RESEARCH ARTICLE

Understanding genetic diversity in blackgram [*Vigna mungo* (L.) Hepper] collections of Indian National Genebank

Gayacharan [ORCID] · **Kuldeep Tripathi** · **Muraleedhar S. Aski** · **Neeta Singh** · **Ashok Kumar** · **Hanuman Lal**

Abstract Blackgram [*Vigna mungo* (L.) Hepper] is a protein rich pulse crop of Indian origin. The crop is cultivated in diverse range of agro-ecological regions, cropping systems and cultural environments, since ancient times. This has resulted in build-up of substantial amount of genetic diversity, directly or indirectly associated with survival and useful economic traits. However, the diversity remains underutilized in the crop improvement. This has adversely impacted the crop improvement programs. Therefore, in this study, we have characterized 840 accessions of blackgram for important agro-morphological traits to highlight the useful diversity available in the crop genepool. Statistical analyses have revealed a substantial amount of genetic diversity available in blackgram. Phenotypic and genotypic coefficient of variation showed a wide range of variation for pod length, seeds per pod, seed weight, flowering and maturity related traits. Comparatively high level of genetic gain was observed for days to initial flowering (57.12), flowering period (40.35), days to 50% flowering (37.26), days to end of flowering (31.23), canopy height (31.06) and days to 80% maturity (29.6). The first seven principal components (PCs) explained 92.197% of the total variability in the blackgram germplasm. Plotting of the first two major PCs in 2-dimnentional space highlighted the relationship among traits, and certain associations between traits and observations. Promising accessions with desirable magnitude of trait variation were also identified. The implications of results obtained from this study in blackgram breeding programs may prove useful in enhancing yield and genetic variability in the crop.

Keywords Agro-morphological variation · Breeding value · Urdbean · Heritability · Promising accessions

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Introduction

Blackgram [*Vigna mungo* (L.) Hepper var. mungo] is protein rich highly priced pulse crop originated in South Asia (Pratap and Kumar 2011). It appears that the *Vigna mungo* (L.) Hepper var. mungo has been domesticated in India from its wild progenitor species *V. mungo* var. *silvestris* (Chandel et al. 1984). India is also the primary center of genetic diversity for blackgram (Zeven and de Wet 1982). The crop is

cultivated in diverse agro-ecologies and cropping systems under rainfed conditions (Gupta et al. 2001). It is mainly grown in India and few other countries such as Bangladesh, Pakistan, Sri Lanka, Myanmar, Philippines, Nepal and Thailand (Kaewwongwal et al. 2015). Blackgram is locally known by other vernacular names such as urdbean, ulundu paruppu, minapa pappu, mungo bean, black matpe bean, etc. Blackgram is very similar to green gram [*Vigna radiata* var. *radiata* (L.) R.Wilczek] with respect to its plant and seed morphology, soil and climatic requirement. Nutritional value is comparatively better for blackgram than green gram (Kakati et al. 2010). The most important nutritional component in blackgram is protein which constitutes 21–29% of the total dry grain weight (Girish et al. 2012; Kavitha et al. 2013). Other major nutrients in blackgram are fat (4.4 to 5.6 g/100 g), phosphorus (380 to 420 mg/100 g), calcium (107 to 134 mg/100 g), iron (3 to 4.4 mg/100 g) (Kavitha et al. 2013). Anti-nutritional factors (ANFs) such as flatulence producing oligosaccharides, protease inhibitors, saponins and phytic acids are comparatively higher in blackgram, which reduce nutrient availability and palatability of the food (Jain et al. 2009; Suneja et al. 2011). However, concentration of nutritional as well as ANFs varies significantly among blackgram genotypes (Suneja et al. 2011). This opens up the scope of developing excellent blackgram crop through screening diverse germplasm and identification of the better genotypes with high nutritional values and low ANFs.

India is the largest producer (ca. 54% of the global production) as well as consumer of blackgram (Singh et al. 2016). In India, blackgram accounts for ca. 17% of the total area under pulse cultivation (Gupta et al. 2021). For the last decade blackgram annual grain production varied between 1.7 and 3.49 million tonnes (Ministry of Agriculture and Farmers Welfare, Government of India). Blackgram production remains highly unstable and average productivity is alarmingly stagnant at ca. 0.5 ton/ha (Gupta and Parihar 2015; Singh et al. 2016; Patidar and Sharma 2017). Despite the crop being grown since ancient times, high nutritional values, soil ameliorating properties, a very little research have been done to improve the crop as compared to other pulse crops such as chickpea, pea, cowpea, lentil, pigeonpea and greengram. Lack of suitable ideotypes, poor harvest index, susceptibility to biotic and abiotic stresses and changing climatic

conditions are very discouraging for traditional growers of blackgram. Blackgram is also being outcompeted by similar *Vigna* crops such as greengram (*Vigna radiata*) and cowpea [*V. unguiculata* (L.) Walp.] with better agronomical traits. This is primarily because of inadequate utilization of variability in blackgram breeding programs (Gupta and Parihar 2015; Sridhar et al. 2020; Gupta et al. 2021). The pedigree analysis of blackgram varieties indicates that 30% of the varieties are developed utilizing only ten parents, of which T-9 is the most frequently used (Gupta and Parihar 2015). In addition to that there may be a gradual loss of genes associated with yield and resistance due to overriding role of natural selection (Arulbalachandran et al. 2010). Therefore, immediate corrective measures such as enhanced utilization of local landraces, closely related wild species in breeding programs are required. Here comes the role of seed genebanks which safeguards the genetic diversity of cultivated as well as crop wild relatives. The FAO database on ex situ collections of plant genetic resources shows that 6483 blackgram collections are conserved in various genebanks across the world (http://www.fao.org/wiews). The largest blackgram collections (3154 accessions) are available at National Bureau of Plant Genetic Resources of Indian Council of Agricultural Research, New Delhi (Rana et al. 2016). There are several other seed genebanks having significant number of blackgram conserved, some of them are: Department of Agriculture (DOA) genebank, Thailand (1201 acc.), Bio-resources Conservation Institute, Pakistan (944 acc.), AVRDC-The World Vegetable Centre, Taiwan (849 acc.), NARO Genebank, Japan (449), Southern Regional Plant Introduction Station, USDA-ARS, University of Georgia, USA (304 acc.), etc. Despite such a good number of collections conserved in genebanks across the globe, there has been only a very few studies to understand and utilize the blackgram diversity for the crop improvement. Genetic diversity studies, though with limited number of germplasms, indicates that significant amount of variation exists in locally cultivated blackgram germplasm (Ghafoor et al. 2001, 2008; Gupta et al. 2001; Souframanien and Gopalakrishna, 2004; Kaewwongwal et al. 2015). Therefore, in this study a significant proportion of blackgram collections of the Indian National Genebank was evaluated and characterized for important agronomic traits. The study revealed the useful information about the nature

of variability and promising germplasm lines for important economic traits, which may be highly helpful in executing successful blackgram breeding programs.

Materials and methods

Source of blackgram germplasm

Total 840 accessions of blackgram were randomly selected and obtained from the Indian National Genebank, Indian Council of Agricultural Research—National Bureau of Plant Genetic Resources, New Delhi. The passport information (as shown in Online Resource (1) indicated that the blackgram accessions used in this study originated from almost each Indian state except from a few states of North-western and North-eastern Himalayan regions (Fig. 1). The accessions were grown during rainy season, July–October, 2016 and single representative plant seed was harvested to avoid any admixture of seeds in the original gene bank accessions. The pure seed was used for this experiment during rainy season, July–October, 2017.

