



RESEARCH ARTICLE

# Variability, association and cluster analysis of backcross derived lines in black gram [*Vigna mungo* (L.) Hepper]

Prasanna Mahabaleshwar Hegde<sup>1</sup>, Manivannan Narayana<sup>1\*</sup>, Kumaresan Dharmalingam<sup>1</sup>, Saminathan Vangity Ramasamy<sup>2</sup> & Senthilraja Govindasamy<sup>2</sup>

<sup>1</sup> Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641003, India

<sup>2</sup> Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore 641003, India

\*Email: [nm68@tnau.ac.in](mailto:nm68@tnau.ac.in)

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## Abstract

A BC<sub>2</sub>F<sub>4</sub> population of black gram was derived from a cross between a high-yielding variety (MDU 1) and a bruchid-resistant donor (TU 68). Twenty-four backcross-derived lines (BLs) of this population were evaluated for yield and yield components. Eight BLs had at par seed yield with MDU 1. Variability studies showed high PCV (%), GCV (%), heritability (%) and GAM (%) for plant height, number of branches per plant, number of clusters per plant, number of pods per plant and seed yield per plant. Correlation analysis for yield components revealed that all traits significantly and positively correlated with seed yield per plant. Path analysis on seed yield per plant indicated that the number of pods and hundred seed weight had a high and moderate direct effect on seed yield per plant. Hence, these two traits should be used as seed yield improvement programme selection indices. Cluster analysis grouped the progenies into three major clusters. Most of the progenies that are at par with MDU 1 for yield were clustered together. The promising high-yielding progenies identified may be tested for their bruchid resistance and released as a variety after the large-scale evaluation.

## Keywords

black gram; backcross derived lines; variability; correlation; path analysis; cluster analysis

## Introduction

Black gram (*Vigna mungo* [L.] Hepper) is an important pulse crop grown in India. It is a short-duration crop belonging to the family Leguminosae. It is one of the major pulse crops grown in India, Pakistan, Myanmar, Bangladesh and Afghanistan. India is the top producer and consumer of black gram, with 70% of the world's supply (1). In India, it has been grown in an area of 40.02 lakh hectares with a production of 26.31 lakh tonnes and a productivity of 657kg/ha (2). The rainy season covers about 60% of the crop area. Post-rainy season farming is rising due to early maturing varieties. The ideal climate for Black gram is between 27-30°C, with moderate rainfall and loamy soil with high water holding capacity (3).

Black gram is a very nutritious crop used in cosmetics, nutraceuticals, pharmaceutical preparations and sustainable agricultural systems. Black gram is a major protein supplement in the vegetarian diet. It has carbohydrates (60%), protein (25%), fat (1.3%) and vital vitamins and minerals (4). It also contains many bioactive components, i.e. nondigestible carbohydrates, carotenoids, tocopherol, tocotrienol, polyphenols, phytosterols, enzyme inhibitors, phytic acid, lectins and saponins. It is a cheap source of protein for the vegetarian

community (5). This crop can grow in harsh environmental conditions, increasing soil fertility through nitrogen fixation (6).

The knowledge of variability among genotypes is a must in any breeding program. Seed yield is a complex trait. It is associated with many component traits. The information on the association between yield and components is essential for the efficient selection program. The clustering pattern helps to assess the diversity among the genotypes studied. Hence, in the present study, variability, correlation, path analysis and clustering were carried out using backcross-derived lines in Black gram.

## Materials and Methods

A BC<sub>2</sub>F<sub>4</sub> population of black gram was derived from a cross between a high-yielding variety, MDU 1 and a bruchid-resistant donor, TU 68. MDU-1 was released from Agriculture College and Research Institute, Madurai, Tamil Nadu Agricultural University. It is recommended for the October season in Tamil Nadu (7). It is a high yielder but susceptible to bruchid storage pests (7). TU 68 was developed at the Bhabha Atomic Research Centre (BARC), Trombay. It was made by crossing TU 94-2 with *Vigna mungo* var *sylvestris* (8). It is highly resistant to bruchid storage pests (8). F<sub>1</sub> of the cross between these genotypes was backcrossed twice with MDU 1 to incorporate bruchid resistance to MDU 1 (9). High-yielding progenies were selected and forwarded from BC<sub>2</sub>F<sub>2</sub> to BC<sub>2</sub>F<sub>4</sub> generation. In the post-rainy season (2023-2024), 24 BC<sub>2</sub>F<sub>4</sub> lines were evaluated with parents at the Centre for Plant Breeding and Genetics, Tamil Nadu Agriculture University, Coimbatore. Each entry was assessed in a non-replicated trial using a 4 m row plot and 30 x 10 cm spacing. All the recommended agronomic practices of Tamil Nadu were followed to raise the crop. Observations were recorded on nine traits viz., plant height (cm), number of branches per plant, number of clusters per plant, number of pods per cluster, number of pods per plant, pod length (cm), number of seeds per pod, hundred seed weight (g) and seed yield per plant (g) on individual plants.

