Genetic Diversity Assessment using D^2 Statistics in Finger Millet

[*Eleusine coracana* (L.) Gaertn.]

Abstract:

Aim: The current investigation was carried out using total of 101 finger millet genotypes was carried out at two locations in four environments to study diversity.

Study design: Randomized Block Design

Place and Duration of Study: 6 Months at Hill Millet Research Station and College Farm, Navsari Agricultural University, Navsari

Methodology: D^2 statistics was used for study of diversity in 101 genotypes

Results: The perusal of the data for diversity studies revealed that intra-cluster distance ranged from 59.93 to 96.56. Highest inter-cluster distance was maximum between clusters VI and XI followed by cluster pair VI and VII.

Conclusions: Based upon high yielding genotypes and large inter-cluster distances, it is advised to attempt crossing of the genotypes from clusters VI and XI as well as VIII and XIII which may produce broad spectrum of favourable genetic variability for yield improvement in finger millet.

Key Words: D^2 statistics, inter and intra cluster variation, cluster means, coefficient of variation

1. INTRODUCTION

Millets are typically warm-weather, small-grained cereals that are annual and in the grass family. Millets are not susceptible to pests and do not need spraying of pesticides. Millets strengthen food security since they are less likely to fail than other cereal crops [1]. Looking to current scenario and significance, India's proposal to observe an International Year of Millets in 2023 was approved by the Food and Agriculture Organisation (FAO) in 2018 and the United Nations General Assembly has declared the year 2023 as the International Year of Millets. Thus further research gains traction in such situation. Most important millets grown in India are pearl millet, great millet and finger millet.

Finger millet (*Eleusine coracana* (L.) Gaertn.) also known as *Nagli* or *Ragi*, is a significant hill millet and a tetraploid and self-pollinating species that was most likely descended from *Eleusine africana*, a wild relative. It is widely grown in third world countries like south Asian countries and Africa. It is grown extensively in the hilly Dangs district and surrounding districts of south Gujarat and tribal belt of middle Gujarat [2].The yield levels are modest, and there hasn't been much work done to genetically boost the fingermillet's capacity for yielding.

Before beginning any breeding project for the development of the crop, an evaluation of the germplasm is required to gather the fundamental data.It can be easier to classify and identify various genotypes by assessing the genetic diversity of acquired material. The plant breeder can select a variety of parents for specialised use in hybridization with the aid of precise knowledge of the kind and degree of genetic divergence. Progenies from various parents chosen for breeding programmes on the basis of genetic divergence analyses are anticipated to exhibit a wide range of genetic variability, allowing more opportunity to isolate transgressive segregates in later generations [3] and promising heterotic effect may be observed in early generation.The degree of genetic diversity in the population has a major impact on the program's ability to improve crops. Polygenic traits are greatly influenced by their environment.

2. MATERIALS AND METHODS

A total of 101 finger millet genotypes were used for the experiment and was carried out at two location *viz*., Hill Millet Research Station, Waghai, The Dangs and College Farm, Navsari Agricultural University, Navsari during *Kharif* - 2021 for two different dates of sowing within a gap of one month thereby creating four environments. Mean data across all three replications in each environment for sixteen characters for each genotype was subjected to diversity studies.

Genetic diversity between genotypes was estimated by using D^2 analysis given by [4]. The D^2 value between i^{th} and j^{th} genotypes for P characters was calculated as:

$$D^2 = \sum_{t=1}^{p} (y_{it} - y_{jt})^2$$

Where,

Y_i = Uncorrelated mean value of i^{th} genotype for 't' character
Y_j = Uncorrelated mean value of j^{th} genotype for 't' character
D^2 = D^2 value between i^{th} and j^{th} genotypes
P = Number of characters

A simultaneous test of significance of difference between the mean values of a number of correlated variables was done [5] by using 'V' statistic, which in turn utilized Wilk's criterion

[6]. The sum of squares and sum of products of error and error plus variety, variance-covariance matrix were used for this purpose [7].

