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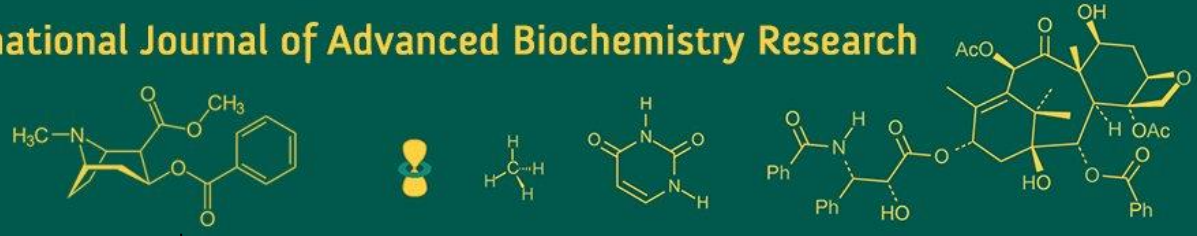

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Phenotypic diversity using D^2 statistics in finger millet [*Eleusine coracana* (L.) Gaertn.]

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Abstract
Finger millet is one of the most important and nutritionally rich crop of India. The present investigation was carried out with 40 genotypes of finger millet in randomized complete block design with three replications at the Experimental Farm of Department of Genetics and Plant Breeding, Anand Agricultural University, Anand during *Kharif* 2020. The genetic divergence assessed by Mahalanobis D^2-statistic, grouped 40 genotypes into 7 clusters. The maximum inter-cluster distance (D =770.59) was found between cluster III and V and minimum (226.07) between V and VII. The intra-cluster distance ranged from 126.59 (cluster-III) to 202.14 (cluster-V). In general, intra- cluster distance was lower than inter cluster distances, indicating that genotypes included within a cluster tended to diverse less from each other. The cluster V shows highest mean values followed by cluster-IV and cluster-VII for most of the desirable traits. Percent contribution of various traits towards total genetic divergence showed that protein content contributed maximum percentage (35.13%) towards total genetic divergence followed by Fe content (25.90%) and harvest index (11.79%). Looking to the high yielding genotypes and large inter-cluster distances, it is recommended to attempt crossing of the genotypes from cluster III (VL-149, VL-708) and cluster V (GPU- 66, MR-6), which may lead to produce broad spectrum of favourable genetic variability for yield improvement in finger millet.

Keywords: Phenotypic diversity, D^2 statistics, finger millet, *Eleusine coracana* (L.) Gaertn

Introduction
Finger millet [*Eleusine coracana* (L.) Gaertn.] sub species coracana belongs to family Poaceae. The cultivated *E. coracana* is a tetraploid (2n = 4X = 36); has morphological similarities to both *E. indica* (L.) Gaertn. (2n = 18) and *E. africana* (O.) Byrne (2n = 36). *Eleusine*, the generic name, is after Eleusine the Greek goddess of cereals. Common name finger millet is derived from the finger like branching of the panicle. It is also known as ragi or nagali or nachani (India), bulo (Uganda), wimbi (Swahili), and tellebun (Sudan) and is an important food crop cultivated widely in arid and semi-arid regions of the world, especially in east Africa, India and other Asian countries including Sri Lanka and China. It is believed that Ethiopian highland region is the centre of origin of E. coracana and it was introduced in India, probably over 3000 years ago (Babu *et al*., 2017) [1].

Finger millet is an important cereal because of its excellent storage properties and the nutritive value of the grains. It is also known as 'Nutritious millet' as the grains are nutritionally superior to many cereals in case of nutrients. Finger millet is a good source of calcium, iron and other minerals. It is consumed both in native and processed form (Gopalan *et al*., 1989; Rao and Murlikrishna, 2001) [2, 3]. Finger millet grain can be stored for years without storage pest infestation which makes it a perfect food grain commodity for famine-prone areas. While grains are used for human consumption as it contains higher fiber which prevents constipation, high cholesterol formation and intestinal cancer. Hence, diabetics are advised to eat finger millet and other small millet instead of rice. The crop residues are excellent source of dry matter for livestock especially in dry season. Finger millet straw makes good fodder and contains up to 61 per cent total digestible nutrients.

