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Morphological Characterization of Farmers' Varieties of Blackgram of Northwestern Himalayan Region

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Abstract: A variety of traditional farmers' varieties (FVs) of blackgram [*Vigna mungo* (L.) Hepper] are cultivated on small scale in low input production systems under rainfed conditions by the local farmers of Himachal Pradesh lying in the northwestern Himalayan region. Morphological characterization of blackgram germplasm consisting of 20 FVs collected from interior and remote areas of different districts of H.P. and two released varieties, HIM MASH 1 and UG 218, was carried out using morphological pod and seed descriptors. The qualitative characters evaluated were intensity of green colour of premature pods, colour of mature pods, pod pubescence, seed colour, seed lusture and seed shape. Quantitative characterization of blackgram germplasm was done for pod length, number of seeds pod^{-1}, seed length, seed width and 100 seed weight. The analysis of variance for quantitative characters revealed a significant variation among the germplasm investigated. Correlation matrix for various quantitative characteristics showed significant positive correlation between some variables. The first two principal components together explained 85.43% of the total variability for the investigated quantitative variables in blackgram germplasm. Agglomerative hierarchical clustering *led to the separation of germplasm into four clusters*. The variability in the germplasm could serve as potential valuable genetic resources for the breeding programs in crop improvement.

Keywords: Clusters, farmers' varieties, morphological characterzation, pod descriptors, principal component analysis, seed descriptors

INTRODUCTION

FVs associated with traditional cultivation practices, constitute an integral component of agrobiodiversity and form the basis of *in situ* diversity assessment, on-farm crop improvement programs, collections for *ex situ* conservation and sustainable agriculture [1,2]. The traditional FVs are selected by the local farmers based on the morphological characters, consumer tastes, suitability for end use, and adaptation to local environmental conditions and specific farming systems. They are of enormous ecological, economic, and cultural value and are crucial for global food and nutritional security [3,4]. The characterization of FVs is of prime importance for quantification and structuring of genetic diversity in the germplasm which is of great interest in planning of crop improvement programs and conservation strategies of diversity [2,5]. As such, a lot of emphasis is being put on the characterization of FVs of various crops using various morphological, biochemical and molecular descriptors or markers. The increasing role of traditional FVs in adaptation to climate change has further necessitated their proper characterization and conservation [3,6]. Himachal Pradesh lying in the northwestern Himalayan region is endowed with rich diversity of FVs of various crops. Blackgram is one of the important protein rich pulse crops of H.P. having soil ameliorating properties [2,7,8]. It is mostly grown as intercrop with maize on

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an area of 0.0079 million ha with production of 0.0036 MT and yield of 449 kg/ha in H.P. [9]. Since time immemorial, the traditional FVs of blackgram is being cultivated in diverse agro-ecological regions of H.P. under various cropping systems and cultural environments. However, the lack of proper characterization of the FVs has led to their underutilization in various crop improvement programs [8]. Moreover, there has been a significant loss of FVs during recent decades and some of them are under a constant threat of extinction with the advent of so-called modernization in agriculture. As such, these FVs need to be characterized properly for various desirable agronomical traits for their conservation and sustainable utilization [8,10,11]. Therefore, the present investigation was carried out for morphological characterization of pods and seeds of FVs of blackgram collected from different locations of H.P. and to evaluate the interrelationship among the quantitative descriptors used in the characterization study for their potential use in crop improvement programs and planning conservation strategies.

