Exploring genetic and molecular diversity in land races of finger millet (Eleusine coracana (L.) Gaertn)

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Abstract

The present study was conducted to characterize landraces of finger millets using DUSN (Distinctness, Uniformity, Stability, and Novelty) descriptors, yield-contributing traits, and molecular markers. Significant variations were observed among landraces, particularly in ear shape, ear head length, and seed colour. The predominance of dark-colored seeds suggested the potential use in developing nutritionally rich, wide varieties. The Shannon diversity index ranged from 0.598 to 1.317, indicating a broad spectrum of variability. Most of the traits exhibited high heritability coupled with high genetic advance, suggesting additive gene action and suitability for improvement through selection. Seed yield showed a significant positive correlation with traits like flag leaf width, productive tillers per plant, harvest index, biological yield, and flowering time, highlighting their importance in yield enhancement. Principal component analysis identified seven principal components with eigenvalues >1, which explained about 76.94% of the total variation. Molecular diversity analysis using SCoT and SSR markers revealed high polymorphism, with markers SCoT2, SCoT3, SCoT7, SCoT8, SCoT9, SCoT11, SCoT14, SCoT20, SCoT26, SCoT31, SSR1, SSR3, SSR4, SSR5, SSR7, SSR8, SSR10, SSR12, SSR19 and SSR20 being particularly informative due to high PIC values. Cluster analysis grouped genotypes into three major clusters, with the greatest genetic divergence observed between clusters II and III, indicating their potential for generating maximum variability in breeding programs. The findings provided valuable information that is useful for genetic improvement in finger millet.

Keywords: Cluster analysis, SCoT, SSR, Polymorphism, Molecular diversity,

1. Introduction

Millets are a highly varied group of small-seeded grasses, widely grown around the world as cereal crops for human food, feed, and fodder for cattle[1]. These crops are climate-resilient and best suited to the semi-arid and tropical regions of the globe[2,3]. Finger millet is grown as an annual crop and is botanically known as *Eleusine coracana*[4]. It is commonly known as Ragi in India, Kodo in Nepal, Bulo in Uganda and Tellebun in Sudan[5]. It was believed to have originated in the East African highlands (Ethiopia and Uganda) and was further spread throughout Africa through domestication[6]. It belongs to the grass family Poaceae, subfamily Chloridoideae and is tetraploid in nature with chromosome number 2n = *4x* = 36 (genome composition *AABB*)[7]. It is a highly self-pollinated crop with 97 to 99% of self-pollination[8], and less than 1% cross-pollination occurs by wind or insects[9]. It is the most important small millet in the tropics, covering 12% of global millet area. In India, it is grown on an area of 2.0 million hectares with a production of 2.15 million tonnes and productivity of 1390 kg/ha, which accounts for 45 % of the world's cultivated area and 55 % of the world's production[10].

Finger millet, a nutritionally-rich minor grain, is renowned for its exceptional calcium content (220–450 mg/100 g), which is 5–30 times higher than that of other grains[11]. It comprises 81.5% carbohydrates, 9.8% protein, 3–20% iron, 4.3% crude fiber, and 2.7% minerals, outperforming cereals like rice, wheat, and maize in crude fiber and mineral content[12]. It supplies vital amino acids, including isoleucine (4.4 g), leucine (9.5 g), methionine (3.1 g), and phenylalanine (5.2 g), alongside vitamins B (niacin, B6, folic acid), and key minerals such as potassium, magnesium, and zinc.

Characterisation and evaluation of germplasm are important links between the conservation and utilisation of plant genetic resources. The usefulness of germplasm in the study of plant genetic resources could play an important role in generating and developing new hybrids and high-yielding varieties with disease-resistant traits to cope with adverse biotic and abiotic challenges. The genotypic characterization could reveal the genetic relatedness within these plant species. This will be useful in the conservation of new species and understanding of their genetic diversity. It may further provide information on the plant taxonomy as well as the ecology of the plant[13]. The availability of genetic diversity is a prerequisite for the genetic improvement of a crop. There is a great genetic variation among varieties and germplasm of finger millet. Besides the availability of genetic diversity, their characterization is essential for effective utilization in crop improvement[14]. Phenotypic characterisation of genotypes is based on morphological markers which expression is much influenced by the environment. In this regard, diversity analysis using molecular marker is advantageous over morphological characterization since the environment has no effect on it and is time and cost-effective, show polymorphism effectively[15].

The evaluation of genetic diversity and the clarification of genetic relationships within and among species are both made possible by the use of molecular markers[16]. DNA-based marker technology now available is non-destructive, unaffected by environmental influences, requires less amount samples, and requires a minimal experimental setup[17]. Start codon targeted polymorphism (SCoT) targets on short ATG start codons in plant genes have been reported[18]. SCoT marker is gaining popularity for its superiority over other dominant DNA marker systems like RAPD and ISSR for higher polymorphism, better marker resolvability and reliable bands which can be used for effective population studies, genetic mapping in different plants and marker-assisted selection (MAS) programs[19]. SCoT markers have been successfully

employed in genetic diversity analysis and fingerprinting of several agricultural and horticultural crop species[20]. SSR genotyping involves the use of simple sequence repeats (SSRs) as DNA markers. These markers are highly polymorphic, meaning they vary greatly between individuals, which makes them useful for distinguishing between different genetic profiles[21].

2. Material and methods

The present study comprised 48 accessions, including 45 pre-selected plant materials and four checks, i.e. VL376, RAU03, and VL352 (Supplementary Table S1). The checks are well-adapted varieties and are grown across India. The experiment was conducted during *Kharif* 2021 in an augmented block design (each block contains nine land races) at the Research Farm, College of Agriculture, Banda University of Agriculture and Technology, Banda, India (25.5 °N, 80.3 ^{0}E, and 113 MSL). Genotyping was performed at the molecular plant breeding laboratory, Department of Genetics and Plant Breeding, BUAT, Banda. Standard agronomic practices were followed to raise the crop[6].

2.1. Morphological traits phenotyping

The morphological traits of finger millet land races were evaluated using DUS guidelines set by the Protection of Plant Varieties and Farmers' Rights Authority (2007) and UPOV standards. Observations on yield and related traits were recorded from ten randomly selected plants per accessions, including days to 50% flowering (days) (DF), plant height (cm) (PH), productive tillers per plant (PTPP), number of leaves per plant (NLPP), flag leaf area (cm^2) (FLA), peduncle length (cm) (PL), ear length (cm) (EL), number of fingers per plant (NFPP), finger length (cm) (FL), finger width (cm) (FW), days to maturity (days) (DM), 1000 seed weight (g) (SW), Harvest index (%) (HI), protein content (%) (PC) and seed yield per plant (g) (SYPP) (Supplementary Table S2).

2.2. Protein estimation

Nitrogen (%) was estimated *via* digestion (Kelplus Classica-DX VATS(E) and protein (%) was calculated by multiplying by (6.25)[22]. This approach provides a reliable estimate of protein levels in the sample, utilizing the established relationship between nitrogen content and protein concentration to quantify the nutritional or functional components present. This method is crucial for analysing biological samples in various research contexts.

2.3. Genomic DNA isolation and quantification

Genomic DNA was extracted from fresh leaves using the modified CTAB method[23] and Quantified with a NanoDrop One Microvolume UV-Vis Spectrophotometer (Thermo Scientific, USA).

2.4. SCoT and SRR analysis and PCR conditions

PCR amplifications were performed on 48 finger millet land races using 55 publicly available primers of SCoT and SSR (Supplementary Table S3). PCR reaction was performed in a 20 µl mix (2 µl template DNA, 2 µl primer at 10 pMol, 10 µl master mix, and 6 µl nuclease-free water) using a Thermo Fisher Veriti Thermal cycler. Amplification conditions were set up as initial denaturation at 95°C for 35 cycles of denaturation at 94°C for 30s, primer-specific annealing at 52–60°C for 30s and extension at 72°C for 2 minutes (35 cycles), followed by final extension at 72°C for 7 minutes. PCR products were separated on a 1.5-3.0% agarose gel,

Invitrogen Ultrapure agarose (Thermo Fisher) with ethidium bromide in 1x TAE buffer and visualised using a Gel Doc system (E-box, Eppendorf).