The average parental variances were used as the environmental variance for each character. Data on 24 BC<sub>2</sub>F<sub>4</sub> lines were analyzed for the genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) (10), heritability, genetic advance as a percent of the mean (GAM) (11), correlation and cluster analysis. TNAU STAT (Tamil Nadu Agricultural University Statistical Package) is a statistical package for analyzing plant breeding and agronomy experiments and it was used for descriptive, correlation and path analysis (12). Lenka and Misra's classification was used to categorize direct and indirect effects (13). DARWin (Dissimilarity Analysis and Representation for Windows) is a phylogenetic and diversity analysis software package. DARWin Ver 6.0 was used for cluster analysis (14).

## Results and Discussion

Among the two parents, MDU 1 recorded a higher seed yield per plant than TU 68. MDU 1 has also excelled in plant height,

pod length, seeds per pod, hundred seed weight and seed yield per plant. TU 68 outperformed in the number of branches, clusters, pods per cluster and total number of pods. TU 68 was derived from the cross between cultivated and wild species. It has more wild traits: a small plant, many branches, small pods, small seeds and few seeds per pod. It has a low seed yield per plant. It has more pods, but they are small. The seeds are small and there are few seeds per pod. Hence, a backcross breeding program was planned to create a genotype like MDU 1 with bruchid resistance of TU 68. With this background, the progenies of BC<sub>2</sub>F<sub>4</sub> were analyzed for seed yield per plant and other components.

### Mean performance

Among progenies, none of the BC<sub>2</sub>F<sub>4</sub> progenies had a superior seed yield than the better parent MDU 1 (Table 1). However, eight lines had on-par seed yield per plant with MDU 1. Of the eight progenies, 51-6-1 matched MDU 1 for seven traits: plant height, pods per cluster, number of pods per plant, pod length, seeds per pod and hundred seed weight and seed yield per plant. This was followed by progenies 30-3-1, 74-6-1, 89-5-1 for six traits, 87-5-1, 95-1-2, 95-5-2 for five traits and 124-1-1 for four traits. Therefore, all these high-yielding progenies may be considered for further evaluation.

Table 2 presents the variability analysis in the BC<sub>2</sub>F<sub>4</sub> population for various traits. The PCV is higher than the GCV for all characteristics. This shows that the environment affects all traits. The GCV was high regarding plant height, branches, clusters, pods and seed yield. Moderate GCV was observed for the number of pods per cluster and hundred seed weight. Similar results for pods per cluster and hundred seed weight were reported in many studies (15-17). Low GCV was observed for pod length and number of seeds per pod. Previous studies also reported low GCV for pod length and seeds per pod (18). High and moderate GCV show high variability in these traits among progenies. The heritability estimates were high for all traits except the number of seeds per pod. The number of seeds per pod had moderate heritability. This is following previous studies (19). Genetic advance as a percent of the mean was high for all traits except pod length and the number of seeds per pod. These findings follow the previous study (20). The results showed significant variability for all characteristics except pod length and seeds per pod. Hence, selection for these traits can be practised to select promising progenies due to additive gene action.

### Correlation Studies

Correlation studies show the interrelationships between the yield and its related traits. The association between yield and component traits is essential for crop improvement to make efficient selection. Table 3 shows the correlation coefficients between the seed yield and its traits. In this study, all traits had a significant and positive correlation with seed yield per plant. Many researchers reported the same results (21-23). All traits showed a significant and positive correlation with all other characteristics. However, no association was found between the number of clusters and the hundred seed weight. Many previous reports showed no association between clusters per plant and hundred seed weight (24-26). Hence, more focus should be given to all the correlated traits during the selection program to obtain a high yield.

