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Studies on Genetic Divergence in Foxtail millet (*Setaria italica* (L.) P. Beauv.) Genotypes Grown over Multi season at the Nagaland ecosystem

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Abstract

Foxtail millet cultivation in India's North Eastern Hill region holds promise due to its adaptation to diverse environments and high-quality grain. Analysis of variance (ANOVA) indicated statistically significant differences ($P<0.05$) among the 30 genotypes for all yield variables. D^2 analysis confirmed high genetic diversity among the genotypes and grouped into nine clusters in the first environment, six clusters in the second, seven clusters in the third, ten clusters in the fourth, and five clusters in the pooled environment combination. In environment-1, Cluster-I, IV, V, and VI are largest and having a maximum of five genotypes each. Environment-2 had Cluster-I as the largest with 20 genotypes. Environment-3 had Cluster-I as the largest with 24 genotypes. While environment-4, Cluster-I had largest with 18 genotypes. Finally, when considering pooled environments together, Cluster-I had the largest with 26 genotypes. The foxtail millet genotypes exhibited a wide range of intra-cluster distances in each environment. Clusters VIII and IX showed the highest inter-cluster distance in Environment-1, while clusters III and IV displayed the maximum distance in Environment-2. In Environment-3, Cluster I and VII exhibited the highest distance, and in Environment-4, clusters II and X had the maximum distance. The pooled environment analysis showed clusters III and V with the highest inter-cluster distance. Mahalanobis' D^2 Statistic revealed the percentage contribution of different traits to genetic diversity in different environments. In Environment-1, plant height had the highest contribution (48.74%), while test weight dominated in Environment-2 (31.03%), Environment-3 (53.56%), Environment-4 (36.78%), and the pooled environment analysis (22.30%).

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Introduction

Foxtail millet (*Setaria italica* (L.) P. Beauv.) is a self-pollinating, C4 cereal crop with a rich history of cultivation dating back to 5000-6000 BC along the Yellow River in China.[1] This ancient grain holds significant importance as both a staple food and a valuable source of fodder. Notably, it displays remarkable adaptability to challenging environment conditions such as drought, extreme temperatures, and high soil salinity. Foxtail Millet stands as one of the oldest cultivated millet varieties globally, with a presence in approximately 23 countries across Asia, Africa, and the America.[2]

Foxtail millet is an important crop grown in several parts of the world. According to the Food and Agriculture Organization (FAO) of the United Nations and International Crops Research Institute for the Semi-Arid Tropics, the global production of foxtail millet was estimated to be around 6 million tons in 2023, with India being the largest producer, accounting for more than 50% of the total production. In India, the cultivation of foxtail millet spans across an area of 0.87 lakh hectares, yielding a total production of approximately 0.66 lakh metric tons, and achieving a productivity rate of 762 kilograms per hectare in the 2015-16 period.[3] The International Year of Millets 2023, a United Nations initiative, aims to raise awareness about the significance of millets as a nutritious and sustainable food source, while promoting their cultivation, consumption, and trade. The year 2023 serves as a platform to share knowledge, best practices, and innovations in millet farming, processing, marketing, and consumption.

Genetic diversity in crop plants is essential for sustainable agriculture and food security. It provides a reservoir of different genes that can be tapped into to develop new varieties with improved traits, such as resistance to pests, diseases, and environment stresses like drought or extreme temperatures.[4] Preserving genetic diversity in crop plants is crucial to guard against potential threats to global food production. Plant genetic diversity has been evaluated through morphological and molecular markers. Mahalanobis D^2 statistics provide a robust method for identifying clustering patterns, helping to establish links between genetic and geographical variations. It also aids in exploring the influence of various quantitative traits in achieving maximum divergence.[5]

Although there have been numerous studies on the genetic variation and characterization of Foxtail millet (*Setaria italica*(L.) P. Beauv.) accessions, there is a lack of comprehensive research that specifically investigates the performance of these accessions in the Nagaland ecosystem, particularly focusing on yield and yield components. The Nagaland ecosystem presents unique environment conditions that may influence the growth and productivity of Foxtail millet, yet there is limited information available on how different accessions respond to these conditions. Therefore, a research gap exists in investigating the genetic variation and characterization of Foxtail millet accessions under multi-environments, with a particular emphasis on the Nagaland ecosystem. This research gap can be addressed by conducting a comprehensive study "Studies on genetic divergence in foxtail millet (*Setaria italica* (L.) P. Beauv.) grown over four environments in Medziphema region of Nagaland". Such research would contribute to a better understanding of the genetic potential of Foxtail millet accessions in Nagaland and enable the development of targeted strategies for improving millet cultivation in this region.

Materials and Methods

Experiment Location

The investigation was carried out during July 2022 to May 2023 for four different dates of sowing with twenty-five-day interval (Table 1.). Each sowing date was chosen to create varying environment conditions, including different temperatures and moisture levels throughout the crop growth stages. Two environments maintained under rained condition and the remaining two environments are maintained under irrigated condition with seven days interval. The experiment was conducted at the Research Farm of the Department of Genetics and Plant Breeding School of Agricultural Sciences, Nagaland University located in Medziphema, India.

Table 1: Environment description of the experimental site

Code	Sowing date	Season	Latitude	Longitude	Altitude	Av. Temp		Av. Hum(%)		Rainfall (mm)	Year
						min	Max	min	Max		
Env-1	01-07-2022	*Kharif*	25º 45' 15.95" N	93º 51' 44.71 E	310 MSL	31.66	22.30	91.75	69.64	51.92	2022
Env-2	26-07-2022	*Kharif* (Late)	25º 45' 15.95" N	93º 51' 44.71 E	311 MSL	32.09	22.84	92.10	69.99	55.19	2022
Env-3	01-01-2023	*Summer*	25º 45' 15.95" N	93º 51' 44.71 E	312 MSL	29.11	17.40	94.48	61.84	15.58	2023
Env-4	26-01-2023	*Summer* (Late)	25º 45' 15.95" N	93º 51' 44.71 E	313 MSL	28.28	15.97	95.29	60.11	8.46	2023

Env=Environment, Av. Temp= Average temperature, Av. Hum=Average humidity

Soil Sampling and Analysis

In all four situations, the top 15 cm of soil was randomly selected from the field. The university lab analyzed this composite sample. The materials were dried in shade and pulverized with a glass mortar and pestle to guarantee nutrient distribution homogeneity and plot representation. After sifting, the sample was tested for chemical characteristics and particle size distribution. These tests measured sand, clay, silt, pH, organic carbon (OC), nitrogen (N), potassium (K), and phosphorus. Results are presented at Table 2.

