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Determining selection criteria in finger millet (*Eleusine coracana*) genotypes using multivariate analysis

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ABSTRACT

Finger millet [*Eleusine coracana* (L.) Gaertn.] grows in upland rainfed conditions customarily at disadvantageous regions of the country. Evaluation of genetic diversity and the choice of parents is the crucial step to augmenting the desired improvement of crops towards grain and fodder yield. In the present study, 31 finger millet genotypes were studied for genetic diversity employing cluster and principal component analysis (PCA) at G. B. Pant University of Agriculture and Technology (GBPUA&T), Pantnagar during 2018 and 2019. Mahalanobis D^2 statistics revealed seven clusters where cluster I represented 24 genotypes, cluster II with two genotypes, and the remaining clusters with a single genotype each. The maximum inter-cluster distance was observed between clusters III and VII (49.783) followed by III and IV (46.737) indicating more diversity between clusters. Five PCs accounted for 77.50% of total genetic variability using PCA. Furthermore, two diverse and complementary parents (PKPS4 and F20) were identified that possessed complement traits, viz. bold seed, high mean for single head weight, grain yield, harvest index, number of finger/spike (PKPS4), and number of tillers/plant (F20). Therefore, PKPS4 and F20 genotypes could be considered as donor parents for different traits to increase grain yield in finger millet.

Keywords: Cluster analysis, D^2 statistics, Finger millet, Principal component analysis

Finger millet [*Eleusine coracana* (L.) Gaertn.] provides food security over the years to the disadvantageous regions of the agro-ecological conditions of the country where no other crop produces satisfactory yield. The ragi grains contain certain indispensable amino acids and micro-nutrients like methionine and calcium that are required for good health (Srivastava and Sharma 2012, Patil *et al.* 2019) and also serve as a cheap and main source of nutrients for undernourished people (Devi *et al.* 2014). Finger millet is predominantly cultivated as an upland crop and has a unique ecotype that does not tolerate the accumulation of stagnant water at the growing site. Barring its importance as a staple food crop, breeding works on finger millet draw little attention to breeders due to low physiological production capacity and poor compatibility with other privileged crops like rice, wheat, etc.

The major constraints associated with the production of grains in finger millet are narrow genetic bases due to self-pollinated nature coupled with attainment of a plateau in yield potential of popular high yielding finger millet cultivars (Swetha 2011). Since it is difficult to trace and evaluate the entire diversity in a gene pool of a crop, it has become imperative for a plant breeder to set an optimum number of plant traits to explain the variability present in the population more effectively during crop growth from sowing to harvest (Ulaganathan and Nirmalakumari 2015) and aid in the selection of superior genotypes of plants. In order to harvest the benefit of transgressive segregation in ragi on economically important characteristics like yield, the knowledge of the genetic distance between parents is necessary (Joshi *et al.* 2004) because the genotypic improvement of plants in breeding programs mainly depends on the amount of genetic diversity present in the population. The present investigation, therefore, was aimed to evaluate the total diversity existing among finger millet genotypes based on multivariate analysis and to identify the promising parents for augmenting grain yield suitable for upland areas.

MATERIALS AND METHODS

In the present study, 31 finger millet genotypes were collected from different centers, viz. three local accessions from Pithoragarh, Uttarakhand; three (two cultivated and one advanced breeding line) from GBPUA&T, Pantnagar; five from VPKAS, Almora; eight from ICRISAT, Hyderabad and 12 from PCPGR, Pantnagar, Uttarakhand. The experiment

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was conducted at Crop Research Center of GBPUA&T, Pantnagar (29°N, 79.3°E) at an altitude of 243.84 m amsl, in the foothills of the Himalayas during *Kharif* 2018 and 2019, however average mean values were used for data analysis. The soil *p*H at the experimental site ranged from 7.4 to 7.6 and experienced 600 mm mean annual rainfall with average minimum and maximum temperatures of 13^0C and 32^0C respectively. The soil in the experimental area is characterized by silty loam in texture with moderate availability of phosphorous and potassium.

The seeds of the studied material were sown during the first week of June 2018 and 2019 in a randomized complete block design (RCBD) with three replications. The seeds of one genotype were planted in one plot of each replication. Each plot consisted of 3 rows of 1.5 m in length with the spacing of 30 cm × 10 cm. The FYM was applied during the land preparation prior to sowing. The recommended dose of fertilizer [50:40:25; NPK (Kg/ha)] and required prophylactic measures were taken during the cropping season and other agronomic practices were followed for raising good and healthy plant stand on the field and better crop growth. In order to avert the stress situation and to introduce favourable conditions for crop growth, the field was irrigated at regular intervals.