**Table 1.** Mean performance of BC<sub>2</sub>F<sub>4</sub> progenies and parents for seed yield and component traits

Lines	Plant Height (cm)		Number of branches per plant		Number of clusters per plant		Number of pods per cluster		Number of pods per plant		Pod length (cm)		Number of seeds per pod		Hundred seed weight (g)		Seed yield per plant (g)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
30-3-1	13.1 <sup>a</sup>	0.9	7.0 <sup>*</sup>	0.7	9.3 <sup>a</sup>	1.1	3.4 <sup>a</sup>	0.2	28.3 <sup>a</sup>	3.8	4.9 <sup>a</sup>	0.1	7.0 <sup>*</sup>	0.1	4.2 <sup>*</sup>	0.2	7.6 <sup>a</sup>	1.0
51-6-1	12.2 <sup>a</sup>	1.2	7.4 <sup>*</sup>	0.7	8.6 <sup>*</sup>	1.1	3.2 <sup>a</sup>	0.2	28.2 <sup>a</sup>	4.3	4.9 <sup>a</sup>	0.1	6.8 <sup>a</sup>	0.2	4.3 <sup>a</sup>	0.2	6.8 <sup>a</sup>	1.1
53-6-1	11.1 <sup>a</sup>	1.0	7.8 <sup>*</sup>	0.6	9.1 <sup>a</sup>	1.2	3.0 <sup>a</sup>	0.3	25.7 <sup>a</sup>	5.0	4.7 <sup>a</sup>	0.2	6.9 <sup>*</sup>	0.2	4.1 <sup>a</sup>	0.3	5.6 <sup>*</sup>	1.2
54-6-1	15.9 <sup>*</sup>	0.9	8.5 <sup>*</sup>	0.7	9.7 <sup>a</sup>	0.9	3.0 <sup>a</sup>	0.2	25.7 <sup>a</sup>	3.1	4.9 <sup>a</sup>	0.1	6.5 <sup>*</sup>	0.3	4.0 <sup>*</sup>	0.2	5.4 <sup>*</sup>	0.8
55-15-1	9.0 <sup>*</sup>	0.6	6.0 <sup>a</sup>	0.7	7.7 <sup>a</sup>	1.6	2.9 <sup>a</sup>	0.2	18.0 <sup>*</sup>	3.7	4.7 <sup>a</sup>	0.1	7.1 <sup>*</sup>	0.1	4.2 <sup>*</sup>	0.1	3.3 <sup>*</sup>	0.6
60-8-1	15.2 <sup>a</sup>	0.8	6.1 <sup>a</sup>	0.5	7.2 <sup>*</sup>	0.8	3.1 <sup>a</sup>	0.1	20.7 <sup>*</sup>	2.2	4.8 <sup>a</sup>	0.1	7.0 <sup>*</sup>	0.1	4.0 <sup>*</sup>	0.2	4.5 <sup>*</sup>	0.7
61-13-1	13.6 <sup>a</sup>	0.9	4.1 <sup>a</sup>	0.5	8.5 <sup>*</sup>	0.6	3.3 <sup>a</sup>	0.2	21.7 <sup>*</sup>	2.0	4.8 <sup>a</sup>	0.1	7.4 <sup>a</sup>	0.2	3.8 <sup>*</sup>	0.2	4.1 <sup>*</sup>	0.5
64-10-1	11.8 <sup>*</sup>	0.4	6.2 <sup>*</sup>	0.3	6.1 <sup>*</sup>	0.4	3.4 <sup>a</sup>	0.1	19.4 <sup>*</sup>	1.6	4.8 <sup>a</sup>	0.1	7.3 <sup>*</sup>	0.1	4.4 <sup>a</sup>	0.2	5.7 <sup>*</sup>	0.6
65-13-1	13.8 <sup>a</sup>	0.9	7.4 <sup>*</sup>	0.4	7.7 <sup>*</sup>	0.8	3.2 <sup>a</sup>	0.2	24.1 <sup>a</sup>	2.2	4.9 <sup>a</sup>	0.1	7.2 <sup>*</sup>	0.1	4.4 <sup>a</sup>	0.2	6.2 <sup>*</sup>	0.6
69-10-1	14.3 <sup>a</sup>	0.8	6.8 <sup>*</sup>	0.5	9.3 <sup>*</sup>	0.5	3.3 <sup>a</sup>	0.2	25.1 <sup>a</sup>	1.8	5.2 <sup>a</sup>	0.1	7.6 <sup>a</sup>	0.1	4.4 <sup>a</sup>	0.2	6.7 <sup>*</sup>	0.5
72-12-1	11.8 <sup>a</sup>	0.7	5.8 <sup>a</sup>	0.5	7.4 <sup>*</sup>	0.6	3.1 <sup>a</sup>	0.2	23.8 <sup>a</sup>	2.2	4.7 <sup>a</sup>	0.1	7.2 <sup>*</sup>	0.2	4.4 <sup>a</sup>	0.2	5.9 <sup>*</sup>	0.7
74-6-1	17.2 <sup>*</sup>	1.2	8.9 <sup>*</sup>	0.5	11.3 <sup>a</sup>	1.2	3.3 <sup>a</sup>	0.2	31.9 <sup>a</sup>	4.0	5.2 <sup>a</sup>	0.1	7.7 <sup>a</sup>	0.2	4.3 <sup>*</sup>	0.1	7.1 <sup>a</sup>	0.9
