

CHARACTERIZATION OF BLACKGRAM GERMPLASM [*Vigna mungo* (L.) HEPPEY] FOR YIELD AND RESISTANCE TO YELLOW MOSAIC DISEASE

Sandeep Kaur Dhaliwal¹, Ranjit Kaur Gill^{1*}, Satinder Kaur², Jagmeet Singh¹, Guriqbal Singh Dhillon², Ashok Kumar³, Kuldeep Singh⁴ and Dhammaprakash P Wankhede⁵

¹Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana-141 004, India

²School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana-141 004, India

³Punjab Agricultural University Regional Research Station, Gurdaspur-143 521, India

⁴International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Hyderabad-502 324, India

⁵Division of Genomic Resources, National Bureau of Plant Genetic Resources, New Delhi-110 012, India

ABSTRACT

Black gram is a highly nutritious legume crop primarily cultivated for its grains in Asian countries. Despite its significant importance due to its health benefits and organoleptic properties, its productivity remains low. The exploration and cataloging of genetic diversity are crucial initial steps towards maximizing production potential. The objective of this study was to evaluate 482 germplasm accessions of black gram for 14 quantitative traits (including grain yield and related characteristics) and 13 qualitative traits (such as plant growth, leaf structure, branching pattern, and pod morphology). Analysis of the phenotypic data revealed extensive variation in both quantitative and qualitative traits among the germplasm under study. Several germplasm accessions, including IC0619217, IC0279521, IC0472035, IC0393537, IC0321149, IC0331231, IC0530627, and IC0330889, exhibited numerous desirable traits that surpassed those of commercial checks. Heritability and genetic advance as a percentage of population means (GAM) were highest for PDM (60.795), GyPp (58.82), and PpP (56.89). Principal component analysis and correlation analysis indicated strong associations of traits such as PdL, PSD, PdpP, PpP with GyPp, while, days to maturity showed a negative association with grain yield. Furthermore, germplasm with traits such as insect tolerance (pod and leaf pubescence), drought tolerance (lanceolate leaves), and heat tolerance (central branching) were present in germplasm. This selected germplasm holds promise for use in urdbean genetic improvement programs.

Keywords: Accessions, Principle component analysis, Quantitative traits

Black gram [*Vigna mungo* (L.) Hepper], a member of the Fabaceae family (the bean or legume family), is an autogamous, diploid legume crop ($2n=2x=22$) with an estimated genome size of 0.59 pg/1C (574 Mbp) (Arumuganathan and Earle, 1991). Originating in India, black gram, also known as *urd*, holds a significant position in agriculture. India ranks first globally in both the area (5.0 million hectares) and production (3.8 million tonnes annually) of this highly prized pulse (Anonymous, 2020). The grains of black gram are highly nutritious, containing minerals, vitamins, amino acids, carbohydrates (60%), protein (25-26%), and fat (1.5%). This rich nutritional profile, comprising proteins, antioxidants, micronutrients, and bioactive compounds, positions black gram as a balanced dietary option. It serves as an effective measure against malnutrition, offering a cost-effective alternative to expensive micronutrient supplements in developing countries like India. Moreover, black gram is valued for its short duration and photoperiod insensitivity, making it suitable for multi-cropping between two nutrient-exhausting

cereal crops. Its ability to fix atmospheric nitrogen through root nodulation replenishes soil nutrient levels and improves soil physical properties.

The crop was domesticated from the ancestral form of *V. mungo* var. *silvestris* (Lukoki *et al.*, 1980). While the progenitor of the crop was primarily out-crossing in nature, domesticated black gram has evolved into a highly self-pollinated species. Consequently, present-day black gram exhibits a narrow genetic base due to extensive inbreeding over the years. Earlier researchers have made limited efforts to broaden its genetic diversity by crossing it with diverse genotypes, as much of the genetic diversity within the primary gene pool of the crop remains unexplored (Ghafoor *et al.*, 2001).

Germplasm exploration, collection, conservation, and evaluation are crucial components of any plant breeding program aimed at incorporating desirable traits into cultivated varieties. Systematic evaluation of germplasm is essential to understand various morphological and developmental characteristics, as well as to identify resistance against abiotic and biotic stresses. The United Nations (UN) designated 2010 as the "Year of Biodiversity," emphasizing the importance

*Corresponding author: rkgillpbg@pau.edu

Date of receipt: 07.08.2023, Date of acceptance: 29.02.2024

of conserving, evaluating, and utilizing biological diversity for overall human development. Plant species with little or no history of improvement often benefit the most from collections of raw or unimproved germplasm (Ferguson *et al.*, 2007). Numerous examples exist of the utilization of germplasm accessions in improving major food crops such as rice (Vasudevan *et al.*, 2014), wheat (El-Bouhssini *et al.*, 2009), maize (Williams *et al.*, 2008), chickpea (Upadhyaya and Ortiz, 2001), and cotton (Abd-El salam, 2014), among others.

The present study represents a significant effort towards evaluating the germplasm of black gram, which will contribute to the development of a core collection for the crop. Core collections offer a representation of the genetic diversity of a crop in a non-repetitive manner, thereby enabling breeders to select genotypes for hybridization.

Statistical analysis of phenotypic data using genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), and heritability plays a crucial role in identifying the heritable fraction of traits, aiding in the formulation of selection strategies (Allard, 1960). Additionally, correlation, regression, principal component analysis, and path analysis are valuable tools for dissecting variation and enhancing our understanding of each trait, particularly yield, and its relationship with other traits (Ghafoor *et al.*, 2001).