Table 2: Characterization of soil properties of the experimental region

Determination	Field-1	Field-2	Field-3	Field-4
Physical analysis	Value			
Sand (%)	42.8	43.4	42.9	45.1
Silt (%)	24.9	26.7	35.1	34.5
Clay (%)	32.2	29.8	21.9	14.2
Textural classes (USDA)	Clay loam	Clay loam	Loam	Sandy Loam
Chemical analysis			**Value**	
pH	4.68	5.49	6.48	5.74
Organic matter (%)	0.89	0.98	0.94	1.03
Available nitrogen (Kg ha-1)	193.56	197.94	195.75	207.20
Available phosphorus (Kg ha-1)	17.08	17.56	16.05	16.85
Available potassium (Kg ha-1)	124.54	128.36	121.87	120.89

Plant Materials

Thirty genotypes of Foxtail millet, which include one check variety (Surya Nandi), were collected from Indian Institute of Millets Research (IIMR), Hyderabad. These 30 selected genotypes included one check variety were used to assess genetic variability, diversity, and stability across different environments. List of 30 genotypes represented in Table 3.

Table 3: List of selected genotypes based on the mean yield

ACC. No	IC. No	Source	Code
ELS 20	IC 0621991	Andhra Pradesh	G1
FOX 4438	IC 0077702	West Bengal	G2
FOX 4394	IC0610541	Andhra Pradesh	G3
FOX 4339	IC 0597715	Andhra Pradesh	G4
ERP 82	IC 0622113	Tamil Nadu	G5
FOX 4384	IC 0610531	Andhra Pradesh	G6
FOX 4396	IC 0610543	Andhra Pradesh	G7
FOX 4403	IC 0610550	Andhra Pradesh	G8
FOX 4428	IC 0850064	Unknown	G9
ESD 79	IC 0618660	Maharashtra	G10
FOX 4336	IC 0597710	Andhra Pradesh	G11
FOX 4386	IC 0610533	Andhra Pradesh	G12
ERP 26	IC0622071	Tamil Nadu	G13
ESD 3	IC 0618597	Maharashtra	G14
ELS 40	IC 0622003	Andhra Pradesh	G15
ERP 90	IC 0622117	Tamil Nadu	G16
FOX 4478	IC 0078006	Uttar Pradesh	G17
FOX 4489	IC 0078200	Tamil Nadu	G18
FOX 4392	IC 0610539	Andhra Pradesh	G19
FOX 4390	IC 0610537	Andhra Pradesh	G20
FOX 4330	IC 0596783	Arunachal Pradesh	G21
ESD 75	IC 0618657	Maharashtra	G22
ESD 46	IC 0618634	Maharashtra	G23
ERP 57	IC 0622094	Tamil Nadu	G24
FOX 4341	IC 0597722	Andhra Pradesh	G25
FOX 4440	IC 0077761	Gujarat	G26
FOX 4420	IC 0613573	Andhra Pradesh	G27
ELS 36	IC 0621999	Andhra Pradesh	G28
ELS 34	IC 0621998	Andhra Pradesh	G29
Surya Nandi	Check	Andhra Pradesh	G30

Experimental Design and Intercultural Practices

The experiment used a randomized complete block design (RCBD) with three replications for all environments. Each of the three replications had 30 plots (1m x 1m) spaced 10 cm apart, with plants and rows 10cm and 22.5cm apart, respectively. The total plot size was 30m x 5m, accommodating 90 beds. Recommended agricultural practices were followed throughout.

Data Collection

To collect data, a total of fourteen quantitative characteristics of foxtail millet were considered. These characteristics were chosen based on descriptions and guidelines provided by PPV & FR in 2001 (DUS). For each characteristic, data were gathered from five randomly selected plants within each genotype and replication.the quantitative data encompassed various traits, including days to 50% flowering (DF), days to maturity (DM), plant height (PH) (cm), panicle length (PL) (cm), flag leaf length (FL) (cm), flag leaf width (FW) (cm), peduncle length (PDL) (cm), total tiller numbers per plant (NT), panicle width (PW) (cm), biological yield (BY) (g), harvest index (HI) (%), test weight (TW) (g), fodder yield per plant (FY) (g) and grain yield per plant (GY) (g).

Statistical Analysis

The analysis of variance (ANOVA) was conducted using the OPSTAT open-source software to assess the pooled data. The factors considered for variance testing were genotype (G), environment (E), and the interaction between genotype and environment (G×E). Mahalanobis' D^2 statistic serves as a guide for plant breeders, helping them navigate the vast terrain of genotype diversity to develop new cultivars. Cluster groupings are determined following the outlined approach by (Singh and Choudhary, 2010), and the intra-cluster and inter-cluster distances are calculated. The genetic distance 'D' between clusters is determined by taking the square root of the average D^2 values. Using Tocher's method, the genotypes are grouped into clusters based on the ascending order of magnitudes of their D^2 values. Percent contribution towards total divergence was calculated by Mahalanobis D^2 statistic.[6] D^2 analysis is done by INDOSTAST software.

Table 4: Combined Analysis of variance for pooled data

		Mean Squares							
S. No	**Source of Variation**	**Seasons DF=3**	**Rep within Season DF=8**	**Genotypes DF=29**	**Year X Season DF=87**	**Pooled Error DF=232**	**CD for Seasons**	**CD for Geno -types**	**CD for Season X Genotypes**
1	Days to 50% flowering	63,745.27*	2.61	111.69*	34.26*	1	0.63	1.06	2.12
2	Days to maturity	2,39,669.82*	5.16	129.99*	37.73*	1	0.88	1.06	2.12
3	Plant height (cm)	38,415.20*	2.52	125.17*	43.80*	1	0.61	1.06	2.12
4	Panicle length (cm)	262.10*	0.52	36.42*	6.41*	1	0.28	1.06	2.12
5	Flag leaf length (cm)	60.75*	4.87	45.19*	10.65*	2.8	0.85	1.77	3.55
6	Flag leaf width (cm)	1.07*	0.27	1.31*	0.31*	0.06	0.2	0.27	0.53
7	Peduncle length (cm)	989.87*	0.84	23.51*	5.25*	1	0.36	1.06	2.12
8	No. of basal tillers	93.98*	3.98	5.40*	1.79*	1	0.77	1.06	2.12
9	Panicle width cm	188.85*	2.43	16.90*	3.11*	1	0.6	1.06	2.12
10	Biological yield (g)	1,236.78*	1.01	34.30*	5.04*	1	0.39	1.06	2.12
11	Harvest index (%)	2,636.12*	1.71	6.42*	2.97*	1	0.51	1.06	2.12
12	Fodder yield per plant (g)	423.90*	0.91	36.07*	4.50*	1	0.37	1.06	2.12
13	Test weight	1.05*	0	0.36*	0.01*	0	0.01	0.06	0.11
14	Grain yield per plant (g)	490.15*	4.43	60.49*	13.12*	3.2	0.82	1.9	3.79