87-5-1	21.8 <sup>*</sup>	1.4	9.8 <sup>*</sup>	0.4	11.0 <sup>a</sup>	0.9	3.2 <sup>a</sup>	0.1	30.4 <sup>a</sup>	3.0	5.1 <sup>a</sup>	0.1	7.2 <sup>*</sup>	0.2	4.4 <sup>*</sup>	0.1	7.2 <sup>a</sup>	0.8
89-5-1	21.1 <sup>*</sup>	0.8	9.2 <sup>*</sup>	0.5	10.3 <sup>a</sup>	0.8	3.4 <sup>a</sup>	0.1	28.0 <sup>a</sup>	2.7	5.0 <sup>a</sup>	0.1	7.3 <sup>a</sup>	0.2	4.1 <sup>*</sup>	0.2	6.9 <sup>a</sup>	0.8
95-1-2	14.0 <sup>a</sup>	1.3	8.8 <sup>*</sup>	0.5	14.0 <sup>a</sup>	1.0	3.0 <sup>a</sup>	0.2	35.5 <sup>a</sup>	3.3	4.6 <sup>*</sup>	0.1	6.4 <sup>*</sup>	0.2	3.4 <sup>*</sup>	0.2	6.9 <sup>a</sup>	0.9
95-2-4	20.1 <sup>*</sup>	1.1	8.2 <sup>*</sup>	0.3	10.5 <sup>a</sup>	0.8	3.1 <sup>a</sup>	0.1	28.4 <sup>a</sup>	2.5	5.0 <sup>a</sup>	0.1	7.6 <sup>a</sup>	0.1	4.0 <sup>*</sup>	0.1	6.6 <sup>*</sup>	0.7
95-3-5	14.4 <sup>a</sup>	0.9	7.6 <sup>*</sup>	0.3	10.3 <sup>a</sup>	0.6	2.9 <sup>*</sup>	0.1	26.1 <sup>a</sup>	1.9	4.6 <sup>*</sup>	0.1	6.7 <sup>*</sup>	0.1	3.3 <sup>*</sup>	0.1	4.8 <sup>*</sup>	0.5
95-5-2	22.1 <sup>*</sup>	1.2	8.5 <sup>*</sup>	0.4	10.2 <sup>a</sup>	0.9	3.3 <sup>a</sup>	0.1	31.9 <sup>a</sup>	3.3	4.9 <sup>a</sup>	0.1	7.3 <sup>*</sup>	0.1	3.8 <sup>*</sup>	0.1	7.2 <sup>a</sup>	0.8
95-7-1	12.9 <sup>a</sup>	1.0	7.9 <sup>*</sup>	0.4	10.1 <sup>a</sup>	0.5	3.0 <sup>a</sup>	0.1	27.4 <sup>a</sup>	2.3	4.9 <sup>a</sup>	0.1	7.4 <sup>a</sup>	0.2	3.3 <sup>*</sup>	0.1	5.1 <sup>*</sup>	0.5
98-4-1	19.0 <sup>*</sup>	1.0	7.9 <sup>*</sup>	0.4	9.1 <sup>*</sup>	0.7	3.1 <sup>a</sup>	0.1	24.6 <sup>a</sup>	2.2	4.9 <sup>a</sup>	0.1	7.1 <sup>*</sup>	0.1	3.8 <sup>*</sup>	0.2	4.9 <sup>*</sup>	0.5
99-4-1	18.1 <sup>*</sup>	1.0	7.5 <sup>*</sup>	0.5	9.2 <sup>a</sup>	0.9	3.1 <sup>a</sup>	0.1	25.7 <sup>a</sup>	3.0	4.6 <sup>*</sup>	0.1	7.5 <sup>a</sup>	0.1	3.6 <sup>*</sup>	0.1	5.6 <sup>*</sup>	0.7
109-6-1	15.8 <sup>*</sup>	0.7	8.0 <sup>*</sup>	0.4	9.4 <sup>a</sup>	0.7	3.3 <sup>a</sup>	0.1	27.3 <sup>a</sup>	2.6	4.8 <sup>a</sup>	0.1	7.0 <sup>*</sup>	0.2	4.0 <sup>*</sup>	0.2	5.4 <sup>*</sup>	0.6
112-2-1	16.4 <sup>*</sup>	0.7	6.7 <sup>*</sup>	0.3	7.1 <sup>*</sup>	0.5	3.2 <sup>a</sup>	0.1	21.4 <sup>*</sup>	1.8	4.9 <sup>a</sup>	0.1	7.6 <sup>a</sup>	0.1	3.9 <sup>*</sup>	0.1	5.0 <sup>*</sup>	0.5
124-1-1	18.3 <sup>*</sup>	0.9	8.7 <sup>*</sup>	0.5	10.8 <sup>a</sup>	0.7	3.1 <sup>a</sup>	0.2	30.2 <sup>a</sup>	2.7	4.6 <sup>*</sup>	0.1	7.3 <sup>*</sup>	0.1	4.2 <sup>*</sup>	0.1	6.9 <sup>a</sup>	0.7
MDU1 (P1)	13.3	0.4	4.9	0.4	11.5	0.6	3.5	0.2	29.2	2.4	5.0	0.1	7.8	0.1	4.8	0.1	8.9	0.6
TU 68 (P2)	6.3	0.7	6.6	0.5	14.5	0.5	4.0	0.0	42.3	2.3	4.4	0.1	7.0	0.0	3.9	0.2	5.9	0.6
C.D. (P=0.05)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

