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UNVEILING GENETIC DIVERSITY IN FOXTAIL MILLET (*SETARIA ITALICA* L.) THROUGH MORPHOLOGICAL AND MOLECULAR INSIGHTS

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ABSTRACT

Thirty foxtail millet genotypes were evaluated under a Randomized Block Design (RBD) with three replications to study variability and molecular diversity using ISSR markers. Data were recorded for 10 traits. ANOVA revealed significant differences among genotypes for all traits. PCV values were slightly higher than GCV, suggesting minimal environmental influence. High heritability coupled with high genetic advance as % of mean was observed for productive tillers per plant, panicle weight, grain yield per plant, and straw yield per plant. ISSR analysis with 10 primers generated 60 bands (average 6 per primer), of which 45 (75%) were polymorphic. Cluster analysis grouped the 30 genotypes into two major clusters.

Key words: Foxtail Millet, Genetic variability, Molecular Diversity

Introduction

Millets are one of the cereals asides the major wheat, rice, and maize. Millets are major food sources for millions of people, especially those who live in hot, dry areas of the world. They are grown mostly in marginal areas under agricultural conditions in which major cereals fail to give substantial yields (Adekunle, 2012). The four major types are Pearl millet (*Pennisetum glaucum*), which comprises 40% of the world production, Foxtail millet (*Setaria italica*) Proso millet or white millet (*Panicum miliaceum*) and Finger Millet (*Eleusine coracana*) (Amadou *et al.,* 2012). Foxtail millet is a diploid C_4 panicoid crops having a comparatively small genome size of approximately 515 Mb, short life cycle and inbreeding nature (Lata *et al.,* 2013). Foxtail millet is a gluten-free, nutrient-rich whole-grain food. Compared with rice and wheat, it contains various vitamins, minerals and high level of protein. In addition to its rich nutrition, it also has a lower glycemic index than the staple food made of rice and flour. Moreover, some polyphenols in foxtail millet have adjuvant therapy for diabetes, cancer and cardiovascular diseases. Therefore, foxtail millet has potential application of functional foods (Yang *et al.,* 2022).

Morphology and anthesis behaviour make foxtail millet one of the most difficult species to cross pollinate. It is largely self-pollinated, with cross pollination averaging about 4 per cent (Li *et al.,* 1935). During summer, anthesis of foxtail occurred between 18:00 h and 07:00 h, starting the third day, after the emergence of the spike. Each spike required from 10 to 13 d to complete anthesis. Opening and closing of a single flower occurred in about 7 min (Siles *et al.,* 2001)

Assessing genetic diversity in foxtail millet germplasm is crucial for conservation, expanding the genetic base, and exploiting heterosis. This is especially important in inbred species where genetic diversity has declined. As reported by Deb (2009), maintaining crop diversity can ensure agricultural sustainability and food security. The

relative genetic diversity of a species population can be determined using morphological and molecular markers. Phenotypic traits are of limited importance because they are frequently affected by environmental conditions and the developmental status of the plant (Tatineni *et al.,* 1996). Molecular markers are popular for investigating genetic diversity because of their high efficiency, low sample number requirements, and low number of limitations on the growth stage (Bjorklund *et al.,* 2009). Therefore, keeping in view above facts the present investigation was carried out to assess the varibility parameters and molecular diversity analysis using ISSR markers.

Materials and Methods

Morphological characterization

Thirty foxtail millet genotypes including three checks viz., DHFT-109-3, SIA-31-56 and Local check were evaluated in three replications using a Randomised Block Design (RBD) at the Hill Millet Research Station, Navsari Agricultural University, Waghai (The Dangs), during Summer (*Zaid*) season, 2024. The location of the experimental site falls under "South Gujarat Heavy Rainfall Zone, AES-III". The climate of this zone is typically tropical. The genotypes were sown in plot size of 3 × 1.8 m^2. Recommended agronomic practices were implemented. For observations, five randomly selected competitive plants selected randomly from each five rows plot in each replication excluding border except for days to 50 % flowering and days to maturity, where it was recorded on population basis. For 1000-grain weight, a random sample of 1000 grains was counted from the threshed seed and the weight was recorded in grams. Observations were recorded for 10 characters. The mean data were statistically analyzed for analysis of variance (Panse and Sukhatme 1978). Heritability in broad sense (h^2_b) was calculated and classified into low (below 30%), medium (30- 60%) and high (above 60%) and genetic advance as percent over mean (at 5%) were categorized into low (0-10%), moderate (10-20%) and high (-20%) (Johnson *et al.,* 1955). Estimates of PCV and GCV were categorized into low (<10%), medium (10-20%) and high (>20%) (Burton and DeVane, 1953).