Then the value of 'V' statistic was worked out using Wilk's lambda criterion[5]described the multivariate analysis of genetic divergence using Mahalonobis D^2 Statistics. Transformation of the original mean of various characters (Xi's) to uncorrelated variables (Yi's) was carried out by computer. Grouping of the genotypes in different clusters was done by using Tocher's method [5]. Unweighted analysis of variance using the mean values of different characters was carried out. This was calculated according to [8] and, inter-cluster coefficient of variation was calculated. After the formation of the clusters, average intra cluster distance (D) was alsocalculated.Clusters were taken one by one and their distances from other clusters were calculated. The clusters and their mutual relationships were presented diagrammatically. Based on D^2 values (inter-cluster distance), the scale given by Rao [5] for rating of the distance was adopted and the cluster diagram was prepared.

3. RESULTS AND DISCUSSION

Multivariate test using Wilk's criterion was carried out to test the difference among all the finger millet entries. Wilk's criterion was significant and eventually the differences among entries were also significant.

Mahalanobis D^2 statistic was computed between all possible pairs of all finger millet genotypes and the genetic diversity present among the genotypes was assessed and given as follows:

Distribution of genotypes into clusters

All the finger millet genotypes under study were distributed into 13 clusters using dissimilarity matrix generated using D^2 statistics and clustering using Torcher method. Composition of each cluster is shown in Table 1. Analysis showed that maximum number of genotypes *i.e*. 56 genotypes were included in cluster I followed by cluster II which had 17 genotypes in its composition. Cluster IV had third highest constituents *i.e*. 11 genotypes which was followed by cluster VI having 8 genotypes.

Remaining clusters *i.e*. III, V, VII, VIII, IX, X, XI, XII and XII each had only one genotype, thus were monogenotypic clusters. These genotypes are likely to have extreme form of expression for particular character. Genotypes of different geographical areas were fall in one group and also the genotypes of the same geographical area were clubbed into different groups indicating there is no formed relationship between geographical diversity and genetic diversity.These results are in close agreement with [9], [10], [11] and [12].

Table 1: Distribution of all genotypes of finger millet into thirteen different clusters on the basis of Mahalanobis D^2 statistics

Cluster	Total no. of genotypes	Genotypes
I	56	WN – 630, WN – 664, WN – 665, VL – 352, GPU – 45, GN – 8, WN – 467, WN – 544, WN – 563, WN – 569, WN – 591, WN – 835, WN – 836, WN – 837, WN – 838, WN – 840, WN – 842, WN – 843, WN – 844, WN – 847, WN – 850, WN – 851, WN – 852, WN – 853, WN – 855, WN – 858, WN – 859, WN – 860, WN – 861, WN – 864, WN – 865, WN – 866, WN – 867, WN – 868, WN – 870, WN – 871, WN – 872, WN – 873, WN – 874, WN – 875, WN – 876, WN – 877, WN – 878, WN – 879, WN – 880, WN – 881, WN – 882, WN – 886, WN – 890, WN – 892, WN – 894, WN – 895, WN – 896, WN – 897, WN – 900, WN – 903
II	17	WN – 561, WN – 566, WN – 575, WN – 592, PR – 202, WN – 586, WN – 594, DN – 6, WN – 845, WN – 854, WN – 856, WN – 857, WN – 863, WN – 869, WN – 883, WN – 884, WN – 885
III	1	WN – 889
IV	11	WN – 587, WN – 601, WN – 609, WN – 657, WN – 846, WN – 849, WN – 862, WN – 893, WN – 898, WN – 599, WN – 899
V	1	WN – 902
VI	8	WN – 562, WN – 572, WN – 577, GPU – 67, WN – 583, WN – 834, WN – 848, WN – 891
VII	1	WN – 887
VIII	1	GNN – 6
IX	1	WN – 841
X	1	WN – 888
XI	1	WN – 901
XII	1	WN – 839
XIII	1	WN – 593

In heterosis breeding, genotypes of diverse groups were known to play very important role of potential parents and when each genotype of different clusters were inter crossed, which are likely to produce heterotic combinations. These heterotic combinations should be advanced to further generations and there is high possibility of obtaining transgressive segregant which can be further utilized to advance the generation and stabilize such segregant which is high yielding and has other desirable traits and can be released as variety for cultivation.

The intra and inter cluster distances (D) between all possible pairs of thirteen clusters were computed and presented in Table 2 as well as shown in Fig. 1.

