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Genetic diversity analysis in blackgram (*Vigna mungo*) genotypes using microsatellite markers for resistance to *Yellow mosaic virus*

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Abstract: One hundred and two blackgram genotypes were evaluated for *Yellow mosaic virus* (YMV) resistance based on disease scoring, molecular characterisation, yield and morphological observation. An analysis of variance and a correlation analysis were performed on nine biometric traits. The genotypes showed the highest genetic variation for a single plant yield (genotypic coefficient variation 42.72%). The genotypes were grouped into 18 clusters based on the morphological data. The genetic divergence among the blackgram genotypes was differentiated by 60 alleles using 14 polymorphic simple sequence repeat markers. The polymorphism information content value varied from 0.37 to 0.79. The multivariate analyses of the simple sequence repeat marker scorings divided the germplasm into five divergent clusters wherein, clusters viz., II, III and V accommodated 61 genotypes and all these genotypes were resistant to moderately resistant for YMV. The tolerant accessions identified from this present investigation can be directly used for further crop improvement programmes.

Keywords: black gram germplasm; YMV resistance; polymorphic information content

Pulses are the second largest class of food crops grown globally on about 22 million hectares, annually producing 12–16 million tonnes of vegetarian protein source (FAOSTAT 2014). The contribution of legume pulses viz., the pigeon pea, blackgram and greengram, is notable to the total pulse

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production. India is the leading producer and consumer of blackgram, producing 1.76 million tonnes from 3.26 million hectares (AICRP 2015). Blackgram or urdbean (*Vigna mungo* L. Hepper), a member of the Asian *Vigna* crop group is a staple crop in Central and South East Asia and is extensively used in India and is now grown in the southern United States, the West Indies, Japan and other tropic and subtropic regions (Delic et al. 2009). Blackgram is a member of the Fabaceae family and the genus *Vigna* of tribe Phaseoleae. Blackgram is a perfect ingredient of a balanced diet with protein (25–26%), carbohydrates (60%), fat (1.5%), minerals, amino acids and vitamins (Karamany 2006).

The productivity of blackgram remains low due to biotic and abiotic stresses. Plant viral diseases cause serious economic losses in pulse crops (Kang et al. 2005). Among the diseases, *Yellow mosaic virus* (YMV) disease was given special attention because of the severity and ability to cause yield losses up to 85 percent with a host range of Fabaceae members including the blackgram, mung bean, moth bean and pigeon pea (Nene 1972; AVRDC 1998; Varma & Malathi 2003; Biswas & Varma 2012).

YMV resistant blackgram varieties have not been successfully developed through conventional breeding methods due to the rapid outburst of new YMV isolates and also due to the complexity of the mechanism in controlling the YMV resistance. Screening at endemic and epidemic hotspot regions has also produced incompatible results. Hence, plant breeders and pathologists are in need of new crop improvement tools in elucidating the molecular mechanism identifying tolerant genotypes. A single dominant gene (Sandhu et al. 1985; Gupta et al. 2005), double recessive genes (Verma & Singh 1988; Ammavasai et al. 2004) and complementary recessive genes (Shukla & Pandya 1985) were reported as contributing to the YMV resistance.

The average grain yield of blackgram ranges about 450–800 kg/ha, which is lower than its potential yield. To improve the yield potential of cultivated blackgram varieties, it is essential to utilise the available genetic diversity present in the genetic resources and it could be the only possible means to attain sustainable development and nutritional security (Ramya et al. 2013; Yasin 2015). When compared to morphological markers, molecular markers can survey allelic diversities among genotypes and they are efficient tools for germplasm characterisation without the influence of environmental factors. Molecular genetic diversity could be used to exemplify and classify reliable resistance germplasm sources for use in crop breeding processes (O'Neill et al. 2003).

Hence, the present investigation was framed to identify the resistant germplasm sources through field evaluation and to differentiate them at the molecular level through micro satellite markers (simple sequence repeat markers) linked with YMV resistance in the blackgram.

MATERIAL AND METHODS

Plant material

The field experiments were carried out at Department of Plant Breeding and Genetics, Agricultural College and Research Institute, Killikulam, Tamil Nadu, India during the kharif season from 2015 to 2017 to evaluate 102 blackgram accessions collected from various resources viz., National Bureau of Plant Genetic and Resources (NBPGR), New Delhi, Agricultural College and Research Institute, Madurai and Agricultural College and Research Institute, Killikulam, which susceptible checks (ADT 3 and APK 4) and resistant check (VBN 4) in a randomised block design with two replications. These 102 diverse accessions were chosen from the total gene bank collections based on passport data. The pedigree details of the blackgram accessions used in the present investigation are listed in Table S1 in electronic supplementary material (ESM) (for the supplementary material see the electronic version).

Sampling

Five random plants were taken from each replication to observe the quantitative traits viz., the days to 50% flowering, plant height (cm), primary branches per plant, clusters per plant, pods per plant, seeds per pod, hundred seed weight (g), single plant yield (g) and protein content. The protein content was estimated using the micro Kjedahl method (Konda et al. 2009). The mean values of five plants were computed and taken for analysis.

YMV screening

To screening for YMV, the genotypes were raised along with two susceptible checks in an YMV hot spot area at Panpozhi, Tamil Nadu, India. Observations on the disease incidence were taken on five randomly selected plants of each entry and

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the mean of each entry was taken to assign the category. The disease was recorded on a 1–9 arbitrary scale according to Alice and Nadarajan (2007).

Statistical analysis using morphological data

The data were subjected to a statistical analysis using (Mahalanobis 1936) D^2 statistics and Tocher's method as described by Rao (1952) to determine the group constellation. The various genetic parameters viz., genotypic coefficient of variance (GCV), phenotypic coefficient of variance (PCV) were calculated by adopting the formulae given by Johnson et al. (1955). The genotypic correlation coefficient was calculated based on the formula given by Snedecor (1961).

Extraction of genomic DNA and quantification

DNA was extracted from the leaf samples of the 102 blackgram genotypes following the cetyl triammonium bromide method (Gupta et al. 2015). Young fresh 10–15-day-old leaf samples were taken for the DNA extraction. The DNA quality was checked on an 0.8% (w/v) agarose gel and the concentration was determined using a spectrophotometer (Eppendorf Biophotometer D30; Eppendorf, Germany). The DNA dilution for genotyping was prepared in molecular grade water to a concentration of 10 ng/L and stored at –20 °C.