2.5. Statistical analysis

Morphological data were analyzed using R Software (v4.4.3). to study the diversity. Genetic parameters, including major allele frequency, allele number, gene diversity, heterozygosity, polymorphic information content (PIC) and inbreeding coefficient, were estimated using Power Marker v3.25 [6]. Cluster analysis was performed using the unweighted pair-group method with arithmetic average (UPGMA) on Jaccard's similarity coefficient, via FREE TREE and TREE VIEW. Analysis of the molecular variation (AMOVA) was conducted using GenALEx v6.5 to partition genetic variation among regions, populations, and within populations.

3. Results

3.1. Morphological characterisation

A distinctiveness test was conducted through visual scoring of 48 land races during the growing season. Significant variation was observed in both qualitative and quantitative traits ((Supplementary Table S4), Key traits sowing notable diversity included plant pigmentation at leaf juncture (0.598), stem clum branching (0.682), ear shape (1.211), finger branching position (0.667), flag leaf blade length (0.770 cm), number of productive tillers per plant (0.329), plant height at maturity (0.598), seed color (1.177), ear head length (1.317 cm), finger length (0.852), seed shape (0.834), and seed surface (0.940) (Supplementary Table S4).

3.2. Analysis of variance (ANOVA)

The ANOVA conducted for block mean squares was non-significant across all traits except for DF. The Genotype vs. Check revealed significant mean squares for all traits at the significance level ($p < 0.01\%$), excluding DF, PTPP, EL, FW, and SW in Table 1. Additionally, the ANOVA of treatment and genotypic mean squares showed significant differences for all traits except PTPP and SW at the significance level ($p < 0.01$). Finally, the checks themselves were significant for all traits examined except for SW, indicating that the control group exhibited substantial effects on most traits measured in this study in Table 1.

Table 1: ANOVA of an augmented block design for sixteen characters in finger millet genotypes.

Characters	Source of Variation					
	Block	Treatment	Genotype	Check	Genotype vs Check	Error
df	8	47	44	2	1	12
DF (days)	15.13*	91.62 **	61.6 **	557.92 **	13.69	3.63
DM (days)	4.37	87.31 **	46.98 **	671.12 **	110.72 **	1.91
PH (cm)	2.17	395.84 **	194.95 **	3170.53 **	910.94 **	9.08

PTPP	1.26	0.37	0.34	0.99*	0.03	0.2
NLPP	3.93	45.3 **	11.32 **	406.6 **	456.9 **	2.73
FLA (cm2)	3.25	139.52 **	121.63 **	164.7 **	851.24 **	7.03
PL (cm)	0.04	2.32 **	1.82 **	9.62 **	2.62 **	0.21
EL (cm)	0.75	3.37 **	2.94 **	10.78 **	0.09	0.46
NFPP	0.89	2.99 *	2.41 *	7.7 **	14.09 **	0.93
FL (cm)	0.09	2.22 **	1.55 **	7.63 **	15.44 **	0.23
FW (cm)	0.5	1.34 **	1.06 **	5.89 **	0.27	0.28
SW (g)	0.85	0.56	0.57	0.47	0.7	0.39
BYPP (g)	1.12	160.08 **	73.03 **	1178.58 **	934.8 **	1.79
SYPP (g)	0.21	0.47 **	0.5 **	4.17**	1.05**	0.12
HI (%)	0.78	9.09 **	6.86 **	41.11 **	11.13 **	0.75
PC (%)	0.001	4.7 **	4.05 **	2.51 **	39.48 **	0.00089

Days to 50% flowering (days) (DF), plant height (cm) (PH), productive tillers per plant (PTPP), number of leaves per plant (NLPP), flag leaf area (cm^2) (FLA), peduncle length (cm) (PL), ear length (cm) (EL), number of fingers per plant (NFPP), finger length (cm) (FL), finger width (cm) (FW), days to maturity (days) (DM), 1000 seed weight (g) (SW), Harvest index (%) (HI), protein content (%) (PC) and seed yield per plant (g) (SYPP). ** Significant at $p < 0.01$

3.3. Genetic variability of morphological traits

The estimations of genetic and phenotypic variance are significant to determine the variability present within the genotypes. The estimation of phenotypic and genotypic coefficient of variation among the evaluated yield and yield component trains in Table 2. The phonotype coefficient of variation (PCV), genotypic coefficient of variation (GCV), coefficients of variation, range, mean, heritability (broad sense), expected genetic advance (GA), and genetic advance as a percentage of the mean (GAM) for the studied traits (Table 2). PCV ranged from DM (6.61%), DF (8.9%), PH (16.11%), FL (18.94%), PC (20.94%), EL (21.67%), PL (22.66%), BYPP (23.6%), PTPP (24.83%) NFPP (25.04%), SYPP (26.98%), FLA (27.06%), NLPP (29.45%), FW (29.78%), HI (34.24%) and SW (44.72%) in Table, while GCV varied from DM (6.48%) DF (8.64%), PH (15.73%), PTPP (15.91%), FL (17.47%), NFPP (19.64%), EL (19.92%), PC (20.94%), PL (21.33%), BYPP (23.31%), SYPP (23.44%), SW (24.99%), NLPP (25.66%), FW (25.47%), FLA (26.26%), and HI (32.33%) in Table 2.

The estimation of heritability will reflect the potential success of selection for exploiting existing genetic variability. The heritability ranged from PC (99.98%) to SW (31.23%), with highest heritability values for PC (99.98%), followed by BYPP (97.55%), DM (95.94%), PH (95.34%), FLA (94.22%), DF (94.12%), HI (89.13%), PL (88.61%), FL (85.1%), EL (84.48%), NLPP (75.92%), SYPP (75.51%), FW (73.17%), and NFPP (61.51%) in Table 2. The genetic advance was observed at 5% intensity ranged from PH (27.46) to PTPP (0.09), with notable values for PH (27.46), FLA (21.44), BYPP (17.2), DF (15.24), DM (13.57), NLPP (5.27), HI (4.82), PC (4.15), EL (2.99), PL (2.46), FL (2.19), NFPP (1.97), FW (1.55), SYPP (1.1), SW (0.48), and PTPP (0.09) in Table 2. The high (GAM) was observed for HI (62.97%), FLA (52.59%), BYPP (47.5%), NLPP (46.12%), FW (44.95%), PC (43.19%), SYPP (42.02%), EL (37.77%), FL (33.25%), NFPP (31.77%), PH (31.7%), SW (28.81%), and PTPP (21.02%) in Table 2. Traits such as PH, DF, DM, NLPP, FLA, BYPP, and HI showed high heritability

along with high GA, indicating additive gene action. In contrast, SW and FW showed high heritability but low GA in Table 2.

Table 2: Range, general mean, GCV, PCV, heritability, genetic advance and genetic advance as per cent of the mean for yield and yield component in finger millet genotype.

Characters	Range		General mean	GCV (%)	PCV (%)	Heritability(b.s.)	GA	GA as % of mean
	Min.	Max.						
DF (days)	73.2	104.75	88.16	8.64	8.9	94.12	15.24	17.28
DM (days)	96.75	124.75	103.65	6.48	6.61	95.94	13.57	13.09
PH (cm)	42.6	119.7	86.65	15.73	16.11	95.34	27.46	31.7
PTPP	1.26	4.95	2.35	15.91	24.83	41.05	0.49	21.02
NLPP	6.2	30	11.42	25.66	29.45	75.92	5.27	46.12
FLA (cm2)	0.97	1.65	40.76	26.26	27.06	94.22	21.44	52.59
PL (cm)	2.92	9.67	5.95	21.33	22.66	88.61	2.46	41.43
EL (cm)	5.03	12.58	7.9	19.92	21.67	84.48	2.99	37.77
NFPP	2.8	9.81	6.21	19.64	25.04	61.51	1.97	31.77
FL (cm)	3.89	9.32	6.58	17.47	18.94	85.1	2.19	33.25
FW (cm)	1.96	5.96	3.46	25.47	29.78	73.17	1.55	44.95
SW (g)	0.85	1.7	1.68	24.99	44.72	31.23	0.48	28.81
BYPP (g)	17	62.8	36.2	23.31	23.6	97.55	17.2	47.5
SYPP (g)	1.73	4.33	2.62	23.44	26.98	75.51	1.1	42.02
HI (%)	4.43	16.12	7.65	32.33	34.24	89.13	4.82	62.97
PC (%)	6.6	16.28	9.62	20.94	20.94	99.98	4.15	43.19

Days to 50% flowering (days) (DF), plant height (cm) (PH), productive tillers per plant (PTPP), number of leaves per plant (NLPP), flag leaf area (cm^2) (FLA), peduncle length (cm) (PL), ear length (cm) (EL), number of fingers per plant (NFPP), finger length (cm) (FL), finger width (cm) (FW), days to maturity (days) (DM), 1000 seed weight (g) (SW), Harvest index (%) (HI), protein content (%) (PC) and seed yield per plant (g) (SYPP).