\* - significantly different from MDU 1 at (P=0.05); a - at par with MDU 1

**Table 2.** Variability parameters of BC<sub>2</sub>F<sub>4</sub> population for various characters

Character	Mean	Range	PCV (%)	GCV (%)	h <sup>2</sup> (%)	GAM (%)
Plant Height (cm)	15.9	4 - 37	33.88	32.22	90.45	63.13
Number of branches per plant	7.6	2 - 13	29.63	23.44	62.57	38.19
Number of clusters per plant	9.5	2 - 25	40.25	36.79	83.55	69.27
Number of pods per cluster	3.2	1 - 5	20.43	16.75	67.16	28.27
Number of pods per plant	26.6	1 - 70	47.43	38.18	65.92	63.86
Pod length (cm)	4.8	3 - 6	8.75	7.49	73.20	13.20
Number of seeds per pod	7.2	5 - 9	10.40	7.32	49.55	10.62
Hundred seed weight (g)	4.0	0.6 - 6.0	19.90	16.58	69.41	28.45
Seed yield per plant (g)	5.9	1 - 17.9	54.01	43.21	64.00	71.21

**Table 3.** Correlation between seed yield and component traits in BC<sub>2</sub>F<sub>4</sub> progenies

Characters	Plant Height (cm)	Number of branches per plant	Number of clusters per plant	Number of pods per cluster	Number of pods per plant	Pod length (cm)	Number of seeds per pod	Hundred seed weight (g)
Number of branches per plant	0.56**							
Number of clusters per plant	0.43**	0.63**						
Number of pods per cluster	0.28**	0.26**	0.32**					
Number of pods per plant	0.41**	0.58**	0.86**	0.58**				
Pod length (cm)	0.29**	0.24**	0.24**	0.39**	0.34**			
Number of seeds per pod	0.24**	0.17**	0.13**	0.33**	0.26**	0.32**		
Hundred seed weight (g)	0.14**	0.13**	0.01ns	0.23**	0.13**	0.25**	0.23**	
Seed yield per plant (g)	0.43**	0.56**	0.74**	0.59**	0.88**	0.38**	0.37**	0.38**

\*, \*\* significant at 0.01 and 0.05 level respectively