Results

Analysis of Variance

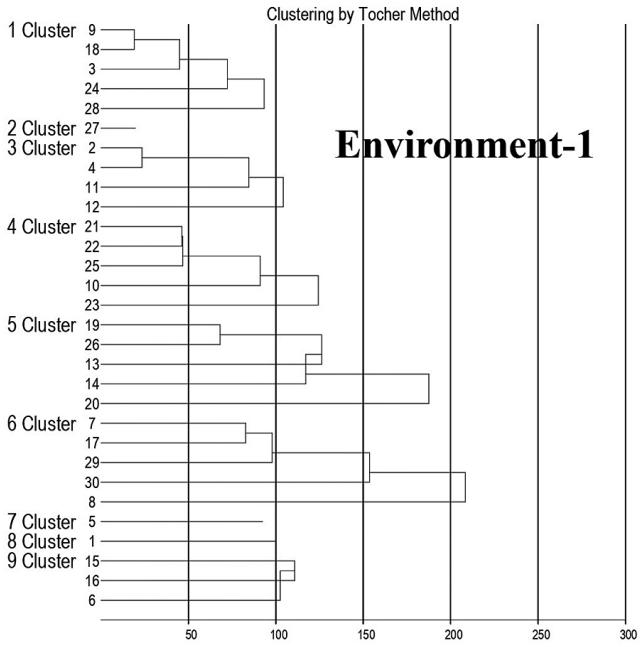
The pooled analysis of variance (ANOVA) was used to examine the interactions between different genotypes and environments. Table 4 presents the results of the combined ANOVA for all genotypes across various environments, focusing on yield and its components. As indicated in Table 4, there were

significant variations observed among the different environments (E), genotypes (G), and the interaction between genotypes and environments (G×E). In fact, all the variables studied showed highly significant differences ($p \leq 0.05$) in terms of the environment, genotype, and genotype-environment interaction. These significant differences suggest that there is a substantial amount of genetic variation among the evaluated genotypes.

Genetic Diversity by Mahalanobis' D^2 Statistic

In the world of plant breeding, the genetic diversity of genotypes is often measured by Mahalanobis' D^2 method. In this study, Mahalanobis' D^2 Statistic was used to group the genotypes into clusters based on their similarities and differences in various traits. These clusters help plant breeders understand the genetic diversity among the foxtail millet genotypes and how they perform under different environment conditions. This study aimed to identify suitable parents for hybridization by analyzing the genetic diversity of 30 foxtail millet genotypes across four environments.

In the study, we observed 30 foxtail millet genotypes in four different environments. The results of the D^2 analysis confirmed the presence of high genetic diversity among the genotypes. We found that there were many differences in the traits among these genotypes. In the first environment, 30 genotypes grouped into nine clusters (Fig 1 (A).) based on their similarities using by Tocher method, followed by six clusters in environment-2 (Fig 1. (B)), seven clusters in environment-3 (Fig 1. (C)), ten clusters in environment-4 (Fig 1. (D)) and five clusters in the pooled environment combination (Fig 1. (E)).