Perusal of the data from table revealed that intra-cluster distance ranged from 59.93 to 96.56. Highest intra-cluster distance was found in cluster VI (D = 96.56) followed by cluster IV having distance of 75.89. Cluster II had third highest cluster distance of 63.92 which was

followed by cluster I (59.93). Remaining clusters had only one member so their intra-cluster distance would be zero.

The minimal and maximal inter-cluster distance was found to be 36.94 to 242.49. Highest inter-cluster distance was maximum between clusters VI and XI (D = 242.49) which was followed by cluster pair VI and VII (D = 229.68). Next comes cluster pair IX and XII (D = 215.85). Minimum inter-cluster distance was found in between clusters III and VII which were followed by cluster pair VIII and IX. Next lowest inter-cluster was in between clusters III and XI. Similar results were also found by [11] and [12].

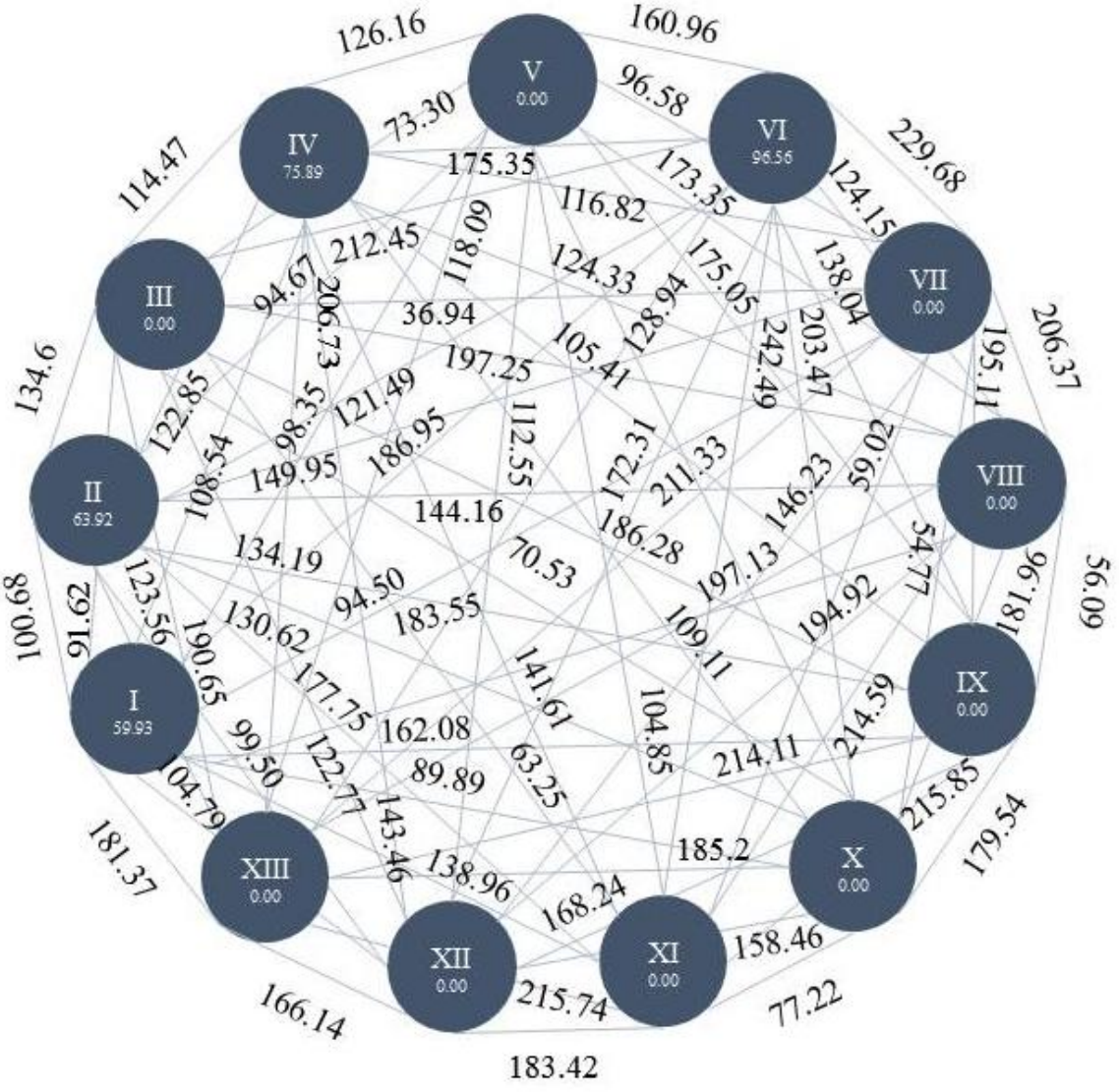

Fig 1. Intra and Inter cluster distance map in D^2 statistics

Table 2: Average intra and inter-cluster distance among 13 clusters formed.