DNA isolation and purification

All the procedure starting from DNA isolation to PCR amplification was conducted at the Molecular Breeding Laboratory of the Department of Genetics and Plant Breeding, N. M. College of Agriculture, NAU. Genomic DNA was extracted from 5 g of leaf tissue ground in liquid nitrogen using the CTAB method described by Doyle and Doyle (1990). The finely ground leaf powder was transferred to 2 ml microcentrifuge tubes, and 1.5 ml of 3% CTAB extraction buffer was added. The mixture was thoroughly mixed and incubated at 65°C for 60 minutes in a water bath. DNA was purified using an equal volume of chloroform: isoamyl alcohol (24:1), followed by centrifugation. The upper aqueous phase was transferred to a fresh tube, and DNA was precipitated by adding an equal volume of 100% chilled isopropanol and gently inverting. The DNA pellets were air-dried at room temperature, washed with 200 µl of 70% ethanol, and centrifuged. The dried pellets were dissolved in 200 µl of 1X Tris-EDTA (TE) buffer and treated with 10 µl of DNase-free RNase. The DNA samples were then stored at -20°C until further use. The quality of isolated genomic DNA was assessed by 0.8% agarose gel electrophoresis conducted at 80/ V. DNA bands were visualized under UV light using a Gel Doc™ XR+ imaging system and photographed. Quantification of DNA was performed using a NanoDrop spectrophotometer (Thermo, USA). Pure DNA was considered as one having an A_{260}/A_{280} ratio of 1.8 to 2.0.

PCR amplification

15 ISSR primers were screened. Out of 15, only ten were found to amplify with foxtail millet genotypes. PCR reactions for primers were carried out in a reaction volume of 10 µl which contained Emerald AmpGT PCR master mix 5 µl, RNAase-free water 2 µl, primer 2 µl and genotype DNA 1 µl. PCR tubes were placed in the Thermal cycler for cyclic amplification which was carried out using a Thermal cycler (BIORAD, USA). The cycling conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation (94 °C) for 1 min, annealing (50 to 54°C) for 1 min, primer extension (72 °C) for 1 min, followed by an extension at

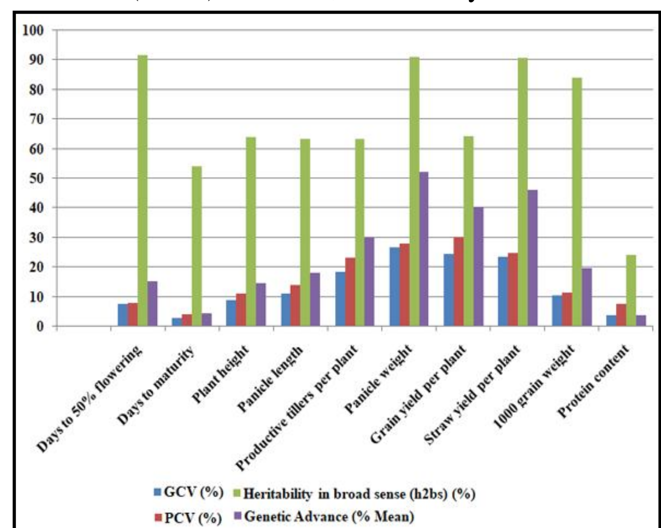

Fig. 1: GCV (%), PCV (%), Heritability and genetic advance (% mean) for ten characters in foxtail millet.

Table 1: Analysis of variance of experimental design for yield and yield contributing characters in foxtail millet.