Thirteen agro-morphological traits were considered for recording data from the field at appropriate stages of crop growth. Data were recorded on 10 randomly selected plants of each plot in each replication for plant height (cm), number of productive tillers/plant, leaf area (cm^2), number of fingers/head, number of spikes/finger, single head weight (g), seed volume (seeds/cm^2; the number of seeds occupy the space of 1 cm^2 area) and fodder yield (straw)/plant (kg). Seeds of a small size are designated by low seed volume as they represent the larger number in a space of 1 cm^2 area; contrarily high seed volume stands for a bold seed that covers the same area of space with less seed number. Whereas, the field data for days to 50% flowering, number of heads/plot, days to final harvest, harvest index (%) and grain yield/plot (kg) were recorded on a plot basis in each replication. The D^2 statistics (Mahalanobis 1928; Rao 1952; Harman 1976) and PCA were used to analyze the field data for grouping varieties based on their divergence to identify the promising parents. The principal component analysis and agglomerative hierarchical clustering (Ward's method) were done using STAR version 2.0.1 (STAR 2014).

RESULTS AND DISCUSSION

Genetic diversity employing Mahalanobis D^2 statistics: D^2 statistics were used for grouping of genotypes based on their divergence and to make the preliminary distinction (Hoque *et al.* 2015, Bandyopadhyay *et al.* 2017, Tripathi *et al.* 2017, Palaniyappan *et al.* 2020) among finger millet genotypes. Thirty-one genotypes, accessed from different locations, were clustered into seven groups (Table 1). The constellation of grouping exhibited a maximum number (24) of genotypes in cluster I followed by cluster II with 2 genotypes, whereas the rest of the clusters were represented by one genotype each. The intra-cluster heterogeneity of cluster number II (21.426) was high indicating considerable diversity existed among genotypes belonging to this group and might be served as a guideline to select desirable parents for recombination breeding programs within the same cluster (Table 1). However, Karad and Patil (2013) grouped 65 finger millet genotypes into five groups based on 12 morphological traits. In another study, using Euclidean distance, Patil *et al.* (2017) reported genetic divergence on 65 finger millet accessions and grouped them into five clusters.

Cluster VII showed the highest mean values for number of fingers/plant, days to harvest, single head weight, harvest index, fodder yield/plant and grain yield/plot; however, possessed bold seed size i.e. greater seed volume as indicated by least number of seeds/cm^2 (Table 2). Cluster VI had the maximum number for days to 50% flowering, leaf area and number of heads/plot; but showed the least number of days for maturity suggesting the genotypes required minimum grain filling period to attain maturity or less post-flowering period. Cluster V represented early flowering genotypes with less number of spikes/finger. Cluster IV was characterized by a tall plant with a low single head weight and leaf area. Cluster III represented late maturing genotypes with a high number of tillers/plant; but showed less seed volume i.e., the greater number of seeds/cm^2. Cluster II exhibited the

Table 1 Grouping of 31 finger millet genotypes into seven clusters, average inter (non-diagonal) and intra (diagonal) cluster distances based on Mahalanobis D^2 statistics (D^2 values)

Cluster	I	II	III	IV	V	VI	VII
Genotype	24[a]	2[b]	1[c]	1[d]	1[e]	1[f]	1[g]
I	17.955	28.230	32.678	25.982	28.398	28.065	28.816
II		21.426	37.324	31.100	39.983	38.041	34.357
III			0.000	46.737	45.220	43.764	49.783
IV				0.000	39.349	33.562	42.695
V					0.000	50.021	43.526
VI						0.000	44.402
VII							0.000

[a] clustering of F3, F4, F9, F10, F12, F15, F33, F37, F89, F98, F99, F113, F114, F115, F116, F117, F118, F160, F165, F200, F202, F203, PRM1, PRM2; [b] F142, F201; [c] F20; [d] F119; [e] F139; [f] F196; [g] PKPS4.