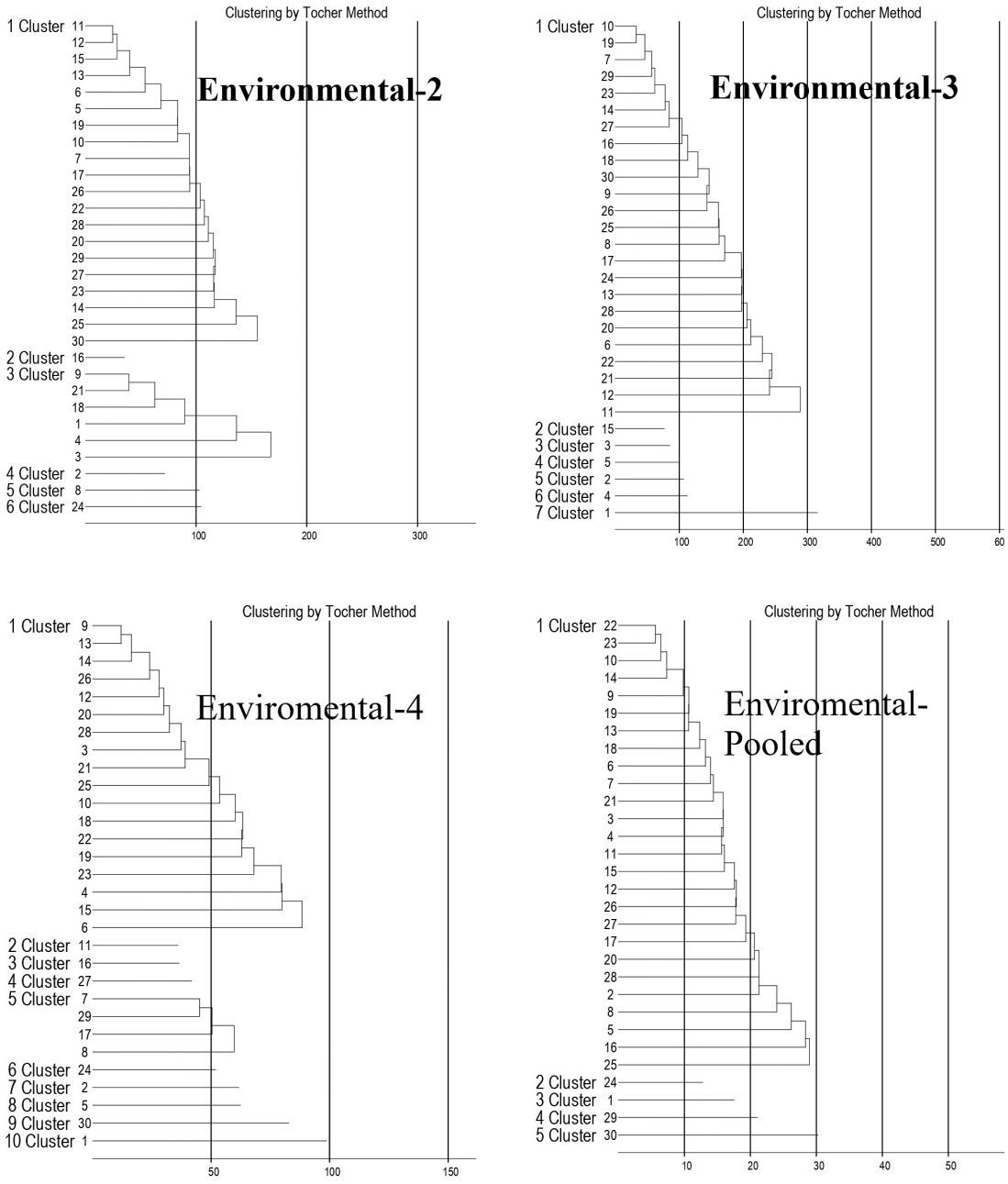


Fig 1: A, B, C, D, E Clustering by Tocher Method over five environments

Across various environment conditions (Tables 5-9), different clusters were identified based on genotype distribution. In Environment-1 (Table 5), four clusters (Cluster-I, IV, V, and VI) exhibited a maximum of five genotypes each. Cluster-III contained four genotypes, while Cluster-IX had three. Cluster-II, VII, and VIII were solitary clusters.

Moving to Environment-2 (Table 6), Cluster-I emerged as the largest, housing 20 genotypes. Following this, Cluster-II contained six genotypes, and Cluster-II, IV, V, and VI stood as solitary clusters. Within Environment-3 (Table 7), Cluster-I remained the largest with 24 genotypes, while mono solitary clusters (Cluster-II, III, IV, V, VI, and

VII) were also observed. Environment-4 (Table 8) reiterated Cluster-I as the largest, encompassing 18 genotypes. Additionally, Cluster-V was identified with four genotypes, and several solitary clusters (Cluster-II, III, IV, VI, VII, VIII, IX, and X) were apparent. However, upon pooling the data from all environments together (Table 9), Cluster-I persisted as the largest with 26 genotypes. This was followed by several mono solitary clusters (Cluster-II, III, IV, and V).

Table 5:Clustering by Tocher Method in Environment-1

Cluster	No. of genotypes	List of genotypes
Cluster. 1	5	G9, G18, G3, G24, G28
Cluster. 2	1	G27
Cluster. 3	4	G2, G4, G11, G12
Cluster. 4	5	G21, G22, G25, G10, G23
Cluster. 5	5	G19, G26, G13, G14, G20
Cluster. 6	5	G7, G17, G29, G30, G8
Cluster. 7	1	G5
Cluster. 8	1	G1
Cluster. 9	3	G15, G16, G6

Table 6:Clustering by Tocher Method in Environment-2

Cluster	No. of genotypes	List of genotypes
Cluster.1	20	G11, G12, G15, G13, G6, G5, G19, G10, G7, G17, G26, G22, G28, G29, G27, G23, G14, G25, G30
Cluster.2	1	G16
Cluster.3	6	G9, G21, G18, G1, G4, G3
Cluster.4	1	G2
Cluster.5	1	G8
Cluster.6	1	G24

Table 7: Clustering by Tocher Method in Environment-3

Cluster	No. of genotypes	List of genotypes
Cluster.1	24	G10, G19, G7, G29, G23, G14, G27, G16, G 18, G30, G9, G26, G25, G8, G17, G24, G13, G28, G20, G 6, G22, G21, G12, G11
Cluster.2	1	G15
Cluster.3	1	G3
Cluster.4	1	G5
Cluster.5	1	G2
Cluster.6	1	G4
Cluster.7	1	G1

Table 8: Clustering by Tocher Method in Environment-4

Cluster	No. of genotypes	List of genotypes
Cluster. 1	18	G9, G13, G14, G26, G12, G20, G28, G3, G21, G25, G10, G18, G22, G19, G23, G4, G15, G6
Cluster. 2	1	G11
Cluster. 3	1	G16
Cluster. 4	1	G27
Cluster. 5	4	G7, G29, G17, G8
Cluster. 6	1	G24
Cluster. 7	1	G2
Cluster. 8	1	G5
Cluster. 9	1	G30
Cluster. 10	1	G1

Table 9: Clustering by Tocher Method in pooled Environment

Cluster	No. of genotypes	List of genotypes
Cluster. 1	26	G22, G23, G10, G14, G9, G19, G13, G18, G6, G7, G21, G3, G4, G11, G15, G12, G26, G27, G17, G20, G28, G2, G8, G5, G16, G25
Cluster. 2	1	G24
Cluster. 3	1	G1
Cluster. 4	1	G29
Cluster. 5	1	G30

Analyzing both intra-cluster and inter-cluster distances provides researchers with valuable insights into data structures, unveiling inherent patterns and associations among observations. In Environment-1 (Table 10), the foxtail millet genotypes displayed a wide range of intra-cluster distances, spanning from 0.00 to 14.26. Notably, Cluster-VI exhibited the highest intra-cluster distance of 14.26, comprising five genotypes. Transitioning to Environment-2 (Table 11), the intra-cluster distances spanned from 0.00 to 12.31. Cluster-III stood out with the highest intra-cluster distance of 12.31, consisting of six genotypes. Within Environment-3 (Table 12), intra-cluster distances varied from 0.00 to 14.25. Cluster-I displayed the highest intra-cluster distance of 14.25, housing 24 genotypes. Moving on to Environment-4 (Table 13), intra-cluster distances ranged from 0.00 to 8.13. Cluster-I showcased the highest intra-cluster distance of 8.13, containing 18 genotypes. Lastly, in the comprehensive pooled environment analysis (Table 14), inter-cluster distances ranged from 4.59 to 0.00. Cluster-I emerged with the highest intra-cluster distance of 8.13, comprising 26 genotypes. These findings indicate that the genotypes within these clusters possess greater genetic diversity, encompassing a broader range of desirable traits. Opting for parents from these clusters can significantly increase the likelihood of producing hybrid progeny with enhanced performance and adaptability in breeding programs.