3.4. Correlation Coefficient Analysis

The Pearson correlation analysis (p = 0.05) revealed both positive and negative associations among the sixteen evaluated traits in finger millet landraces (Figure 1). Yield and yield-related traits exhibited positive correlations with seed yield per plant (SYPP), specifically with harvest index (HI) at 0.49***, plant tiller percentage (PTP) at 0.71***, and seed weight (SW) at 0.83***. Additionally, significant positive correlations were found with biomass yield (BY) at 0.29* and days to flowering (DF) at 0.30* (Figure 1). Conversely, negative associations were observed with flag leaf area (FLA) at -0.04, ear length (EL) at -0.16, flag leaf length (FL) at -0.09, and plant height (PH) at -0.05 (Figure 1). Furthermore, BY was positively correlated with SW at 0.32*, HI with the number of leaves per plant (NLPP) at 0.29*, PTP with DF at 0.32*, and SW with DF at 0.37 (Figure 1). These findings highlight the complex interrelationships among various growth traits, suggesting that improvement in yield and its components can be achieved through careful selection and breeding strategies in finger millet landraces. Understanding these correlations can help in developing varieties that optimise yield by enhancing traits that have a significant positive impact on overall plant productivity.

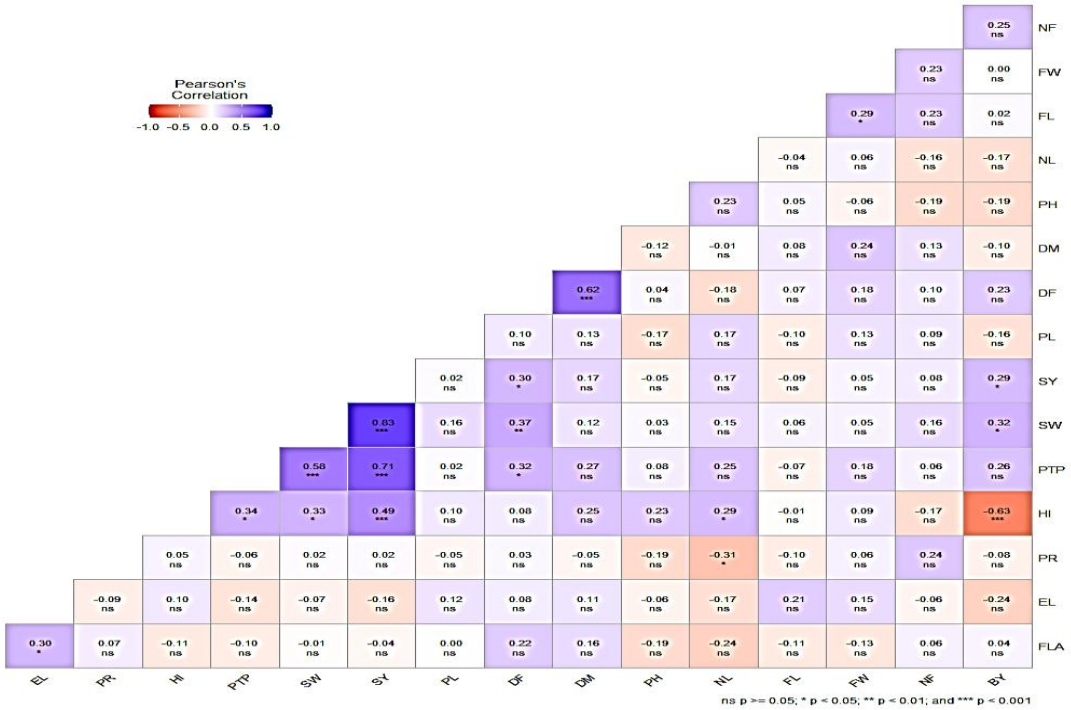


Figure 1: Diagrammatic representation of Pearson's correlation between different yield and yield-related traits in the studied finger millet genotypes. Days to 50% flowering (days) (DF), plant height (cm) (PH), productive tillers per plant (PTPP), number of leaves per plant (NLPP), flag leaf area (cm^2) (FLA), peduncle length (cm) (PL), ear length (cm) (EL), number of fingers per plant (NFPP), finger length (cm) (FL), finger width (cm) (FW), days to maturity (days) (DM), 1000 seed weight (g) (SW), Harvest index (%) (HI), protein content (%) (PC) and seed yield per plant (g) (SYPP).

3.5. Path coefficient analysis

Path coefficient analysis revealed that PL, HI, PTPP, SW, BYPP, DF, NLPP, and NFPP exerted a direct effect on SYPP, whereas PH, FL, FW, EL, DM, FLA, and PC negative direct effect on SYPP in Figure 2. These traits were thus key contributors to seed yield per plant. DF showed positive indirect effects on seed yield via PL, BYPP, HI, PTPP, and NFPP, but negative indirect effects through FW, FLA, SW, DM, PC, and NLPP. The NLPP exhibited the highest

positive indirect effect on SYPP through FLA, PL, HI, and other traits, while FW showed the highest positive indirect effect on seed yield per plant via FLA, PL, and HI in Figure 2.

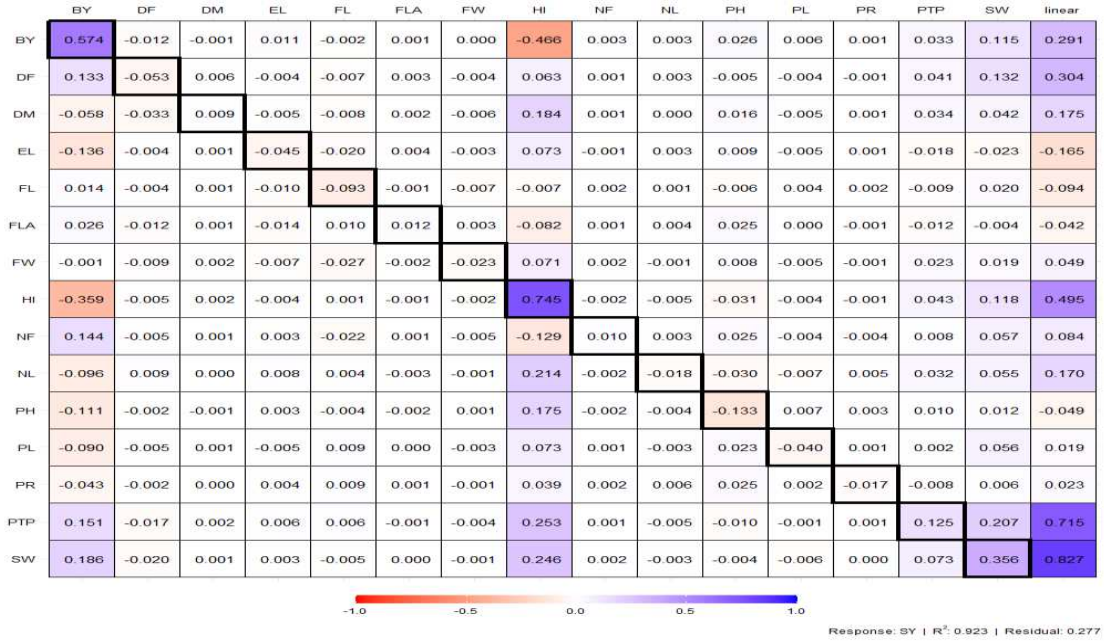


Figure 2: Graphical representation of path coefficient analysis for yield and yield components in finger millet genotypes. Days to 50% flowering (days) (DF), plant height (cm) (PH), productive tillers per plant (PTPP), number of leaves per plant (NLPP), flag leaf area (cm^2) (FLA), peduncle length (cm) (PL), ear length (cm) (EL), number of fingers per plant (NFPP), finger length (cm) (FL), finger width (cm) (FW), days to maturity (days) (DM), 1000 seed weight (g) (SW), Harvest index (%) (HI), protein content (%) (PC) and seed yield per plant (g) (SYPP). ** significant at p <0.01