Table 2 Estimates of principal component analysis and cluster mean values of 13 traits in finger millet genotypes

Character*	Principal component analysis#					D^2 statistic		Cluster numbers							Mean	CV
	PC1	PC2	PC3	PC4	PC5	Ranked@	Contribution (%)	I	II	III	IV	V	VI	VII		
DTF	0.353	-0.354	-0.383	0.573	-0.051	34	7.311	70.6	75.5	71.0	93.0	62.0	97.0	67.0	72.1	7.9
PH	-0.054	-0.202	-0.836	-0.035	0.076	41	8.817	96.3	101.0	102.0	117.0	57.0	115.0	95.0	96.7	5.7
PT/P	0.172	0.679	-0.153	-0.155	0.510	44	9.462	3.0	3.0	12.0	1.0	3.0	5.0	4.0	3.4	47.7
LA	-0.073	0.494	-0.295	0.497	-0.323	20	4.301	21.2	16.0	25.7	13.4	14.5	31.9	26.6	21.1	9.1
F/H	0.111	0.432	-0.122	-0.311	-0.488	40	8.602	6.3	10.0	8.0	3.0	9.0	6.0	15.0	6.8	39.6
S/F	-0.149	0.296	-0.527	0.185	-0.270	51	10.967	38.6	35.0	40.0	28.0	31.0	49.0	41.0	38.2	14.1
DFH	-0.444	0.272	-0.238	0.212	0.643	30	6.451	147.3	135.0	158.0	146.0	96.0	131.0	158.0	145.0	3.1
H/P	-0.926	-0.165	-0.193	-0.182	-0.013	53	11.397	169.9	21.5	21.0	144.0	76.0	244.0	230.0	156.0	3.6
SHW	0.053	0.765	0.393	0.286	-0.088	31	6.666	3.5	3.1	4.8	2.2	3.7	2.5	5.2	3.5	5.8
SV	0.367	0.221	-0.398	-0.680	-0.079	27	5.806	38.4	44.0	54.0	40.0	49.0	42.0	37.0	39.7	11.8
FY/P	-0.956	0.161	-0.006	-0.048	-0.044	30	6.451	1448.5	465.0	550.0	770.0	670.0	1625.0	2490.0	1.4	3.7
HI	-0.834	-0.181	0.146	0.030	-0.143	40	8.602	38.8	14.4	18.2	40.8	41.8	37.0	47.8	37.0	5.6
GY/P	-0.972	0.126	0.013	-0.062	-0.074	24	5.161	573.6	67.5	100.0	315.0	280.0	615.0	1190.0	0.5	9.5
Eigenvalues	3.94	1.95	1.68	1.36	1.14											
Standard deviation	1.98	1.40	1.29	1.16	1.07											
Proportion of variance (%)	30.33	15.04	12.91	10.47	8.76											
Cumulative proportion (%)	30.33	45.36	58.27	68.73	77.50											

*DTF, Days to 50% flowering; PH, Plant height; PT/P, Productive tillers/plant; LA, Leaf area; F/H, Fingers/head; S/F, Spikes/finger; DFH, Days to final harvest; H/P, Heads/plot; SHW, Single head weight; SV, Seed volume; FY/P, Forage yield/plant; HI, Harvest index; GY/P, Grain yield/plot; # PC, Principal component; @ Number of times ranked first; CV, Coefficient of variation.

least values for harvest index and grain yield/plot. The inter-cluster distance has appeared highest (49.783) between cluster III and VII explaining the presence of a greater diversity between them. The D^2 analysis for the characters among the 31 finger millet genotypes revealed that number of fingers/plant, harvest index, number of heads/plot, number of spikes/finger, number of tillers/plant and plant height appeared as the major contributors towards total divergence (Table 2). Previously, Anuradha *et al.* (2017) reported six clusters while studying 25 finger millet genotypes and described different ranges of eight morphological traits for six clusters. The genotypes belonging to cluster VII (PKPS4); cluster VI (F196); cluster IV (F119) and cluster III (F20) were found more diverse and could be utilized as parents for the hybridization programs to obtain maximum heterosis in grain yield production.

Principle component analysis: The PCA would help to strengthen the findings from the D^2 analysis and exhibits the principal contributors towards variability between the genotypes (Mahajan and Mehan 1980, Sheela *et al.* 2020) by reducing large data sets and finding a small number of important independent variables without disturbing its original variability. In this investigation, five PCs having eigenvalues greater than one (Table 2) were extracted from the original data accounting for 77.50% of total variation amongst the 31 finger millet genotypes evaluated for 13 quantitative traits. The high contribution of the first few PCs in total variability based on various plant traits had already been reported in the literature by Anteneh *et al.* (2019).