In the analysis of different environments, using Mahalanobis' D^2 Statistic, inter-cluster distances were observed to vary among foxtail millet genotypes, providing insights into their genetic relationships. In Environment-1 (Table 11.), the inter-cluster distances ranged from 35.09 to 11.25. Clusters VIII and IX showed the maximum inter-

cluster distance (35.09), followed by clusters VII and IX (34.99), clusters I and IX (30.07), clusters VI and IX (27.53), clusters III and IX (26.87), clusters II and IX (26.5), clusters IV and IX (22.52), and clusters V and IX (18.97). Similarly, in Environment-2 (Table 12.), the inter-cluster distances ranged from 22.94 to 11.71. Clusters III and IV exhibited the maximum inter-cluster distance (22.94), followed by clusters II and V (21.72), clusters V and VI (16.53), clusters IV and V (16.39), and clusters III and I (16.04). In Environment-3 (table 13.), the inter-cluster distances ranged from 25.56 to 10.59. Cluster I and VII showed the maximum inter-cluster distance (25.56), followed by clusters VI and VII (25.00), clusters V and VII (24.36), clusters I and V (23.64), clusters III and V (20.22), clusters I and VI (19.94), clusters II and VII (19.94), clusters III and VII (19.85), and clusters IV and VII (19.61). In Environment-4 (table 14.), the inter-cluster distances ranged from 21.35 to 6.46. Clusters II and X exhibited the maximum inter-cluster distance (21.35), followed by clusters V and X (20.61), clusters IV and X (19.4), clusters III and X (19.25), clusters III and VII (19.61), clusters V and VII (18.55), clusters V and VIII (18.12), clusters VII and IX (18.12), clusters VI and X (17.94), clusters IV and VII (16.99), clusters II and IX (16.88), clusters II and VIII (16.71), clusters IX and X (16.71), and clusters VIII and IX (16.71). Finally, in the pooled environment analysis (Table 15.), inter-cluster distances ranged from 8.75 to 6.01. Clusters III and V displayed the maximum inter-cluster distance (8.75), followed by clusters III and IV (8.01), clusters II and V (7.92), clusters I and V (7.02), and clusters IV and V (6.85).

Table 10: Cluster Distances in Environment-1

	Cluster. 1	**Cluster. 2**	**Cluster. 3**	**Cluster. 4**	**Cluster. 5**	**Cluster. 6**	**Cluster. 7**	**Cluster. 8**	**Cluster. 9**
Cluster. 1	9.54	12.23	14.09	15.04	18.28	25.62	13.89	12.78	30.07
Cluster. 2		0.00	14.79	13.94	17.27	17.82	13.26	16.00	26.50
Cluster. 3			10.76	16.02	16.32	25.78	15.98	19.63	26.87
Cluster. 4				10.83	15.71	20.53	19.33	18.28	22.52
Cluster. 5					13.61	24.99	24.27	24.23	18.97
Cluster. 6						14.26	24.56	26.12	27.53
Cluster. 7							0.00	11.25	34.99
Cluster. 8								0.00	35.09
Cluster. 9									11.81

Table 11: Cluster Distances in Environment-2

	Cluster. 1	**Cluster. 2**	**Cluster. 3**	**Cluster. 4**	**Cluster. 5**	**Cluster. 6**
Cluster.1	10.9	14.0	16.0	13.2	13.9	14.1
Cluster.2		0.0	12.9	19.1	21.7	20.2
Cluster.3			12.3	15.5	22.9	20.1
Cluster.4				0.0	16.4	11.7
Cluster.5					0.0	16.5
Cluster.6						0.0

Table 12: Cluster Distances in Environment-3

	Cluster. 1	Cluster. 2	Cluster. 3	Cluster. 4	Cluster. 5	Cluster. 6	Cluster. 7
Cluster.1	14.25	17.82	18.50	18.72	23.64	19.94	25.56
Cluster.2		0.00	13.48	11.74	13.54	10.61	19.94
Cluster.3			0.00	10.59	20.22	14.51	19.85
Cluster.4				0.00	12.27	16.63	19.61
Cluster.5					0.00	19.38	24.36
Cluster.6						0.00	25.00
Cluster.7							0.00

Table 13: Cluster Distances in Environment-4

	Cluster. 1	Cluster. 2	Cluster. 3	Cluster. 4	Cluster. 5	Cluster. 6	Cluster. 7	Cluster. 8	Cluster. 9	Cluster. 10
Cluster. 1	8.13	11.20	11.24	10.09	11.64	11.08	12.88	11.14	11.77	14.68
Cluster. 2		0.00	15.07	11.95	12.17	12.50	14.60	16.71	16.88	21.35
Cluster. 3			0.00	6.46	11.25	13.84	19.61	13.92	14.65	19.25
Cluster. 4				0.00	9.98	11.90	16.99	13.73	14.30	19.40
Cluster. 5					8.51	13.92	18.55	18.12	12.52	20.61
Cluster. 6						0.00	13.87	13.79	14.77	17.94
Cluster. 7							0.00	10.66	18.12	12.32
Cluster. 8								0.00	16.46	9.94
Cluster. 9									0.00	16.71
Cluster. 10										0.00

Table 14: Cluster Distances in Pooled Environment

	Cluster. 1	Cluster. 2	Cluster. 3	Cluster. 4	Cluster. 5
Cluster. 1	4.59	6.26	6.01	6.45	7.02
Cluster. 2		0.00	6.49	6.77	7.92
Cluster. 3			0.00	8.01	8.75
Cluster. 4				0.00	6.85
Cluster. 5					0.00

Cluster mean, represents the average values of all the variables for the data points belonging to a particular cluster. In Environment-1 (Table 15), Clusters II, VII, and VIII each consist of single genotypes. G27 in Cluster II stands out with long flag leaf, a high harvest index, and grain yield per plant. G5 and G1 in Clusters VII and VIII respectively show higher peduncle length and greater biological yield. Cluster I with 5 genotypes, demonstrates higher days to flowering, days to maturity and grain yield per plant. Cluster III has 4 genotypes, these genotypes are exhibits highest cluster mean of peduncle length, number of base tillers, and highest test weight. Cluster V has 5 genotypes that are exhibited an average performance of all traits. Cluster VI had 5 genotypes, which are exhibits lowest values of all traits. Cluster IX had 3 genotypes, which are exhibits highest number of tillers per plant.