Experimental design

The experiment was conducted in an Augmented Block Design (ABD) along with three morphologically distinct and high yielding blackgram varieties viz. KU6, Mash114 and PU11-14 used as checks to replicate in each block to estimate phenotype variation attributing to genetic factors with respect to the population studied. There were 14 blocks and each block contained 60 accessions and three checks. Each accession was grown in 3 m paired row with 45 cm row spacing and one row gap between each accession. The experiment was undertaken during rainy season at the Experimental Farm of ICAR-NBPGR, Issapur, New Delhi (28° 34′ 21″ N; 76° 52′ 11″ E).

Traits studied

The genotypes were characterized based on 29 morphological traits including 15 qualitative and 14 quantitative traits following standard descriptors for *Vigna mungo* developed by Bioversity International (https://www.bioversityinternational.org/fileadmin/ bioversity/publications/Web_version/299/ch2.htm). The representative plants were used for taking observations. Qualitative traits observed are plant growth habit, terminal leaf type, leaf pubescence, pod pubescence, hypocotyl pigmentation, leaf colour, leafiness, petiole colour, stem colour, leaf senescence, mature pod colour, pod attachment to peduncle, seed color, seed lustre and seed shape. Quantitative traits are canopy height (cm), number of primary branches, days to initial flowering, days to 50% flowering, days to end of flowering, flowering period (days), days to initial maturity, days to 80% maturity, pod length (cm), number of seeds per pod, 100 seed weight (g), seed length (mm), seed breadth (mm), seed roundness (ranges between 0–1, where 1 represents a perfect circle). Three representative pods of three plants were used for taking observation of pod length (cm) and number of seeds per pod and average values were used for the analysis. Seed length, seed breadth and roundness of the seed are image analysis-based data. Image analysis is based on the observation of five representative seeds of each accession and the average values were taken for the analysis.

Statistical analysis

The morphological data collected for the above said traits was analyzed to estimate various statistical parameters. Data on qualitative traits was used to classify the entire accessions in designated categories of descriptor states. The quantitative data was used to estimate mean, range, phenotypic coefficient variation (PCV%), skewness, Kurtosis, correlation and clustering analysis using XLSTAT software (https://www. xlstat.com/en/). The histograms were derived using quantitative data to highlight the distribution pattern of observations for each quantitative trait. The principal components (PCs) were estimated using Pearson's correlation coefficient to identify traits playing significant role in blackgram diversity. The communalities for each trait were calculated to estimate the proportion of the traits' variance which can be explained by PCs. The communalities were calculated as per described by Gayacharan et al (2020). The correlation matrix and Agglomerative Hierarchical Clustering (AHC) was done using Euclidian dissimilarity matrix and Ward's agglomeration method. As the large number of accessions could not be displayed in the dendrogram, entropy based automated

Fig. 1 Representation of black gram germplasm collections from 24 Indian states and union territories
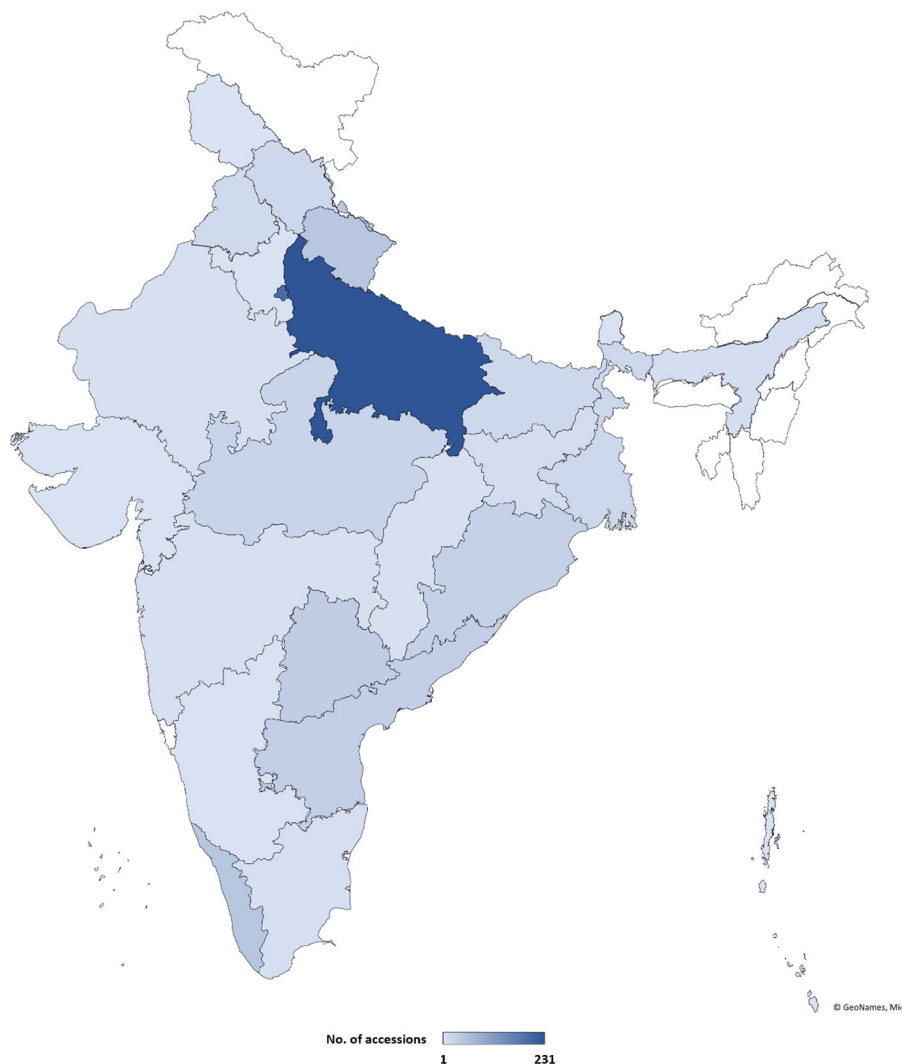
truncation was applied to obtain truncated dendrogram showing distinct clusters. The R package integrated with XLSTAT was used to obtain the graphical illustrations.

The analysis of variance (ANOVA) for quantitative traits' data in augmented block design was estimated as described by Rathore et al (2004). The critical difference (CD) values (at 5% level) for each trait were calculated to identify critically different accessions from superior check to make selections of promising accessions. Further genetic variability parameters of breeders' importance viz. phenotypic variance (Vp) and genotypic variance (Vg) were estimated. The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were calculated as

per the method given by Burton, (1952). The broad sese heritability (h^2) was calculated using the method given by Lush (1940). The h^2 was further classified into the categories viz. low ($< 30\%$), medium (30–60%) and high ($> 60\%$) as per Robinson (1966). Genetic advance over mean or genetic gain (GG) was calculated as per Johnson et al. (1955).