Simple sequence repeat marker-based genotyping

YMV resistance specific 29 simple sequence repeat (SSR) primer pairs (Gupta et al. 2013) were selected for the molecular studies. A polymerase chain reaction (PCR) reaction was carried out in a total volume of 10 µL containing 50 ng DNA, 0.2 mM of each dNTP, 2.0 mM $MgCl_2$, 1.0 IU Taq polymerase and 400 nM of each primer using a thermal cycler (GeneAmp PCR system 9700; Applied Biosystems, USA) with 40 cycles of initial denaturation at 94 °C for 5 min; denaturation at 94 °C for 30 s; annealing at 55 °C for 30 s and extension at 72 °C for 1 minute. The final extension was set at 72 °C for 10 min and stored at (4 °C). The list of microsatellite markers specific to the YMV resistance used for surveying the genotypes are furnished in the table (Table S2 in ESM). The PCR products were electrophoretically separated on 3.5% MetaPhor agarose gel containing 0.01% ethidium bromide, prepared in 1× TAE (tris-acetic acid-EDTA). After electrophoresis, the amplification products were visualised in a gel documentation system.

Data analyses

Polymorphism survey. Microsatellite SSR markers were used to survey the polymorphism variation between the genotypes at the DNA level. Once the gel electrophoresis was finished, the gel was visualised to score the allelic variations of the amplified DNA products for each primer. Clear cut polymorphic bands were scored visually by comparing them with a standard 100 bp ladder. The scores were obtained in the form of a matrix with the allele's size scored in columns, which indicates variation in the migration of the amplicons for each genotype. The scores of each marker were used to determine the polymorphism information content (PIC). The scores were analysed to construct a dendrogram using the software DARwin (version 5.0; http://darwin.cirad.fr/).

Polymorphic information content. The polymorphic information content or expected heterozygosity scores for each SSR marker was calculated based on the formula given by Anderson et al. (1993):

$$PIC = 1 - \Sigma P_i^2 \quad (1)$$

where: P_i – the frequency of the marker i and the summation extends over n alleles.

Multivariate analysis. A multivariate analysis (cluster analysis) was carried out using the software DARwin (version 6.0; http://darwin.cirad.fr/). The alleles scored in Excel files were later converted to a text editor and a dissimilarity matrix was constructed using DARwin (version 5.0). The dissimilarity matrix was used for the construction of the dendrograms based on the neighbour-joining method. Hierarchical clustering for quantitative traits of the mutants was similarly formed using the software SPSS (version 6.0).

Principal component analysis. The use of established multivariate statistical algorithms is a key strategy in classifying germplasm, for ordering the variability of a large number of accessions and analysing the genetic relationships among breeding materials. Among these, a cluster analysis, principal component analysis (PCA), principal coordinate analysis and multidimensional scaling are most commonly employed (Mohammadi & Prasanna 2003). The mean values regarding the quantitative traits were calculated and a PCA on the Box-Cox transformed values was performed with the SPSS software (version 6.0). In the PCA, the data were

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used to generate eigenvalues, the percentage variation accumulated by the component, the load coefficient values between the original characteristics to the respective principal components. The principal components that accounted for the highest variation were used to plot the scatter diagram of the cultivars.

RESULTS AND DISCUSSION

Analysis of variance

An analysis of variance (ANOVA) was carried out for all nine studied characteristics. All the genotypes differed significantly among themselves for all the studied characteristics (Table 1). The treatment mean sum of squares due to the genotypes was found to be highly significant for all the studied characteristics which would ultimately indicate the diverse nature of the selected genotypes.

Mean performance

The mean performance showed variation for most of the characteristics under study (Table 2). The mean performance of the genotypes for days to 50% flowering ranged from 30.00 (VBG 12034, VBG 10010) to 44.50 (KKB 14011) days. The highest mean value for the plant height was recorded for the genotype KU 1239 (59.90 cm) and the lowest was observed for the genotype ABG 11036 (22.40 cm). The mean performance of the trait primary branches per plant was 5.84 and the range varied from 3.20 (IC 281978) to 11.60 (ABG 11030). A mean of 7.88 with the range of 3.20 (IC 282002) to 18.40 (IC 281982) was recorded for clusters per plant. The highest mean obtained for the trait pods per plant was 49.60 (KU 11667) and the lowest mean value was 15.20 (VBG 11024). The range recorded for the single plant yield varied from 4.02 g (IC 343947) to 29.10 g (IC 343962) with an average of 11.13 g. The highest mean value observed for the protein content was 28.44 g (ABG 11015).

Variability parameters

The success of any breeding programme depends largely on the extent of the genetic variability present in the base population (Table 2). The highest genetic variation was observed in the single plant yield (GCV 42.72% and PCV 42.96%); clusters per plant (GCV 35.45% and PCV 35.89%); pods per plant (GCV 26.99% and PCV 27.16%) and primary branches per plant (GCV 26.39% and PCV 27.15%). Moderate PCV and GCV were observed for the traits: plant height (GCV 18.09% and PCV 18.23%); hundred seed weight (GCV 14.29% and PCV 15.91%) and protein content (GCV 11.52% and PCV 12.05%). Similar findings had been reported for the traits – plant height and hundred seed weight – by Panigrahi et al. (2014). High heritability estimates were observed for all the characteristics. High heritability was recorded for the single plant yield (98.9%), pods per plant (98.7%), plant height (98.5%), clusters per plant (97.6%), primary branches per plant (94.5%), days to fifty percent flowering (91.4%), protein content (91.4%), hundred seed weight (80.7%) and seeds per pod (69.8%). A high genetic advance as percentage of mean (GAM) was recorded for the hundred seed weight (87.53%) followed by the primary branches per plant (72.14%), clusters per plant (55.24%), plant height (52.85%), protein content (22.69%), seeds per pod (26.46%) and single plant yield (22.69%). In the present investigation, high heritability coupled with high GAM was recorded for the hundred seed weight, primary branches per plant, clusters per plant, plant height, protein content per pod and single plant yield indicating that the additive gene action is involved in the genetic control of these