Source	d.f.	DF	DM	PH	PL	PTP	PW	GYP	SYP	GW	PC
Replication	2	6.86	3.81	19.36	7.41	0.08	0.06	6.31	3.28	0.07	1.52
Genotypes	29	80.40	28.99	15.51	15.39	1.15	20.31	13.48	33.15	0.38	1.07
Error	58	2.37	6.86	5.22	2.48	0.19	0.65	2.10	1.10	0.02	0.54

DF: Days to 50% flowering; DM: Days to maturity; PH: Plant height; PL: Panicle length; PTP: Productive tillers per plant; PW: Panicle weight; GYP: Grain yield per plant; SYP: Straw yield per plant; GW: 1000 grain weight; PC: Protein content

72 °C for 10 min. The amplified products were analyzed by horizontal electrophoresis on 1.8 % agarose gel stained with ethidium bromide (0.5 ìg/ml) at 100 V for 2 hours and 30 minutes. The gel was visualized under UV in the gel documentation system (Gel Doc™ XR$^+$ Imaging system).

Data analysis

The amplified ISSR profiles of all genotypes for each primer were documented using a gel documentation system. Bands were scored as present (1) or absent (0) across the amplified profiles. This binary data was recorded separately for each primer. Each amplified band was considered as a unique locus. The binary data were used to calculate genetic similarities based on Jaccard's coefficient (Jaccard, 1908) and UPGMA (Unweighted Pair Group Method using Arithmetical Averages) dendrogram was generated to determine the genetic relationship of foxtail millet genotypes. Cluster analysis of standardized morphological data was performed using the Euclidean distance coefficient and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) in NTSYS-pc version 2.11T (Sokal and Michener, 1958). For ISSR data, a similarity matrix was generated using the SIMQUAL module based on Jaccard's coefficient. A dendrogram was constructed using UPGMA through the SAHN tool in NTSYS.Parameters of genetic variability were calculated which included, number of monomorphic and polymorphic loci, total bands, polymorphism information content (PIC), resolving power (Rp) effective multiplex ratio (EMR), marker index (MI) and polymorphism percentage.

Results

Morphological Variability

Analysis of variance (ANOVA) was performed in a Randomized Block Design (RBD) to assess ten quantitative traits—including days to 50% flowering, days to maturity, plant height, panicle length, productive tillers per plant, panicle weight, grain yield, straw yield, 1000-grain weight, and protein content, across 30 genotypes, to identify significant genetic variation among the genotypes. The variation due to replication was not found to be significant for all the traits under study as shown in Table 1. All the ten characters under study had shown a wide range of phenotypic variation.

Phenotypic and genotypic coefficients of variation were estimated. Higher estimates of both phenotypic and genotypic coefficients of variation were observed for panicle weight, grain yield per plant, and straw yield per plant. In the case of productive tillers per plant, a moderate genotypic coefficient of variation and a high phenotypic coefficient of variation, along with a large difference between the two. Moderate values of both PCV and GCV were recorded for panicle length and 1000-grain weight. On the other hand, traits such as days to 50 per cent flowering, days to maturity and protein content showed low PCV and GCV values.

Table 2: Measures of variability parameters of ten characters in foxtail millet.

Characters	Range of variation		Mean	GV	PV	EV	GCV (%)	PCV (%)	HB	GAM
	Min.	Max.								
Days to 50% flowering	50.00	73.67	66.08	26.01	28.38	2.37	7.72	8.06	91.65	15.22
Days to maturity	84.00	100.33	95.98	7.38	14.23	6.86	2.83	3.93	54.24	4.20
Plant height	103.50	193.62	173.56	240.17	375.39	135.22	8.93	11.16	63.98	14.71
Panicle length	12.60	22.71	18.74	4.30	6.78	2.48	11.11	13.94	63.45	18.23
Productive tiller per plant	2.34	5.39	3.05	0.32	0.51	0.19	18.55	23.31	63.30	30.40
Panicle weight	5.17	15.29	9.61	6.55	7.20	0.65	26.63	27.91	91.03	52.33
Grain yield per plant	3.86	12.09	7.99	3.80	5.89	2.10	24.37	30.37	64.41	40.29
Straw yield per plant	9.59	21.10	13.89	10.68	11.78	1.10	23.53	24.70	90.70	46.16
1000 grain weight	2.45	3.84	3.29	0.12	0.14	0.02	10.53	11.49	84.03	19.88
Protein content	10.41	12.47	11.12	0.17	0.72	0.54	3.76	7.63	24.30	3.82