Finger millet is a tufted annual crop, growing to a height of 30-150 cm and maturing in 75-160 days. Leaves are narrow, grass-like and capable of producing many tillers and nodal branches. The panicle consists of a group of digitally arranged spikes often referred to as fingers. The spikelets are made up of 4-10 florets arranged serially on the finger. All florets are perfect flowers with the exception of the terminal ones which may sometimes be infertile.

The grain is oblong to round and oval, reddish brown in colour with the grains surface finely corrugated. Typically, a tropical, rainfed crop, it is one of the best suited for dry farming. Finger millet is very adaptable and thrives at higher elevations than most other tropical cereals. (Vilas *et al.,* 2015) [4]. In India, it is cultivated on 1.19 million ha with a production of 1.98 million tonnes and average productivity of 1661 kg per ha (Anonymous, 2018) [5]. Major finger millet growing states in India are Karnataka followed by Uttrakhand, Maharashtra, Tamil Nadu, Andhra Pradesh, Orissa, Gujarat, Jharkhand and Bihar. Considering the increased demand of finger millet for food purposes and decreasing area under this crop due to competing crops like rice, maize and soybean, there is an immediate need for genetic enhancement of finger millet productivity.

The basic information on the existence of genetic variability and diversity in a population and the relationship between different traits is essential for any successful plant breeding programme. Genetic improvement through conventional breeding approaches depends mainly on the availability of diverse germplasm and presence of enormous genetic variability the characterization and evaluation are the important pre-requisites for effective utilization of germplasm and also to identify sources of useful genes. An insight into the nature and magnitude of genetic variability present in the gene pool is of immense value for starting any systematic breeding programme because the presence of considerable genetic variability in the base material ensures better chances of evolving desirable plant type.

Estimation of genetic divergence between the parents is crucial because a cross between genetically diverse parents is likely to create a high level of heterosis and more variability may be anticipated in segregating generations. Thus further, a classification of genotypes based on diversity analysis will allow the breeder to choose the parents with the greatest genetic diversity and utilize a few of the selected diverse parents in the hybridization programme. Evaluation of genetic divergence aids in reducing the number of breeding lines that have to be maintained. The genetic distance between different genotypes can be measured effectively using Mahalanobis (1936) [6] D^2 statistics. Finally, their genetic distance-based grouping offers a clear image of how the genotypes interact and helps in the selection of suitable genotypes for use in the hybridization programme. The evaluation of genetic diversity is commonly employed by breeders as an alternative to the process of germplasm selection since it enables lines to be organized into groups that, when inter-crossed, would provide the most promising results and requires less time and resources. D^2 analysis is a helpful method for measuring the degree of genotypic divergence between biological populations and determining how much each component contributed to the overall divergence at both the intra and inter-cluster levels. Earlier scientists also studied diversity analysis in finger millet and classified the genotypes in different clusters (Negi *et al*., 2017) [7].

Materials and Methods

Planting material and experimental site

The experimental material for present investigation comprised of 40 finger millet genotypes, which were procured from Hill Millet Research Station, Navsari Agricultural University, Waghai and Zonal Agricultural Research Station (ZARS), Kolhapur. The name and source of genotypes included in the present investigation are listed in Table 1. These all genotypes were evaluated at the experimental farm of Department of Genetics and Plant Breeding, B. A. College of Agriculture, Anand Agricultural University, Anand is located in Agro-Climatic Zone III (Middle Gujarat) of Gujarat state and is situated at 22o 35' North latitude, 72o 55' East longitude and at an altitude of 45.01 meters above mean sea level. The soil of the experimental site is sandy loam, locally known as "Goradu soil" of "charotar" tract. It is alluvial in origin, deep, well drained and fairly moisture retentive. The climatic condition of the experimental area represents semi-arid tropical conditions.