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
I	59.93	100.68	91.62	108.54	98.35	186.95	94.50	183.55	162.08	89.89	138.96	104.79	181.37
II	100.68	63.92	134.60	122.85	94.67	121.49	149.95	144.16	134.19	130.62	177.75	99.50	123.56
III	91.62	134.60	0	114.47	73.30	212.45	36.94	197.25	186.28	70.53	63.25	122.77	190.65
IV	108.54	122.85	114.47	75.89	126.16	175.35	116.82	124.33	105.41	109.11	141.61	143.46	206.73
V	98.35	94.67	73.30	126.16	0	160.96	96.58	173.35	175.05	84.97	104.85	112.55	118.09
VI	186.95	121.49	212.45	175.35	160.96	96.56	229.68	124.15	138.04	203.47	242.49	172.31	128.94
VII	94.50	149.95	36.94	116.82	96.58	229.68	0	206.37	195.11	54.77	59.02	146.23	211.33
VIII	183.55	144.16	197.25	124.33	173.35	124.15	206.37	0	56.09	181.96	214.59	194.92	197.13
IX	162.08	134.19	186.28	105.41	175.05	138.04	195.11	56.09	0	179.54	215.85	168.24	214.11
X	89.89	130.62	70.53	109.11	84.97	203.47	54.77	181.96	179.54	0	77.22	158.46	185.20
XI	138.96	177.75	63.25	141.61	104.85	242.49	59.02	214.59	215.85	77.22	0	183.42	215.74
XII	104.79	99.50	122.77	143.46	112.55	172.31	146.23	194.92	168.24	158.46	183.42	0	166.14
XIII	181.37	123.56	190.65	206.73	118.09	128.94	211.33	197.13	214.11	185.20	215.74	166.14	0

The intra and inter cluster distances were considerably high revealing that genetic diversity was high among genotypes.Cluster VI had highest intra-cluster distance. Higher intra cluster distance indicated higher diversity among the genotypes. Similar result for cluster III was reported by [13].

As far as inter-cluster distance was concerned, high value of inter-cluster distance pointed out towards high amount of diversity between the clusters involved. Hence, from the above discussions, it was concluded that the genotypes from the cluster VI and XI were more divergent than any other cluster. Same results were reported by [14] and [15].

Magnitude of heterosis largely depended on the degree of genetic diversity in the parental lines. Hence, the genotypes belonging to the distinct cluster (VI and XI) could be used in hybridization programme for obtaining a wide spectrum of variability among the segregates. From the results of this investigation, it was found that number of clusters contained at least one genotype with the desirable traits, which ruled out the possibility of selecting directly one genotype for immediate use. Therefore, hybridization between the selected genotypes from divergent clusters is essential to judiciously combine all the targeted traits into a single cultivar or strain which has maximum yield and desirable biochemical parameters.

Contribution of various characters towards genetic divergence

Contribution of various characters towards genetic divergence is presented in the Table 3. The analysis for estimating the contribution of various characters towards the expression of genetic divergence indicated that the traits *viz*., Calcium content (48.00%) followed by iron content (26.24%), zinc content (19.41%) and protein content (5.92%). Two characters contributed very low towards divergence were 1000 seed weight (0.42%) and main ear head length (0.02%).

Looking to the data, all the biochemical parameters contributed maximum to the total genetic divergence, nearly about 99.57 % while only 2 morphological parameters contributed divergence and that too very minimal of 0.44 %. Similar results were also obtained by [16] and [17].

Cluster means for different characters

Cluster mean for all the sixteen characters were presented in Table 4. The results clearly indicated appreciable difference among cluster means for most of the characters.