In Environment-2 (Table 15.), clusters II, IV, V, and VI are solitary clusters those are containing G16, G2, G8, and G24 genotypes, respectively. These exhibits

high to moderate performance of yield traits. Cluster III had 6 genotypes, which are exhibits highest cluster mean values of days to flowering, days to maturity, biological yield, harvest index, fodder yield and grain yield per plant. Cluster I had 20 genotypes which are exhibits average cluster mean value of all traits. In Environment-3 (Table 16.), clusters II, III, IV, and V is solitary. Those are containing G15, G3, G5, G2, G4, and G1, respectively. These exhibits high fodder yield and grain yield per plant. Cluster-I have 24 genotypes those are exhibits average cluster mean of all traits.

In Environment-4 (Table 16.), Cluster I have 18 genotypes those are exhibits the lowest cluster mean of peduncle length and remain traits exhibit average cluster means. Cluster V has 4 genotypes, those are exhibiting the lowest days to flowering. Cluster II is mono solitary (G11) which is exhibits the lowest days to maturity, panicle length and harvest index. Cluster-III, IV, VI, VII, VIII, IX, and X are mono solitary clusters and those are containing G16, G27, G7, G24, G2, G5, G30, and G1 respectively and exhibits high to moderate performance of yield traits. In pooled environment (Table 19.), cluster-II, III, IV and V are solitary, these are containingG24, G1, G29 and G30 respectively. G24 exhibits lowest cluster means of the most of traits. G1 exhibits highest cluster means of the most of traits. G29 and G30 both are exhibits lowest days to flowering, and days to maturity. Cluster-I have 26 genotypes which are exhibits average cluster mean values.

Table 15: Cluster means on Environment 1, 2, 3.

	DF	DM	PH	PL	FL	FW	PDL	NT	PW	BY	HI	FY	TW	GY
Cluster Mean in Environment-1														
Cluster.1	74.79	114.99	133.42	14.47	23.77	2.23	22.47	3.7	2.15	41.07	50.49	20.39	2.83	20.67
Cluster.2	70.97	110.4	133.13	13.47	28.13	2.8	24.57	4.2	2.47	37.33	54.17	17.1	2.87	20.23
Cluster.3	74.72	112.71	124.47	11.12	22.21	1.79	18.48	4.13	1.69	35.17	46.34	18.88	3.22	16.3
Cluster.4	72.39	112.78	118.43	19.27	22.04	1.45	19.7	3.71	1.81	38	50.69	18.74	2.9	19.27
Cluster.5	73.08	113.63	108.3	10.67	21.24	2.05	20.31	3.85	1.9	34.73	50.97	17.25	2.94	17.49
Cluster.6	65.37	104.36	126.67	15.28	21.69	2.43	22.23	4.09	2.07	34.87	48.1	18.06	2.83	16.81
Cluster.7	71.17	110.6	147.67	13.3	26	1.7	27.6	4.23	2.5	41.5	42.53	23.83	3.13	17.67
Cluster.8	70.73	115.73	145.73	15.37	22.6	1.63	27.07	3.27	1.77	42.1	43.1	23.97	2.87	18.13
Cluster.9	69.62	110.12	87.04	13	23.26	2.18	23.33	4.43	1.61	35.5	48.93	18.18	3.06	17.32
Cluster Mean in Environment-2														
Cluster.1	70.3	109.47	107.21	13.68	20.66	1.77	19.33	3.57	1.76	29.4	44.01	16.5	2.7	12.9
Cluster.2	76.9	118.93	93.6	14.57	23.6	2.13	17.1	3.9	1.63	29.63	44.1	16.57	2.43	13.07
Cluster.3	80.01	119.77	110.73	13.76	21.77	1.63	20.25	3.52	1.73	38.19	42.37	22.06	2.86	16.14
Cluster.4	71.53	109.83	125.63	12.77	22.9	1.8	23.4	4.03	1.6	30.07	45.63	16.37	3.17	13.73
Cluster.5	65.97	97.17	113.7	17.97	20.4	2.17	23.7	3.7	2.1	35.6	44.37	19.73	2.67	15.83
Cluster.6	66.33	106.33	126.67	11.47	18.4	1.47	27.37	3.53	1.37	19.6	49.1	10.13	2.87	9.47
Cluster Mean in Environment-3														
Cluster.1	69.82	109.79	107.58	14.42	20.67	1.92	19.66	3.36	1.82	30.23	43.56	17.04	2.68	13.19
Cluster.2	75.77	115.13	86.23	13.17	22.07	2.5	22.93	3.33	1.7	30.17	40.4	18.03	2.96	12.2
Cluster.3	85.93	121	93.57	8.97	20.37	1.2	17.13	3.03	1.2	36.57	36.87	23.03	2.74	13.53
Cluster.4	79.27	114.17	121.23	14.17	25.43	1.33	22.57	3.03	1.97	41.87	46.63	22.37	2.84	19.5
Cluster.5	73.23	106.83	124.23	17.23	25.27	1.8	24.23	4.1	1.63	32.23	44.83	17.8	3.12	14.47
Cluster.6	76.13	113.87	63.73	6.8	17.27	1.27	14.87	3.07	1.4	23.3	42.27	13.5	2.96	9.83
Cluster.7	76.8	129.93	131.7	15.2	22.2	1.3	23.43	3.37	1.57	46.47	44.6	25.77	2.83	20.73

Table 16: Cluster means on Environment 4 and pooled.