The impact of environmental factors and genotype x environment interactions can often confound the genetic fraction of total variation. Employing the strategy of Best Linear Unbiased Estimation (BLUE) for final data analysis proves reliable, especially when yearly data exhibits significant variation and the aim is to minimize residual variation. Within this framework, fixed effects (where genotypes are considered fixed) can be accurately calculated using BLUE, while Best Linear Unbiased Prediction (BLUP) is employed for estimating random effects (Piepho *et al.*, 2008). The adjusted means derived from BLUEs provide corrected averages, compensating for data imbalances and offering reliable estimates for the formulation of selection strategies.

There are limited reports of evaluation of black gram germplasm (Ghafoor *et al.*, 2001; Aftab *et al.*, 2018; Thirumalai and Murugan, 2020). A significant portion of black gram germplasm remains unexplored and underutilized in crop breeding endeavors, hindering the realization of its maximum production potential. Consequently, the present study was designed to assess the resistance of germplasm accessions to yellow mosaic disease (YMD). These accessions were collected from diverse agro-climatic regions of India and abroad by the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India, and were

evaluated for 14 quantitative and 13 qualitative traits.

MATERIAL AND METHODS

Plant material and field experiments

The germplasm panel consisted of 482 accessions of black gram (*Vigna mungo*), sourced from the National Bureau of Plant Genetic Resources (NBPGR) in New Delhi, India. These 482 accessions were resistant to yellow mosaic disease (YMD) and were selected from a total of 2614 germplasm lines obtained from NBPGR (Fig. 1). The panel was planted and evaluated at the Pulses Research Farm, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana (30.9°N, 75.85°E), for two consecutive years, 2019 and 2020. The germplasm was planted according to an augmented block design, with five commercially successful varieties serving as checks within each block, and these checks were randomized. The seeds were sown in a 2m long row at plant x row spacing of 10 cm x 30 cm, respectively, with two rows per accession. The checks used in the present study included KU96-3 (C1), Mash 114 (C2), IPU2-43 (C3), PU11-14 (C4), and Mash 479 (C5).

Phenotypic data collection

The data were recorded for both qualitative and quantitative traits by selecting three random plants from each line. A total of 14 quantitative traits were studied, including terminal leaf length (TLL), terminal leaf width (TLW), plant height (PH), plant stem diameter (PSD), petiole length (PoL), peduncle length (PdL), number of primary branches (PB), number of pods per plant (PpP), number of peduncles per plant (PdP), days to 80 per cent maturity (DM), pod length (PL), number of seeds per pod (SpP), plant dry biomass (PDM), and grain yield per plant (GYpP).

Data for 13 qualitative traits were also collected, including hypocotyl colour (HyC), seedling vigour (SV), terminal leaflet type (TLT), leaf pubescence (LP), growth habit (GH), leafiness (L), leaf colour (LC), branching pattern (BP), raceme position (RP), pod attachment to peduncle (PAP), pod pubescence (PP), leaf senescence (LS), and pod colour at maturity (PCM).

All germplasm lines, along with checks, were characterized for a total of twenty-seven descriptors following the standard operating procedures (SOPs) for characterizing germplasm accessions in minor pulses provided by NBPGR.

Statistical analysis

Pairwise correlations among different traits were studied, with special emphasis on grain yield. Descriptive analysis and variability studies were done

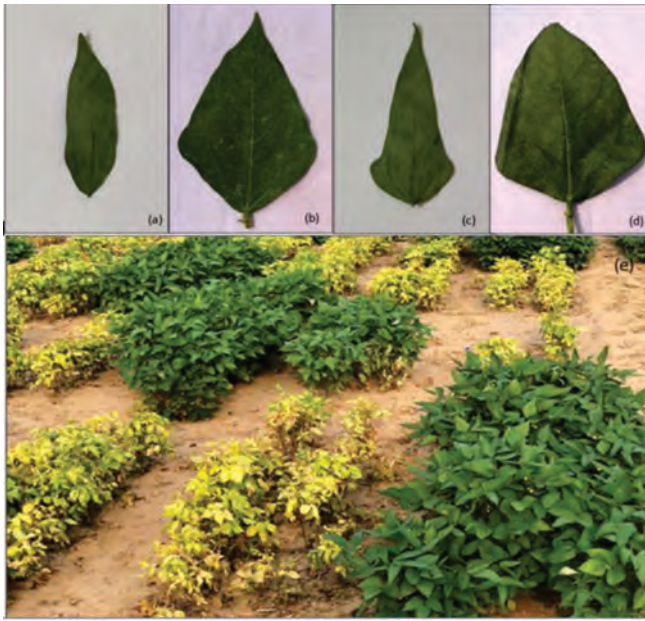


Fig. 1(a). Terminal leaf Ovate-lanceolate type, (b) Terminal leaf rhombic type, (c) Terminal leaf lanceolate type, (d) Terminal leaf ovate type, (e) Germplasm accessions showing reaction to yellow mosaic disease.