GV: Genotypic variance; PV: Phenotypic variance; EV: Environment variance; HB: Heritability (h^2bs) (%); GAM: Genetic Advance (% Mean); GCV (%): Genotypic coefficient of variation; PCV (%): Phenotypic coefficient of variation

Table 3: DNA concentration and A260/A280 ratio of foxtail millet genomic DNA.

Sr. No.	Genotypes	Nucleic acid concentration(ng/µl)	A260/ A280
1	W.Fx. -1	468	1.91
2	W.Fx. -2	941.9	1.86
3	W.Fx. -3	341.4	1.92
4	W.Fx. -4	1089.1	1.95
5	W.Fx. -5	848.2	1.96
6	W.Fx. -6	290.3	1.99
7	W.Fx. -7	1080	1.92
8	W.Fx. -8	1005.3	1.98
9	W.Fx. -9	1021.4	1.92
10	W.Fx. -10	759.1	2.01
11	W.Fx. -11	237.0	1.87
12	W.Fx. -12	926.9	2.02
13	W.Fx. -13	603.0	2.01
14	W.Fx. -14	612.7	2.02
15	W.Fx. -15	887.4	1.95
16	W.Fx. -16	673.3	1.91
17	W.Fx. -17	687.6	1.96
18	W.Fx. -18	624.8	2.04
19	W.Fx. -19	568.1	1.98
20	W.Fx. -20	820.6	1.93
21	W.Fx. -21	247.0	2.01
22	W.Fx. -22	1008.7	2.07
23	W.Fx. -23	1097.6	1.99
24	W.Fx. -24	449.6	2.06
25	W.Fx. -25	773.5	1.99
26	W.Fx. -26	735.1	1.92
27	W.Fx. -27	540.9	1.83
28	DHFT-109-3	697.2	1.96
29	SIA-31-56	859.7	1.91
30	Local check	853.6	1.96
	Average	724.96	

Most of character studied in this research had high value of heritability along with genetic advance (Table 2). High heritability found for Characters like days to 50% flowering (91.65%), plant height (63.98%), panicle length (63.45%), productive tillers per plant (63.30%), panicle weight (91.03%), grain yield per plant (64.41%), straw yield per plant (90.70%) and 1000 grain weight (84.03%). Moderate heritability value found for trait days to maturity (54.24%).

Higher broad sense heritability coupled with high genetic advance over percent mean was reported for characters like, panicle weight, productive tillers per plant, straw yield per plant and grain yield per plant. High broad sense heritability with medium genetic advance over per cent mean was reported in characters *viz*, days to 50 % flowering, 1000 grain weight, panicle length, plant height. Medium broad sense heritability with low genetic advance over percent mean was reported for only one character *i.e.* days to maturity.

Molecular Diversity Analysis

Primer analysis:

PCR based molecular markers *i.e.* ISSR were used in present study. Quantitative analysis showed the average concentration of DNA extracted from foxtail millet leaves, which was 724.96 ng/µl, quantified on nanodrop. The genotype W.Fx. -4 showed the highest DNA concentration of 1089.1 ng/µl followed by W.Fx. -9 showed (1021.4 ng/µl). Lowest concentration of DNA (237 ng/µl) was recorded for genotype W.Fx. -11 (Table 3). A total of 15 ISSR primers from the UBC series were initially screened to identify primers suitable of producing polymorphic amplification profiles among the foxtail millet genotypes. Out of these, only ten primers produced reproducible and polymorphic banding patterns suitable for further analysis as shown in Fig. 3. The selected 10 ISSR primers collectively amplified a total of 60 bands, with amplicon size ranging from 188.19 to 1996.24 bp. Among these, primer UBC-818 produced the highest number of bands (9), whereas primer UBC-823 produced minimum (4). Of the 60 amplified bands, 15 were monomorphic (25%) and 45 were polymorphic (75%). On an average, each primer generated 6 bands, with an average of 4.5 polymorphic bands per primer as shown in Table 4.