Results

Genetic variability in blackgram germplasm

The quantitative data on agro-morphological traits reveled substantial amount of variation in the

genebank collections of blackgram (Table 1). Pod length, seeds per pod, seed weight, flowering and maturity related traits revealed wide range of variation. For example, days to 50% flowering ranged from 31 to 72 days and days to 80% maturity ranged from 52 to 100 days. The phenotypic coefficient of variation (PCV) revealed substantial variability for traits such as flowering period (52.33%), canopy height (20.55%), days to end of flowering (21.19%), days to initial flowering (27.81%), days to 50% flowering (19.39%) and no. of primary branches/plant (18%). Population means of quantitative traits which gives an idea about the typical characteristics of blackgram are given in the Table 1. Skewness and Kurtosis values indicated the deviations from the Normal distribution pattern of observation for quantitative data. Highest phenotypic (Vp) and genotypic (Vg) variance were observed for canopy height followed by days to end of flowering and flowering period. The broad sense heritability (h^2) was observed from high to low (Table 1). Genetic gain (GG), another important parameter of breeders' interest varied from 57.12% (days to initial flowering) to 1.25% (seed roundedness).

Frequency distribution of qualitative and quantitative traits

The phenotypic variability in blackgram germplasm was revealed by 58 descriptor states of 15 qualitative traits (Table 2). Traits such as seed colour, hypocotyl pigmentation, terminal leaflet shape, leaf pubescence and pod pubescence petiole colour, canopy height, number of primary branches, and flowering related

Table 1 The genetic variability parameters for quantitative variables

Traits	Range	Mean ± Std	Skewness (Pearson)	Kurtosis (Pearson)	Vg	Vp	GCV	PCV	h^2 (%)	GG
Canopy height	10.0–83.33	30.16 ± 9.28	0.92	10.41	28.18	38.41	17.60	20.55	73.36	31.06
No. of primary branches	1.67–8.00	4.10 ± 1.25	0.78	2.19	0.38	0.55	15.09	18.00	70.29	26.07
Days to initial flowering	30.0–70.0	41.97 ± 14.98	1.22	0.74	135.85	136.25	27.77	27.81	99.71	57.12
Days to 50% flowering	31.0–72.0	43.81 ± 15.68	1.10	0.42	67.32	72.16	18.73	19.39	93.29	37.26
Days to end of flowering	37.0–95.0	54.86 ± 19.75	0.74	−0.44	96.67	135.14	17.92	21.19	71.54	31.23
Flowering period (days)	3.0–41.0	12.89 ± 7.51	1.34	1.33	17.03	45.50	32.02	52.33	37.43	40.35
Days to initial maturity	45.0–90.0	60.06 ± 18.37	1.21	0.87	74.23	79.54	14.34	14.85	93.32	28.55
Days to 80% maturity	52.0–100.0	68.89 ± 20.07	1.06	0.29	101.83	105.78	14.65	14.93	96.27	29.60
Pod length (cm)	2.87–5.5	4.20 ± 1.00	0.06	0.69	0.11	0.17	8.00	9.90	65.33	13.32
Number of seeds/pod	3.67–8.33	5.92 ± 1.91	0.07	0.12	0.45	0.58	11.36	12.86	78.06	20.68
100 seed weight (g)	2.17–6.71	3.90 ± 0.97	0.27	1.35	0.23	0.28	12.33	13.46	83.97	23.28
Seed length (mm)	4.24–6.7	4.94 ± 1.41	0.76	2.49	0.08	0.14	5.88	7.62	59.47	9.34
Seed breadth (mm)	3.38–4.98	4.07 ± 1.27	0.35	0.46	0.07	0.12	6.33	8.35	57.52	9.89
Seed roundness (0–1)	0.66–0.92	0.81 ± 0.04	− 0.03	0.42	0.00	0.00	1.68	4.68	12.94	1.25

Std.: standard deviation from mean, *Vp*: phenotypic variance, *Vg*: genotypic variance, *GCV*: genotypic coefficient of variation, *PCV*: phenotypic coefficient of variation, h^2: broad sense heritability, *GG*: genetic gain

Table 2 Qualitative traits and the frequencies for their alternative forms (descriptor states) in the blackgram population

Traits	Descriptors' states (frequencies*)
Hypocotyl pigmentation	Green purple (1.42), Purple (69.75), Dark Purple (28.83)
Growth habit	Erect (8.54), Semi-erect (89.8), Spreading (1.66)
Terminal leaflet shape	Ovate (0.83), Ovate-lanceolate (62.63), Lanceolate (36.18), Rhombic (0.24), Ovate (0.12)
Leaf pubescence	Glabrous (12.69), Very sparsely pubescent (64.29), Sparsely pubescent (0.59), Moderately pubescent (19.69), Densely pubescent (2.73)
Pod pubescence	Glabrous (5.93), Sparsely pubescent (11.74), Moderately pubescent (79.72), Densely pubescent (2.61)
Leaf colour	Light green (3.2), Intermediate green (79.72), Dark green (17.08)
Leafiness	Sparse (4.51), Intermediate (82.8), Abundant (12.69)
Petiole colour	Green (3.2), Green with purple spots (27.05), Greenish purple (30.37), Purple (34.28), Dark purple (5.1)
Stem colour	Light green (10.08), Dark green (1.78), Light purple (87.31), Dark purple (0.83)
Leaf senescence	Not visible (0.12), Slightly visible (22.89), Moderately visible (74.73), Conspicuous concurrent (2.25)
Mature pod colour	Straw (0.24), Tan (0.95), Brown (0.95), Brown and black (0.83), Black (97.03)
Pod attachment to peduncle	Pendant (0.83), Erect (96.32), Sub-erect (2.85)
Seed colour	Light green (3.32), Green–brown (6.76), Brown (2.61), Chocolate (38.32), Black (34.88), Mottled (11.63), Other (2.49)
Seed luster	Absent (93.36), Present (6.64)
Seed shape	Globose (0.83), Ovoid (51.36), Drum-shaped (47.81)

*Values in brackets are the observed percentage for the descriptor state

traits reveled good amount of phenotypic variability (Tables 1 and 2, Fig. 2). Overall, blackgram germplasm is characterized by prominent phenotypes such as purple hypocotyl pigmentation (69.75%), semi-erect growth habit (89.8%), ovate lanceolate terminal leaflet shape (62.63%), very sparsely leaf pubescence (64.29%), moderately pubescent pods (79.72%), intermediate green colour leaf (79.72%), intermediate leafiness canopy (82.8%), purple colour petiole (34.28%), light purple colour stem (87.31%), moderate level of senescence (74.73%), black colour mature pods (97.03%), erect pod attachment to peduncle (96.32%), chocolate coloured seed coat (38.32%), seed coat without surface luster (93.36%) and ovoid seed shape (51.3%).

In quantitative traits, flowering period, which is the difference between initial flowering to the end of flowering, was measured to find out synchronous nature of flowering and maturity. 47.92% of blackgram accessions recorded flowering period of 10 days or less than 10 days. 62.87% of the blackgram germplasm have canopy height ranging between 25 to 32 cm (Fig. 3). Around 89.6% of the accessions have 3–5 primary branches/plant. For around 65% of

blackgram accessions initial flowering took place within 34 to 43 days after sowing and 66.5% accessions reached to 50% flowering within 35 to 45 days after sowing. Accessions for which initial pod maturity occurred within 50–60 days after sowing accounts for 67.72% of the total blackgram collections. Around 87% of blackgram germplasm took around 20–29 days to reach its 80% maturity from days to 50% flowering. 78.3% of the blackgram germplasm recorded 3.9 to 4.6 cm long pods. 76.5% accessions have pod length ranging between 5.3–6.7 seeds/pod. Frequency distribution for quantitative traits including seed breadth, seed length and seed roundness are highlighted in Fig. 3.