3.6. Cluster analysis

To evaluate the genetic divergence among 48 genotypes, Mahalanobis' D^2 statistics were calculated for all possible pairs of genotypes. This analysis facilitated the assessment of genetic diversity within the population. Based on the results of cluster analysis, the genotypes were classified into five distinct and non-overlapping clusters. Among these, five clusters where the highest accessions were identified, with cluster III with 21 accessions (Supplementary Table S5). Cluster I included 4 accessions, followed by cluster II with 12, cluster IV with 3 and cluster V with 8 accessions (Supplementary Table S5). The highest inter-cluster distance was observed between cluster III and IV (6.533), followed by cluster I and IV (6.475) and cluster I and III (6.425). The lowest distance occurred between cluster II and V (4.907) (Table 3). The highest intra-cluster distance was found in cluster I (5.295), while the lowest intra-cluster in cluster II (3.846 (Table 3). The cluster mean performance for various traits (Table 3), cluster I showed the highest mean for PH, NLPP, PL, and HI. Cluster II recorded superior means for EL, cluster III having the highest mean with DM, FLA, NFPP and PC. Cluster IV had the highest mean for DF, PTPP, FW, SW, BYPP and SYPP (Table 3).

Overall, cluster IV demonstrated the best performance for most yield-related traits, and cluster V the lowest values for DF, DM, FLA and EL (Table 3).

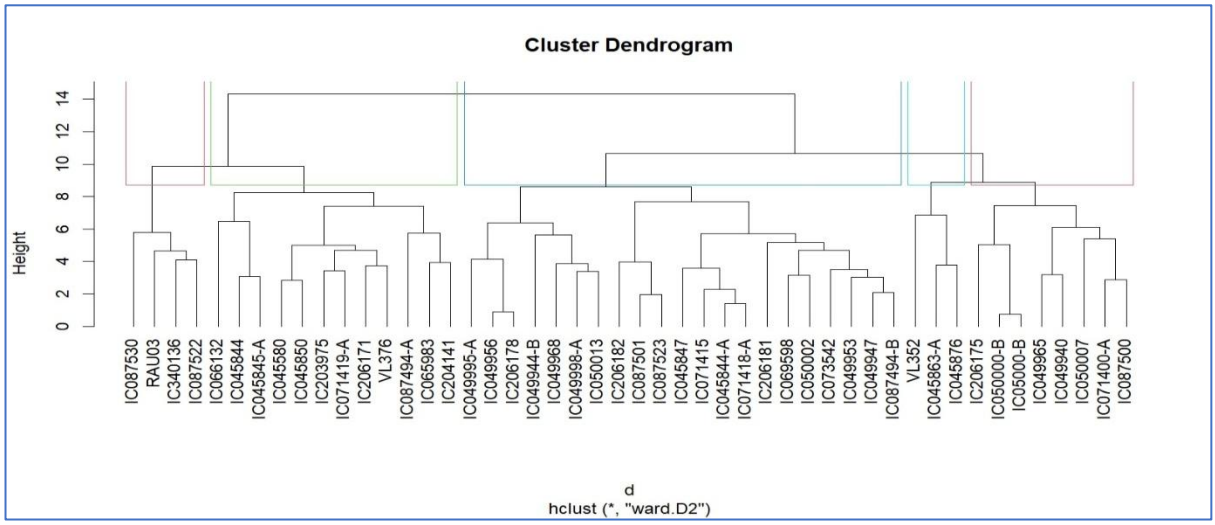


Figure 3: Cluster Dendrogram for 48 genotypes

Table 3: **Estimates of average intra- and inter-cluster distances for five clusters in finger millet and the clustered mean.**

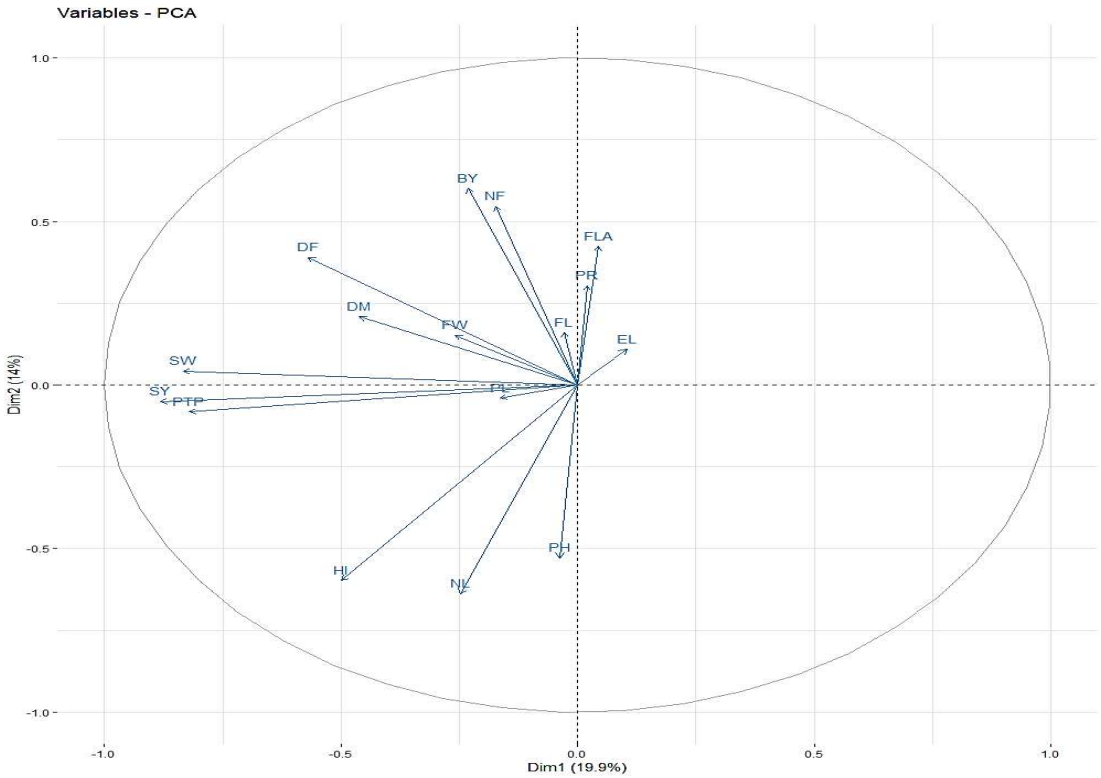
Cluster	I	II	III	IV	V
I	**5.295**	5.551	6.425	6.475	5.294
II		**3.846**	5.489	6.411	4.907
III			**5.061**	6.533	5.495
IV				**5.133**	5.827
V					**4.065**
Clustered Mean					
DF (days)	85.63	85.54	90.24	95.45**	85.21*
DM (days)	105.39	100.13	108.44**	107.18	99.93*
PH (cm)	98.23**	90.16	63.79*	85.05	90.07
PTPP	2.15	2*	2.04	3.09**	2.38
NLPP	16.57**	8.49*	8.73	11.38	10.81
FLA (cm2)	38.56	43.38	54.73**	41.96	34.46*
PL (cm)	6.64**	5.39*	6.03	5.91	5.79
EL (cm)	8.12	11.27**	8.69	7.71	6.4*
NFPP	5.36*	5.6	7.32**	6.71	6.13
FL (cm)	5.83*	7.77**	6.09	6.57	6.72
FW (cm)	3.02*	3.74	3.53	3.82**	3.37
SW (g)	1.56	1.29	1.27*	2.79**	1.42
BYPP (g)	26.86*	32.29	38.91	41.95**	37.71
SYPP (g)	2.45	2.16*	2.25	3.75**	2.37
HI (%)	9.68**	7.07	5.84*	9.48	6.51
PC (%)	8.758	8.79	10.98**	9.32	10.05