Similarity Index based on Jaccard's Coefficient:

Jaccard's similarity coefficients among the thirty genotypes ranged from 0.14 to 0.75. The lowest genetic distance (0.14) was observed between genotypes W.Fx.-10 and W.Fx.-8, as well as between W.Fx.-11 and W.Fx.-8, followed by a similarity coefficient of 0.16 between W.Fx.-27 and W.Fx.-8. Conversely, the greatest genetic dissimilarity (0.75) was recorded between W.Fx.-14 and

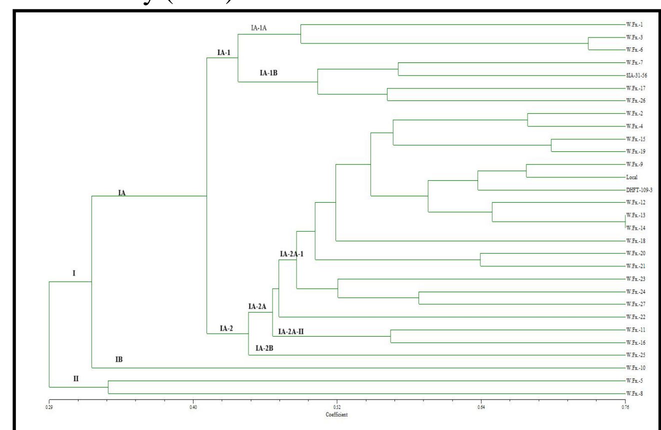

Fig. 2: Cluster analysis among Foxtail millet genotypes based on ISSR marker.

Table 4: Details of ISSR marker analysis.

Sr. No.	Primer	Total bands	Polymorphic band	Polymorphic %	Band size (bp)	PIC	RPI	MI
1	UBC-810	6	5	83.33	272.72- 1996.24	0.914	5.45	76.17
2	UBC-811	6	5	83.33	263.33- 752.46	0.668	4.01	55.67
3	UBC-812	6	4	66.66	192.30- 1969.52	0.628	3.77	41.87
4	UBC-813	7	5	71.42	233.88- 1267.98	0.813	5.70	58.07
5	UBC-816	6	5	83.33	309.82- 1460.11	0.711	4.27	59.25
6	UBC-818	9	7	77.78	198.85- 1989.71	0.78	7.02	60.67
7	UBC-819	6	4	66.67	188.19- 771.78	0.667	4.00	44.46
8	UBC-823	4	3	75	385.22- 1451.72	0.725	2.90	54.375
9	UBC-829	5	3	60	264.69- 790.74	0.445	2.22	26.7
10	UBC-830	5	4	80	439.69- 1740.03	0.922	4.61	73.76
Total		**60**	45	747.54	-	7.27	44.00	550.98
Average		**6**	4.5	74.75	-	0.72	4.40	55.09

W.Fx.-13, and between W.Fx.-15 and W.Fx.-14, followed by 0.72 between W.Fx.-3 and W.Fx.-6 as shown in Table 6.

Cluster Analysis:

The UPGMA clusters are depicted in Fig. 2. Thirty foxtail millet genotypes were grouped into two main clusters I and cluster II. Maximum number of genotypes was grouped in cluster I (28), which was further divided into two sub clusters IA and IB. Main cluster II has less number of genotypes as compared to Main cluster I (2) as shown in Table 5.

Discussion

Morphological Variability

All 30 genotypes varied significantly in all the traits measured by an analysis of variance. Similar results for most of characters were also reported by Yogeesh *et al.,* (2015), Kavya *et al.,* (2017), Sachan (2017), Amarnath *et al.,* (2018), Anuradha and Patro (2019), Mohan *et al.,* (2019) and Singh *et al.,* (2023).