Principal component analysis (PCA)

The PCA, a multivariate analysis was used to investigate the correlation among the traits and between traits and observations. PCA was done based on the Pearson's correlation coefficient matrix. Communalities were calculated to estimate the proportion of variance explained by PCs. It was found that communalities for traits viz. canopy height and no. of primary

Fig. 2 Highlights of seed variability for seed size, seed coat colour and seed surface luster. Each accession is labelled with its genebank identifier number, 100 seed weight (SW) and presence (1) or absence (0) of seed surface lustre (SL). Accessions are arranged in descending order of their 100 seed weight

branches/plant were comparatively low and rest of the traits were having higher communalities value. 92.2% of the total variance was explained by first seven PCs. PC1 has highest contribution (35.629%) to the total variance mainly contributed by flowering and maturity related traits. 15.515% of the total variance was explained by PC2 which was mainly attributed to seed associated traits viz. 100 seed weight, seed length and seed breadth (Table 3). First tow PCs were also plotted in 2-D space to understand the relationship among
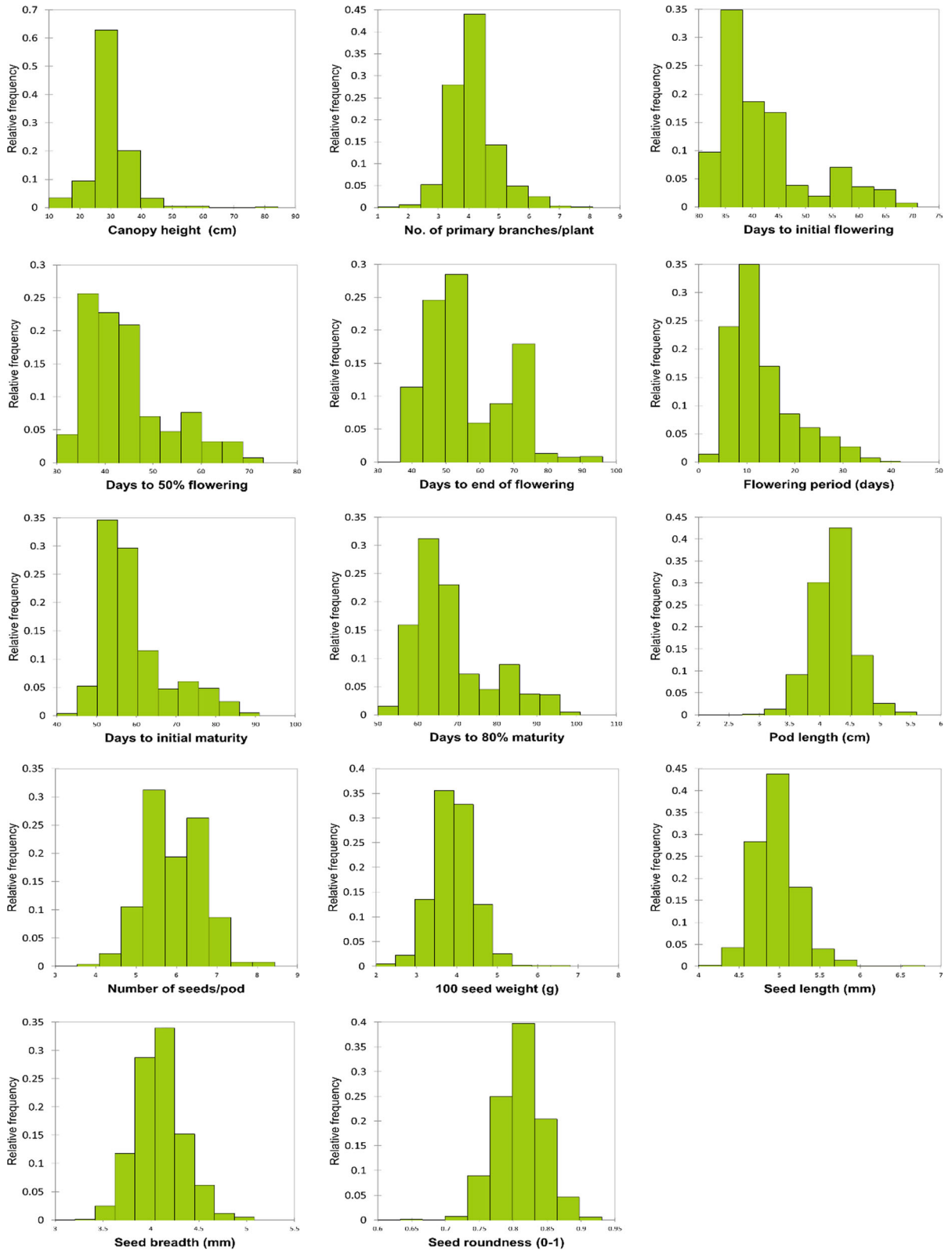
◀ **Fig. 3** Frequency distribution of quantitative traits. Each distribution has 10 groups in its variable axis

traits and observations (Fig. 4). Most of the observations were plotted towards the center but scattered in both the axis with some outliers. The flowering and maturity related traits were found to have positive correlation. Similarly seed related traits were also positively correlated. However, some of the traits such as pod length, flowering period, no. of seeds/pod, etc. were relatively plotted close to the origin, which indicated that the traits would be better explained by lower order of PCs (Fig. 4). In 2-D scatter plot for PC1 vs. PC2 certain accessions were also found to be associated with traits. For example, IC316139, IC485645, IC449269, IC449257, IC449270, etc. were found to be associated with days to 50%, days to 80% maturity, days to initial flowering and initial maturity. IC485605_2, IC530468, IC96884, etc. were found associated with 100 seed weight, seed breadth and seed length.

Hierarchical clustering of blackgram germplasm

The agglomerative hierarchical clustering (AHC) was done based on 14 quantitative traits' data using Euclidian's dissimilarity matrix and Ward's function for agglomeration. The entire blackgram germplasm was grouped in two major clusters (Fig. 5). The first group in blue colour revealed less variance (143.3) within the group of accessions than another group of accessions (474.1) which are shown red in colour in the dendrogram. The sub-cluster blue in colour is less flattened than the sub-cluster red in colour. More flattened cluster indicates the presence of less amount of variance within sub-cluster. Within class variance was estimated to 45.23% while between classes the variance was 54.77%. The sub-cluster (red) consists of 263 accessions with minimum and maximum distance to centroid 4.18 and 54.26 respectively. While the sub-cluster (blue) consists of 580 accessions with minimum and maximum distance to centroid 2.18 and 40.08 respectively (additional data are given in Online Resource 2). The clustering pattern of accessions was checked for their association for their geographical

Table 3 Estimated major principal components, factor loadings and communalities for the respective variables and PCs