Days to 50% flowering (days) (DF), plant height (cm) (PH), productive tillers per plant (PTPP), number of leaves per plant (NLPP), flag leaf area (cm^2) (FLA), peduncle length (cm) (PL), ear

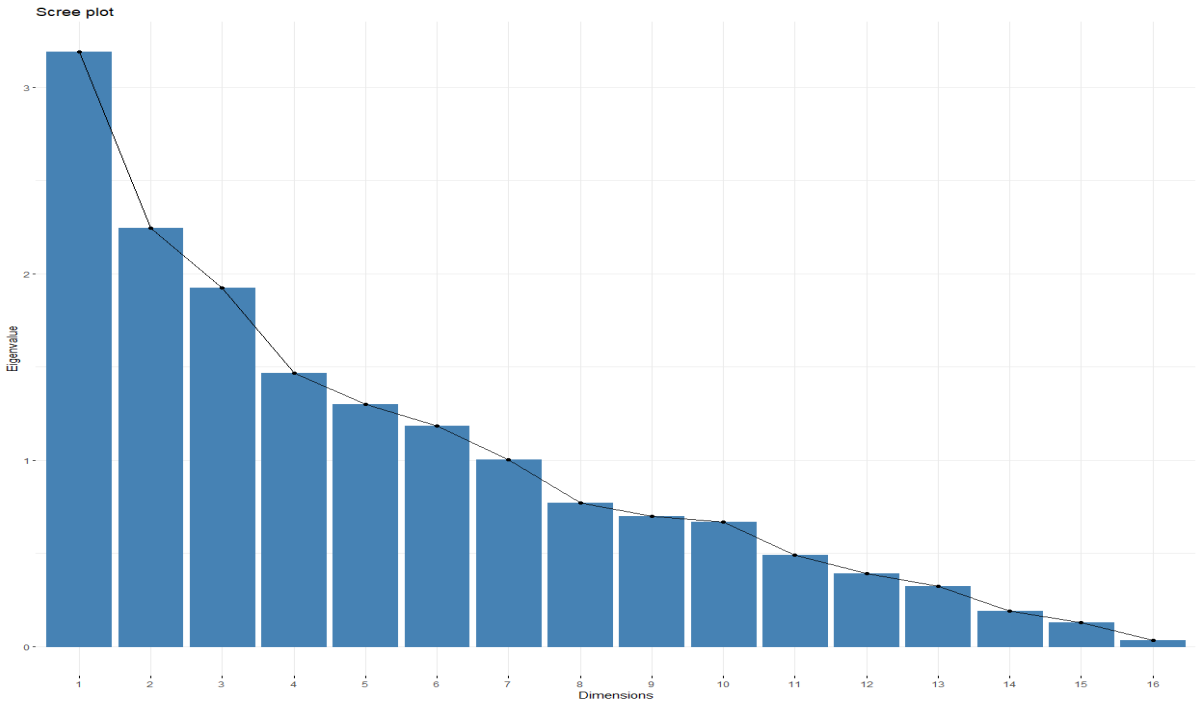
length (cm) (EL), number of fingers per plant (NFPP), finger length (cm) (FL), finger width (cm) (FW), days to maturity (days) (DM), 1000 seed weight (g) (SW), Harvest index (%) (HI), protein content (%) (PC) and seed yield per plant (g) (SYPP). ** highest value and * lowest value.

3.7. Principal component analysis (PCA)

The estimates from the principal component analysis for the quantitative traits are presented in Supplementary Table S6. The table reveals that the data from twelve traits were transformed into twelve principal components. PCA for 16 traits yield and yielded related traits seven components with eigenvalues >1, which contributed 76.941% of total variation (Supplementary Table S6). PC1 contributed the highest variation (19.935%) and followed by PC2 (14.019%). Trait vectors in the PCA plot showed that longer vectors indicate stronger influence, and acute angles between vectors suggest positive correlations. The biplot revealed a strong positive association of SYPP with SW, PTPP, DM, FW, FL, DF, NFPP, BYPP, PL, HI, NLPP, and PH (acute angle >90°), suggesting for yield selection. The scree plot indicates that the first principal component (PC1) contributed the most to the total variation among the traits analysed in Figure 4B. This suggests that PC1 captures key underlying patterns in the data. Additionally, findings from the scatter plot (Figure 4A) identified several traits as significant contributors to diversity and variability, including seed yield per plant (SYPP), seed weight (SW), plant tiller percentage (PTPP), harvest index (HI), biomass yield per plant (BYPP), number of leaves per plant (NLPP), number of fertile tillers per plant (NFPP), and days to flowering (DF). These traits play a crucial role in the overall morphological and physiological diversity of the finger millet landraces studied. By focusing on these key traits, researchers and breeders can prioritise their efforts in developing high-yielding varieties with diverse attributes that enhance productivity.



A



B

Figure 4: A-Scatter plot of sixteen characters of finger millet using PC1 and PC2, **B-**The scree plot shows the eigenvalues of differnet principal component (bar in the fingur) as shown by PCA of the finger millet germplams based on quanitative morphological traits.

3.8. Molecular diversity analysis using SCoT and SSR markers

This study investigated molecular diversity in 48 finger millet land races using a combined approach to Start Codon Targeted (SCoT) and Simple Sequence Repeat (SSR) markers. Together, 35 SCoT primer and the Twenty SSR primer pair were employed to assess genetic variation across the land races respectively (Supplementary Table S3). The combined marker system generated a robust dataset. SCoT primer amplified a total of 184 bands, averaging four bands per marker (Supplementary Table S7), while SSR primer produced 59 alleles with an average of 3.105 alleles per locus (Supplementary Table S8). The number of bands per SCoT primer ranged from two (SCoT 3, SCoT 11) to seven (SCoT 7), and the number of alleles per SSR primer ranged from two to six. Several SCoT primers (SCoT 1, 2, 3, 4, 7, 9, 10, 11, 12, 16, 17, 18, 18, 20, 21, 22, 23, 25, 26, 27, 28, 29, 30, 31, 32 and 33) showed complete polymorphism (Supplementary Figure 1), while six SSR markers (SSR1, SSR2, SSR4, SSR7, SSR10, SSR11, SSR12, SSR13, SSR16, SSR18 and SSR 20) (Supplementary Figure 2), exhibited high polymorphism information content (PIC >0.5), indicating their suitability for diversity studies. PIC values across both marker systems demonstrated effective polymorphism detection. SCoT markers showed PIC values ranging from 0.07 (SCoT 4) to 0.48 (SCoT 1, 3, 9, 11, and 14), with an average 0.364, whereas SSR markers ranged from 0.04 (SSR20) to 0.604 (SSR10), averaging 0.3463 gene diversity based on SSR data varied from 0.0408 (SSR15) to 0.6424 (SSR3) with a significant correlation ($r= 0.55$, $P<0.01$) between allele number and gene diversity. Population structure analysis, based on the SCoT and SSR, revealed three distinct genetic clusters among the genotypes, with several accessions showing admixture. This integrated use of plotted data illustrates distinct genetic structure among the sample individuals, with colour-coded bars representing different populations. The blue regions dominate, indicating a strong presence of a specific genetic lineage, while the green and red bars reveal the contributions of minor populations, suggesting variability within the dataset SCoT (Figure 5) and SSR (Figure 6)). Some individuals exhibit significant admixture with multiple colours (blue, green and red), indicating genetic blending. The markers provided a comprehensive and complementary view of genetic diversity in finger millet, reinforcing their utility in breeding, conservation, and germplasm enhancement programs.