Heritable genetic effects and non-heritable environmental influence contribute to variability presented in the germplasm. The GCV flows the heritable portion, while the PCV is an expression of both genetic as well as the environmental effects on the trait. Higher PCV than the GCV indicates influence of environment on the expression of trait. Higher estimates of both PCV and GCV indicates a wide scope for improvement through selection in the desired direction. Moderate GCV and a high PCV suggests a significant environmental influence and so direct selection would be less effective. Moderate values of both PCV and GCV reflects the presence of substantial inherent variability and suggesting that these traits can be improved through careful selection. Low PCV and GCV values indicates limited genetic variation and ineffective selection due to less variation present.

Genotypic variation alone cannot determine selection efficiency. Heritability indicates how much of the observed variation is genetic and heritable, helping predict selection success. High heritability means traits are less affected by the environment and mainly controlled by additive genes, so they can be improved through simple phenotypic selection. Similar results for heritability were reported by Yogeesh *et al.,* (2015), Kavya *et al.,* (2017), Sachan (2017), Mohan *et al.,* (2019) and Singh *et al.,* (2023).

Heritability values coupled with genetic advance are more reliable and useful genetic parameters in predicting the genetic gain under selection than heritability estimates alone (Kundu *et al.,* 2008). Higher broad sense heritability coupled with high genetic advance over percent mean confirmed the role of additive gene action, so the traitscan be improved through direct phenotypic selection. The findings were in accordance with Yogeesh *et al.,* (2015), Sachan (2017) and Amarnath *et al.,* (2018). High broad sense heritability with medium genetic advance over per cent mean, suggested the effect of additive gene action and offers a limited improvement through direct phenotypic selection. Similar findings were also reported

Table 5: Clusters of Foxtail millet genotypes using UPGMA dendrogram.

Main cluster	Sub cluster		Genotypes
I	IA	IA-1	W.Fx. -1, W.Fx. -3, W.Fx. -6, W.Fx. -7, W.Fx. -17, W.Fx. -26, SIA-31-56
		IA-2	WFx. -2, WFx. -4, W.Fx. -9, WFx. -11, W.Fx. -12, W.Fx.-13, W.Fx. -14, W.Fx. -15, W.Fx. -16, W.Fx. -18, W.Fx. -19, W.Fx. -20, W.Fx. -21, W.Fx. -22, W.Fx. -23, W.Fx. -24, W.Fx. -25, W.Fx. -27, DHFT-109-3 and Local check
	IB		W.Fx. -10
II			W.Fx. -5, W.Fx. -8

Table 6: Jaccard's similarity matrix based on ISSR analysis.