Table 1. Analysis of variance for the different traits of blackgram (randomized block design with two replications)

Source of variation	df	Days to 50% flowering	Plant height (cm)	Primary branches per plant	Clusters/ plant	Pods/ plant	Seeds/ pod	100 seed weight (g)	Protein content (g)
Replication	1	0.31	3.87	0.73	1.94	1.16	0.06	0.03	3.70
Genotypes	101	22.73**	122.05**	4.89**	15.81**	123.04**	0.87**	0.99**	14.79**
Error	101	1.01	0.91	0.13	0.19	0.77	0.15	0.1	0.66
Standard error (deviation)	1.01	0.71	0.67	0.26	0.31	0.62	0.27	0.23	0.57
Critical difference	–	2.00	1.90	0.73	0.87	1.74	0.78	–	–

**Significant at 1% level of significance; data are presented as mean sum of squares

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Table 2. Mean performance of the germplasm accessions and variability parameters for the various traits

	Days to 50% flowering	Plant height (cm)	Primary branches per plant	Clusters per plant	Pods per plant	Seeds per pod	100 seed weight (g)	Single plant yield (g)	Protein content (g)
Mean	36.40	43.02	5.84	7.88	28.97	6.51	4.67	11.13	23.07
Range	30.00–44.50	22.40–59.90	3.20–11.60	3.20–18.40	15.20–49.60	5.00–8.00	3.17–6.60	4.02–29.10	17.20–28.44
Standard error (deviation)	0.71	0.68	0.26	0.31	0.62	0.28	0.23	0.35	0.58
CD (0.05)	2.00	1.90	0.74	0.87	1.75	0.78	0.65	0.99	1.62
CD (0.01)	2.65	2.52	0.98	1.16	2.31	1.04	0.86	1.31	2.14
PCV (%)	9.47	18.23	27.15	35.89	27.16	11.02	15.91	42.96	12.05
GCV (%)	9.05	18.09	26.39	35.45	26.99	9.20	14.29	42.72	11.52
Heritability (%)	91.40	98.50	94.50	97.60	98.70	69.80	80.70	98.90	91.40
GAM	17.83	36.99	52.85	72.14	55.24	15.83	26.46	87.53	22.69

CD – critical difference; GAM – genetic advance as percentage of mean; GCV – genotypic coefficient of variance; PCV – phenotypic coefficient of variance

traits. Selection can be recorded for the improvement of these characteristics in a future crop improvement programme. The result is in agreement with the findings of Baisakh et al. (2014) for the characteristics primary branches per plant, pods per plant, hundred seed weight, seed weight and yield per plant and the findings of Reddy et al. (2011) for the traits plant height, number of primary branches per plant, number of clusters per plant and single plant yield in the blackgram.

Correlation

The genotypic correlation coefficient between the different studied characteristics is presented in Table 3. From the intra correlation studies, the seed yield per plant had a significant and positive association of the studied genotypes in the blackgram with the traits viz., clusters per plant (0.348) and pods per plant (0.429). Days to 50% flowering had a positive and significant association with the plant height (0.307); the trait plant height had a positive and significant association with the hundred seed weight (0.225) and the protein content (0.210). The clusters per plant had a positive and significant association with the number of pods per plant (0.514). This result was in close agreement with the earlier results of Konda et al. (2009) and Kumar et al. (2014).

Genetic divergence analysis

The genetic divergence analysis was carried out by calculating D^2 values from the means of 102 genotypes of blackgram for nine characteristics. The genotypes were grouped into 18 clusters (Table 4 and Figure 1). Among the 18 clusters, cluster I had a maximum number of six genotypes followed by clusters viz., II (16), IX (14), III (8) and clusters viz., IV, V, VI, VII, VIII, X, XI, XII, XIII, XIV, XV, XVI, XVII and XVIII had one genotype each. The intra and inter cluster D^2 values are presented in Table S3 in ESM. The intra-cluster distance value ranged from 0.00 to 23.08. The maximum intra cluster D^2 value was observed in cluster IX (23.08) followed by cluster III (19.98) and cluster II (17.95). The inter cluster distance values ranged from 15.20 to 72.84. The maximum inter cluster distance was observed between cluster XVI and XV (72.84) followed by XIV and XVI (66.07) which indicated the maximum divergence among the genotypes of these clusters. Cluster IX recorded the maximum intra cluster distance which indicated more

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Table 3. Genotypic correlation coefficients in the blackgram

Character	Days to 50% flowering	Plant height (cm)	Primary branches per plant	Clusters/ plant	Pods/ plant	Seeds/ pod	100 seed weight (g)	Protein content (g)	Single plant yield (g)
Days to 50% flowering	1.000	0.307*	–0.004	–0.087	–0.012	0.073	0.007	0.110	–0.020
Plant height (cm)	–	1.000	0.131	0.048	0.031	0.119	0.225*	0.210*	0.107
Primary branches per plant	–	–	1.000	–0.007	0.089	0.070	–0.323*	0.032	0.003
Clusters/plant	–	–	–	1.000	0.514*	–0.245*	–0.135	–0.059	0.348*
Pods/plant	–	–	–	–	1.000	–0.270*	–0.136	0.100	0.429*
Seeds/pod	–	–	–	–	–	1.000	0.100	–0.022	–0.086
100 seed weight (g)	–	–	–	–	–	–	1.000	–0.030	0.390
Protein content (g)	–	–	–	–	–	–	–	1.000	0.068
Single plant yield (g)	–	–	–	–	–	–	–	–	1.000

*Significant levels at 5%

Table 4. Clustering pattern of the studied genotypes in the blackgram based on the D^2 analysis