### Path analysis

Path coefficient analysis will assist in determining the direct and indirect effects of all observed independent traits on dependent traits like yield. In the present investigation, path analysis on seed yield was estimated and presented in Table 4. The residual effect (0.37) indicated that most of the essential traits affecting seed yield were included in the path analysis. The trait number of pods per plant has shown a high positive direct effect on seed yield per plant. Many other studies reported the same result (27-30). Hundred seed weight has shown a moderate positive direct effect on seed yield. Many previous studies reported the same results in Black gram (18, 23, 31). All other traits show negligible positive direct effects on seed yield per plant. In the case of indirect effects, all traits have shown negligible effects through all other traits except the number of pods per plant on seed yield. Branches per plant, clusters per plant and pods per cluster had a high indirect effect through the number of pods per plant. Plant height and pod length also moderately indirectly impacted the

number of pods per plant. These results follow the previous results (32). Hence, selecting the number of pods per plant will be more rewarding in the seed yield improvement programme.

### Cluster analysis

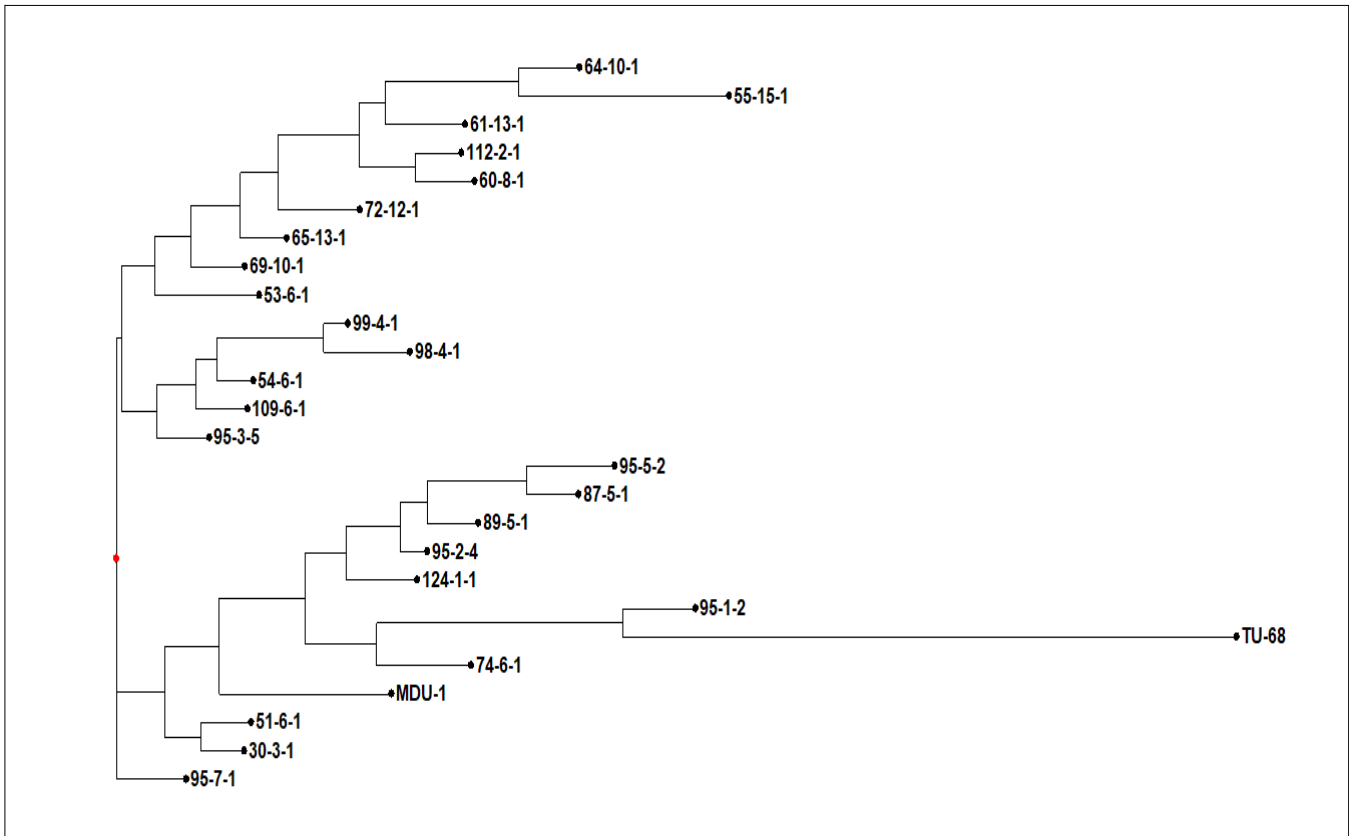
The neighbour-joining method grouped the progenies and parents into three clusters (Fig. 1). The first cluster had 14 genotypes. The second cluster had 11 genotypes, including MDU 1 and TU 68. All eight progenies at par with MDU 1 for seed yield were clustered. The genotype 95-7-1 alone forms a separate cluster. The cluster analysis indicated that the backcross breeding program created moderate variability among the progenies. Almost 50% of the progenies are clustered away from the parental types. Based on yield and components, the backcross-derived lines similar to MDU 1 may be tested for bruchid resistance and released as a bruchid-resistant variety after large-scale evaluation.