using the summary tools package (Comtois, 2020) in R software. Analysis of variation, variability analysis, and calculation of adjusted means (BLUES) were performed using META-R version 6.0 (Alvarado *et al.*, 2016). Comparisons were made between genotypes and their respective recurrent parents, separately for each environment. Adjusted means (BLUES) of replications were utilized for comparisons in the respective environments. The best linear unbiased estimates (BLUES) were obtained by fitting linear mixed-effects models using the lme4 package v1.1-26 (Bates *et al.*, 2015) in R v4.0.3 (R Core Team, 2019) using

$$Y_{ik} = \mu + Year_i + Line_k + \varepsilon_{ik}$$

where Y_{ik} is the trait of interest, μ is the mean effect, $Year_i$ is the effect of the i^{th} year, $Line_k$ is the effect of the k^{th} line, and ε_{ik} is the error associated with the i^{th} year and the k^{th} line, which is assumed to be normally and independently distributed, with mean zero and homoscedastic variance σ^2 . Adjusted values on the basis of ANOVA were calculated and used for statistical analysis. The genotypic coefficient of variability (GCV) was calculated using the equation

$$GCV = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \times 100$$

where σ_g^2 is the genotypic variance, \bar{x} is the mean of the all genotypes. The phenotypic coefficient of variability (PCV) was calculated using the equation

$$PCV = \frac{\sqrt{\sigma_p^2}}{\bar{x}} \times 100$$

where σ_p^2 is the phenotypic variance. The environmental coefficient of variability (ECV) was calculated using the equation

$$ECV = \frac{\sqrt{\sigma_e^2}}{\bar{x}} \times 100$$

where σ_e^2 is the genotypic variance. The broad sense heritability was estimated using the formula by Allard (1960)

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

The broad-sense heritability estimated the quality of the breeding program for the traits and the environments. The genetic advance over mean was calculated using the formula as per Allard (1960):

$$GAM = \frac{h^2 \times K \times \sigma_p^2}{\bar{x}}$$

where, K is selection differential at 5% intensity of selection ($K=2.06$). The correlations were calculated as simple pairwise Pearson's correlations among traits. It is defined as:

$$r = \frac{COV_{xy}}{\sigma_x^2 + \sigma_y^2}$$

where, COV_{xy} is the covariance between trait x and trait y, σ_x^2 is the variance of trait x, σ_y^2 is the variance of trait y.

Further, principal component analysis was performed to determine the number of principal components needed to explain the variation across the years, along with fitted values from linear mixed-effects models. This analysis was performed using FactoMineR v2.4 (Le *et al.*, 2008) and Factoextra v1.0.7 (Kassambara and Mundt, 2020) in R v4.0.3. The principal components were visualized for two years as biplots of eigen vectors.

RESULTS AND DISCUSSION

Quantitative traits

The germplasm panel comprising 482 genotypes exhibited diversity across all 14 quantitative traits studied, as indicated by the range, standard deviation, and coefficient of variation observed over two years using adjusted means via BLUES (Table 1). The dataset surpassed the commercial checks, demonstrating a wide range of values for all traits: terminal leaf length (TLL: 5.94-10.05 cm), terminal leaf width (TLW: 3.10-7.64 cm), plant height (PH: 15.12-61.88 cm), plant stem diameter (PSD: 3.23-8.01 cm), petiole length (PoL: 1.17-3.24 cm), peduncle length (PdL: 1.97-10.37 cm),

number of pods per plant (PpP: 3.51-10.77), number of primary branches (PB: 16.92-147.14), number of peduncles per plant (PdpP: 7.60-56.50), days to maturity (DM: 74.62-81.67), pod length (PL: 3.21-5.12 cm), number of seeds per pod (SpP: 4.94-8.11), plant dry biomass (PDM: 13.38-70.18 g), and grain yield per plant (GYpP: 0.23-23.76 kg). A symmetrical distribution was observed around the mean for most traits, except for slight positive skewness observed for plant dry biomass (PDM) (Table 1 and Fig. 4). Distribution peaks exhibited leptokurtosis for TLW, PdL, PpP, PdpP, PL, PDM, and GYpP, while platykurtosis was observed for TLL, PH, PSD, PoL, PB, DM, and SpP.

The wide range of values observed for all quantitative traits in the germplasm under study clearly indicates its potential for trait enhancement. Genotypes exhibiting maximum phenotypic values, such as accession numbers IC0619217 (23.75) and IC0279521 (20.145) with high yield, IC0472035 (10.05) with the highest TLL, IC0393537 (9.85) with the highest TLW, IC0321149 (10.77) with the maximum number of primary branches, IC0331231 (5.12) with the longest pod length, IC0530627 (8.10) with the maximum number of seeds per pod, and IC0330889 (70.18) with the highest dry matter content, hold promise for breeding improved cultivars. These germplasm lines surpassed the commercial checks for all traits, indicating their potential for use in crossing programs to transfer desirable traits.

The normal distribution of phenotypes for various traits suggests that the germplasm panel under study

is diverse. Previous studies on phenotypic diversity in black gram for agronomic traits (Ghafoor *et al.*, 2001; Aftab *et al.*, 2018) evaluated a smaller number of genotypes, with Ghafoor *et al.* (2001) studying 484 genotypes collected locally from Pakistan only. In contrast, the germplasm in our study was collected from diverse agro-climatic zones of India and abroad, thus possessing a broader range of variation.

Furthermore, all genotypes in our study were resistant to YMD, aligning with a similar study conducted by NBPGR, where 334 germplasm lines were screened and 4 lines showed resistance against YMD, as reported by Bag *et al.* (2014). Therefore, our study presents phenotypic data for 482 germplasm lines completely resistant to YMD.