Cluster number	Number of genotypes	Genotypes
I	50	IC 281986, IC 281989, IC 436758, VBG 11033, IC 281992, ABG 11013, VBG 13023, IC 281995, VBG 11043, IC 343947, ABG 11028, IC 281978, VBG 11046, VBG 11024, IC 436727, IC 343943, VBG 12039, VBG 11044, IC 436724, ABG 11015, VBG 12093, IC 436784, IC 281982, VBG 12034, IC 398970, IC 436765, VBG 12122, VBG 14013, IC 281984, VBG 11028, IC 281990, ADT 3, APK 1, IC 343967, VBG 12122 , IPU 0233, KU 12668, VBG 12056, VBN 4, IC 282008, IC 436536, IC 281980, VBG 11027, IC 436736, ABG 11011, IC 343939, IC 413304, VBG 11050, IC 281977, VBG 12034
II	16	IC 281792, VBG 11040, IC 281991, ADT 5, VBG 11042, VBG 10024, IC 282002, VBG 12062, VBG 11020, VBG 12042, IC 281994, IC 436720, VBG 11018, IC 282001, VBG 11041, ABG 11037
III	8	VBG 10010, VBG 10024, KU 11680, VBG 13019, KU 1239, VBG 11 046, VBG 13017, VBG 10053
IV	1	ABG 11032
V	1	VBG 11037
VI	1	VBG 11045
VII	1	IC 281993
VIII	1	VBG 12005
IX	14	IC 343885, IC 335331, IC 281999, ABG 11004, KU 11667, ABG 11011, KKB 05011, KKB 14011, ABG 11035, IC 398989, ABG 11030, VBG 14003, VBG 11029, ABG 11036
X	1	IC 436811
XI	1	KKB 06012
XII	1	IC 282002
XIII	1	IC 281792
XIV	1	IC 343962
XV	1	IC 281987
XVI	1	KKB 14003
XVII	1	KKB 14001
XVIII	1	IC 281982

divergence followed by cluster III and cluster II. The maximum inter cluster distance was observed between clusters XVI and XV followed by clusters XIV and XVI. The crosses which involve the parents from these divergent clusters will yield a relatively good amount of heterosis in F_1, a high frequency of transgressive segregants and genetic variability in subsequent generations.

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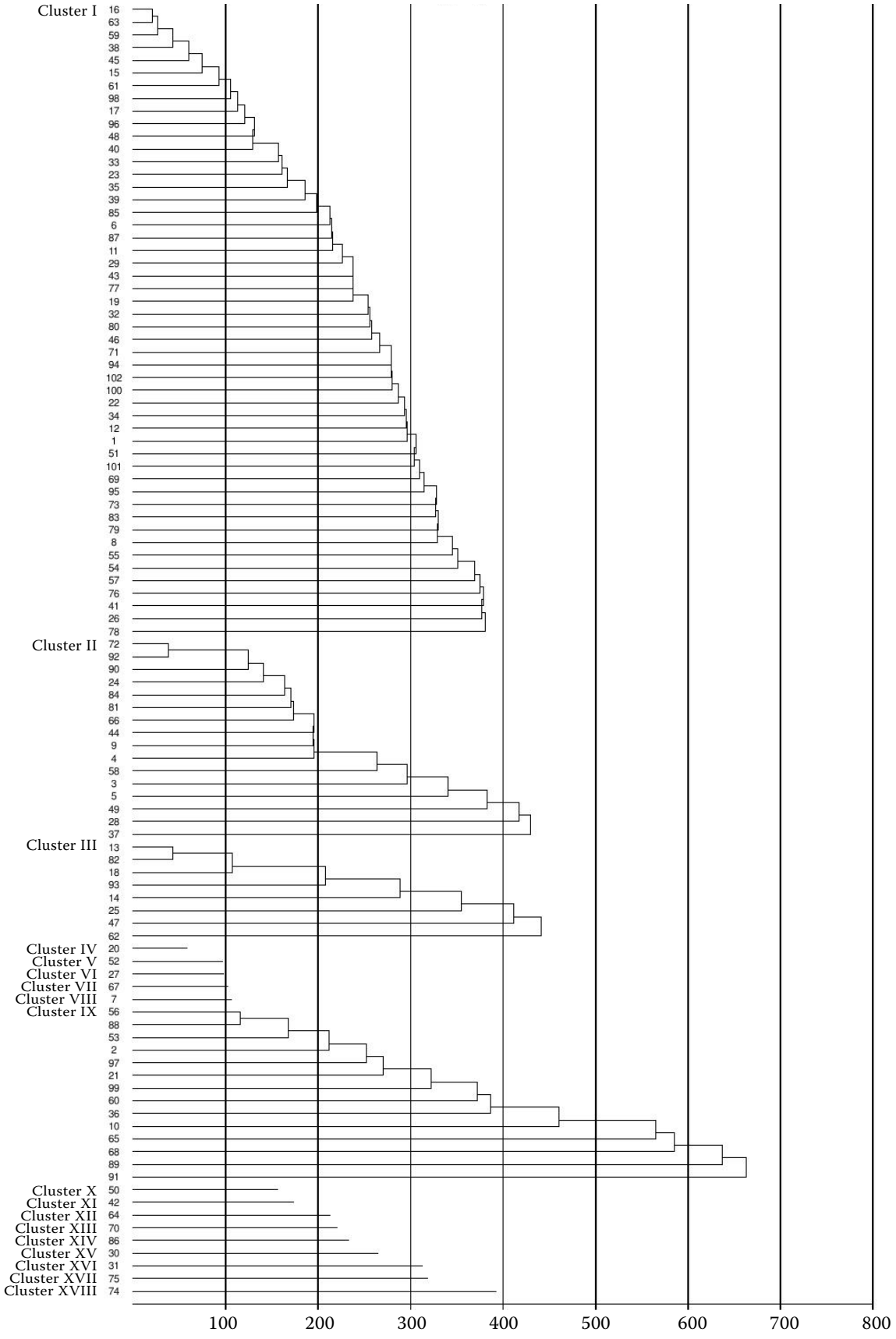

Figure 1. Clustering pattern in the blackgram by Tocher's method

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The molecular characterisation in blackgram

A total of 102 blackgram genotypes were subjected to analysis using 29 SSR markers, of which only 14 markers indicated polymorphism among the genotypes. A total of 60 alleles were observed with an average of 4.29 alleles per locus (Table 5). The number of alleles per locus ranged from two to seven. The range of the amplicons varied for each marker. The range of CEDG 139 is 90–200 bp, CEDG 008 is 50–120 bp, CEDG 268 is 120–135 bp, CEDG 092 is 180–200 bp, CEDG 198 is 190–210 bp, CEDG 013 is 90–100 bp, CEDG 133 is 50–200 bp, CEDG 141 is 210 bp, CEDG 284 is 110–210 bp, CEDG 014 is 70–190 bp, CEDG 067 is 60–250 bp, CEDG 245 is 90–200 bp, CEDG 044 is 80–120 bp and CEDG 180 is 90–163 bp. The level of polymorphism was evaluated by calculating the PIC. The PIC values ranged from 0.37 (CEDG 044) to 0.79 (CEDG 141). A PIC value of more than 0.5 was observed in the SSR markers viz., CEDG 141 (0.79), CEDG 008 (0.71), CEDG 284 (0.69), CEDG 180 (0.69), CEDG 014 (0.67), CEDG 067 (0.64), CEDG 139 (0.64), CEDG 198 (0.60), CEDG 268 (0.53), CEDG 092 (0.53) and CEDG 133 (0.52) and these markers exhibited more polymorphism. The average effective number of the alleles was 2.483 and the average heterozygosity was 0.016 which was found to be very low.