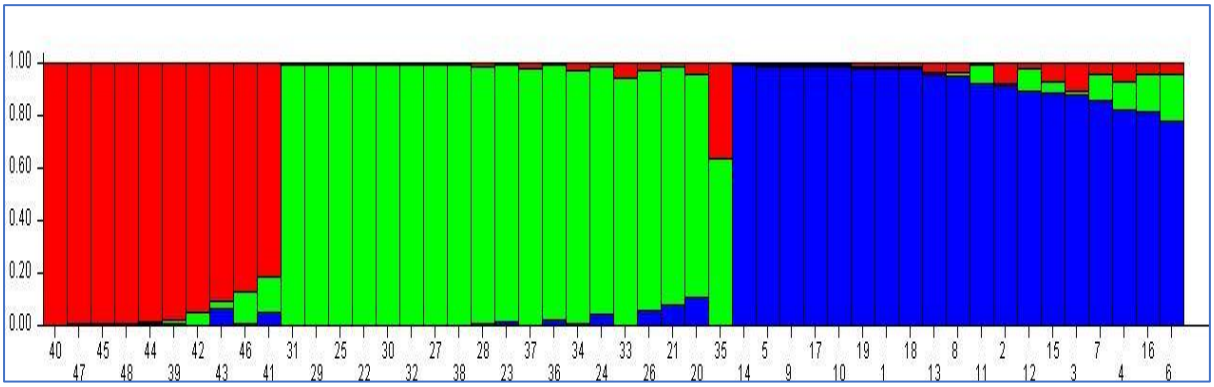


Figure 5: Population structure of molecular marker SCoT (1- IC049947, 2-IC049953, 3-IC045580, 4-IC071400-A, 5-IC069598, 6-IC071415, 7-IC050000-B, 8-IC087501, 9-IC206175, 10-IC087494-A, 11-IC087500, 12- IC045850, 13-IC049998-A, 14-IC049968, 15-IC045844, 16-IC050007, 17-IC05000-B, 18-IC087530, 19- IC203975, 20-IC206181, 21-IC206182, 22-IC049965, 23- IC050013, 24-IC087523, 25-IC045845-A, 26-IC049944-B, 27-IC073542, 28-IC050002, 29-IC045844-A, 30-IC071418-A, 31-IC049995-A, 32-IC049956, 33

- IC206171, 34-IC065983, 35-IC087494-B, 36-IC206175, 37-IC045847, 38-IC204141, 39-IC066132, 40-IC340136, 41-IC071419-A, 42-IC049940, 43-IC045863-A, 44- IC045876, 45-IC087522, 46-VL376, 47-RAU03, 48-VL352)

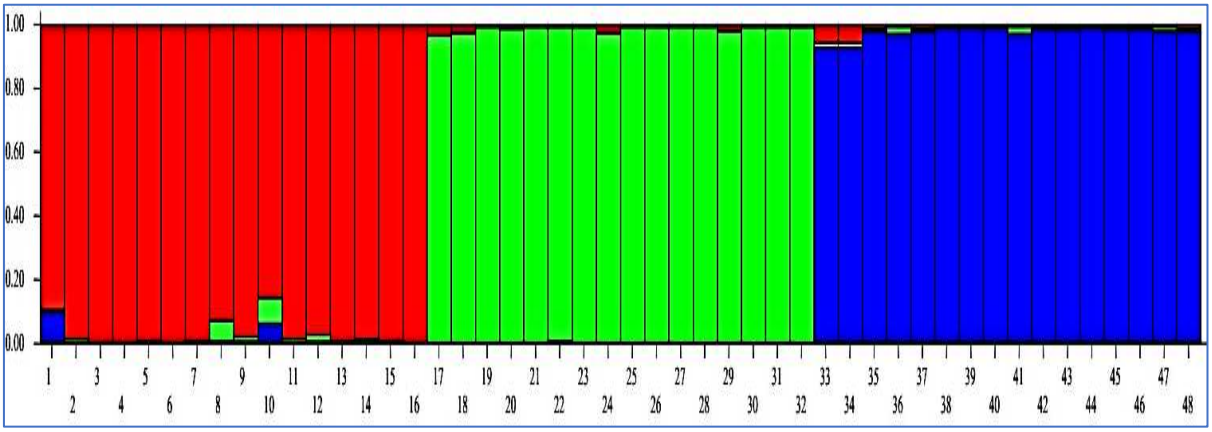
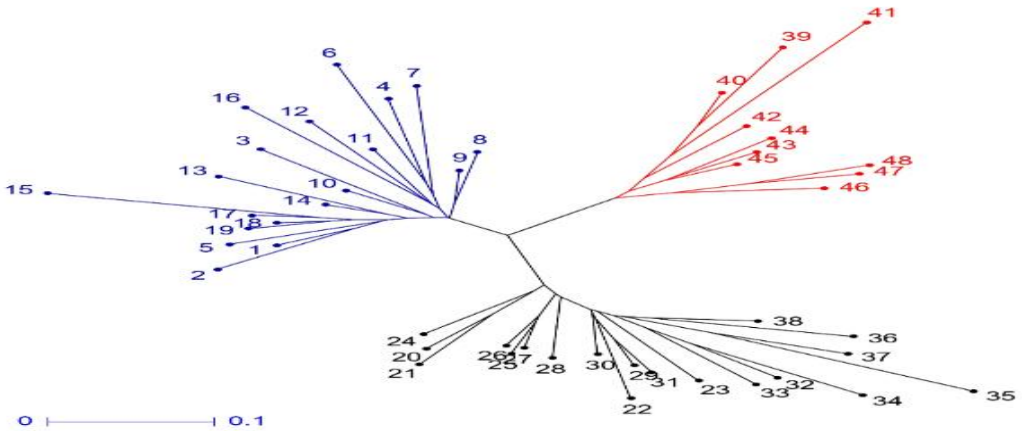


Figure 6: Population structure 48 finger millet genotypes (1- IC049947, 2-IC049953, 3-IC045580, 4-IC071400-A, 5-IC069598, 6-IC071415, 7-IC050000-B, 8-IC087501, 9-IC206175, 10-IC087494-A, 11-IC087500, 12- IC045850, 13-IC049998-A, 14-IC049968, 15-IC045844, 16-IC050007, 17-IC05000-B, 18-IC087530, 19- IC203975, 20-IC206181, 21-IC206182, 22-IC049965, 23- IC050013, 24-IC087523, 25-IC045845-A, 26-IC049944-B, 27-IC073542, 28-IC050002, 29-IC045844-A, 30-IC071418-A, 31-IC049995-A, 32-IC049956, 33 - IC206171, 34-IC065983, 35-IC087494-B, 36-IC206175, 37-IC045847, 38-IC204141, 39-IC066132, 40-IC340136, 41-IC071419-A, 42-IC049940, 43-IC045863-A, 44- IC045876, 45-IC087522, 46-VL376, 47-RAU03, 48-VL352)

3.8.1. Cluster analysis

STRUCTURE software grouped 48 finger millet genotypes into three populations, with some accessions showing admixture. The UPGMA cluster analysis based on SCoT marker, which used Jaccard's similarity coefficients, showed three main clusters: I (19 landraces), II (10 landraces), and III (19 landraces) within the germplasm (Figure 7A), whereas, SSR marker showed two main clusters named I & II within the germplasm. Notably, Cluster I is split further into two sub-clusters: IA and IB. To elaborate, Cluster I was made up of 47 genotypes. Specifically, IA contained 32 genotypes while IB had 15 genotypes. In contrast, there was just one genotype in Cluster II (Figure 7B). The population structure analysis done with the STRUCTURE software suggested there are three distinct subpopulations (K = 3). Each of these subpopulations consisted of sixteen genotypes each (Figure 7B).