Most traits exhibited a high to moderate genotypic coefficient of variance (GCV), except for TLL and DM, which displayed low GCV values (Table 2). A contrasting trend was observed in the environment coefficient of variance (ECV) compared to GCV. The highest GCV and phenotypic coefficient of variance (PCV) were recorded for GYpP (38.54, 52.26) followed by PDM (34.29, 40), averaged over two years, respectively. Only DM showed a low PCV, while the remaining traits exhibited high to medium PCV values. Notably, DM demonstrated a very high broad-sense heritability (HBS) of 90.12%, followed by PL (78.12%), PH (77.99%), and SpP (77.31%). HBS was lower than 50% for PdpP (44.86%) and PSD (48.08%), while the rest of the traits surpassed this benchmark. Genetic advance as a percent of population

Table 1. Genetic variability of phenotypic traits in five standard checks and germplasm set

Trait	Env	C ₁	C ₂	C ₃	C ₄	C ₅	Range	Std. Dev	Mean	CV	Skewness	Kurtosis
TLL	BLUEs	7.9	8.1	8.3	8.2	8.3	5.9-10.1	0.8	8.0	0.1	0.2	-0.4
TLW	BLUEs	5.3	5.8	5.5	5.5	5.5	3.1-7.6	0.7	5.4	0.1	-0.2	0.1
PH	BLUEs	41.3	44.6	44.0	42.9	41.2	15.1-61.9	7.4	41.3	0.2	-0.1	-0.02
PSD	BLUEs	6.2	6.3	6.5	6.1	6.0	3.2-8.0	0.9	5.4	0.2	0.1	-0.5
PoL	BLUEs	2.2	2.3	2.3	2.2	2.3	1.2-3.2	0.4	2.1	0.2	0.0	-0.3
PdL	BLUEs	5.6	5.3	6.0	6.3	6.3	1.9-10.4	1.3	5.9	0.2	0.3	0.3
PB	BLUEs	9.4	9.7	9.9	9.4	9.4	3.5-10.7	1.2	7.5	0.2	0.0	-0.3
PpP	BLUEs	76.8	81.1	76.9	78.1	77.6	16.9-147.1	16.6	55.4	0.3	0.5	1.4
PdpP	BLUEs	33.5	34.5	32.6	32.5	32.1	7.6-56.5	7.3	23.7	0.3	0.6	0.8
DM	BLUEs	76.9	76.5	75.9	76.9	77.1	74.6-81.6	1.4	77.6	0.0	0.0	-0.4
PL	BLUEs	4.2	4.4	4.3	4.2	4.3	3.2-5.1	0.3	4.1	0.1	0.3	0.6
SpP	BLUEs	6.7	6.9	6.8	6.9	6.8	4.9-8.1	0.6	6.5	0.1	0.1	-0.1
PDM	BLUEs	35.4	35.5	39.0	35.4	33.8	13.3-70.2	8.3	29.6	0.3	1.0	1.9
GYpP	BLUEs	11.1	12.6	10.0	11.3	11.6	0.2-23.7	3.7	7.5	0.5	0.6	0.3

• C₁-C₅ are the five checks.

• TLL: Terminal Leaf Length, TLW: Terminal Leaf Width, PH: Plant Height, PSD: Plant Stem Diameter, PoL: Petiole Length, PdL: Peduncle Length, PB: Number of Primary Branches, PpP: Number of Pods per Plant, PdpP: Number of Peduncles per Plant, DM: Days to Maturity, PL: Pod Length, SpP: Number of Seeds per Pod, PDM: Plant Dry Biomass, GYpP: Grain Yield per Plant

means (GAM) was highest for PDM (60.795%), GYpP (58.82%), and PpP (56.89%). Other traits also exhibited high to medium GAM values, while DM (5.62%) had a lower GAM value.

The PCV estimates the total phenotypic variation, while GCV represents the heritable component of the overall phenotypic variation, aiding in the understanding of heritable variation. A high value for GCV and heritability indicates that selection would be beneficial. Similar results were observed from GAM, suggesting high genetic gains from selection in traits with high to moderate GAM and low gains for DM. These findings are consistent with previous studies (Isaacs *et al.*, 2000; Aftab *et al.*, 2018), which reported high to moderate GAM for the traits under investigation.

Scree plot and PCA

The total explained phenotypic variance was partitioned into different principal components (Dim), as illustrated in the scree plot (Fig. 2). The first two principal components, Dim1 and Dim2, accounted for 25.3% and 15.5% of the explained phenotypic variation, respectively, while the remaining principal components collectively contributed to the remaining variance, as evidenced by the flat line after the first two dimensions. Consequently, Dim1 and Dim2 were selected for interpreting phenotypic variation among traits and genotype clustering (Fig. 3). The PCA biplot of trait vectors suggested that TLL, TLW, PdL, PSD, PdpP, PpP, and GYyP were strongly associated with

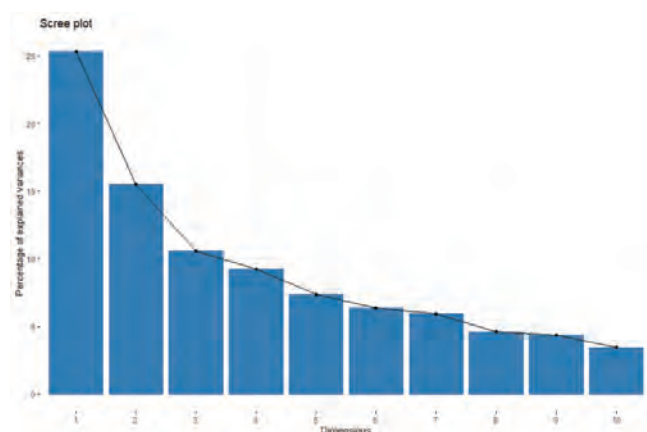


Fig. 2. Scree plot of 10 principal components

Dim1. Among these, TLL and TLW exhibited a strong association with each other in the biplot, while PdL, PSD, PdpP, and PpP were more strongly associated with GYyP. On the other hand, DM showed a strong association with Dim2 and was negatively associated with GYyP and related traits.