Genetic variability studies

The scored alleles of the blackgram genotypes and SSR primers were tabulated into a matrix for further data analyses. The multivariate clustering analysis was performed in DARwin (version 5.0) using the neighbour joining method to generate a dendrogram for the 102 blackgram genotypes (Figure 2). The clusters formed based on the molecular data were consistent with the grouping of genotypes screened against YMV. The distribution of the genotypes into five clusters is shown in Table 6. Cluster II had a maximum number of genotypes (32 accessions), followed by cluster I (25 accessions), cluster V (22 accessions), cluster IV (16 accessions) and cluster III (seven accessions).

Clusters viz., II, III and V had a total of sixty-one genotypes and all these genotypes were resistant to moderately resistant to YMV based on the genotypic screening and field screening. Cluster IV had sixteen genotypes and all these genotypes were moderately resistant to YMV. Cluster I had twenty-five genotypes and all these genotypes were susceptible or moderately susceptible to YMV. According to the phenotypic clustering, 18 clusters were formed, in which there were twenty-two genotypes from the first cluster, five genotypes from the second cluster, four genotypes from the third cluster,

Table 5. Average intra (diagonal) and inter cluster (between) distance of the blackgram genotypes based on the D^2 analysis

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII
I	17.34	24.32	25.11	19.97	20.02	20.68	20.66	26.05	28.55	22.89	20.98	27.88	28.80	43.23	46.13	39.11	33.87	30.85
II	–	17.95	29.38	22.21	27.46	24.05	25.75	32.70	33.24	34.07	27.93	23.10	27.71	27.79	31.34	48.07	39.43	36.60
III	–	–	19.98	33.18	31.15	28.62	33.93	31.16	36.12	25.68	27.89	37.03	40.19	47.67	48.62	37.14	28.24	37.02
IV	–	–	–	0.00	22.16	23.61	15.63	26.36	25.22	29.35	26.62	22.99	20.45	36.24	40.91	44.91	37.11	35.90
V	–	–	–	–	0.00	9.89	16.22	37.39	37.71	31.07	25.49	20.36	27.98	45.62	45.20	51.44	45.10	38.57
VI	–	–	–	–	–	0.00	21.43	39.12	39.28	32.87	24.88	20.26	30.81	40.91	38.92	51.97	44.87	40.40
VII	–	–	–	–	–	–	0.00	30.58	30.42	32.20	28.85	20.09	15.20	41.51	45.03	49.61	40.73	34.95
VIII	–	–	–	–	–	–	–	0.00	18.11	22.77	28.57	41.43	33.06	48.66	56.86	24.32	22.97	25.59
IX	–	–	–	–	–	–	–	–	23.08	28.37	30.27	40.60	32.23	47.09	55.21	33.15	31.51	29.78
X	–	–	–	–	–	–	–	–	–	0.00	21.72	42.95	42.07	55.20	58.68	24.36	29.94	25.99
XI	–	–	–	–	–	–	–	–	–	–	0.00	34.57	35.25	46.45	51.10	34.57	39.24	21.69
XII	–	–	–	–	–	–	–	–	–	–	–	0.00	19.98	30.78	31.51	60.27	48.51	45.39
XIII	–	–	–	–	–	–	–	–	–	–	–	–	0.00	36.04	43.06	54.71	43.01	39.45
XIV	–	–	–	–	–	–	–	–	–	–	–	–	–	0.00	16.28	66.07	55.55	52.55
XV	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.00	72.84	60.23	60.94
XVI	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.00	28.34	31.10
XVII	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.00	42.11
XVIII	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.00

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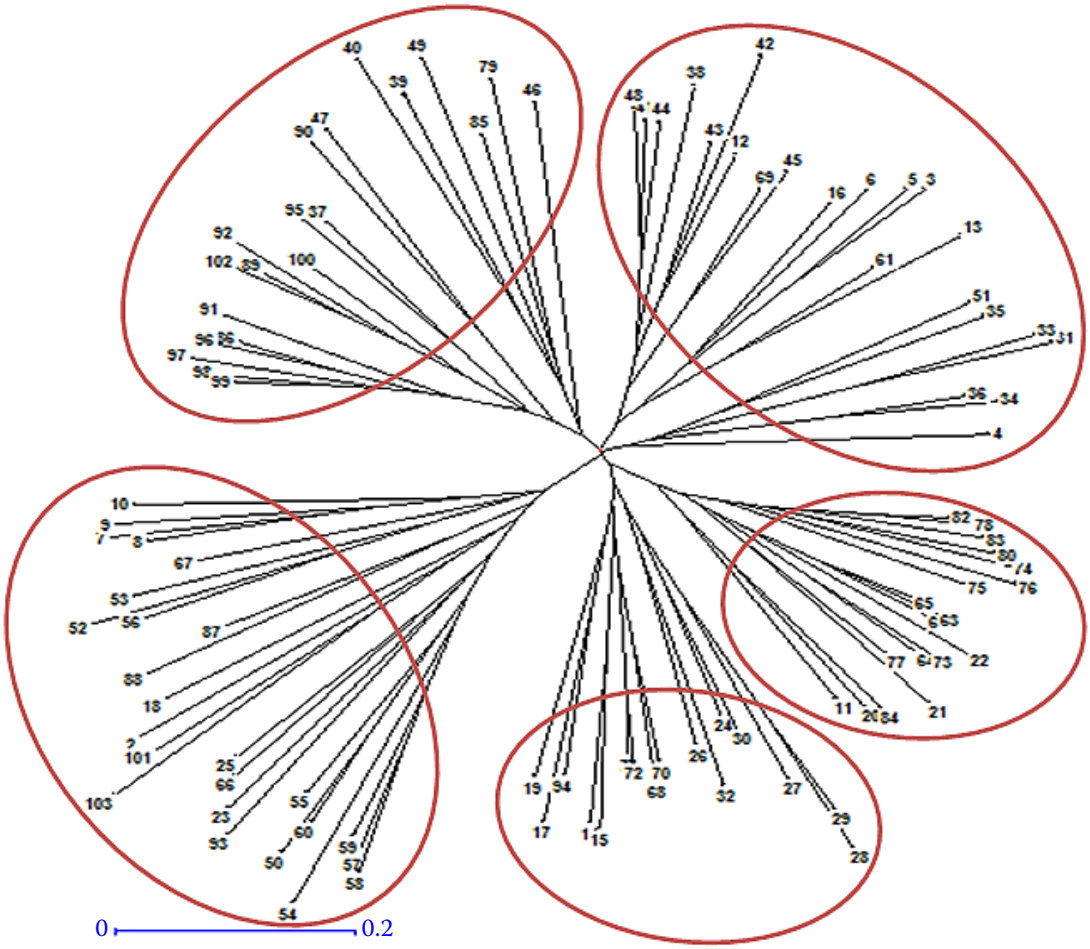