The major contributors to the PCs were determined from the loading factor values. The PC1 accounted for about 30.33% of the total variation. The maximum variation in PC1 was primarily due to days to 50% flowering (0.353) and seed volume (0.367) that were linked in a positive direction but showed negative loadings of grain yield/plot (-0.972), fodder yield/plant (-0.956), number heads/plot (-0.926) and harvest index (-0.834). The PC1 was regarded as a major component for yield since it included several traits, which were associated with grain yield/plant. The PC2 explained 15.04% of the total variation and had highly positive scores from single head weight (0.765) and number of tillers/plant (0.679). Days to 50% flowering possessed the same magnitude of eigenvectors both in PC1and PC2 but in opposite directions to each other. Uniformity in positive scores for eigenvectors was registered in PC1, PC2, PC3, and PC4 for single head weight; contrarily negative scores for eigenvectors were maintained for plant height and number of heads/plot among the first four components of PC1, PC2, PC3 and PC4 which explained together 68.73% variability (Table 2). This has elucidated the presence of physiological competition for equal sharing of photosynthates among sink capacity of finger millet genotypes. Genotype F20 significantly contributed in a positive direction in PC1, however inversely in PC2. Genotype PKPS4 contributed significantly in a negative direction in both PC1 and PC2 (Fig 1A). Genotypes F37, F139, and F142 had also contributed positively to both PC1 and PC2. This illustrates the contribution of each genotype in the total variation among different principal components.

The loading plot (Fig 1B) depicted the direction of the variables as per the sign of score coefficients. Number of heads/plot, harvest index, plant height and days to 50% flowering aligned in opposite direction signifying negative correlation with single head weight. The other characters, viz. grain yield/plant, fodder yield/plant and days to final harvest showed a positive loading with single head weight in PC2 suggesting that a concomitant increase in grain and fodder yield potential was observed with an increase in single head weight. Number of productive tillers/plant, number of fingers/head, number of seed/cm^2 (i.e. less volume of seeds) showed direct positive loading with single head weight both in PC1 and PC2. This illustrates that selection for these traits would be effective under a normal environment and would play a major decisive role

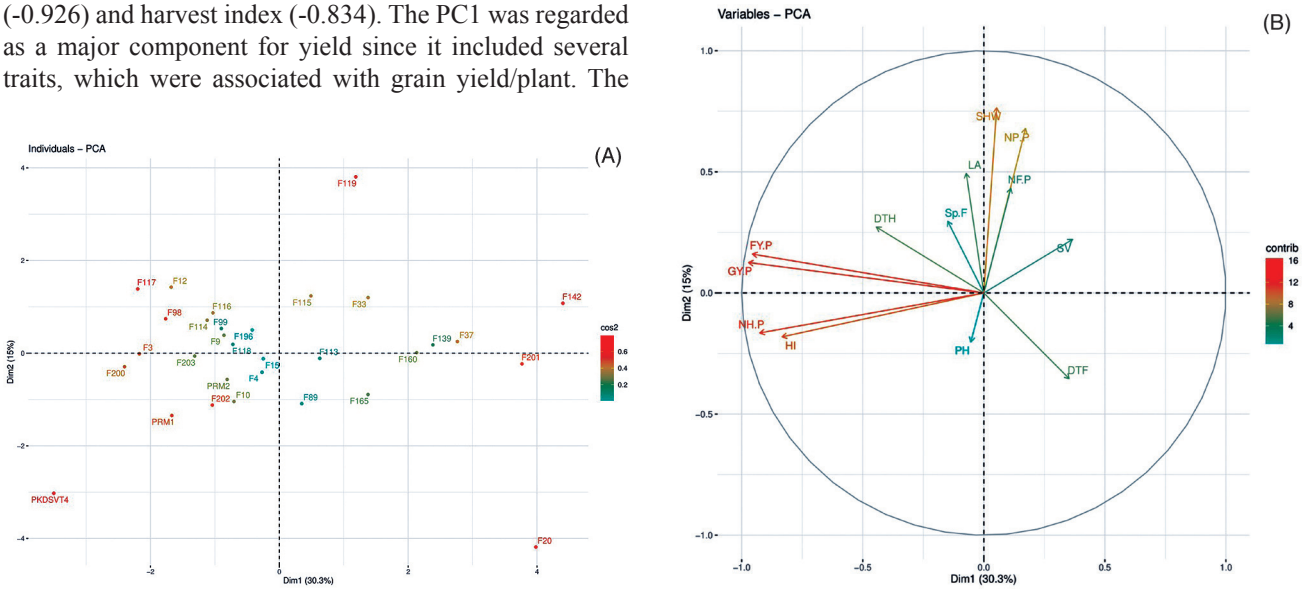

Fig 1 The principal component analysis showing the distribution pattern of 31 finger millet genotypes (A) and 13 agro-morphological traits (B); Dim1 and Dim2 are the first and second principal components, respectively.