Each genotype was planted in a single row 3.0 m long, 30 cm apart, and 10 cm plant-to-plant. To prevent damage and border effects, the experiment was encircled by boundary rows. To achieve a satisfactory output, agronomical and plant protection techniques were undertaken.

Trait Phenotyping

Days to 50% flowering (DF), Days to maturity (DM) were taken on plot basis as per Khatri *et al.,* (2023) [8]. Plant Height (cm), Productive Tillers per Plant, Main Ear Length (cm), Finger Length (cm), Fingers per Ear, 1000 Grain Weight (g), Grain Yield per Plant (g), Harvest Index (%), measured as per Negi *et al.* (2017) [7]. Protein Content (%) estimated as per Patel *et al.* (2023) [8], Calcium Content (mg/100 gm) measured as per Gunguniya *et al.* (2023) [10]. Iron Content (ppm) estimated as per Khatri *et al.* (2023) [8].

Statistical Data Analysis

Genetic divergence was estimated by using D^2 statistics of Mahalanobis (1936) [6] and Grouping of the genotypes in different clusters was done by using Tocher's method (Rao, 1952) [11]. The per cent contribution of characters towards genetic divergence was calculated according to Singh and Choudhary (1985) [12].

Results and Discussion

Genetic Divergence

The knowledge regarding the extent of variability and genetic diversity is of much importance while making improvement in a complex trait like yield. Therefore, while improving grain yield, selection of parents having wide genetic divergence for number of characters is of prime importance, which is assessed by multivariate technique D^2 -statistics developed by Mahalanobis (1936) [6]. The analysis of variance revealed that the differences among the mean square due to genotypes were significant for all the characters indicating the presence of high amount of genetic variability for all the characters studied (Table not given). The value of V- statistic (2306.262) which follows χ^2 distribution for 507 degrees of freedom showed highly significant differences among the genotypes for aggregate of 13 characters. Thus, one can proceed for further diversity analysis. The D2 values between all 780 pairs ranged from 15.20 (between KMR-204 and OEB-532) to 804.35 (between VL-149 and GPU-66), which indicated the presence of high genetic diversity among the genotypes for all the traits (these D2 values were taken from matrix table, which is not given here).

Table 1: List of genotypes used in the study

Sr. No.	Genotype	Source	Sr. No.	Genotypes	Source
1	KOPN-1204	ZARS, Kolhapur	21	VR-847	Hill Millet Research Station, NAU, Waghai
2	KOPN-1211	ZARS, Kolhapur	22	VR-936	Hill Millet Research Station, NAU, Waghai
3	KOPN-1213	ZARS, Kolhapur	23	PR-202	Hill Millet Research Station, NAU, Waghai
4	KOPN-1214	ZARS, Kolhapur	24	GPU-66	Hill Millet Research Station, NAU, Waghai
5	KOPN-1227	ZARS, Kolhapur	25	GPU-28	Hill Millet Research Station, NAU, Waghai
6	KOPN-1228	ZARS, Kolhapur	26	MR-6	Hill Millet Research Station, NAU, Waghai
7	KOPN-1230	ZARS, Kolhapur	27	KMR-340	Hill Millet Research Station, NAU, Waghai
8	Phule Kesari	ZARS, Kolhapur	28	KMR-204	Hill Millet Research Station, NAU, Waghai
9	GPU-45	ZARS, Kolhapur	29	KMR-603	Hill Millet Research Station, NAU, Waghai
10	GPU-67	ZARS, Kolhapur	30	OEB 532	Hill Millet Research Station, NAU, Waghai
11	VL-352	Hill Millet Research Station, NAU, Waghai	31	Indira Ragi-1	Hill Millet Research Station, NAU, Waghai
12	VL-315	Hill Millet Research Station, NAU, Waghai	32	Chhattisgarh Ragi-2	Hill Millet Research Station, NAU, Waghai
13	VL-149	Hill Millet Research Station, NAU, Waghai	33	RAU-8	Hill Millet Research Station, NAU, Waghai
14	VL-324	Hill Millet Research Station, NAU, Waghai	34	GN-1	Hill Millet Research Station, NAU, Waghai
15	VL-376	ZARS, Kolhapur	35	GNN-6	Hill Millet Research Station, NAU, Waghai
16	VL-314	Hill Millet Research Station, NAU, Waghai	36	GNN-7	Hill Millet Research Station, NAU, Waghai
17	KOPN-235	ZARS, Kolhapur	37	GN-8	Hill Millet Research Station, NAU, Waghai
18	KOPN-942	ZARS, Kolhapur	38	WN-587	Hill Millet Research Station, NAU, Waghai
19	Phule Nachani	ZARS, Kolhapur	39	WN-593	Hill Millet Research Station, NAU, Waghai
20	VR-708	Hill Millet Research Station, NAU, Waghai	40	WN-594	Hill Millet Research Station, NAU, Waghai