Figure 2. Phylogenetic tree construction based on the molecular diversity analysis

Cluster I – VBG 11020, ABG 11011, IC 436784, VBG 12005, IC 282007, VBG 11037, VBG 12056, IC 343939, IC 343962, IC 281982, VBG 11043, KU 12668, APK 1, ADT 3, ADT 5, ABG 11030, IC 343967, VBG 11040, IC 413304, IC 436758, IC 282001, IC 281999, IC 281994, IC 343885, VBG 11050

Cluster II – KU 11 680, IC 281986, VBG 13019, KU 1239, VBG 11028, IC 282008, IC 281993, VBG 11046, KKB 14003, VBG 11044, IC 398970, VBG 14015, VBG 11041, VBG 11045, IC 398989, ABG 11028, VBG 11027, ABG 11032, IC 281977, VBG 12121, ABG 11011, VBG 10053, IC 282002, IC 281982, KKB 14001, IC 281980, IC 436736, VBG 10024, VBG 12122, IC 282004, VBG 10010, IC 281989

Cluster III – IC 436811, IC 281978, VBG 12122, IC 281987, IC 436724, VBG 12039, IC 436720

Cluster IV – IPU 0233, KKB 14011, ABG 11004, VBG 12042, VBG 11018, ABG 11013, VBG 12062, VBG 14003, VBG 12034, VBG 12034, KKB 06012, ABG 11037, IC 436765, VBG 13017, VBG 13023, VBG 14013

Cluster V – IC 281995, KU 11667, IC 343947, IC 436536, VBG 12093, IC 281991, VBN 4, IC 335331, ABG 11 036, KKB 05011, IC 281990, ABG 11035, VBG 11029, IC 281984, ABG 11015, VBG 11033, IC 436727, VBG 11042, VBG 11024, IC 281992, IC 343943, IC 281792

five genotypes from the nineth cluster and one genotype from the thirteenth cluster that are resistant to moderately resistant based on both the phenotypic and genotypic screening.

Principal component analysis

In this study, a PCA was employed for the biometric traits. The PCA, based on the morphological characteristics, extracted four components contributing 61.3% of the variance to the total variance. The traits viz., number of primary branches per plant, number of clusters per plant and number of pods per plant were positively associated with component 1 (Figure 3).

The data were subjected for an ANOVA and it was found that the germplasm differed significantly

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Table 6. The distribution of the blackgram genotypes in the different clusters based on the molecular characterisation

Cluster number	Number of genotypes	Genotypes
I	25	VBG 11020, ABG 11011, IC 436784, VBG 12005, IC 282007, VBG 11037, VBG 12056, IC 343939, IC 343962, IC 281982, VBG 11043, KU 12668, APK 1, ADT 3, ADT 5, ABG 11030, IC 343967, VBG 11040, IC 413304, IC 436758, IC 282001, IC 281999, IC 281994, IC 343885, VBG 11050
II	32	KU 11 680, IC 281986, VBG 13019, KU 1239, VBG 11028, IC 282008, IC 281993, VBG 11046, KKB 14003, VBG 11044, IC 398970, VBG 14015, VBG 11041, VBG 11045, IC 398989, ABG 11028, VBG 11027, ABG 11032, IC 281977, VBG 12121, ABG 11011, VBG 10053, IC 282002, IC 281982, KKB 14001, IC 281980, IC 436736, VBG 10024, VBG 12122, IC282004, VBG 10010, IC 281989
III	7	IC 436811, IC 281978, VBG 12122, IC 281987, IC 436724, VBG 12039, IC 436720
IV	16	IPU 0233, KKB 14011, ABG 11004, VBG 12042, VBG 11018, ABG 11013, VBG 12062, VBG 14003, VBG 12034, VBG 12034, KKB 06012, ABG 11037, IC 436765, VBG 13017, VBG 13023, VBG 14013
V	22	IC 281995, KU 11667, IC 343947, IC 436536, VBG 12093, IC 281991, VBN 4, IC 335331, ABG 11036, KKB 05011, IC 281990, ABG 11035, VBG 11029, IC 281984, ABG 11015, VBG 11033, IC 436727, VBG 11042, VBG 11024, IC 281992, IC 343943, IC 281792

for all nine studied characteristics. These findings are in accordance with the findings of Singh et al. (2012), Makeen et al. (2009) and Kavani (2007) who also observed significant variability for the yield and its components in the blackgram. The mean performance can be used as a criterion to select desirable plants and to eliminate undesirable types. The genotypes viz., VBG 11018, VBG 11020, IC