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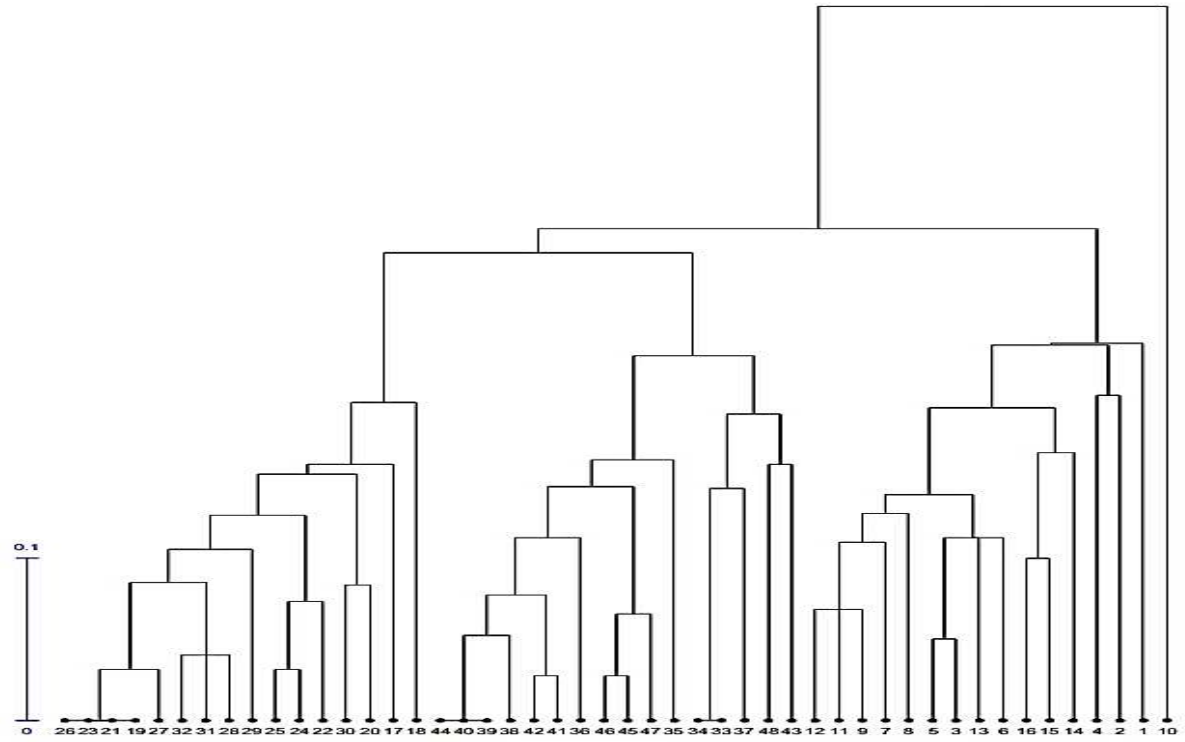

B

Figure 7: Cluster analysis of 48 finger millet genotypes using STRUCTURE software **A (SCoT) and B (SSR), (**1- IC049947, 2-IC049953, 3-IC045580, 4-IC071400-A, 5-IC069598, 6-IC071415, 7-IC050000-B, 8-IC087501, 9-IC206175, 10-IC087494-A, 11-IC087500, 12-IC045850, 13-IC049998-A, 14-IC049968, 15-IC045844, 16-IC050007, 17-IC05000-B, 18-IC087530, 19- IC203975, 20-IC206181, 21-IC206182, 22-IC049965, 23- IC050013, 24-IC087523, 25-IC045845-A, 26-IC049944-B, 27-IC073542, 28-IC050002, 29-IC045844-A, 30-IC071418-A, 31-IC049995-A, 32-IC049956, 33 - IC206171, 34-IC065983, 35-IC087494-B, 36-IC206175, 37-IC045847, 38-IC204141, 39-IC066132, 40-IC340136, 41-IC071419-A, 42-IC049940, 43-IC045863-A, 44- IC045876, 45-IC087522, 46-VL376, 47-RAU03, 48-VL352)

3.8.2. Principal Coordinate Analysis (PCoA)

PCoA showed the dissimilarity matrix of the forty-eight finger millet genotypes. A visual map emerged from this analysis, displaying how similar individuals cluster closely together. By examining this map, we evaluated whether the previously identified groups (from cluster analysis) accurately reflect the genetic differences. This indicates how similar genotypes are, based on their eigenvalues & eigenvectors. The distribution of these genotypes in SCoT marker was seen across all four quadrants of the plot, as shown in Figure 8A. Quadrant 1 included seven genotypes, quadrant 2 included nineteen genotypes, quadrant 3 included ten genotypes and quadrant 4 included twelve genotypes whereas, SSR marker was seen across all four quadrantes of the plot, as shown in Figure 8B. Quadrant 1 included fourteen genotypes, quadrant 2 included fourteen genotypes, quadrant 3 included sixteen genotypes and quadrant 4 included four genotypes.

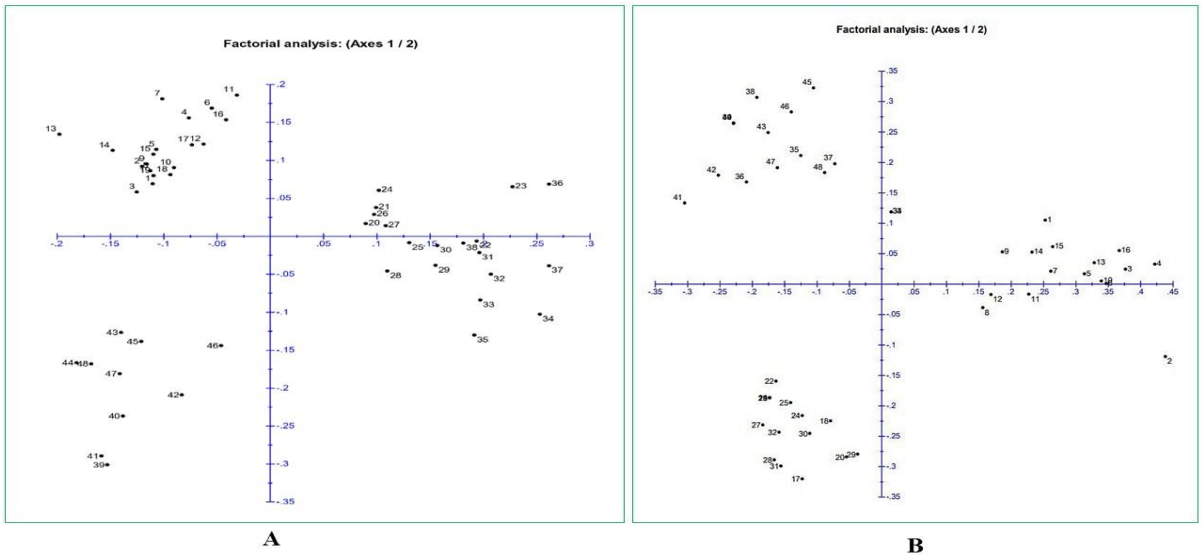

Figure 8: Principal coordinate analysis of representative 48 finger millet genotypes **A (SCoT) and B (SSR)**, **(**1- IC049947, 2-IC049953, 3-IC045580, 4-IC071400-A, 5-IC069598, 6-IC071415, 7-IC050000-B, 8-IC087501, 9-IC206175, 10-IC087494-A, 11-IC087500, 12-IC045850, 13-IC049998-A, 14-IC049968, 15-IC045844, 16-IC050007, 17-IC05000-B, 18-IC087530, 19- IC203975, 20-IC206181, 21-IC206182, 22-IC049965, 23- IC050013, 24-IC087523, 25-IC045845-A, 26-IC049944-B, 27-IC073542, 28-IC050002, 29-IC045844-A, 30-IC071418-A, 31-IC049995-A, 32-IC049956, 33 - IC206171, 34-IC065983, 35-IC087494-B, 36-IC206175, 37-IC045847, 38-IC204141, 39-IC066132, 40-IC340136, 41-IC071419-A, 42-IC049940, 43-IC045863-A, 44- IC045876, 45-IC087522, 46-VL376, 47-RAU03, 48-VL352)