	W.Fx.1	W.Fx.2	W.Fx.3	W.Fx.4	W.Fx.5	W.Fx.6	W.Fx.7	W.Fx.8	W.Fx.9	W.Fx.10	W.Fx.11	W.Fx.12	W.Fx.13	W.Fx.14	W.Fx.15	W.Fx.16	W.Fx.17	W.Fx.18	W.Fx.19	W.Fx.20	W.Fx.21	W.Fx.22	W.Fx.23	W.Fx.24	W.Fx.25	W.Fx.26	W.Fx.27	DHFT-109-3	SIA-31-56	Local check
W.Fx.1	1.00																													
W.Fx.2	0.53	1.00																												
W.Fx.3	0.51	0.59	1.00																											
W.Fx.4	0.51	0.67	0.42	1.00																										
W.Fx.5	0.31	0.38	0.45	0.29	1.00																									
W.Fx.6	0.46	0.53	0.72	0.42	0.52	1.00																								
W.Fx.7	0.40	0.30	0.40	0.45	0.50	0.44	1.00																							
W.Fx.8	0.41	0.24	0.34	0.25	0.33	0.40	0.34	1.00																						
W.Fx.9	0.48	0.54	0.40	0.64	0.25	0.40	0.43	0.21	1.00																					
W.Fx.10	0.28	0.30	0.22	0.38	0.20	0.22	0.31	0.14	0.44	1.00																				
W.Fx.11	0.28	0.50	0.34	0.52	0.20	0.30	0.20	0.14	0.53	0.27	1.00																			
W.Fx.12	0.43	0.44	0.35	0.63	0.30	0.31	0.45	0.22	0.60	0.35	0.45	1.00																		
W.Fx.13	0.42	0.44	0.37	0.60	0.28	0.33	0.45	0.23	0.65	0.41	0.41	0.69	1.00																	
W.Fx.14	0.52	0.50	0.44	0.64	0.28	0.36	0.43	0.25	0.69	0.33	0.53	0.60	0.75	0.10																
W.Fx.15	0.48	0.58	0.58	0.56	0.39	0.48	0.39	0.27	0.65	0.28	0.57	0.52	0.61	0.75	1.00															
W.Fx.16	0.45	0.46	0.37	0.52	0.20	0.37	0.28	0.20	0.50	0.22	0.56	0.42	0.38	0.56	0.57	1.00														
W.Fx.17	0.42	0.44	0.41	0.51	0.36	0.46	0.50	0.27	0.45	0.37	0.38	0.40	0.47	0.52	0.44	0.38	1.00													
W.Fx.18	0.47	0.48	0.41	0.51	0.32	0.41	0.37	0.32	0.52	0.37	0.38	0.55	0.56	0.48	0.52	0.41	0.47	1.00												
W.Fx.19	0.37	0.61	0.50	0.50	0.40	0.50	0.32	0.26	0.51	0.25	0.55	0.46	0.41	0.51	0.69	0.47	0.41	0.54	1.00											
W.Fx.20	0.35	0.52	0.38	0.59	0.30	0.38	0.35	0.19	0.56	0.31	0.52	0.47	0.47	0.60	0.56	0.52	0.51	0.47	0.54	1.00										
W.Fx.21	0.35	0.44	0.30	0.51	0.25	0.30	0.34	0.17	0.48	0.30	0.45	0.51	0.47	0.52	0.45	0.42	0.47	0.51	0.42	0.63	1.00									
W.Fx.22	0.42	0.40	0.34	0.50	0.26	0.34	0.30	0.22	0.51	0.27	0.41	0.50	0.50	0.62	0.28	0.51	0.35	0.39	0.45	0.53	0.46	1.00								
W.Fx.23	0.48	0.41	0.43	0.48	0.21	0.34	0.38	0.24	0.50	0.38	0.33	0.45	0.53	0.54	0.54	0.42	0.40	0.44	0.42	0.41	0.33	0.33	1.00							
W.Fx.24	0.55	0.48	0.38	0.55	0.23	0.38	0.35	0.26	0.52	0.38	0.42	0.55	0.55	0.60	0.48	0.42	0.51	0.55	0.42	0.44	0.47	0.50	0.52	1.00						
W.Fx.25	0.38	0.44	0.41	0.51	0.32	0.33	0.29	0.27	0.48	0.22	0.38	0.43	0.47	0.52	0.52	0.48	0.31	0.35	0.41	0.47	0.32	0.46	0.44	0.36	1.00					
W.Fx.26	0.42	0.44	0.41	0.47	0.32	0.41	0.45	0.32	0.41	0.25	0.38	0.43	0.42	0.48	0.48	0.35	0.56	0.38	0.45	0.47	0.43	0.35	0.48	0.47	0.38	1.00				
W.Fx.27	0.41	0.46	0.40	0.45	0.30	0.40	0.31	0.16	0.47	0.41	0.34	0.42	0.54	0.55	0.47	0.37	0.54	0.45	0.40	0.54	0.50	0.41	0.51	0.58	0.45	0.50	1.00			
DHFT-109-3	0.38	0.48	0.38	0.51	0.25	0.38	0.30	0.21	0.64	0.34	0.48	0.55	0.60	0.60	0.56	0.45	0.43	0.51	0.50	0.51	0.47	0.42	0.48	0.47	0.47	0.55	0.50	1.00		
SIA-31-56	0.43	0.36	0.48	0.44	0.37	0.53	0.57	0.32	0.50	0.33	0.29	0.48	0.53	0.45	0.45	0.29	0.53	0.39	0.38	0.33	0.32	0.35	0.45	0.48	0.39	0.53	0.46	0.48	1.00	
Local check	0.48	0.54	0.43	0.57	0.21	0.43	0.27	0.24	0.67	0.34	0.50	0.52	0.58	0.54	0.58	0.50	0.36	0.48	0.47	0.52	0.40	0.43	0.54	0.52	0.48	0.44	0.46	0.62	0.45	1.00