MATERIALS AND METHODS

Plant material and experimental location

The traditional FVs of blackgram were collected from the farmers of the interior and remote areas of H.P. during 2018-2019 while carrying out research expeditions under DST sponsored project. The collections were made from the stores of the farmers and assigned the alphanumeric nomenclature M1 (blackgram 1), M2 (blackgram 2) and so on. The seeds of each FV were cleaned and stored in air-tight plastic containers and kept in short-term seed bank of the Department of Genetics and Plant Breeding, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur. The collections were purified by growing each collection during *Kharif* 2019 and 2020 at the research farm of CSKHPKV at *Krishi Vigyan Kendra*, Bilaspur and collecting seeds from individual plants of each collection at maturity to avoid any admixture of seeds. The field trial for morphological characterization of pods and seeds was conducted in the month of July at the farmer's field at village Hullu, Tehsil Padhar, District Mandi during *Kharif* 2021 using the pure seed. The experiment was carried out in a Randomized Complete Block (RCB) design with two replications per treatment for 20 collections and two released varieties, HIM MASH 1 and UG 218. Each replication of each FV consisting of two rows was grown in 3 × 0.9 sq m plot, with inter-row spacing of 45 cm and intra-row spacing of 15 cm. The seeds were sown at a depth of 2-3 cm. Weeds were removed manually from the field. Farmers' package and practices for blackgram were followed. The pod characteristics were recorded at relevant growth stages. Each row was harvested in the month of October-November depending upon the maturity period of various FV collections and the seeds collected for quantitative and qualitative characterization.

Qualitative characterisation of pods and seeds

Qualitative characterisation of pods and seeds of blackgram of each FV was done on the basis of visual observations under natural daylight. The pod pubescence and intensity of green colour were observed for premature fully grown pods. The colour of mature pods was recorded at harvest maturity. Seed colour, seed lusture and seed shape were observed for mature seeds of each FV.

Quantitative characterization of pods and seeds

The quantitative characterization involved the measurement of pod length (PL; cm), number of seeds pod^{-1} (NSP), seed length (SL; mm), seed width (SW; mm) and 100-seed weight (SWt; g). The PL was recorded by randomly selecting five plants in each replication for each genotype. The mean length of ten randomly selected pods from each of the selected plants was measured using a scale and the average was taken for PL. PL was measured in the middle portion of the main stem at harvest maturity stage. The NSP was obtained from ten randomly selected plants. SL and SW were measured on 20-randomly selected fully developed healthy and undamaged seeds per replication using a digital vernier calliper (Aerospace 0-150 mm) reading to 0.01 mm. SL and SW were measured as the highest parallel to the hilum and from hilum to the opposite

side, respectively. The SWt was measured in three repetitions from each replication using an electronic balance (KERN 572) weighing to 0.001 g.

Statistical analysis

Statistical analysis was conducted using Data analysis ToolPak of Microsoft excel for mean, standard error, standard deviation, variance, histogram to highlight frequency distribution pattern and ANOVA. The critical difference (CD) for the characteristics exhibiting significance at 5 per cent probability level was calculated as follows:

$$CD\ (<0.05) = 1.414 \times \sqrt{(EMS/r)} \times t$$

where EMS is error mean sum of squares, r is number of replications and t is critical value for student's t distribution at 5 per cent probability (α) and error degree of freedom. Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) indicating the magnitude of variability were calculated by the formula suggested by Burton [12]. The broad sense heritability (h^2) was calculated using the method suggested by Lush [13] and further classified into three categories, namely, low (<30%), medium (30-60%) and high (>60%) as suggested by Robinson [14]. Correlation matrix using Pearson's correlation coefficient, principal component analysis and agglomerative hierarchical clustering was carried out by XLSTAT statistical and data analysis solution.

RESULTS AND DISCUSSION

Qualitative characterization of pods and seeds

The blackgram germplasm investigated was prominently characterized by pubescent (95.45%) and dark green (81.81%) fully grown premature pods, and black coloured mature pods (100%). The mature seeds of all the genotypes were black coloured and the majority had dull-seed coat lusture (90.90%). The germplasm prominently had drum-shaped seeds (86.36%) followed by oval (9.10%) and globose (4.54%). The qualitative characterization of blackgram germplasm clearly revealed that one descriptor state was highly frequent than the other for all characteristics investigated thereby indicating that the larger proportion of the population is represented by the dominant type phenotype which may be the result of selection preference by the farmers or consumers for various reasons like biotic and abiotic adaptive roles [15].

Quantitative characterization of pods and seeds

The data of quantitative characterization of pods and seeds for different characteristics, namely PL, NSP, SL, SW and SWt for FVs of blackgram and released varieties, HIM MASH 1 and UG 218 is presented in Table 1. The analysis of variance revealed significant differences for all the quantitative characteristics in the germplasm studied, thus indicating the existence of wide genetic diversity among them. The dimension of blackgram seeds is highly influenced by various factors like genotype, agroecology conditions and applied agro-techniques during traditional farming system in rural areas and is reported to vary with region of domestication [8,15]. Frequency distribution graphs of 20 FV collections and 2 released varieties, HIM MASH 1 and UG 218 for quantitative characters are presented in Fig 1.