A noteworthy observation from the biplot analysis was that grain yield and related traits were closely associated with Dim1 and with each other, whereas days to maturity was negatively associated with grain yield and was more closely related to Dim2. Similar results were obtained in the study by Aftab *et al.* (2018), where late maturity was associated with the second principal component and placed far away from grain yield in the

Table 2. Genetic variability of quantitative traits in blackgram germplasm

Trait	GCV	PCV	ECV	HBS	GA	GAM
TLL	8.0	10.4	6.7	58.4	1.0	12.5
TLW	11.1	14.0	8.4	63.0	1.0	18.2
PH	23.6	26.7	12.4	78.0	17.7	43.1
PSD	12.1	17.5	12.4	48.1	1.0	17.5
PoL	18.5	21.2	10.3	75.9	0.7	33.2
PdL	23.7	27.8	14.5	72.8	2.5	41.6
PB	18.7	22.4	12.1	69.6	2.4	32.4
PpP	31.4	35.8	17.1	76.9	32.0	56.9
PdpP	22.3	33.4	24.8	44.9	7.4	30.8
DM	2.9	3.0	0.9	90.1	4.3	5.6
PL	8.4	9.5	4.3	78.1	0.6	15.5
SpP	12.5	14.1	6.3	77.3	1.5	22.9
PDM	34.3	40.0	20.4	72.2	17.4	60.8
GYpP	38.5	52.3	35.1	54.6	4.4	58.8

- Genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), environment coefficient of variation (ECV), broad sense heritability (Hbs), genetic advance (GA), genetic advance as percent of population means (GAM)
- TLL: Terminal Leaf Length, TLW: Terminal Leaf Width, PH: Plant Height, PSD: Plant Stem Diameter, PoL: Petiole Length, PdL: Peduncle Length, PB: Number of Primary Branches, PpP: Number of Pods per Plant, PdpP: Number of Peduncles per Plant, DM: Days to Maturity, PL: Pod Length, SpP: Number of Seeds per Pod, PDM: Plant Dry Biomass, GYpP: Grain Yield per Plant

biplot. However, contrary results may be obtained due to a sudden decrease in temperature overlapping with the harvesting of black gram in early November in North India, resulting in many pods in late maturity groups remaining unripe, leading to lower yields.

The length of trait vectors indicated that large variation was present for GYp, PH, TLW, TLL, PDM, PpP, and PdpP in the germplasm, while low variability was found in traits like PoL, PB, and SpP. These findings were supported by Islam *et al.* (2019), Thirumalai and Murugan (2020), and Sridhar *et al.* (2020), who also noted that grain yield exhibited maximum variability and related traits were closely associated with it. Three clusters of genotypes were formed in the biplot corresponding to the two years and BLUEs of both years. The genotypes were mainly concentrated around the origin of the biplot, as inferred from BLUEs.

Pair-wise correlation coefficient between traits

It was inferred from pairwise correlations between traits that GYp was positively correlated with TLL, TLW, PH, PSD, PoL, PdL, PB, PpP, PdpP, PL, and PDM (Fig. 4). Among these traits, grain yield was significantly positively correlated with PpP (0.689) and PdpP (0.595), while it exhibited negative correlations with DM (-0.17) and SpP (-0.016). Additionally, a significant positive correlation was observed between PpP and PdpP (0.813). DM showed negative correlations with all traits, as evidenced by the regression lines presented in Fig. 4.

The regression lines for GYp as the dependent variable were flatter in the year 2019 compared to 2020. In 2020, the lines were diagonal, indicating a positive regression coefficient for all traits with GYp in this year, except for DM. The regression plot followed the same trend as the correlation analysis. The distribution curves overlapped and showed symmetrical distribution for all traits in both years, except for DM, where less significant overlapping was observed.

The pairwise correlation coefficient between traits aids breeders in identifying positively and negatively correlated traits. Consequently, breeders can formulate strategies to select desirable traits, favouring positively associated characters. Undesirable positive correlations can be addressed through recombination. For example, in our study, plant height was positively correlated with grain yield, which may be considered an undesirable association. On the other hand, negative correlations were observed between days to maturity and seeds per pod with grain yield, a finding consistent with studies by Dhasarathan *et al.* (2021) and Panigrahi *et al.* (2014). Dhasarathan *et al.* (2021) investigated mutants in the background of cultivars ADT 3, Co 6, and TU 17-9, reporting negative correlations of days to 50% flowering and seeds per pod with grain yield in the mutant population of cultivar ADT 3.

Qualitative traits

The evaluation of germplasm for morphological traits divided them into various categories based