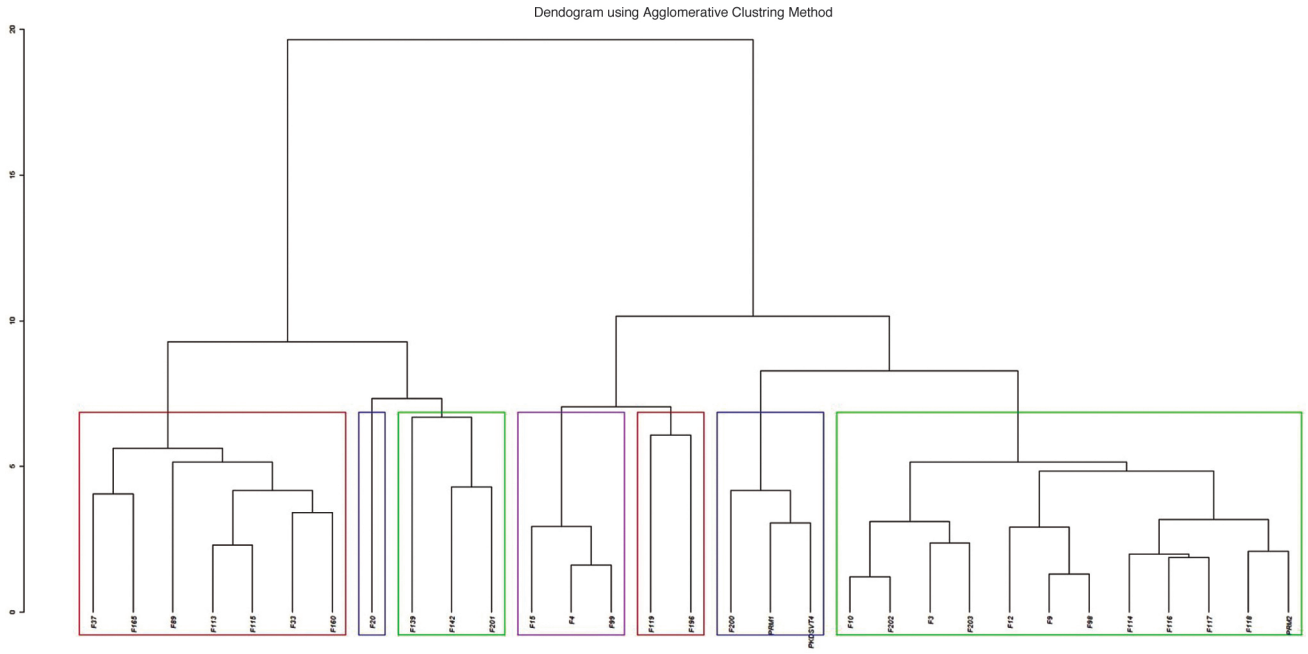

Fig 2 Dendrogram and agglomerative hierarchical clustering (Ward's method) of 31 genotypes of finger millet. Genotypes were grouped into seven main clusters based on similarity euclidean distances and represented by different colors.

in the improvement of finger millet genotypes because the physiology of crops remained favorable for the growth and development of plants. The score plot in PCA (Fig 1) and D^2 statistic (Table 2) revealed that the genotype PKPS4 was distantly related to F20. The selection of these two diverse genotypes would be effective for improving grain yield in finger millet because they possessed the most deserving complement traits of each other. PKPS4, for instance, had bold seed (i.e. less number of seed/cm^2) with higher mean values for single head weight, grain yield, harvest index and number of finger/spike while F20 showed a high number of tiller/plant (Table 2). The clustering pattern of genotypes was illustrated employing a dendrogram (Fig 2).

Selection of these two diverse genotypes, therefore, could be considered as parents in hybridization programs to overcome the limitation associated with the development of promising high yielding cultivars in finger millet suitable for upland areas. The testing of resultant genotypes into non-traditional areas could also help to expand finger millet cultivation at various agro-climatic zones in India.

The results of this study, therefore, provide valuable information and pave the way in designing future breeding programs for finger millet towards the development of the desired improvement on grain and fodder yield while expressing its component traits under a stress-free environment at the growing stage. Nevertheless, the molecular study will be required to upgrade the process of selection of parents, introgression of new gene combinations into genotypes, and development of high yielding cultivars of finger millet suitable for upland areas.

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