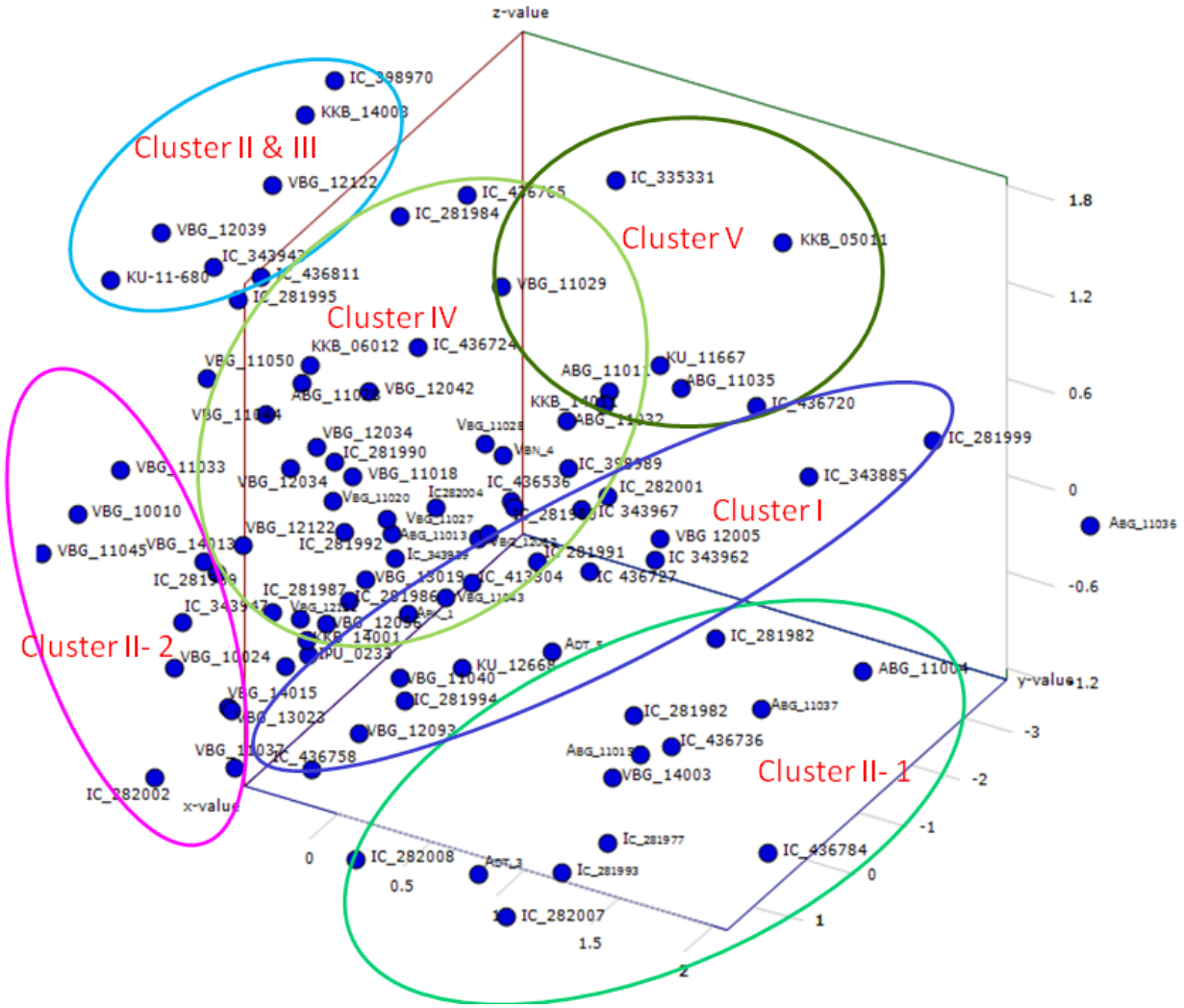

Figure 3. PCA from the phenotypic traits and disease resistance association with the SSR markers

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281987, IC 281994, IC 282007, IC 335331 and IC 343885 were significantly superior for the seed yield per plant. Overall, the genotypes IC 343885 and IC 335331 exhibited significantly superior performance on the single plant yield, clusters per plant and pods per plant. The genotypes KKB 14001 and KKB 14003 and IC 343885 showed desirable performance on the pods per plant and clusters per plant. Therefore, these genotypes can be used in future breeding programmes to improve the seed yield and other characteristics.

The range of values for all the studied characteristics showed the existence of variability among the genotypes. Hence, there is tremendous scope for further improvement. In the present investigation, high PCV and GCV estimates (Table 2) were observed for the traits – single plant yield, clusters per plant, pods per plant and primary branches per plant. It indicated the existence of variability for these characteristics in the blackgram. Hence, direct selection based on these traits would be effective in improving this crop. Similar findings were reported by Ramya et al. (2013), and Sowmini and Jayamani (2013). Konda et al. (2009) reported high PCV and GCV values for the clusters per plant and pods per plant.

From the association analysis, it was found that the seed yield was positively and significantly correlated with the traits viz., clusters per plant and pods per plant. This result was in close agreement with the earlier results of Kumar et al. (2014).

One hundred and two genotypes were resolved into eighteen clusters based on the D^2 analysis. Among the eighteen clusters, cluster I had a higher number of genotypes (50) followed by cluster II with sixteen genotypes, cluster IX with fourteen genotypes and cluster III with eight genotypes. The clusters viz., IV, V, VI, VII, VIII, X, XI, XII, XIII, XIV, XV, XVI, XVII and XVIII had one genotype each. Cluster IX recorded the maximum intra cluster distance which indicated more divergence followed by cluster III and cluster II. While the minimum intra cluster distance was observed in clusters viz., IV, V, VI, VII, VIII, X, XI, XII, XIII, XIV, XV, XVI, XVII and XVIII. The maximum inter cluster distance was observed between clusters XVI and XV followed by clusters XIV and XVI. The crosses which involve the parents from these divergent clusters will yield a relatively good amount of heterosis in F_1, a high frequency of transgressive segregants and genetic variability in subsequent generations. The minimum inter cluster distance was recorded between clusters VII and XIII followed by clusters IV and VI suggesting that the genotypes of these clusters were not genetically that diverse.