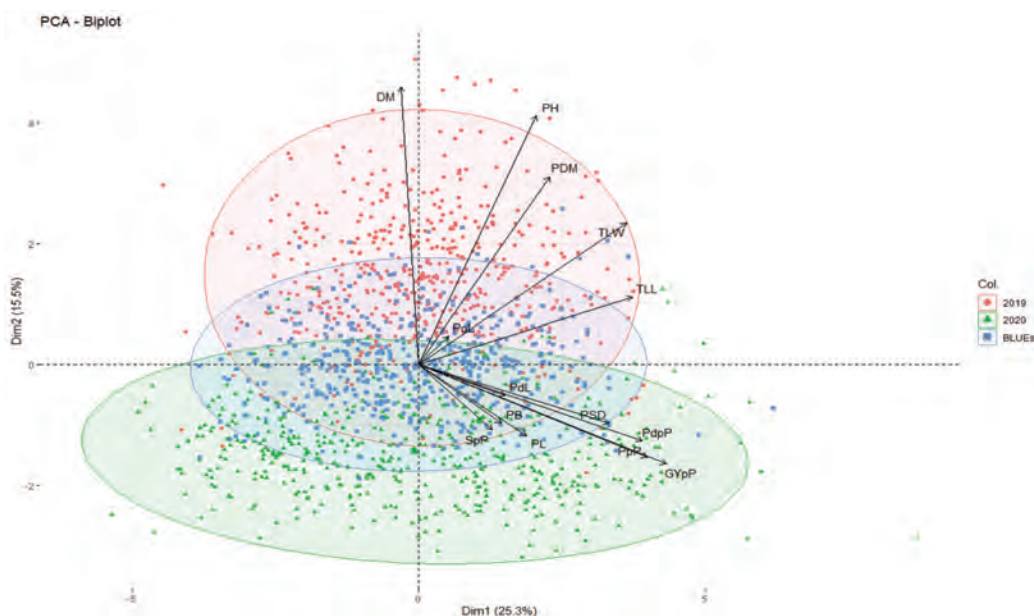


Fig. 3. Principle component biplot of *Vigna mungo* accessions formed using first two principle components (Dim1 and Dim2). Individual genotypes are represented by dots coloured red (year 2019), green (year 2020) and blue (BLUEs). Individual quantitative traits are represented by vectors.

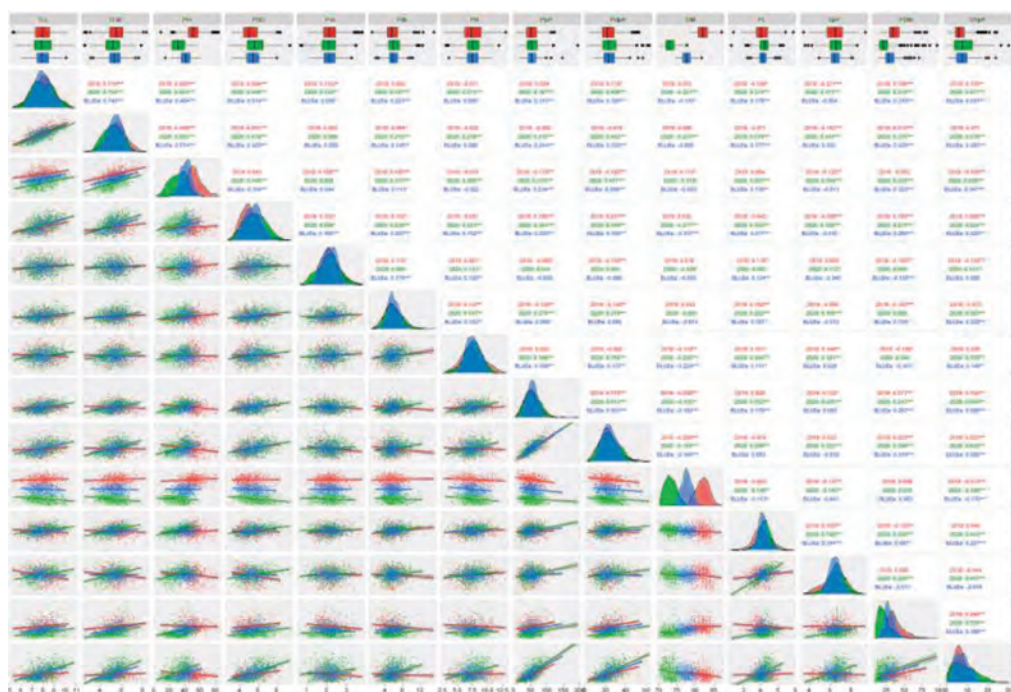


Fig. 4. Evaluation of germplasm across *kharif* seasons 2019, 2020 and BLUEs. Red colour represents trait response in year 2019, green colour represents trait response in year 2020 and blue colour represent trait response under BLUE. Top row indicates distribution of the traits as box-plots. Upper right triangle depicts pair-wise correlation coefficient values with different colours presented individually as explained above. The diagonal indicates density distribution curves of each trait. Lower left triangle indicates individual traits in pair-wise dot plots along with regression lines.

on descriptors and details are provided in following subheadings (Fig. 6).

Plant growth characters

The evaluation of plant growth characters in the present study involved growth habit, seedling vigour, and hypocotyl colour. Analysis of the data revealed that 99% of plants exhibited an erect growth habit, with 2% categorized as semi-erect types. Additionally, 92% of plants displayed vigorous seedling vigour, while 3.91% exhibited intermediate seedling vigour, and 0.41% were classified as poor in vigour. Regarding hypocotyl colour, the majority of plants (99.79%) displayed a purple colour, with only one plant exhibiting mixed hypocotyl colouration.

Leaf characters

Terminal leaflet type, leaf pubescence, leafiness, leaf colour, and leaf senescence were recorded in the germplasm collection. Three types of terminal leaflets were identified: ovate (88%), ovate-lanceolate (0.82%), and rhombic (10.99%). Glabrous leaves were observed in 1.24% of plants, while 97.71% of genotypes exhibited sparse pubescence, and only 1.03% had puberulent leaves. Abundant leafiness was predominant (93.15%), with a few genotypes displaying intermediate (6.01%) and sparse leafiness (0.82%). Leaf colour was

primarily dark green (85.47%), followed by very dark green leaves (9.54%), while only 4.35% of plants had light green leaves. Additionally, two genotypes exhibited intermediate leaf colour, and very light green leaves were present in one plant. Slight leaf senescence was observed in 466 genotypes (96%), while 4 genotypes displayed moderate senescence, and 11 genotypes exhibited moderately high leaf senescence. Concurrent leaf senescence was present in only 1 genotype.

Branching characters

The branching pattern analysis revealed two distinct types: 95.22% of plants exhibited a central branching pattern, while 4.77% displayed a top pattern of branching. Interestingly, no plants showed pods above the canopy level. Additionally, the majority of plants (99.58%) exhibited intermediate positions of pods, with only two plants showing no visible pods above the canopy.