Table 1: Data of quantitative characterisation of seeds of 20 farmers' varieties of blackgram of northwestern Himalayan region and two released varieties, HIM MASH 1 and UG 218

Genotype	*Village/District*	*PL*	*NSP*	*SL*	*SW*	*SWt*
M1	Hullu/Mandi	5.23	7.10	5.36	4.16	5.65
M2	Shahot/Mandi	5.56	6.70	5.58	4.13	5.92
M3	Sarahan/Mandi	5.05	6.70	5.57	4.15	5.57
M4	Shala/Mandi	5.24	6.65	5.65	4.08	5.87
M5	Kao/Mandi	4.86	6.75	4.89	3.73	4.43
M6	Bakhrot/Mandi	5.26	7.00	5.53	4.14	5.53
M7	Payjanu/Mandi	5.00	6.55	5.38	3.98	5.30
M8	Payjanu/Mandi	5.28	7.05	4.96	3.97	5.22
M9	Bandla/Bilaspur	5.45	7.25	5.52	4.07	5.67
M10	Ashamajhari/ Bilaspur	5.01	6.75	5.15	3.97	5.19
M11	Chanalag/Bilas-pur	5.26	7.50	5.22	3.96	5.53
M12	Bandla/Bilaspur	4.96	7.05	4.95	3.93	5.44
M13	Patta/Bilaspur	5.19	6.90	5.34	3.95	5.46
M14	Ganvi/Shimla	5.20	6.95	5.51	4.14	5.60
M15	Ghudet/Chamba	5.36	7.35	5.39	3.92	5.21
M16	Bharmour/ Chamba	5.40	7.50	5.34	3.86	4.78
M18	Bharmour/ Chamba	5.45	7.40	5.33	3.83	5.09

Genotype	*Village/District*	*PL*	*NSP*	*SL*	*SW*	*SWt*
M19	Bajhera/Kangra	5.08	6.90	5.00	3.77	4.55
M20	Bajhera/Kangra	4.96	6.75	5.05	3.73	4.63
M21	Bajhera/Kangra	4.86	6.35	5.05	3.90	4.38
HIM MASH 1	HPKV, Palampur	5.22	6.60	5.23	3.83	5.57
UG 218	HPKV, Palampur	5.13	6.70	5.31	4.04	5.20
CD (<0.05)		0.28	0.23	0.22	0.17	0.34

PL, pod length (cm); NSP, number of seeds per pod; SL, seed length (mm); SW, seed width (mm) and SWt, 100 seed weight (g); CD (<0.05), critical difference, resultant mean difference between two genotypes compared that exceeds the CD value is significant at P = 0.05.

The summary statistics for various quantitative characteristics of blackgram germplasm which give useful information on genetic parameters of breeder's importance are presented in Table 2. Parameters like mean, range, variance, distribution and correlation along with genetic parameters need to be taken into account for making any selections [8]. The PL recorded showed a minimum of 4.86 cm in M5 from village Kao (Mandi) to 5.56 cm in M2 from village Shahot (Mandi). The NSP varied from 6.35 in M21 from village Bajhera (Kangra) to 7.50 in M11 from village Chanalag (Bilaspur) and M16 from village Bharmour (Chamba). The SL varied from 4.89 mm in M5 from village Kao (Mandi) to 5.65 mm in M4 from village Shala