Parameters	PC1	PC2	PC3	PC4	PC5	PC6	PC7	Communalities
Eigenvalue	4.988	2.172	1.596	1.250	1.147	0.960	0.794	
Variability (%)	35.629	15.515	11.401	8.930	8.191	6.859	5.671	
Cumulative %	35.629	51.145	62.546	71.476	79.667	86.526	92.197	
Factors Loadings								
Canopy height (cm)	0.125	0.071	0.118	− 0.134	− 0.571	**0.687**	− 0.367	0.379
No. of primary branches	0.430	0.102	− 0.026	0.073	− 0.296	0.295	**0.790**	0.289
Days to initial flowering	**0.945**	− 0.171	0.071	− 0.009	− 0.156	− 0.166	− 0.053	0.951
Days to 50% flowering	**0.956**	− 0.149	0.059	− 0.014	− 0.093	− 0.140	− 0.049	0.948
Days to end of flowering	**0.928**	− 0.017	− 0.040	− 0.045	0.313	0.164	− 0.041	0.963
Flowering period (days)	0.396	0.184	− 0.157	− 0.066	**0.734**	0.491	− 0.004	0.758
Days to initial maturity	**0.948**	− 0.168	0.069	− 0.009	− 0.127	− 0.148	− 0.068	0.947
Days to 80% maturity	**0.957**	− 0.128	0.016	− 0.030	0.027	− 0.074	− 0.057	0.935
Pod length (cm)	0.069	0.228	**0.869**	− 0.060	0.056	− 0.002	0.027	0.818
Number of seeds/pod	− 0.130	0.094	**0.862**	0.015	0.177	− 0.007	0.055	0.801
100 seed weight	0.137	**0.765**	0.014	− 0.058	− 0.091	0.011	− 0.063	0.616
Seed length (mm)	0.168	**0.821**	− 0.148	− 0.408	− 0.052	− 0.185	0.004	0.893
Seed breadth (mm)	0.259	**0.829**	− 0.140	0.318	− 0.040	− 0.139	− 0.051	0.877
Seed roundness (0–1)	0.115	0.136	0.033	**0.972**	− 0.008	0.075	− 0.100	0.977

PCs are arranged in decreasing order of importance. Factor loadings in bold correspond to variable with high representation quality to the respective PC for which the squared cosine is the largest
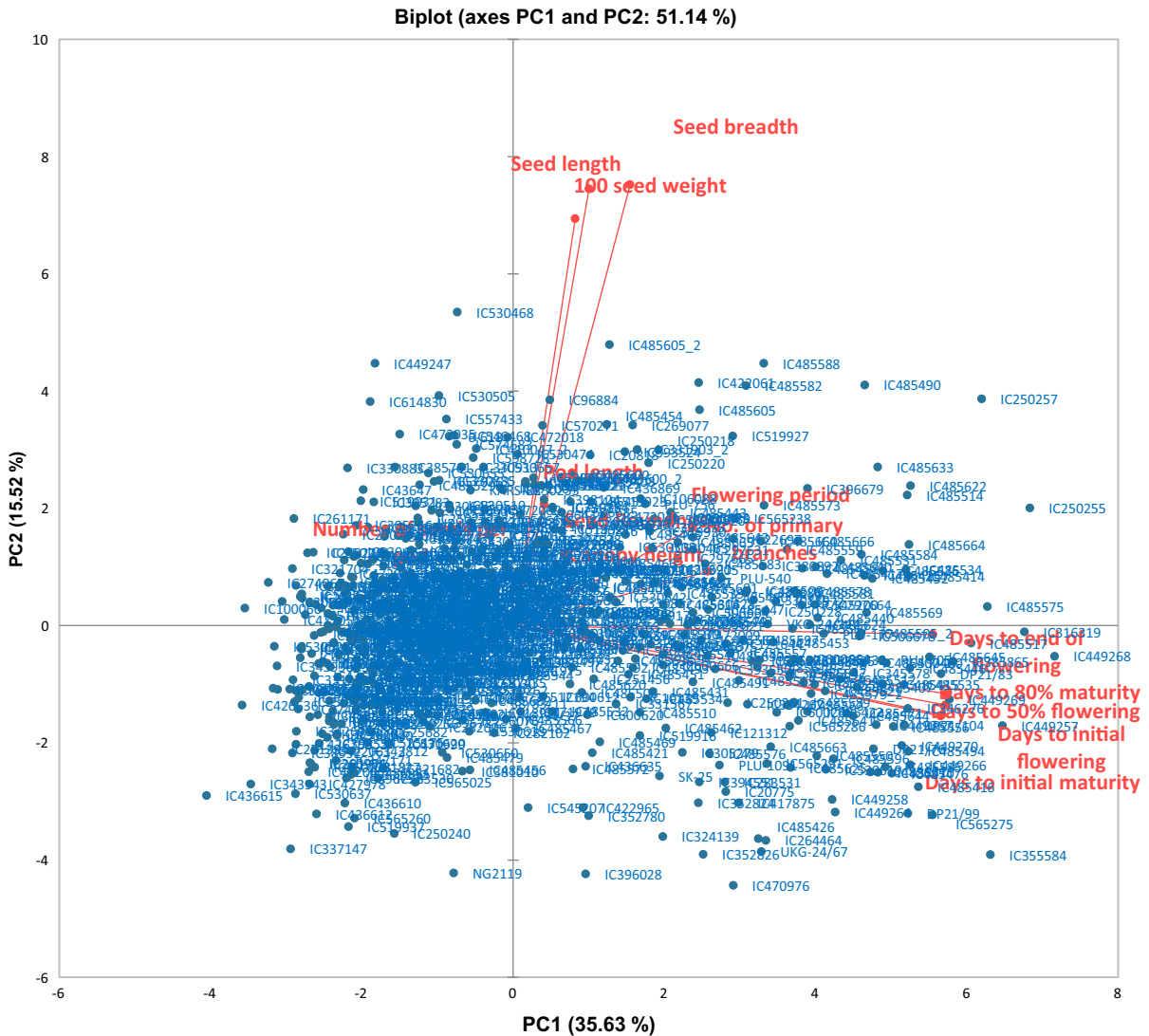
Fig. 4 The 2D bi-plot of PC1 vs PC2 illustrating relationship among variables and accessions. Variables with longer vector length are well represented in the first two PCs while variables with smaller vector length belongs to lower order of PCs. Variables plotted close together are positively correlated and variables in different quadrants (wider angle) are negatively correlated. Accessions plotted near variables have greater influence on that variable

origin and it was found that accessions are approximately randomly distributed among the two clusters. However, some biasedness has been observed. Most of the accessions from Assam state are grouped in the first cluster (red), while most of the accessions from Andaman & Nicobar Islands, Andhra Pradesh, Himachal Pradesh and Telangana states are grouped in second cluster (blue) (see passport data in Online Resource 1). Accessions of each sub-cluster were also checked for their association with trait studied. Although, clustering was done based on the

quantitative data, certain descriptor states of qualitative traits were biasedly distributed among the two clusters. For example, 494 accessions out of 580 accessions in cluster 2 (blue) were having moderately visible leaf senescence while only 69 accessions with slightly visible leaf senescence. Out of 263 accessions in cluster 1 (red), 124 accessions with slightly visible senescence and 136 accessions with moderately visible senescence were grouped. Accessions with sub-erect type pod attachment to the peduncle is mainly found in cluster 2 (blue). Cluster 1
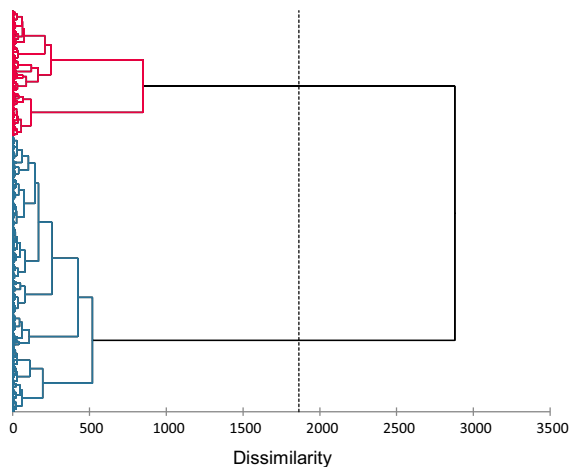
Fig. 5 Agglomerative hierarchical clustering (AHC) using Euclidian's distance and Ward's function of agglomeration. The cut off Euclidian's dissimilarity value (dotted line in x-axis) is 1863

predominantly consists of accessions with drum shaped seed while cluster 2 consists of mainly seed with ovoid shape. Majority of the accessions having early flowering and maturity have grouped with cluster 2.