Composition of Clusters

Grouping of the genotypes was carried-out by following the Tocher's method (Rao, 1952) [11] with the assumption that the genotypes within cluster have smaller D^2- values among themselves than those from genotypes belonging to different clusters. In all, 7 clusters were formed from 40 genotypes. The composition of clusters is given in Table 2. The cluster I was the largest cluster having 27 genotypes (Table 2). Cluster II was the second largest cluster with five genotypes. The cluster III, IV and V ranked third with 2 genotype each, while cluster VI and VII were solitary cluster with single genotype. The mono-genotypic cluster indicated that genotypes belonging to these clusters had huge diversity from the rest as well as each other. Thus, those genotypes have entirely different genetic make-up from the others.

Karad and Patil (2013) [13] classified 65 diverse genotypes of finger millet into five clusters by the same method. Thirty-five germplasm lines of finger millet were grouped in six clusters by Negi *et al.* (2017) [7]. Thirty-six genotypes of finger millet were grouped into seven clusters by Patel *et al.* (2020) [15]. Subramanya and Ravikumar (2020) [16] studied 33 diverse genotypes of finger millet and grouped them into ten cluster. In the current study, clustering pattern showed that the genetic diversity was not fully associated with geographical diversity, so there was no formal relationship between geographical diversity and genetic diversity. This could be because there were other forces than geographical separation like natural and artificial selection, exchange of breeding material, genetic drift and environmental variation responsible for genetic diversity.

Inter and Intra-Cluster Distances

The inter-cluster and intra-cluster distances are shown in Table 3. The maximum inter-cluster distance (D = 770.59) was found between cluster III and V, followed by cluster III and IV (D =651.86) and II and VI (643.40). The minimum inter-cluster distance was observed between cluster V and VII (226.07) followed by cluster VI and VII (D = 236.65) and cluster IV and V (240.95) indicating that genotypes of these clusters were genetically close and had maximum number of gene complexes. The intra-cluster distance (D) ranged from 126.59 (cluster- III) to 202.14 (cluster- V). The clusters VI and VII contained single genotype and therefore, intra-cluster distance was zero. Inter-cluster distances were higher than intra-cluster distance indicating the presence of wider genetic diversity between the clusters rather than within the clusters. Based on inter-cluster distances, the clusters III×V, III×IV and II×VI were found to be more diverse in decreasing order of their magnitude. Hence, the genotypes from these clusters can be utilized as potential parents and crossing among them would be suggested to generate a wide range of variability for effective selection of various characters.