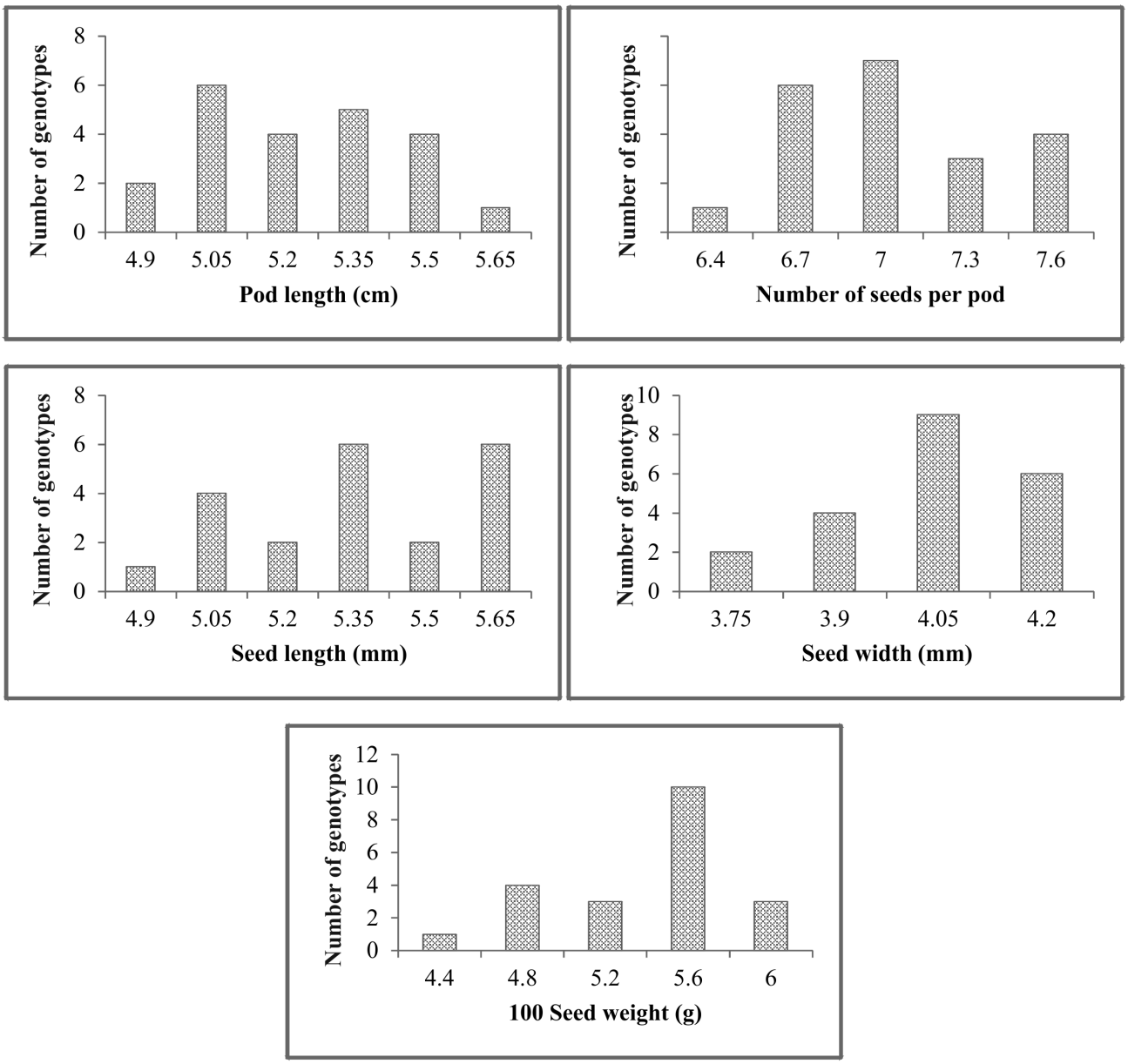

Figure 1: Frequency distribution of 20 farmers' varieties of blackgram of northwestern Himalayan region and two released varieties, HIM MASH 1 and UG 218 for quantitative seed characteristics

Table 2: Summary statistics for quantitative characteristics of 20 farmers' varieties of blackgram of northwestern Himalayan region and two released varieties, HIM MASH 1 and UG 218

Characteristics	*Mean±S.E.*	*Range*	*S.D.*	*V*	*GCV (%)*	*PCV (%)*	*h^2 (%)*
Pod length (cm)	5.17±0.05	4.86-5.56	0.20	0.04	3.04	4.36	48.69
Number of seeds pod^{-1}	6.93±0.07	6.35-7.50	0.32	0.10	4.40	4.80	83.80
Seed length (mm)	5.29±0.05	4.89-5.65	0.23	0.05	3.95	4.63	72.72
Seed width (mm)	3.96±0.03	3.73-4.16	0.14	0.02	2.97	3.86	59.01
100 Seed weight (g)	5.26±0.10	4.38-5.92	0.45	0.20	8.16	9.01	82.15