Pod characters

The analysis of pod attachment to the peduncle revealed pendant attachment in 3 genotypes, while 98.75% of plants exhibited a sub-erect attachment, and 1% of genotypes displayed an erect type attachment. Pod pubescence varied among the collection, with dense pubescence observed in 44% of genotypes,

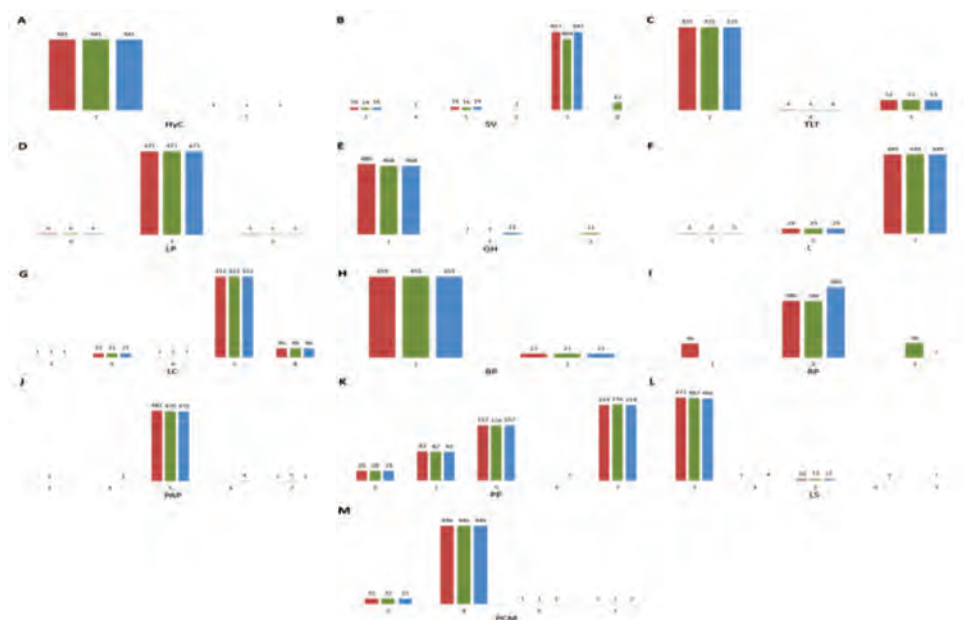


Fig. 6. Line graph of individual qualitative traits showing distribution of different genotypes against trait classes. Red colour represents year 2019, green colour represents year 2020 and blue colour represents BLUE.

moderate pubescence in 32%, sparse pubescence in 17% of accessions, and a glabrous type present in 5.89% of genotypes. In terms of pod colour, tan pods were recorded in 6.84% of plants, while brown-coloured pods dominated the collection, accounting for 92.53% of the fraction. Brown-black pods were found in only one plant, and black-coloured pods were observed in 2 plants.

Various types of plant growth characters (growth habit, seedling vigour, and hypocotyl colour), leaf characters (terminal leaflet type, leaf pubescence, leafiness, leaf colour, and leaf senescence), branching characters (branching pattern, raceme position), and pod characters (pod attachment to peduncle, pod pubescence, pod colour) were observed within the germplasm under study. This documented variation holds potential for future utilization in the development of desirable genotypes with specific morphological traits. For instance, the prevalence of an erect growth habit among the present set of genotypes is advantageous for smooth cultural practices, intercropping, and mechanical harvesting. Furthermore, the majority of lines exhibited good seedling vigour, which is crucial for crop establishment under harsh climatic conditions.

Significant variation in leaf characters suggests diverse photosynthetic efficiencies among genotypes (White & Montes-R, 2005). Although predominantly ovate, the presence of lanceolate and rhombic leaf types indicates potential drought tolerance, which could be further investigated for cultivar development in rainfed areas. Leaf pubescence, a key trait for

insect resistance (Lill *et al.*, 2006), was predominantly sparse among the germplasm, although genotypes with pubescent leaves were identified (IC0250187, IC0250196, IC0250195) and most of the germplasm had sparse pubescence. Branching pattern and raceme position are vital traits determining sunlight interception and photosynthetic efficiency. Most genotypes exhibited a central branching pattern, with a few displaying top branching. Comparative analysis of different branching patterns and raceme positions could provide insights into ideal canopy types for high productivity.

Pod and seed characters are important from a consumer's perspective, with a large variation observed in pod pubescence and less variation in pod colour and brown colour was found to be predominant. This consistency in variability corresponds with the results of earlier research, as demonstrated by Jayamani *et al.* (2014) in accessions of *Vigna mungo* var. *silvestris*, and Islam *et al.* (2019), who also observed diversity in qualitative traits among landraces of black gram. This suggests the opportunity for continued exploration and selection of desirable qualitative traits. Some genotypes identified with desirable qualitative traits include IC0250187, IC0250196, and IC0250195.

Authors' contribution

Conceptualization and designing of the research work (DPW, KS, RMN); Execution of field/lab experiments and data collection (JS, SKD); Data analysis and interpretation (GS); Preparation of manuscript (SKD, RK, SK), germplasm accessions provided by NS, GC and DPW.

Declaration: All the authors declare that they do not have any conflict of interests.