Correlation among quantitative traits

The correlations among the quantitative traits were estimated using Pearson's correlation coefficient at (0.05 significance level) to understand the magnitude and direction of relationship among the traits. The correlations among days to initial maturity, days to 80% maturity and days to initial flowering and days to 50% flowering are highly significant and positive. Flowering period is positively correlated with days to end of flowering ($r = 0.68$), days to 80% maturity ($r = 0.34$), 100 seed weight ($r = 0.12$), seed length ($r = 0.14$) and seed breadth ($r = 0.16$). Seed roundness is significantly positive correlated with number of primary branches ($r = 0.08$), days to initial maturity ($r = 0.08$), 100 seed weight (0.08) and flowering related traits. Number of seeds/pod was found to have negatively correlated with no. of primary branches/plant ($r = 0.07$), days to initial flowering ($r = 0.01$), days to 50% flowering ($r = 0.01$), days to initial maturity ($r = 0.01$), days to 80% maturity ($r = 0.012$) and pod length ($r = 0.059$). Days to 50% flowering is positively correlated with canopy height ($r = 0.09$).

Canopy height is also positively and significantly correlated with no. of primary branches/plant ($r = 0.13$) (Fig. 6). For more details see the pairwise scatter plots for each combination of variables given in Fig. 6. The colour in scatter plots indicates the positive (green colour), negative (blue colour) or neutral (red colour) relationship among the traits. The scatter chart also indicates the strength of relationship among the traits (Fig. 6).

Identification of promising accessions

Promising accessions are those accessions which have desired phenotype of a particular trait or for multiple traits having economic value. The promising accessions were identified based on promising limit (PL) value, which is trait specific. If a trait with higher range is desirable, the CD value and best check value for that trait is added to calculate the PL value and the accessions having higher values than PL are considered as promising and if the lower range are desirable for a trait, CD value is subtracted to the best check value to calculate the PL of that trait and accessions having lower value than the PL are considered as promising accessions. Promising accessions having 46 to 83.33 cm of canopy height were identified (Table 4). Accessions viz. IC438379 (5.5), IC396679 (5.4), IC485490 (5.4), IC250229 (5.37) and IC485664 (5.37) were having longer pods than the best check Mash114 (4.27). Superior accessions having more than 7.5 grains/pod such as IC472051_2, IC565238, IC396679, IC398743, IC570256, IC598728, etc. were identified. Similarly superior accessions having higher seed weight were also identified (Table 4).

Discussion

Germplasm characterization and evaluation are the two main activities of any crop genebank. It helps in identification of useful germplasm of current needs and also understanding the existing variability in the crop gene pool. Blackgram is one of the most important pulse crops and it is being grown in India since ancient times (Nene 2006). Blackgram collections used in this study are traditional cultivars originated from diverse agro-ecologies and cropping systems spread across the country. Therefore, as expected the results shows that a good amount of
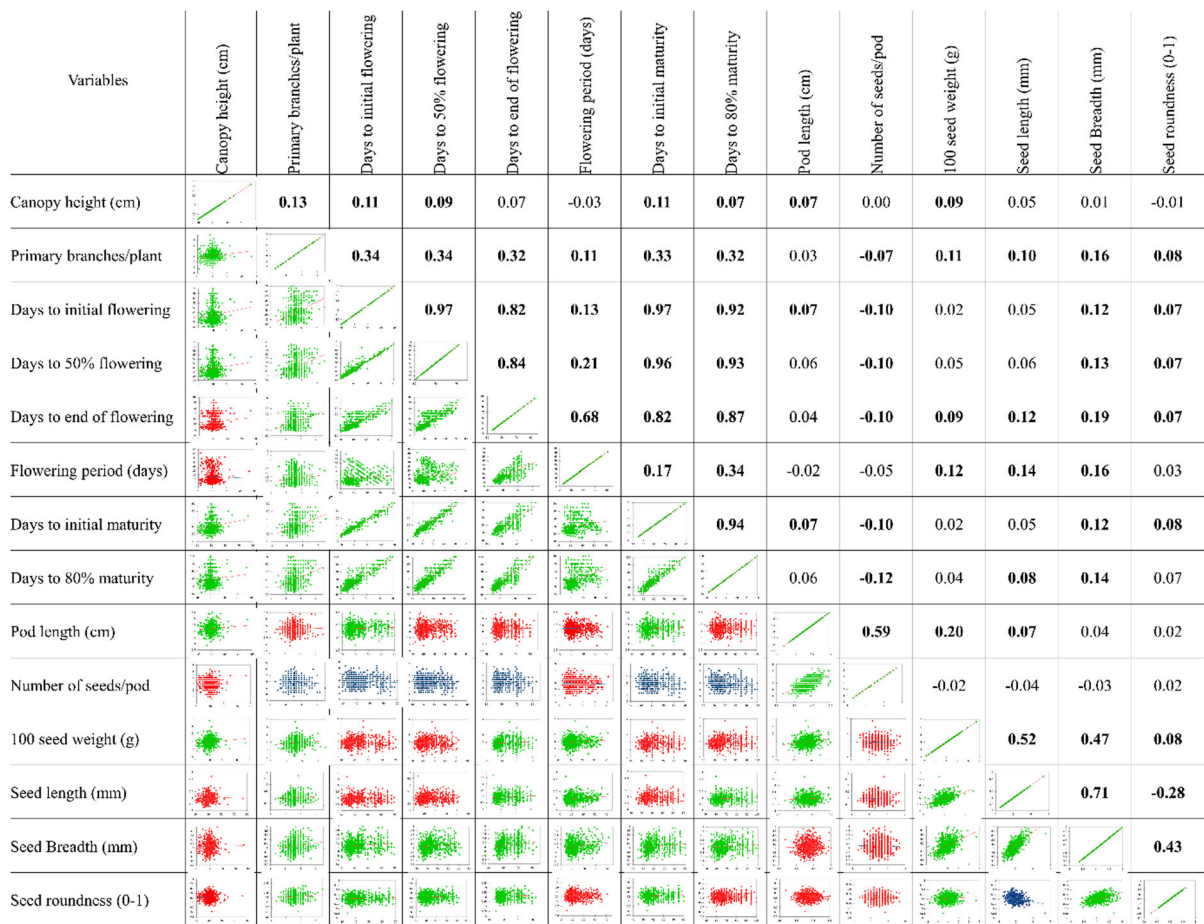
Variables	Canopy height (cm)	Primary branches/plant	Days to initial flowering	Days to 50% flowering	Days to end of flowering	Flowering period (days)	Days to initial maturity	Days to 80% maturity	Pod length (cm)	Number of seeds/pod	100 seed weight (g)	Seed length (mm)	Seed Breadth (mm)	Seed roundness (0-1)
Canopy height (cm)		0.13	0.11	0.09	0.07	-0.03	0.11	0.07	0.07	0.00	0.09	0.05	0.01	-0.01
Primary branches/plant			0.34	0.34	0.32	0.11	0.33	0.32	0.03	-0.07	0.11	0.10	0.16	0.08
Days to initial flowering				0.97	0.82	0.13	0.97	0.92	0.07	-0.10	0.02	0.05	0.12	0.07
Days to 50% flowering					0.84	0.21	0.96	0.93	0.06	-0.10	0.05	0.06	0.13	0.07
Days to end of flowering						0.68	0.82	0.87	0.04	-0.10	0.09	0.12	0.19	0.07
Flowering period (days)							0.17	0.34	-0.02	-0.05	0.12	0.14	0.16	0.03
Days to initial maturity								0.94	0.07	-0.10	0.02	0.05	0.12	0.08
Days to 80% maturity									0.06	-0.12	0.04	0.08	0.14	0.07
Pod length (cm)										0.59	0.20	0.07	0.04	0.02
Number of seeds/pod											-0.02	-0.04	-0.03	0.02
100 seed weight (g)												0.52	0.47	0.08
Seed length (mm)													0.71	-0.28
Seed Breadth (mm)														0.43
Seed roundness (0-1)														