S.E., standard error; S.D., standard deviation; V, variance; GCV, genotypic coefficient of variation; PCV, phenotypic coefficient of variation; h^2, broad sense heritability

(Mandi). The SW recorded varied from 3.73 mm in M5 from village Kao (Mandi) to 4.16 mm in M1 from village Hullu (Mandi). The SWt recorded varied from 4.38 g in M21 from village Bajhera (Kangra) to 5.92 g in M2 from village Shahot (Mandi) and further indicated the size variation of the seeds among the genotypes. The estimates of GCV and PCV revealed least environmental influence on the expression of NSP, SWt and SL. The broad sense heritability (h^2) which measures the phenotypic variance due to genetic background of a genotype, was observed to be high for NSP, SWt and SL while medium for SW and PL. Higher h^2 is reported to lead to higher genetic gain in response to artificial selections [16].

Quantitative characteristic correlation

Correlation matrix for various morphological quantitative characteristics among 20 collections of FVs of blackgram and two released varieties was estimated using Pearson's correlation coefficient (at 0.05 significance level) to understand the magnitude and direction of relationship between pairs of quantitative characteristics (Table 3). The correlations among all the characteristics were positive except the correlation between number of seeds pod^{-1} and seed width. Significant positive correlation existed for 100 seed weight with seed width, seed length and pod length. Seed width was also significantly positively correlated with seed length. Pod length had significant positive correlation with number of seeds pod^{-1} and seed length also. The correlation is one of the most common and most useful statistics that describes the degree of relationship between two variables [17] and can be utilized for the crop improvement programmes by breeding one of them and improving several performances [8]. Okii [18] suggested that strongly correlated quantitative traits are possibly under the influence of the same genes which could be selected simultaneously based on one of the traits during selection.

Principal Component Analysis (PCA)

PCA helps to quantify the degree of variability between populations and evaluate the potential breeding value of the germplasm through characteristics loaded on various components [8]. Principal Component 1 (PC1) and Principal Component 2 (PC2) had eigen value greater than 1 and together represented 85.43% of the total variability for the investigated quantitative variables in blackgram germplasm (Table 4, Figure 2). A negligible proportion of variability was accounted for by the remaining three principal components thus revealing their less importance. SWt, SL, SW and PL were major contributors to the variation in PC1. Likewise, NSP was the major contributor to PC2. The PCA

Table 3: Correlation matrix [Pearson (n)] for various quantitative charcteristics in blackgram germplasm

Variables	*Pod length*	*Number of seeds pod^{-1}*	*Seed length*	*Seed width*	*100 Seed weight*
Pod length	1.00				
Number of seeds pod^{-1}	0.59*	1.00			
Seed length	0.59*	0.07	1.00		
Seed width	0.35	-0.03	0.74*	1.00	
100 Seed weight	0.54*	0.11	0.72*	0.78*	1.00

* Significant correlations at p = 0.05

revealed that all the characteristics are important for the spatial representation of the blackgram germplasm investigated.

Table 4: Principal components, eigen values and principal component loadings of the variables for the respective principal components

Parameters	*PC1*	*PC2*	*PC3*	*PC4*	*PC5*
Eigen value	2.95	1.33	0.35	0.24	0.14
Variability (%)	58.91	26.52	7.00	4.86	2.71
Cumulative variability %	58.91	85.43	92.43	97.29	100.00
Principal component loadings					
Pod length (cm)	0.76*	0.53	0.28	0.15	0.19
Number of seeds pod^{-1}	0.30	0.90*	-0.27	-0.15	-0.09
Seed length (mm)	0.89*	-0.18	0.30	-0.24	-0.17
Seed width (mm)	0.83*	-0.40	-0.27	-0.18	0.20
100 seed weight (g)	0.89*	-0.20	-0.19	0.33	-0.15