Table 2: Grouping of 40 genotypes of finger millet in various clusters on the basis of D2 statistics

Sr. No.	Clusters	No. of genotypes	Name of the genotypes
1	I	27	KMR-204, OEB 532, GN-1, VL-315, VL-352, Indira Ragi-1, RAU-8, WN- 587, WN-593, KOPN-942, Chhattisgarh Ragi-2, GPU-28, GNN-7, VL-314, VR-847, KOPN-1211, KMR 603, GN-8, WN-594, GNN-6, GPU-67, KOPN-1213,PR-202, KOPN-1204, KOPN-1214, Phule Kesari, KOPN-1227
2	II	5	VL-324, VR-936, GPU-45, Phule Nachani, VL-376
3	III	2	VL-149, VR-708
4	IV	2	KOPN-1230, KOPN-235
5	V	2	GPU-66, MR-6
6	VI	1	KOPN-1228
7	VII	1	KMR-340

Table 3: Average intra-cluster (diagonal) and inter-cluster distance for 40 genotypes of finger millet

	I	II	III	IV	V	VI	VII
I	141.81						
II	252.28	129.93					
III	272.63	457.68	126.59				
IV	286.78	281.96	651.86	184.19			
V	342.36	434.67	770.59	240.95	202.14		
VI	339.51	643.40	376.36	410.61	507.07	0.00	
VII	255.87	510.44	509.11	325.48	226.07	236.65	0.00

Cluster Means of Various Characters

The cluster V manifested highest mean values for days to maturity (133.83 days), finger length (8.65 cm), calcium content (203.12 mg/100 gm), productive tillers per plant (3.67), harvest index (4.16%) presented in Table 4. The cluster VII showed lowest mean value for harvest index (2.09%), while manifested highest mean values for productive tillers per plant (3.67), main ear length (11.39 cm) and grain yield per plant (25.80 g). The cluster II exhibited highest mean values for fingers per ear (8.06) and protein content (8.53%). The cluster IV showed highest mean values for days to 50% flowering (95.17 days) and 1000 grain weight (2.97 g), while lowest mean value for fingers per ear (6.20). According to Table 4, The cluster III exhibited lowest mean values for days to 50% flowering (70.50 days), days to maturity (94.00 days), plant height (85.77 cm), main ear length (8.43 cm), and finger length (6.50 cm), while manifested highest mean value for Fe content (23.63 ppm). The cluster VI exhibited highest mean values for plant height (121.00 cm) and lowest for productive tillers per plant (2.27), 1000 grain weight (2.16 g), protein content (3.17%), calcium content (135.62 mg/100 gm), and grain yield per plant (13.80 g). There were different characters which showed superiority with respective clusters so, for further breeding programme, breeder should select genotypes from particular clusters according to need of crop improvement programme.

Characters Contribution towards Genetic Divergence

The analysis of variance for each character was carried out using mean of the 40 genotypes. Estimation of inter and intra cluster variances, along with ratio of inter cluster variance to the total variance (R2) and inter cluster coefficient of variation (CV_b) for 13 characters were worked out and presented in Table 4 Maximum value of R^2 was observed for days to maturity (0.846) followed by harvest index (0.833) and protein content (0.694) and minimum value for R^2 was observed for 1000 grain weight (0.202), followed by days to 50% flowering(0.224) and productive tillers per plant(0.276). From inter cluster coefficient of variation (CVb), it was observed that the 1000 grain weight contributed maximum (41.6%) towards the total divergence in yield followed by harvest index (36.5%). Apart from above mentioned traits, other characters *viz.*, days to maturity (13.7%), plant height (12.2%), productive tillers per plant (11.3%), main ear length (12.9%), finger length (11.9%), grain yield per plant (14.8%), protein content (10.9%) and iron content (16.4%) had moderate while, days to 50 per cent flowering (8.8%) and fingers per ear (8.8%) had low contribution towards the total divergence.

Table 4: Mean values of different characters of finger millet grouped into seven clusters

