusters plant$^{-1}$, NPC: Number of pods cluster$^{-1}$, NPP: Number of pods plant$^{-1}$, PL: Pod length (cm), NSP: Number of seeds pod$^{-1}$, SYP: Seed yield plant$^{-1}$ (g), 100-SW: 100- seed weight (g), HI: Harvest index (%)

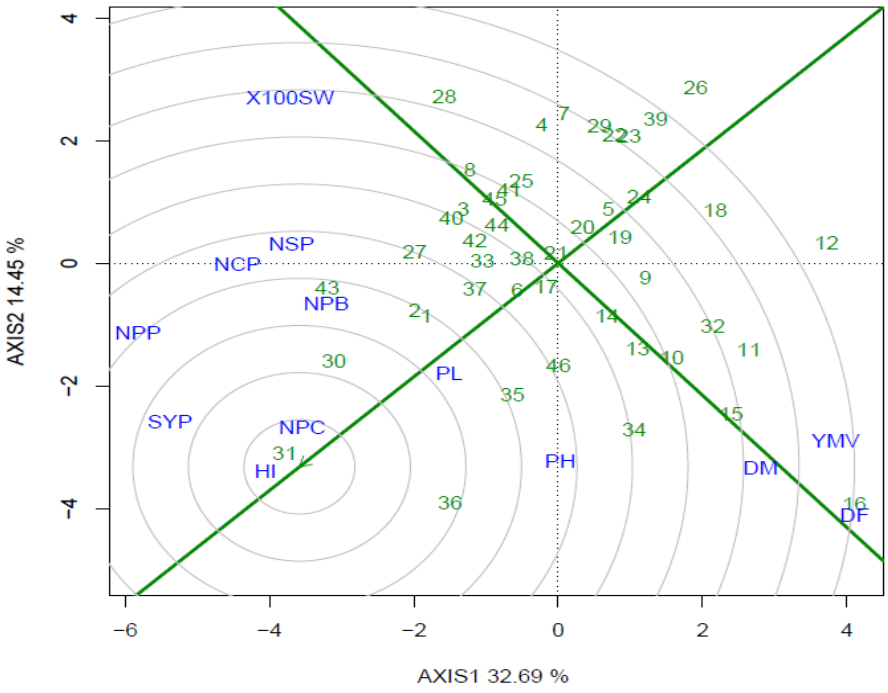


**Fig. 6. Multi trait profile based genotype ranking**

DF: Days to 50% flowering, DM: Days to maturity, PH: Plant height (cm), NPB: Number of primary branches plant$^{-1}$, NCP: Number of clusters plant$^{-1}$, NPC: Number of pods cluster$^{-1}$, NPP: Number of pods plant$^{-1}$, PL: Pod length (cm), NSP: Number of seeds pod$^{-1}$, SYP: Seed yield plant$^{-1}$ (g), 100-SW: 100- seed weight (g), HI: Harvest index (%)

1-LBG-884, 2- LBG-946, 3-TBG-141, 4-TBG-129, 5-TBG-138, 6-TBG-139, 7-TBG-130, 8-GBG-1, 9-LBG-752 , 10-LBG-22, 11-LBG-685, 12- LBG-645, 13-MBG-207, 14-MBG-223, 15-MBG-1037, 16-LBG-623, 17-LBG-904, 18-LBG-932, 19-LBG-918, 20-LBG-922, 21-LBG-933, 22-GBG-79, 23-GBG-92, 24-GBG-108, 25-TBG-140, 26-TBG-135, 27-TBG-136, 28-TU-40, 29-PU-31, 30-IPU-2-43, 31-TBG-104, 32-LBG-709, 33-LBG-787, 34-MBG-1050 , 35-MBG-1051, 36-MBG-1058, 37-MBG-1061, 38-P-728, 39-P-1032, 40-TU-67 , 41-VBG-11-301, 42-VBN-5, 43-VBN-6, 44-VBN-7, 45-VVG-09-005, 46-VBN- 4

TU-40 ≈ TBG-129 ≈ GBG-108 ≈ LBG-685 > TBG-130 ≈ PU-31≈ GBG-79 > GBG-92 >LBG-932 > P-1032 >LBG-623 > TBG-135 ≈ LBG-685.

The genotypes showing zero disease incidence could be used as potential donors in YMV resistance breeding programs of blackgram. The genotypes, TBG-104 and VBN-6 were ideal cultivars that can be most desirable parents for breeding programs aimed at developing short duration, high yielding varieties with YMV tolerance. Among all, CEDG180 could be considered as a potential marker for marker assisted breeding programs in blackgram aimed at developing YMV resistant varieties. Visual comparison of trait profiles among the 46 blackgram genotypes using GT biplot analysis identified the genotypes *viz.,* TBG-104 and VBN-6 as ideal cultivars that serve as most desirable parents in breeding programs aimed at developing short duration, high yielding varieties with YMV tolerance.

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