	DF	DM	PH	PL	FL	FW	PDL	NT	PW	BY	HI	FY	TW	GY
Cluster Mean in Environment-4														
Cluster. 1	72.81	111.75	107.71	13.24	20.54	1.89	18.76	3.61	1.79	32.22	42.24	18.6	2.74	13.63
Cluster. 2	66.4	98.83	98.7	7.67	17.83	1.77	20.33	3.27	1.5	26.63	37.73	16.57	2.93	10.03
Cluster. 3	73.37	116.4	82.27	12.77	23.63	2.2	20.97	3.93	1.5	28.63	40.4	17.03	2.43	11.63
Cluster. 4	70.33	110.33	91.63	12.27	24.1	1.9	21.1	5.07	1.37	29.87	47.83	15.33	2.63	14.53
Cluster. 5	65.62	101.88	113.67	15	20.78	2.02	23	3.63	1.66	28.37	45.99	16.81	2.55	11.8
Cluster. 6	73.33	113.33	119.63	12.77	25.33	2	19.27	2.27	1.47	12.07	52.53	5.67	2.87	6.4
Cluster. 7	76.77	106.6	131.4	16.63	24.83	1.77	25.43	3.97	1.6	32.07	41	18.93	3.17	13.13
Cluster. 8	81.47	116.63	104	13.97	25.23	1.57	23.27	3.63	1.63	38.4	42.1	22.2	2.83	16.17
Cluster. 9	72	106.07	132.67	16.8	15.43	1.63	18.9	3.47	2.87	29.87	47.73	15.63	2.47	14.27
Cluster. 10	80.5	123.23	132.43	15.47	22.53	1.87	26.93	3.33	1.77	48.07	43.7	27.07	2.8	21
Cluster Mean in Pooled Environment														
Cluster. 1	71.92	110.97	109.09	13.78	21.48	1.91	20.04	3.64	1.79	33.01	44.72	18.27	2.8	14.78
Cluster. 2	72.13	112.26	122.22	12.77	22.29	1.73	21.76	2.84	1.49	21.75	49.33	10.97	2.88	10.78
Cluster. 3	76.46	122.99	133.6	14.5	22.16	1.5	24.78	3.42	1.61	46.04	43.75	25.91	2.83	20.14
Cluster. 4	66.24	106.64	120.38	13.92	22.51	1.95	24.63	4.61	1.82	24.23	44.46	13.4	2.59	10.82
Cluster. 5	71.63	106.08	131.69	16.96	16.73	1.63	19.99	3.41	2.58	28.87	47	15.26	2.52	13.61

In Mahalanobis' D^2 Statistic, the percentage contribution to genetic diversity is represented by the eigenvalues associated with the principal components used in the analysis. The eigenvalues provide information about the amount of variance explained by each principal component. In this study, Environment-1 (Table 17.), plant height had the highest contribution to the total genetic divergence (48.74%), appearing 212 times in the first rank. It was followed by days to flowering (21.84%) with 95 times in the first rank, test weight (11.49%) with 50 times in the first rank, and panicle length (7.13%) with 31 times in the first rank. Together, these four traits accounted for 89.2% of the total diversity in Environment-1. In Environment-2 (Table 17.), test weight played the most significant role in the total genetic divergence (31.03%), being ranked first 135 times. Days to flowering (20.69%) followed, ranked first 90 times, then days to maturity (17.24%) in first rank with 75 times, and biological yield (7.13%) in first rank with 31 times. These four traits together contributed to 76.09% of the total diversity in Environment-2. In Environment-3 (Table 17.), test weight had the highest contribution to the total genetic divergence (53.56%), ranked first 233 times. Days to flowering (13.56%) ranked first 59 times, followed by days to maturity (14.94%) ranked first 65 times, and biological yield (5.74%) ranked first 25 times. These four traits collectively accounted for 87.81% of the total diversity in Environment-3. In Environment-4 (Table 17), test weight had the greatest contribution to the total genetic divergence (36.78%), ranked first 160 times. Days to flowering (18.85%) ranked first 82 times, biological yield (15.17%) ranked first 66 times, and plant height (5.52%) ranked first 24 times. Together, these four traits contributed to 76.32% of the total diversity in Environment-4. In the pooled environment analysis (Table 17.), test weight showed the highest contribution to the total genetic divergence (22.30%), ranked first 97 times. Panicle width (17.24%) ranked first 75 times, followed by flag leaf width (10.57%) in first rank with 46 times, and biological yield (9.43%) ranked first 41 times. These four traits collectively accounted for 59.54% of the total diversity in the pooled environment analysis.

Table 17: Per cent contribution of different traits towards total divergence of different environments

Source	TR	CB	TR	CB	TR	CB	TR	CB	TR	CB
	E1		E2		E3		E4		POOLED	
1 Days to 50% flowering	95	21.84%	90	20.69%	59	13.56%	82	18.85%	9	2.07%
2 No. of Days to maturity	24	5.52%	75	17.24%	65	14.94%	19	4.37%	3	0.69%
3 Plant height (cm)	212	48.74%	19	4.37%	21	4.83%	24	5.52%	10	2.30%
4 Panicle length (cm)	31	7.13%	22	5.06%	3	0.69%	13	2.99%	38	8.74%
5 Flag leaf length (cm)	2	0.46%	2	0.46%	5	1.15%	8	1.84%	14	3.22%
6 Flag leaf width (cm)	4	0.92%	16	3.68%	5	1.15%	9	2.07%	46	10.57%
7 Peduncle length (cm)	1	0.23%	2	0.46%	8	1.84%	11	2.53%	29	6.67%
8 No. of basal tillers	0	0.00%	2	0.46%	1	0.23%	13	2.99%	34	7.82%
9 Inflorescence width (cm)	0	0.00%	20	4.60%	10	2.30%	13	2.99%	75	17.24%
10 Biological yield (g)	5	1.15%	31	7.13%	25	5.75%	66	15.17%	41	9.43%
11 Harvest index (%)	5	1.15%	9	2.07%	0	0.00%	2	0.46%	2	0.46%
12 fodder yield per plant (g)	6	1.38%	12	2.76%	0	0.00%	15	3.45%	21	4.83%
13 Test weight	50	11.49%	135	31.03%	233	53.56%	160	36.78%	97	22.30%
14 grain yield per plant (g)	0	0.00%	0	0.00%	0	0.00%	0	0.00%	16	3.68%
Tocher Cut-off Value	126.44			171.46		315.85		98.78		30.28

TR=Times Ranked 1st, CB=Contribution %

Discussion

Analysis of Variance

There was significant variation observed in the pooled analysis of variance for the 14 traits across the 30 foxtail millet genotypes in the four environments. This information provides valuable insights for breeders to make informed decisions on genotype selection, trait prioritization, and targeted breeding strategies. Similar reports are found in foxtail millet.[7,8] In this research, significant divergence was observed among genotype-environment interactions and genotype effects.