PCs are arranged in decreasing order of importance; *Values correspond for the high correlation between variable and the principal component

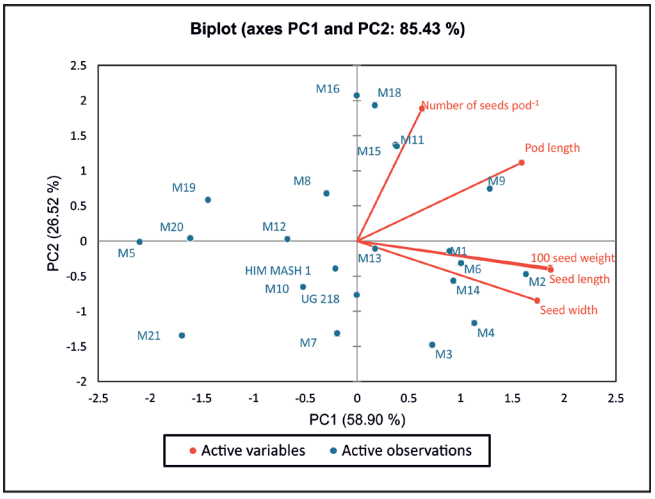

Figure 2: Two-dimensional plot of principal component analysis of 20 farmers' varieties of blackgram of northwestern Himalayan region and two released varieties, HIM MASH 1 and UG 218 using quantitative characteristics

Dendrogram

Dendrogram was obtained by performing Agglomerative Hierarchical Clustering (AHC) using Euclidean distance, Ward's method of agglomeration, automatic truncation and Hartigan index (Figure 3). AHC led to the separation of germplasm into four clusters, namely C1, C2, C3 and C4 having seven genotypes in Cluster C1 (M1, M2, M3, M4, M6, M9 and M14) and Cluster C3 (M7, M8, M10, M12, M13, HIM MASH 1 and UG 218) and four genotypes in Cluster C2 (M5, M19, M20 and M21) and Cluster C4 (M11, M15, M16 and M18) (Figure 3). Based on within-cluster variance, the order of decreasing heterogeneity was C3, C1, C2 and C4. The quantitative traits for hierarchical clustering are reported to be primarily influenced by farmers' selection and agro-ecological conditions rather than geographical location [8,19]. Moreover, the qualitative traits have indirect impact in the variability of quantitative traits.

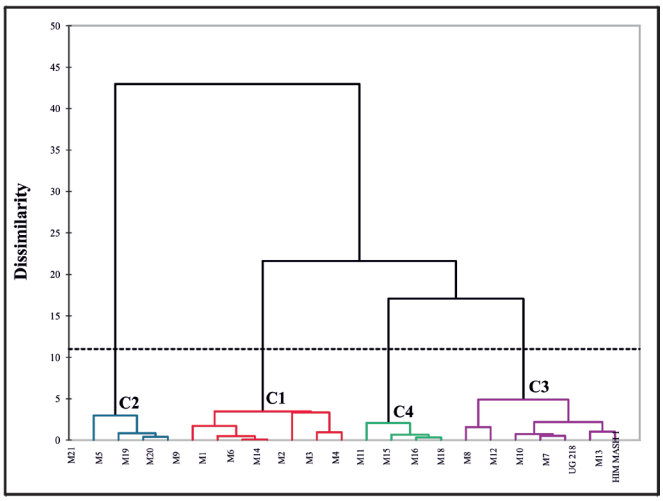

Figure 3: Dendrogram obtained by performing Agglomerative Hierarchical Clustering using Euclidean distance, Ward's method of agglomeration, automatic truncation and Hartigan index for 20 farmers' varieties of blackgram of northwestern Himalayan region and two released varieties, HIM MASH 1 and UG 218

CONCLUSIONS

The present investigation reveals the existence of diversity in the FVs of blackgram collected from interior and remote areas of H.P. based on morphological pod and seed descriptors. Further evaluation of these genotypes using other morphological, biochemical and molecular markers need to be carried out in order to generate more information on their genetic diversity and subsequent utilization in crop improvement programs. The best parents from various clusters may be used in breeding programs to broaden the genetic base of cultivated blackgram.

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