**Table 4.** Path analysis on seed yield per plant in BC<sub>2</sub>F<sub>4</sub> progenies

Characters	Plant Height (cm)	Number of branches per plant	Number of clusters per plant	Number of pods per cluster	Number of pods per plant	Pod Length (cm)	Number of seeds per pod	Hundred seed weight (gm)	Correlation with seed yield per plant
Plant Height (cm)	<b>0.02</b>	0.02	0.03	0.02	0.29	0.00	0.02	0.03	0.43
Number of branches per plant	0.01	<b>0.03</b>	0.05	0.01	0.41	0.00	0.02	0.03	0.56
Number of clusters per plant	0.01	0.02	<b>0.07</b>	0.02	0.60	0.00	0.01	0.00	0.74
Number of pods per cluster	0.01	0.01	0.02	<b>0.06</b>	0.40	0.00	0.03	0.06	0.59
Number of pods per plant	0.01	0.02	0.06	0.03	<b>0.70</b>	0.00	0.02	0.03	0.88
Pod length (cm)	0.01	0.01	0.02	0.02	0.24	<b>0.00</b>	0.03	0.06	0.38
Number of seeds per pod	0.01	0.01	0.01	0.02	0.18	0.00	<b>0.09</b>	0.06	0.37
Hundred seed weight (gm)	0.00	0.00	0.00	0.01	0.09	0.00	0.02	<b>0.25</b>	0.38

Residual effect:- 0.37

Diagonal values indicate direct effects.



**Fig. 1.** Dendrogram of yield and yield components of BILs of black gram

## Conclusion

The results show that eight progenies, i.e. 30-3-1, 51-6-1, 74-6-1, 87-5-1, 89-5-1, 95-1-2, 95-5-2 and 124-1-1, have a similar seed yield and traits as the recurrent parent MDU 1. Variability studies showed that selection can be practised for plant height, number of branches per plant, number of clusters per plant, number of pods per plant and seed yield per plant. Correlation analysis revealed that all traits had a positive association with seed yield per plant. Path analysis on seed yield per plant identified the number of pods and hundred seed weight as important selection indices for the seed yield improvement programme. Cluster analysis grouped progenies into three groups. MDU 1 and most of the high-yielding progenies clustered together. Progenies similar to MDU 1 may be tested for bruchid resistance and released as a bruchid-resistant variety.

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## Authors' contributions

PM carried out experimentation and drafted the manuscript, and MN planned, supervised and edited it. All authors were involved in planning and providing critical feedback on the manuscript. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None.

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