Fig. 6 Pearson's correlation scatter plots' matrix for quantitative traits. Above the diagonal are Pearson's correlation values (*r*). Values in bold are significantly different from 0 with a significance level alpha = 0.05. The colour in scatter plots indicates the positive (green colour), negative (blue colour) or neutral (red colour) relationship among the variables. The scatter charts also indicate the strength of relationship among the variables

variability exists in blackgram germplasm which will be highly useful for the crop improvement. It is also expected that this study will also help in efficient management of ex situ collections genebank and enrichment of the genebank collections by adding the diversity from targeted niche areas (Bisht et al. 1998).

The crop domestication is primarily driven by two factors viz. natural and artificial selections on natural variants. After a long evolutionary history, various forms of characters evolve which leads to shaping a wild crop relative into a landrace or local cultivar specific to its region of origin. In this study type of germplasm used is primarily local cultivars. These local cultivars were characterized using 58 descriptor states (alternative forms) of qualitative traits and fourteen quantitative traits. It was found that in almost all qualitative traits one descriptor state is highly frequent than other (Table 2). This is as expected because qualitative traits are generally oligogenic in nature, therefore, dominant type phenotype represent the larger proportion of the population (Singh et al. 2016). Moreover, the dominant type phenotype may also be the result of selection preference by farmers' or consumers. Qualitative trait such as seed colour, seed luster, seed shape, growth habit, pod attachment to peduncle leafiness and leaf senescence are known to have direct influence of consumers and farmers. Small and shiny seeds are found to have less infestation of bruchids (*Callosobruchus maculatus* and *C. chinensis*) storage pest (Sarikarin et al. 1999; Duraimurugan et al. 2014; War et al. 2017). Leaf pubescence is known to have number of adaptive roles such as lowering leaf

Table 4 List of promising blackgram accessions identified for important economical traits

Characters	CD (5%)	Check accessions	Promising blackgram accessions
Canopy height (cm)	4.12	Mash114 (41.66)	IC297664 (83.33), IC283531 (60), IC530460 (55), IC530461 (55), IC485644 (54), IC427983 (48), IC393524 (48), IC305251 (46.67), IC331232 (46), **PL (45.79)**
No. of primary branches	0.93	PU11-14 (4.0)	IC485663 (8), IC485557 (7.33), IC485556 (7), IC485664 (7), IC485416 (6.67), IC600664 (6.67), IC396679 (6.5), IC321682 (6.33), IC485505 (6.33), IC485582 (6.33), IC485424 (6.33), IC438380 (6.33), IC485446 (6.33), **PL (4.93)**
Days to initial flowering	6.49	KU6 (37.64)	IC436615 (30), IC530641 (30), IC343936 (30), IC342696 (30), IC250244 (30), IC343943 (30), IC426636 (30), IC100066 (30), IC305255 (30), IC321704 (30), IC330883 (30), IC274067 (30), IC305232 (30), IC614830 (30), **PL (31.15)**
Days to 50% flowering	2.50	Mash114 (38.79)	IC343936 (31), IC436615 (32), IC250244 (32), IC343943 (32), IC426636 (32), IC305255 (32), IC261171 (32), IC321704 (32), IC385718 (32), IC305232 (32), IC398744 (33), IC250252 (33), IC250251 (33), IC274067 (33), IC614830 (33), PL (33.68)
Days to end of flowering	14.14	Mash114 (48)	IC436615 (37), IC427977 (38), IC449247 (39), IC447792 (39), IC436512 (39), IC398744 (39), **PL (33.86)**
Flowering period (days)	12.51	PU11-14 (9.21)	IC73523 (3), IC396032 (3), IC485444 (3), IC530638 (4), IC436628 (4), IC396727 (4), IC321705 (4), IC330904 (4), IC296076 (4), IC296265 (4), IC401376 (4), IC565256 (4), **PL (26.37)**
Days to initial maturity	5.95	KU6 (55.43)	IC530641 (45), IC343936 (45), IC342696 (45), IC614830 (46), IC343943 (47), IC426636 (47), IC321704 (47), **PL (49.48)**
Days to 80% maturity	1.85	PU11-14 (64.14)	IC100066 (52), IC274045 (53), IC530641 (55), IC472051_2 (55), IC250225 (55), IC426636 (55), IC251394 (55), IC398744 (55), IC321704 (55), IC331226 (55), IC274067 (55), IC485496 (55), IC614830 (55), **PL (58.82)**
Pod length (cm)	0.58	Mash114 (4.27)	IC438379 (5.5), IC396679 (5.4), IC485490 (5.4), IC250229 (5.37), IC485664 (5.37), IC519911 (5.23), IC570256 (5.13), IC305256 (5.1), IC251913 (5.07), IC584696 (5.07), IC519912 (5.03), IC519905 (5), IC485598 (5), **PL (4.86)**
Number of seeds per pod	0.83	Mash114 (6.29)	IC472051_2 (8.33), IC565238 (8.33), IC396679 (8), IC398743 (8), IC570256 (8), IC598728 (8), IC380892 (7.67), IC331231 (7.67), IC251913 (7.67), IC545207 (7.67), IC398148 (7.67), IC436606 (7.5), **PL (7.12)**
100 seed weight (g)	0.54	Mash114 (4.09)	IC485605_2 (6.71), IC485588 (5.9), IC485605 (5.48), IC472035 (5.38), IC485514 (5.35), IC628067 (5.3), IC472018 (5.29), IC530505 (5.29), IC530468 (5.26), IC546468 (5.24), IC485664 (5.17), IC096884 (5.12), IC436869 (5.08), IC570271 (5.08), IC506656 (5.07), **PL (4.64)**

temperature and transpiration rates, dew harvesting, enhancing water use efficiency, insect resistance (Johnson 1975; Ehleringer and Mooney 1978; Konrad et al. 2015). In general hair density, orientation and size reduces oviposition and larval feeding and therefore, imparts to some level of insect resistance. Blackgram genotypes with narrow, thin and highly pubescent leaves and short erect trichomes are reported to have higher resistance to whitefly (*Bemisia tabaci*), a vector of highly devastating diseases of the crop (Taggar and Gill 2012; Hasanuzzaman et al. 2016). Genotypes with intermediate leafiness are observed with comparatively high frequency (82.8%), probably farmers selected dual purpose genotype having better foliage yield as well as grain yield. Hypocotyl pigmentation is not known to have influence of any kind of selection pressure, but descriptors states' frequencies are probably due to genetic interactions (Singh et al. 2016). Higher frequency of semi-erect type genotypes is probably due to selection pressure as well as the semi-erect type phenotype is known to be dominant over the others. Spreading type genotypes helps in reducing soil erosion and soil moisture loss and also comparatively have higher fodder yield. Black colour matured pods are known to reduce the discoloration of seeds inside and therefore, selection is made for black coloured pods over light-coloured pods and the black colour phenotype is also reported as dominant (Singh et al. 2016). Chocolate and black seed colour were

predominant and almost equally recorded. Majority of the accessions are non-mottled type. Erect type pod attachment to the peduncle was observed in 96.3% blackgram genotypes. Probably this trait has co-evolved in interaction with other traits such as growth habit, peduncle length, etc. These traits along with seed hilum shape, pod pubescence and pod length are the defining characteristics of *Vigna mungo* differentiating it from other *Vigna* species.