Table 3: Per cent contribution of each character towards divergence

Sr. No.	Characters	No. of times ranked first	Per cent contribution towards divergence
1	Days to 50 % flowering	0	0.00
2	Days to maturity	0	0.00
3	Plant height (cm)	0	0.00
4	Productive tillers per plant	0	0.00
5	Fingers per ear head	0	0.00
6	Main ear head length (cm)	1	0.02
7	Finger width (cm)	0	0.00
8	Finger length (cm)	0	0.00
9	1000 Grain weight (g)	21	0.42
10	Grain yield per plant (g)	0	0.00
11	Fodder yield per plant (g)	0	0.00
12	Harvest index (%)	0	0.00
13	Protein content (%)	299	5.92
14	Calcium content (mg/100g)	2424	48.00
15	Iron content (mg/100g)	1325	26.24
16	Zinc content (mg/100g)	980	19.41
	Total	5050	100

Clusters I and II had average values for each and every character and neither had minimal or maximal cluster mean value of any trait. Cluster III had minimum mean values for characters *viz*., plant height (99.75cm), 1000 seed weight (1.95 g), grain yield per plant (4.2g) and harvest index (17.72 %) while maximum mean value was found for the trait fingers per earhead (8.50). Fodder yield per plant (17.44g) had minimum mean value in Cluster IV while traits main ear head length (8.06cm) and finger length (4.88cm) had maximum mean values for the same cluster.

Cluster V had lowest mean value for the trait productive tillers per plant (1.70) while highest mean value was found for none of the trait in cluster V. Cluster VI had lowest mean value for none of the traits while highest for plant height (cm) (121.67) and calcium content (mg/100g) (629.24). Cluster VII had lowest and highest cluster mean for

Table 4: Cluster means, their R^2 value and Inter-cluster variation (CV_b%) value for each of the sixteen traits

Character / Cluster	Days to 50% flowering	Days to maturity	Plant height (cm)	Productive tillers per plant	Fingers per ear head	Main ear head length (cm)	Finger Width (cm)	Finger length (cm)
I	77.26	110.29	109.51	2.23	7.16	7.70	1.02	4.70
II	78.97	110.31	113.65	2.15	7.17	7.66	1.05	4.69
III	73.83	106.58	99.75	1.90	8.50	7.14	1.06	4.32
IV	79.25	112.17	108.08	2.47	7.12	8.06	0.95	4.88
V	76.08	109.83	111.05	1.70	7.20	6.70	0.99	3.67
VI	79.01	109.20	121.67	2.20	7.94	7.62	1.05	4.78
VII	80.33	113.42	105.50	2.60	7.40	7.22	1.03	4.28
VIII	76.67	106.67	115.80	2.05	7.35	6.30	0.96	3.90
IX	77.17	108.50	115.75	2.60	7.00	6.28	1.00	3.85
X	73.75	108.83	114.20	2.40	7.55	6.77	0.95	3.90
XI	75.67	113.17	112.05	1.85	7.05	6.65	0.97	3.75
XII	75.67	105.83	103.95	2.90	8.45	5.82	1.02	3.38
XIII	77.42	111.25	116.85	2.40	7.45	7.85	1.13	4.57
R^2	0.46	0.30	0.50	0.62	0.47	0.41	0.39	0.33
CV_b %	4.21	2.88	10.18	18.47	10.80	14.25	8.99	17.20
Harmonic Mean				1.40				

Table 4: Contd….

Character Cluster	1000 Seed weight (g)	Grain yield per plant (g)	Fodder yield per plant (g)	Harvest index (%)	Protein content (%)	Calcium content (mg/100g)	Iron content (mg/100g)	Zinc content (mg/100g)
I	2.22	6.07	18.03	25.13	7.18	216.27	1.59	1.65
II	2.38	6.68	19.93	24.96	7.02	413.94	1.80	1.82
III	1.95	4.20	19.48	17.72	9.90	165.29	1.88	3.47
IV	2.25	6.42	17.45	26.93	7.35	273.89	3.55	1.99
V	2.08	5.55	21.28	20.62	7.96	322.66	1.53	3.72
VI	2.56	7.61	20.66	26.93	5.93	629.24	2.83	2.16
VII	2.65	5.38	18.00	22.90	8.26	105.94	2.02	3.33
VIII	2.65	7.66	25.24	23.21	5.40	524.30	4.95	2.21
IX	2.22	6.80	20.63	24.56	7.39	457.65	4.63	1.09
X	2.75	6.56	17.44	27.30	4.30	175.07	2.22	3.31
XI	2.10	5.01	20.52	19.74	7.92	123.67	2.45	4.78
XII	2.33	6.30	20.28	23.92	13.70	336.30	1.03	1.47
XIII	2.70	8.03	21.39	27.54	5.77	577.28	0.78	3.82
R^2	0.60	0.54	0.43	0.48	0.80	0.97	0.94	0.83
CV_b %	16.74	25.75	16.97	16.45	33.95	114.66	93.52	55.92
Harmonic Mean				1.40				