Of the total 50 accessions identified as being tolerant to YMV, VBN4 is a variety released from Tamil Nadu Agricultural University (TNAU) showed complete resistance to YMV. A maximum number of tolerant lines were identified from the present investigation, and this was observed due to the initial selection of the trait specific germplasm from the total gene bank collection in conducting the present investigation. Five of the pre-release cultures KKB 05 011, KKB 14 001, KKB 14 011, KKB 06 012, KKB 14 003 are from Agricultural College and Research Institute, Killikulam which were found to be tolerant along with other better sources indicating the availability of pure line cultures for immediate release as varieties. Culture KKB 05 011 was already proven to be high yielding (Pillai et al. 2010; Pillai et al. 2017). Fourteen accessions from NBPGR viz., IC 282004, IC 281792, IC 282008, IC 281993, IC 281989, IC 282002, IC 436811, IC 281984, IC 343943, IC 436765, IC 436724, IC 281978, IC 398970 and IC 335331 were identified as tolerant sources based on both genotypic and phenotypic screening for the first time. These accessions can directly be incorporated in any crop improvement programme for the development of new varieties and released through selections. In an earlier study, Gopi et al. (2016) reported that the cultivar ADT 5 was highly susceptible to YMV. In this study the ADT 5 was also highly susceptible to the YMV.

Though disease resistance is a complex mechanism enacted through intracellular events being regulated by a cascade of genes, YMV resistance is controlled by a single recessive gene (Thakur et al. 1977; Malik et al. 1986; Reddy & Singh 1995; Saleem et al. 1998; Reddy 2009). Very few reports are available on the molecular genetic diversity in the blackgram. Ghafoor et al. (2001) and Gupta et al. (2001) studied the genetic variability of the blackgram based on agro morphological traits. Studies on isozyme markers (Gupta & Gopalakrishna 2009), DNA markers including random amplified polymorphic DNA, inter-simple sequence repeat, amplified fragment length polymorphism, and simple sequence repeat (Gupta & Gopalakrishna 2009; Sivaprakash et al. 2014) have been reported earlier on the blackgram. These reports are insufficient as few germplasm accessions confined to a geo-

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graphical region were investigated. The present investigation concentrated on SSR markers being a true representative of the trait linked variation. Of the total 29 SSR markers used in the present investigation, only 14 markers indicated polymorphism. Of these 14 markers, SSR marker CEDG 141 indicated a higher level of polymorphism. This confirms the earlier findings (Chaitieng et al. 2006). The primers designed for *V. angularis* (Wang et al. 2004) and validated to be associated with the blackgram, indicated a significant level of polymorphism in the blackgram; indicated polymorphism among the contrasting stress responsive accessions. Such extents in the variations are common in germplasm sources enabling breeders to select a suitable accession for pre-breeding and varietal development. Similar studies proved the high level of informativeness revealed by SSR markers (Gupta & Gopalakrishna 2009; Souframanien & Gopalakrishna 2009; Pyngrope et al. 2015). Suvan et al. (2020) screened 60 blackgram genotypes for protein and three minerals (calcium, potassium and phosphorus) along with 11 SSR markers. The germplasm displayed ample variability for protein and the minerals. Malarkodi et al. (2020) validated the genetic diversity of 40 genotypes by using a set of nineteen SSR markers.

In the present study, a total of 60 alleles were observed with an average of 4.29 alleles per locus for the 14 SSR markers. The average number of alleles per locus in the current investigation was less than the report of Suvan et al. (2020).

The average effective number of alleles was 2.483 and the average heterozygosity was 0.016 which was found to be very low due to the self-pollinating nature of legumes. This is accordance with Gediya et al. (2019). The PIC value is a discriminating power of a particular marker in a defined germplasm (Smith et al. 2000). In this study, the PIC values ranged from 0.37 (CEDG 044) to 0.79 (CEDG 141) with an average of 0.59 (Table 7). The PIC was higher than Gupta et al. (2013), where the mean PIC was 0.49 for the SSR markers. Out of 14 markers, 11 markers showed PIC value more than 0.5, which shows they exhibited more polymorphism.

A dendrogram based on DARwin using the neighbour joining method grouped 102 genotypes of the blackgram in five major clusters. The clustering of the genotypes clearly showed the extent of the genetic similarities among the test genotypes. Similar studies utilising polymorphic SSR markers for the construction of dendrogram maps of the blackgram genotypes were reported by Souframanien and Gopalkrishna (2009) and Ganguly and Bhat (2012).

A higher level of variations among the germplasms was already reported in many crops. The genotype clustering analysis and population

Table 7. Polymorphism information content (PIC) of the microsatellite simple sequence repeat (SSR) markers

SSR markers	Total alleles	Amplicon range (bp)	Effective number of alleles	Average heterozygosity	PIC
CEDG 139	4	90–200	2.799	0.000	0.64
CEDG 008	7	50–120	2.836	0.073	0.71
CEDG 268	3	120–135	2.108	0.000	0.53
CEDG 092	4	180–200	1.942	0.024	0.53
CEDG 198	4	190–210	2.470	0.009	0.60
CEDG 013	2	90–100	1.963	0.000	0.49
CEDG 133	4	50–200	2.071	0.009	0.52
CEDG 141	7	210	3.510	0.088	0.79
CEDG 284	6	110–210	2.937	0.019	0.69
CEDG 014	5	70–190	2.944	0.004	0.67
CEDG 067	4	60–250	2.746	0.000	0.64
CEDG 245	3	90–200	1.632	0.000	0.39
CEDG 044	3	80–120	1.583	0.000	0.37
CEDG 180	4	90–163	3.215	0.000	0.69
Total	60	–	–	–	8.24
Average	4.29	–	2.483	0.016	0.59

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structure produced similar results of Dikshit et al. (2007) ascertaining the efficiency of a randomly picked panel of SSR markers in the diversity assessment. Similar studies, utilising polymorphic SSR markers for the construction of a dendrogram of blackgram genotypes were reported by many investigators (Souframanien & Gopalakrishna 2009; Ganguly & Bhat 2012; Gupta et al. 2015; Reddy et al. 2015). As the estimates of the eigenvalues, corresponding to the first four canonical variables, obtained 61.3% of the total variance in the analysed traits, it was possible to satisfactorily explain the variability among the evaluated blackgram genotypes and represent them in a three-dimensional scatter plot (Luz et al. 2016).

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