Quantitative traits such as days to 50% flowering, days to 80% maturity, smaller flowering period (synchronous flowering) are important economic traits and are the target traits of blackgram breeding program. Short duration genotypes are highly important for the development of early maturing cultivars to specific zones where terminal drought is frequent or fitting to a cropping system (Brar et al. 2002). Gupta et al (2020) has reported a few progenies of blackgram derived from intraspecific crosses which matures in 60–65 days. However, accessions such as IC100066 and IC274045 identified in this study which matures within 52 and 53 days respectively can make a better source for the trait development. Similar results but with some differences were also reported in small scale germplasm characterization studied (Ghafoor and Ahmad 2005). Shorter flowering period, a time period between start of flower initiation to cease of new flower bud opening, is highly desirable trait for commercial cultivation of any crop. Although, flowering period for blackgram is comparatively shorter than the other Vigna crops, some promising accessions mentioned in Table 4 have flowering period of three-four days. Such accessions will be of highly useful in developing cultivars with synchronous maturity. Pod length, seeds/pod and seed weight are considered as missing traits in blackgram (Gupta and Parihar 2015). However, a few promising accessions identified for these traits can have a great impact on blackgram breeding programs. Blackgram genotypes generally have ovoid or drum shaped seeds, only a very small proportion of genotypes have globose seed. Round seed is desired for milling purpose. Primarily blackgram is spreading to semi-spreading type plant with canopy height of around 20 -40 cm. Poor plant height and spreading growth habit is one of the major drawbacks of blackgram, as a result the crop could not become popular for commercial scale production. Genotypes identified in this study with better plant height will be highly useful for developing modern cultivars.

Useful information on genetic parameters was derived for statistical parameters of breeders' importance. The estimates of PCV and GCV revealed least environmental influence on the expression of traits such as days to initial flowering, days to 80% maturity, days to 50% flowering, days to initial maturity, 100 seed weight and number of seeds/pod. Heritability (h^2) another important parameter, measures the phenotypic variance due to genetic background of a genotype. In general, higher h^2 leads to the higher genetic gain (GG) in response to artificial selections (Xu et al. 2017). GG helps in prediction of a degree to which a population can respond to artificial or natural selection with reference to a particular trait. Since these genetic parameters are specific to population and expression of a traits is influenced by multiple factors, selection based on a single parameter may be misleading. Therefore, parameters such as mean, range, variance, distribution and correlations among traits also should be taken into account for making any selections. Based on GCV, PCV, h^2 and GG, traits such as days to initial flowering, days to 80% maturity, days to initial maturity, days to 50% flowering, seed weight and seeds/pod were found highly suitable for blackgram breeding programs (Table 1). As source of the germplasm and population size varies among other studies, results also varies to some extent but trends remain similar (Ghafoor et al. 2001; Ghafoor and Ahmad 2005; Punia et al. 2014; Sridhar et al. 2020). Simple selection of promising accessions listed in Table 4 for these traits and their utilization in the breeding programs can have significant impact on the crop improvement.

Hierarchical clustering helps in grouping of objects (accessions) which are more similar within the group than of objects of another group. This is helpful in many ways such as accessing genetic distance between any two accessions, identification of diverse accessions, a smaller set of accessions representing maximum diversity of entire collections, accessing level of diversity in entire collections, understanding pattern of clustering and its relation with traits and origin of germplasm, etc. It is highly useful in bringing desirable variability in progenies with minimum efforts (Rana et al. 2015). In this study most of the accessions were clustered irrespective of their site of origin, except for accessions from Assam, Andaman &

Nicobar Islands, Andhra Pradesh, Himachal Pradesh and Telangana states. In an earlier study based on quantitative traits and molecular markers also showed the similar results (Ghfoor and Ahmad 2005). They had identified only a low level of geographic relationship with blackgram diversity. This indicates that the quantitative traits selected for hierarchical clustering are primarily influenced by farmers' selection and agro-ecological conditions have least role to play in development of these traits. However, qualitative traits such as terminal leaf shape type, seed shape, leaf senescence were also found to have relationship with clustering pattern. Probably certain qualitative traits have indirect impact in the variability of quantitative traits.

PCA is a representation of multi-dimensional complex quantitative data in a lower dimensional space, which helps in understanding the nature of variability. PCA was done to quantify variability, correlation among traits and between traits and observations. This also helps in understanding the trends among the observations. PC1 represents 35.629% of the total variability with eigen value 4.988. As highlighted in the Table 3, traits such as days to initial flowering, days to 50% flowering, days to end of flowering, days to initial maturity and days to 80% maturity are major contributor to the variation in PC1 followed by 100 seed weight, seed length, and seed breadth in PC2. Traits such as flowering period, no. of primary branches per plant and canopy height are of less importance in decreasing order as reveled by low communalities and eigen values (Table 3). Though heritability and genetic gain of these traits are comparatively good, if we look at frequency distribution, Kurtosis and skewness values, it is found that variability range is very narrow. Therefore, selection of these traits with narrow variability in blackgram population may not result in much improvement. Plotting of PC1 against PC2 in 2-dimentional space which accounts total 51.145 of the total variability reveled some correlations among traits and also indicated non-random distribution of objects (Fig. 4). Further, correlations among traits are highlighted in Fig. 6 and similarities among accessions are represented in Fig. 5 and Online Resource 2 information. Some unique correlations were observed such as positive correlation of flowering period with seed weight, seed length and width. Another such significant positive correlation was observed for seed roundness with number of primary branches, days to initial maturity, 100 seed weight and flowering related traits. Similarly seeds per pod was found to have significant negative correlation with no. of primary branches/plant, days to initial flowering, days to 50% flowering, days to maturity. Such information about trait correlations helps in planning crossing programs for crop improvement.

Overall, the nature of genetic diversity in blackgram germplasm explained by this study may be highly helpful in breaking the yield barriers. Diverse and promising accessions identified may serve as the best source for screening and development of resistant cultivars against biotic and abiotic stresses for climate resilient varieties. Early type of genotypes may help in breeding cultivars suitable for rice-fallow crop land and for the crop season in which terminal drought is a problem. This study may be highly helpful in selection of traits of breeders' importance as revealed by statistical parameters such as heritability, genetic gain and PCA. Additionally, hierarchical clustering would be useful in selection of contrasting parents for enhancing variability in blackgram varieties. Selection of promising accession for a particular target trait with these attributes would have greater impact on blackgram breeding program.

Declarations

Conflict of interest The authors declare no potential conflict of interest.

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