the traits calcium content (mg/100g) (105.94) and days to 50 % flowering (80.33), respectively.

Cluster VIII had highest mean values for the traits fodder yield per plant (25.24g) and iron content (4.95mg/100g). Cluster IX had lowest mean values for the traits fingers per ear head (7.00) and zinc content (1.09mg/100g). Cluster X had lowest mean value for the traits days to 50 % flowering (73.75), finger width (0.95cm) and protein content (4.30 %) while highest cluster mean value was found for the trait 1000 seed weight (2.75g). Cluster XI had maximum means for the traits days to maturity (113.42) and zinc content (4.78mg/100g). Cluster XII had lowest mean clause for the traits days to maturity (105.83), main ear head length (5.82cm) and finger length (3.38cm) while productive tillers per plant (2.90) and protein content (13.70 %) had highest cluster means. Final cluster XIII had minimal means for the trait iron content (0.78 mg/100g) while maximal means were found for the traits finger width (1.13 cm), grain yield per plant (8.03g) and harvest index (27.54 %).

On the basis of cluster means for different characters, it can be concluded that high yielding genotypes coupled with other important physiological traits could be selected as parents for hybridization programme. Clustering pattern is generally utilized in selection of diverse parents. It is well established that more the genetic divergence in parents used for hybridization programme, the greater will be the heterotic potential of the consequent segregants and chance of appearance of broad-spectrum variability in segregating generations.Genotypes of any cluster with high mean could be used either for direct adoption or for hybridization, followed by selection. Therefore, based upon inter-cluster distances, it is advisable to attempt crossing between genotypes belonging to cluster VI and XI, to derive highly heterotic hybrids or to derive transgressive segregants for traits of interest to improve finger millet.

Inter-cluster variation and R^2 value for each trait

The analysis of variance for each character was carried out using mean of the 101 genotypes. Estimation of inter and intra cluster variances, along with ratio of inter cluster variance to the total variance (R^2) and inter cluster coefficient of variation (CV_b) for 16 characters were worked out and presented in Table 4. Maximum value of $R^2$was observed for calcium content (0.97) followed by iron content (0.94) and zinc content

(0.83), while minimum value of $R^2$was observed for days to maturity (0.30) and finger length (0.33). Similar results were also reported in chick pea and maize by [18] and [19].

From inter cluster coefficient of variation (CV_b), it was observed that the trait calcium content (114.66 %) had maximum variation among the clusters. The next major variation among the clusters was found for the trait iron content (93.52 %) followed by zinc content (55.92 %), protein content (33.95 %), grain yield per plant (g) (25.75 %) and productive tillers per plant (18.47 %). Apart from above mentioned traits, other characters *viz*., finger length (17.20 %),fodder yield per plant (16.97 %), 1000 seed weight (16.74 %), harvest index (16.45 %), main ear head length (14.25 %), fingers per ear head (10.80 %), plant height (10.18 %), finger width (8.99%), days to 50% flowering (4.21 %), days to maturity (2.88 %) had considerable amount of variation present among the clusters as presented in the Table 4. Similar results were also reported in chick pea and maize by [18] and [19].Current population can be used for biochemical traits as it is highly diverse for same trait.

4. CONCLUSIONS

It has been well established fact that more the genetically diverse parents usedin 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 highyielding parents with a high genetic diversity. Therefore, in the present investigation,based upon high yielding genotypes and large inter-cluster distances, it is advisable toattempt crossing of the genotypes from clusters VI and XI as well as VIII and XIII, which may lead to producebroad spectrum of favourable genetic variability for yield improvement in finger millet.

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