Character Cluster	DF	DM	PH (cm)	PT	MEL (cm)	FL (cm)	FE	TGW (g)	HI (%)	PC (%)	CC (mg/100 gm)	FC (ppm)	GYP (g)
I	84.16	108.84	92.26	3.57	8.96	7.04	7.18	2.67	4.00	5.49	193.73	17.90	20.87
II	85.06	110.86	89.30	3.10	8.90	6.91	8.01	2.71	4.05	8.53	192.99	18.75	19.66
III	70.50	94.00	85.77	3.50	8.43	6.50	6.83	2.34	4.04	5.16	183.50	23.63	15.27
IV	95.17	128.17	107.13	3.53	9.64	7.73	6.20	2.97	3.84	6.56	175.96	20.68	22.36
V	88.50	133.83	102.70	3.67	11.04	8.65	7.33	2.86	4.16	5.98	203.12	12.75	24.20
VI	87.00	116.67	121.00	2.27	10.71	8.25	7.67	2.16	2.82	3.17	135.62	22.6	13.80
VII	86.67	122.67	101.20	3.67	11.39	8.27	7.67	2.47	2.09	3.75	147.9	12.35	25.80
GM	84.49	111.11	93.78	3.49	9.17	7.18	7.25	2.67	3.94	5.84	190.12	18.16	20.63
S.Em±	1.85	2.74	3.44	0.26	0.34	0.26	0.34	0.11	0.40	10.03	1.77	1.87	0.32
CD 5%	5.19	7.69	9.65	0.75	0.97	0.73	0.95	0.31	1.12	28.14	4.98	5.25	0.91
CV%	5.20	5.85	8.70	18.22	8.97	8.67	11.17	10.14	16.31	12.51	23.19	21.56	19.67
R2	0.22	0.84	0.66	0.27	0.67	0.65	0.38	0.20	0.83	0.69	0.43	0.36	0.36
CVb	8.80	13.70	12.20	11.30	12.90	11.90	8.80	41.60	36.5	10.9	20.30	16.40	14.80

DF=Days to 50% flowering; DM= Days to maturity; PH= Plant height; PT= Productive tillers per plant; MEL= Main ear length; FL= Finger length; FE= Fingers per ear; TGW= 1000 grain weight; GYP= Grain yield per plant; HI= harvest index; PC= protein content; CC= Ca content; FC= Fe content; R^2= Ratio of inter cluster variance to the total variance; CVb=Inter cluster coefficient of variation

Table 5: Contribution of various traits towards total genetic divergence

Sr. No.	Characters	Times ranked first	Contribution (%)
1	Days to 50% flowering	47	6.03%
2	Days to maturity	68	8.72%
3	Plant height	16	2.05%
4	Productive tillers per plant	32	4.10%
5	Main ear length	5	0.64%
6	Finger length	1	0.13%
7	Finger per ear	3	0.38%
8	1000 grain weight	9	1.15%
9	Harvest index	92	11.79%
10	Protein content	274	35.13%
11	Ca content	10	1.28%
12	Fe content	202	25.90%
13	Grain yield per plant	21	2.69%

Contribution of percentage of various traits towards total genetic divergence presented in Table 5 which showed that protein content contributed maximum percentage (35.13%) with 275 time ranked first towards total genetic divergence followed by Fe content (25.90%). While, other characters like finger length (0.13%), finger per ear (0.38%) and main ear length (0.64%) contributed minimum towards total genetic divergence.

Conclusion

Intra-cluster distances were lower than the inter-cluster distances. Thus, the genotypes included within a cluster tended to diverse less from each other. High intra-cluster distance indicated about the wider genetic diversity among the genotypes which could be used in yield improvement of finger millet. The genotypes belonging to the clusters separated by high statistical distance could be used in hybridization programme for obtaining a wide spectrum of variation among the segregants. The clustering pattern could be utilized in selection of parents for crossing and deciding the best cross combinations which may generate the highest possible variability for various traits. The genotypes with high values of any cluster can be used either for direct adoption or for hybridization, followed by selection. It has been well established fact that more the genetically diverse parents used in hybridization programme, the greater will be the chances of obtaining high heterotic hybrids and broad-spectrum variability in segregating generations. It has also been observed that the most productive hybrids may come from high yielding parents with a high genetic diversity. Therefore, in the present investigation, based upon high yielding genotypes and large inter-cluster distances, it is advisable to attempt crossing of the genotypes from clusters V and VII which may lead to produce broad spectrum of favorable genetic variability for yield improvement in finger millet.

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