Genetic Diversity by Mahalanobis' D^2 Statistic

In this study, Mahalanobis' D^2 Statistic was utilized to group the genotypes into clusters, considering their similarities and differences in various traits across four environment datasets and pooled data as well. Mahalanobis' D^2 Statistic was employed to facilitate the clustering process, taking into account the multivariate traits and their respective distances to establish meaningful groupings.[9] The observed variation in the number of clusters across environments can be attributed to the influence of different environment conditions on the expression of traits in the genotypes.[10] Environment factors such as temperature, humidity, soil type, and photoperiod can significantly impact the phenotypic expression of traits in plants. As a result, genotypes that exhibit similar trait profiles in one environment may show different trait patterns in another environment, leading to the formation of distinct clusters.[10]

Cluster distance in Mahalanobis' D^2 Statistic refers to the distance between clusters of data points in a multivariate space. It measures the dissimilarity or similarity between different groups of data points based on their mean vectors and covariance matrices.[11] The cluster distance helps to identify how distinct or similar the clusters are, providing insights into the genetic divergence or similarity between groups of genotypes in plant breeding.[12] Inter-cluster distance quantifies the dissimilarity between different groups of genotypes, while intra-cluster distance measures the variability or spread of data points within each cluster.[13]

The inter-cluster distances provide insights into the genetic relationships and relatedness among clusters. Larger inter-cluster distances suggest greater dissimilarity and differentiation between clusters, indicating distinct and genetically diverse

groups.[12] On the other hand, smaller inter-cluster distances suggest closer genetic relationships and similarities between clusters, possibly sharing common traits or ancestry.[13]

Parental selection is a crucial step in plant breeding, aiming to identify diverse and superior genotypes for hybridization. In Environment-1, Cluster-VI with five genotypes and Cluster-III in Environment-2 with six genotypes have the highest intra-cluster distances (14.26 and 12.31, respectively). Similarly, in Environment-3, 4, and the pooled combination, Cluster-I with 24, 18, and 26 genotypes, respectively, exhibits the highest intra-cluster distances. These findings indicate that the genotypes within these clusters possess greater genetic diversity, encompassing a broader range of desirable traits. Opting for parents from these clusters can significantly increase the likelihood of producing hybrid progeny with enhanced performance and adaptability in breeding programs.

In this present study, environment-1, the inter-cluster distances range from 11.25 to 35.09. The presence of diverse genotypes from different geographical regions in this environment might contribute to the significant genetic differentiation observed between clusters.[16] Environment-2, The inter-cluster distances in this environment range from 22.94 to 11.71. The genotypes in this environment may have some level of geographical overlap with those in Environment-1, leading to some similarity in cluster patterns.[17] However, the different environment conditions still contribute to variations in genetic relationships among clusters. Environment-3,inter-cluster distances in this environment vary from 25.56 to 10.59.Similar to Environment-2, there might be some geographical overlap with previous environments, but unique environment factors lead to distinctive genetic relationships and cluster formations.16Environment-4, the inter-cluster distances in this environment range from 21.35 to 6.46, while Pooled Environment Analysis, the inter-cluster distances in this analysis range from 8.75 to 6.01. The pooled analysis includes genotypes from various geographical regions and environment conditions. As a result, the pooled data may show smaller inter-cluster distances compared to some individual environments due to the broader representation of genotypes.[18]

In summary, geographical distribution plays a significant role in shaping the genetic diversity and relationships among foxtail millet genotypes in different environments.[9] Geographical factors can lead to the presence of distinct genotypes with specific adaptations, resulting in diverse cluster formations and inter-cluster distances. The environment conditions in each specific region further influence the genetic expression of these genotypes, leading to variations in intra-cluster distances as well.[12] By considering the geographical distribution and environment factors, researchers and breeders can better understand the genetic relationships among foxtail millet genotypes and make informed decisions for crop improvement and breeding programs tailored to specific regions and environments. Several studies reported similar results on genetic divergence in various millet genotypes using Mahalanobis' D^2 statistic,[14,15] reported highest inter-cluster are suitable for inter-varietal hybridization to obtain desirable recombinants.

In Mahalanobis' D^2 Statistic, the percentage contribution to genetic diversity is represented by the eigenvalues associated with the principal components used in the analysis. The eigenvalues provide information about the amount of variance explained by each principal component. When conducting cluster analysis using Mahalanobis' D^2 Statistic, the data is transformed into a multidimensional space, and the first few principal components are selected to represent the most significant sources of variation in the data.[19] The eigenvalues associated with these principal components indicate the proportion of total variance explained by each component. These percentages represent how much of the total genetic diversity in the data is attributed to each principal component. The higher the percentage contribution of a component, the more important it is in explaining the genetic variation among the genotypes.[20] Understanding the percentage contributions helps researchers prioritize the most influential components and focus on the key sources of genetic diversity in their analysis.

In this study, in Environment-1 plant height is dominance of contributing to genetic divergence may indicate its strong influence on the overall variability observed in this environment. In Environment-2, test

weight played the most significant role in the total genetic divergence (31.03%), being ranked first 135 times. In Environmental-3, test weight (g) had the highest contribution to the total genetic divergence (53.56%), ranked first 233 times. In Environment-4, test weight had the greatest contribution to the total genetic divergence (36.78%), ranked first 160 times. In the pooled environment analysis, test weight showed the highest contribution to the total genetic divergence (22.30%), ranked first 97 times.

Conclusion

Based on the cluster means comparison results from different environments, genotypes G1, G25, G22, G21, and G5 showed the highest yield and yield traits performance across different environments, making them the ideal genotypes for further utilization in breeding programs. The study's findings revealed that certain clusters, such as Cluster-VI in Environment-1, Cluster-III in Environment-2, and Cluster-I in Environment-3, Environment-4, and the pooled combination, exhibit the highest intra-cluster distances, indicating greater genetic diversity and desirable trait variations. Selecting parents from these clusters can enhance the performance and adaptability of hybrid progeny in breeding programs. Additionally, clusters with higher inter-cluster distances demonstrate considerable genetic variation, making them suitable for developing diverse and distinct varieties with a broader range of traits. By strategically selecting clusters based on their genetic characteristics, plant breeders can optimize breeding programs to develop improved and resilient foxtail millet varieties to address various agricultural challenges.

Test weight consistently emerged as a significant contributor to genetic divergence in all environments, indicating its importance in shaping the observed variability. Other traits such as days to flowering, days to maturity, and plant height also played crucial roles in certain environments, emphasizing their impact on the overall genetic diversity. Understanding the reasons behind the diversity of these traits provides valuable insights for crop improvement strategies and targeted breeding efforts to develop foxtail millet varieties with desirable traits and enhanced adaptability in diverse environment conditions.

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Conflict of Interest

The authors declare not to have any conflict of interest.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Authors' Contributions

Conceptualization, D. P. Rao, Writing—original draft preparation and HPC, Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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