4. Discussion

Characterising finger millet genotypes based on qualitative and quantitative traits is vital for selecting superior germplasm in breeding programs. These efforts aim to develop varieties with enhanced nutritional value, local adaptability, and superior quality. The uniqueness of 48 finger millet accessions was assessed using 22 traits aligned with DUS criteria. The majority of finger millet genotypes in this study exhibited an erect plant growth habit (48 genotypes), consistent with findings by Upadhyaya et al.[24] and Patil et al.[25], who observed 92.8% and 80% of such genotypes, respectively. Patil et al.[25] also noted higher yields in varieties with erect growth habits. Being both photo and thermo-sensitive, finger millet's growth and development are directly affected by fluctuations in day length and temperature[26]. Plant pigmentation at the leaf juncture studied by Ankit et al.[27] highlighted its role in reducing canopy temperature and enhancing water and heat tolerance, making it a valuable trait for breeding heat and water-stress-tolerant varieties. Genotypes with pubescence can serve as parents in crossing programs. Ankit et al.[27] observed that stem culm branching is a crucial trait contributing to lodging resistance during the crop's maturity stage, and ear shape is an essential yield-contributing trait. Productive tillers per plant play a crucial role in yield-related traits[28]. Plant height is one of the most important characters that influence lodging[27]. Seed colour is affected not only by genetic factors but also by environmental conditions during ripening[29]. Previous studies have documented significant variation in grain colours among finger millet

genotypes[24]. Ankit et al.[27] highlighted that seed colour is a significant trait that serves as a morphological marker for variety identification, quality assessment, and cultivar acceptance. Varieties with round-shaped seeds are preferred by growers and consumers[28]. Varieties with smooth surface seeds are preferred over rough ones. Accessions with smooth seeds can be utilized in breeding programs for milling varieties. Characterization based on visual morphological traits has proven to be a quick and cost-effective method, even for large numbers of accessions. Similar studies on finger millet were conducted by Bezaweletaw et al.[30]., Reddy et al.[31]., and Patil et al.[10].The analysis of variance revealed significant genetic diversity among the finger millet genotypes, indicating ample scope for selection. Similar observations were made by Singh et al.[32]., Madhavilath et al.[33]., Singamsetti et al.[34]., and Priyadharshini et al.[35].

High PCV and GCV were noted for traits such as HI, FW, FLA, SYPP, and PC, indicating potential for improvement through selection[10,36,37]. Low variability was observed for days to flowering and maturity due to their low GCV[38]. A high degree of broad-sense heritability suggests of more diverse traits for the selection of genotypes. Genetic advance reflects the potential for trait improvement through selective breeding, with higher values indicating faster progress. Actual values depend on the species, population, and variability. These findings align with Suryanarayana et al.[39] and Negi et al.[40]. High heritability paired with high genetic advance was noted for traits like PH, DF, DM, NL, FLA, BYPP, and HI, indicating additive gene action. Gebreyohannes et al.[14] also noted high broad-sense heritability for DF, DM and GYPP. Traits such as 1000 seed weight and FW showed high heritability but low genetic advance, suggesting non-additive gene action. The findings highlight genetic diversity and potential for improvement through selection[38], aligning with studies by Emrey et al.[37], Patel et al.[41]., and Divya et al.[42]. This genetic data aids breeders in prioritising traits for selection and enhancing crop performance.

Pearson's correlation analysis revealed significant relationships among traits in finger millet genotypes, highlighting traits with positive associations with seed yield for indirect selection in yield improvement[41,43,44]. Understanding the influence of traits on seed yield is vital for crop improvement. Traits such as PL, HI, PTPP, SW, BYPP, DF, NL, and NFPP positively impact seed yield. In contrast, traits like PH, FL, FW, EL, DM, FLA, and PC negatively affect it. These are key contributors to seed yield per plant. Selecting these traits can lead to improvement in related characteristics and ultimately enhance seed yield per plant (Patil *et al.* 2023). Similar results were reported by Hema et al.[43]., Madhavilatha et al.[45], Chavan et al.[46]., and Abhilash and Singh[44].

Genetic divergence plays a key role in selecting suitable parents for hybridization programs, with higher heterosis observed in crosses between genetically diverse parents compared to close relatives[47]. In this study, 48 entries were divided into five clusters: Cluster I (4 genotypes), Cluster II (12 genotypes), Cluster III (21 genotypes), Cluster IV (3genotypes) and Cluster V (8 genotypes). Cluster III and IV exhibited the highest inter-cluster distance, indicating significant genetic diversity, while Cluster I showed the highest intra-cluster distance. Genotypes in Clusters III and IV are more divergent and suitable for breeding programs to achieve variability in segregants[41]. Similar findings were reported by Patil et al.[25]. Cluster means for traits revealed that Cluster I exhibited the highest mean for PH, NLPP, PL, and HI. Cluster II displayed the highest means for key yield-contributing traits, including EL and FL. Cluster II recorded superior means for EL, cluster III having the highest mean with

DM, FLA, NFPP and PC. Cluster IV had the highest mean for DF, PTPP, FW, SW, BYPP and SYPP. These findings align with Sharma et al.[23]., Patel et al.[41]., and Vasisth et al.[48].

Principal Component Analysis (PCA) is a vital multivariate statistical method that identifies components explaining maximum variability and ranks genotypes based on PC scores. In this study, PCA revealed seven principal components with eigenvalues above one, accounting for 76.94% of total variation, with PC1 contributing the most. Traits in these components are crucial for ragi improvement programs. Positive associations with seed yield were observed for traits like 1000-seed weight, productive tillers, biological yield, and harvest index, among others. Selecting traits within a principal component can simultaneously enhance associated traits[49]. These findings align with earlier studies by Choudhary et al.[50].

The molecular diversity analysis of 48 finger millet genotypes using 35 SCoT primers revealed their reliability, cost-effectiveness, and reproducibility in assessing genetic diversity and population structure[51]. PIC values ranged from 0.07 to 0.48, with an average of 0.364, indicating the markers' usefulness in detecting polymorphism. STRUCTURE and cluster analyses grouped the genotypes into three populations, highlighting genetic diversity. Similar findings were reported by Panda et al.[52].The SSR analysis yielded a total of 59 alleles. The number of alleles per locus varied, ranging from 2 to 6, resulting in an average of 3.105 alleles for each locus. These results align with earlier studies on finger millet by Arya et al.[53]., Dida et al.[54]., which also showed different levels of polymorphism when using SSR markers. The observed diversity in alleles indicates that the SSR markers used in this study effectively captured genetic variability within finger millet germplasm.

5. Conclusion

The characterization and analysis of finger millet genotypes revealed critical insights into their traits, genetic diversity, and molecular profiling. Traits like erect plant growth habit, seed colour, smooth seed surface, and stem culm branching were highlighted for their importance in breeding programs aimed at improving yield and stress tolerance. High heritability and genetic advance for yield-contributing traits, coupled with positive trait correlations, emphasise their potential for selective breeding and diversity enhancement. Genetic divergence and clustering analyses identified distinct genotype groups, facilitating hybridisation and variability in segregants. The molecular diversity analysis using both SCoT and SSR markers effectively captured genetic variation among finger millet accessions. SCoT markers provided reliable insights into population structure, while SSR markers detected 59 alleles across loci, showcasing significant genetic variability. The number of bands per SCoT primer ranged from two (SCoT 3, SCoT 11) to seven (SCoT 7), and the number of alleles per SSR primer ranged from two to six. Several SCoT primers (SCoT 1, 2, 3, 4, 7, 17, 18, 18, 20, 21, 2226, 27, 28, 29, 30, 31, 32 and 33) showed complete polymorphism, while six SSR markers (SSR1, SSR2, SSR4, SSR7, SSR10, SSR11, SSR12, SSR13, SSR16, SSR18 and SSR 20) exhibited high polymorphism information content (PIC >0.5), indicating their suitability for diversity studies. Markers with high PIC values and gene diversity, such as SSR3, SSR7, and SSR10, were particularly informative for identifying key genetic traits vital for breeding programs. UPGMA and population structure analyses grouped genotypes into clusters and populations, reflecting both genetic similarity and unique traits. These findings establish a solid foundation for breeding strategies and conservation efforts, aiming to improve finger millet's

adaptability, yield, and nutritional value while ensuring sustainable genetic resource management.

Author Contributions

V.K.K., N.K., V.S., K and I.O.: contributed to the conceptualization and methodology; V.K.K..: performed data collection and curation, and prepared the initial draft of the manuscript; V.K.K., N.K., I.O., V.S., C.C., and C.M.S.: conducted statistical analysis; H.K.Y., M.K., D.K.P., S.K., D.K., and V.K.: reviewed and edited the final draft of the manuscript; V.K.K., N.K., and I.O.: contributed to data visualisation; V.S., N.K., M.K., and K provided resources to carry out the experiments. All authors read and approved the final manuscript.

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Not Applicable

Data Availability Statement

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request or All data generated or analysed during this study are included in this published article [and its supplementary information files].

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Conflicts of Interest

The authors declare no conflicts of interest.

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