LITERATURE CITED

- Abd-Elsalam K A 2014. Evaluation of a cotton germplasm collection against *Fusarium* wilt race 3 isolates from Egypt. *Trop Plant Pathol* **39**: 95-103.
- Aftab N, Lal G M, Sheera A, Chandra Bose N and Tripathi A M 2018. Evaluation of genetic variability in black gram (*Vigna mungo* L. Hepper) germplasm. *JPDS* **10**(8): 445-52.
- Allard R W 1960. *Principles of Plant Breeding*, 1st edn. John Wiley & Sons Ltd., New York.
- Alvarado G, Lopez M, Vargas M, Pacheco A, Rodríguez F, Burgueño J and Crossa J 2016. META-R (Multi Environment Trial Analysis with R for Windows), CIMMYT, Mexico.
- Anonymous 2020. *Area, production, productivity of blackgram in India*. Directorate of Economics and Statistics, Ministry of Agriculture and Farmers' Welfare, Government of India.
- Arumuganathan K and Earle E D 1991. Nuclear DNA content of some important plant species. *Plant Mol Biol Rep* **9**: 208-15.
- Bag M K, Gautam N K, Prasad T V, Pandey S, Dutta M and Roy A 2014. Evaluation of an Indian collection of black gram germplasm and identification of resistance sources to mungbean yellow mosaic virus. *Crop Prot* **61**: 92e101.
- Bates D, Mächler M, Bolker B M and Walker S C 2015. Fitting linear mixed-effects models using lme4. *J Stat Softw* **67**: 1-48.
- Comtois D 2020. Summarytools: Tools to quickly and neatly summarize data. R-package version 0.9.6. *Comp Sci* <https://api.semanticscholar.org/CorpusID:226112641>
- Dhasarathan M, Geetha S, Karthikeyan A, Sassikumar D and Meenakshiganesan N 2021. Development of novel blackgram (*Vigna mungo* (L.) Hepper) mutants and deciphering genotype x environment interaction for yield-related traits of mutants. *Agron* **11**: 1287.
- El-Bouhssini M, Street K, Joubi A, Ibrahim Z and Rihawi F 2009. Sources of wheat resistance to sun pest, *Eurygaster integriceps* Puton, in Syria. *Genet Resour Crop Evol* **56**: 1065-69.
- Federer T W 1956. Augmented Designs. *Hawaii Plant Rec* **4**(2): 191-208.
- Ferguson A 2007. The need for characterisation and evaluation of germplasm: Kiwifruit as an example. *Euphytica* **154**: 371-82.
- Ghafoor A, Sharif A, Ahmada Z, Zahid M A and Rabbani M A 2001. Genetic diversity in blackgram (*Vigna mungo* L. Hepper). *Field Crops Res* **69**: 183-90.
- Isaacs S M, Jebaraj S and Ganesh S K 2000. Estimates of genetic variability and heritability in black gram [*Vigna mungo* (L.) Hepper]. *Res Crop* **1**(1): 37-39.
- Islam M T, Rahman S, Malek M A, Ahmed I and Jahan T 2019. Characterization and diversity of blackgram germplasm. *Bangladesh J Agric Res* **44**(2): 239-51.
- Jayamani P, Srimathy M and Sathya M 2014. Characterization of blackgram genotypes based on qualitative traits (*Vigna mungo* L. Hepper). *Madras Agric J* **101**: 12-15.
- Kassambara A and Mundt F 2020. Factoextra: Extract and visualize the results of multivariate data analyses. 1-84. <https://cran.r-project.org/package=factoextra>
- Lê S, Josse J and Husson F 2008. Facto Mine R: an R package for multivariate analysis. *J Stat Softw* **25**: 1-18.
- Lill J T, Marquis R J, Forkner R E, Le C J, Holmberg N and Barber N A 2006. Leaf pubescence affects distribution and abundance of generalist slug caterpillars (Lepidoptera: Limacodidae). *Environ Entomol* **35**: 797-806.
- Lukoki L, Marechal R and Otoul E L 1980. Ancetressauvages des haricots cultives: *Vigna radiata* (L.) Wilczek et *V. mungo* (L.) Hepper. *Bull Jard Bot Natl Belg* **50**: 385-91.
- Panigrahi K K, Mohanty A and Baisakh B 2014. Genetic divergence, variability and character association in landraces of blackgram (*Vigna mungo* L. Hepper) from Odisha. *J Crop Weed* **10**(2): 155-65.
- Piepho H P, Möhring J, Melchinger A E and Büchse A 2008. BLUP for phenotypic selection in plant breeding and variety testing. *Euphytica* **161**: 209-28.
- R Core Team 2019. *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. <https://www.scienceopen.com/book?vid=b164ea90-95d2-43bf-9710-99753c479112>
- Sridhar V, Prasad B V, Shivani D and Rao S S 2020. Genetic divergence studies for yield components in blackgram (*Vigna mungo* L.) genotypes. *Int J Curr Microbiol Appl Sci* **9**(01): 1816-23.
- Thirumalai R and Murugan S 2020. Multivariate analysis in blackgram (*Vigna mungo* (L.) Hepper) genotypes for mungbean yellow mosaic virus (MYMV) resistance. *Plant Arch* **20**(1): 2473-80.
- Upadhyay H D and Ortiz R 2001. A mini core sub set for capturing diversity and promoting utilization of chickpea genetic resources in crop improvement. *Theor Appl Genet* **102**: 1292-98.
- Vasudevan K, Vera Cruz C M, Grisse W and Bhullar N K 2014. Large scale germplasm screening for identification of novel rice blast resistance sources. *Front Plant Sci* **5**: 505.
- White J W and Montes-R C 2005. Variation in parameters related to leaf thickness in common bean (*Phaseolus vulgaris* L.). *Field Crops Res* **91**(1): 7-21.
- Williams W, Matthew D, Krakowsky G L, Windham P, Balint-Kurti L K, Hawkins and Henry W 2008. Identifying maize germplasm with resistance to aflatoxin accumulation. *Toxin Rev* **27**: 319-45.