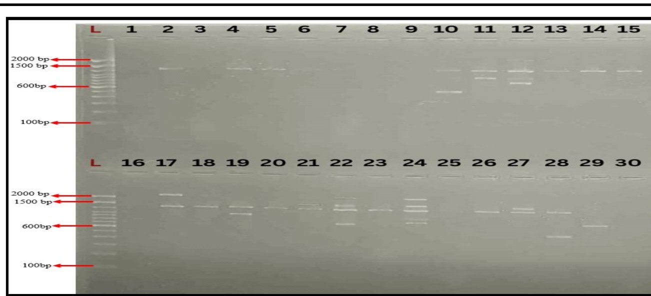

Fig. 3(a): ISSR profile of 30 foxtail millet genotypes using primer **UBC-810**

Fig. 3(f): ISSR profile of 30 foxtail millet genotypes using primer **UBC-818**

Fig. 3(b): ISSR profile of 30 foxtail millet genotypes using primer **UBC-811**

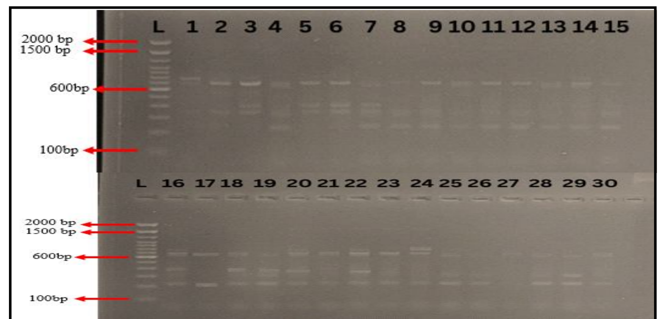

Fig. 3(g): ISSR profile of 30 foxtail millet genotypes using primer **UBC-819**

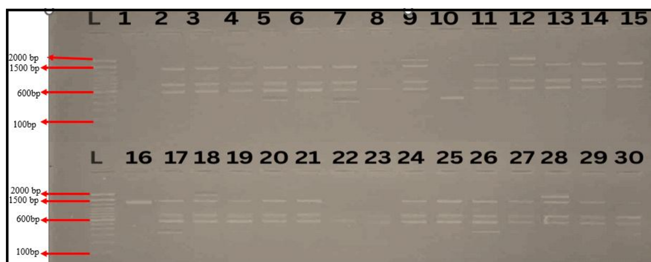

Fig. 3(c): ISSR profile of 30 foxtail millet genotypes using primer **UBC-812**

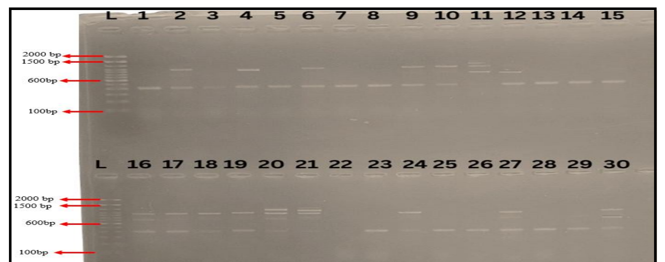

Fig. 3(h): ISSR profile of 30 foxtail millet genotypes using primer **UBC-823**

Fig. 3(d): ISSR profile of 30 foxtail millet genotypes using primer **UBC-813**

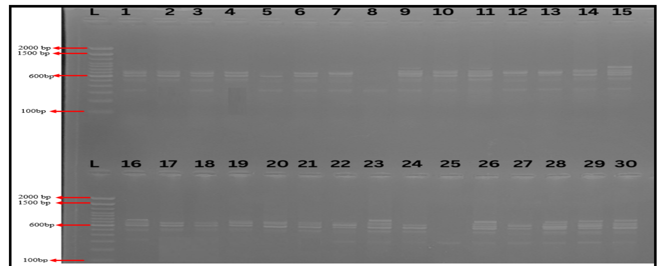

Fig. 3(i): ISSR profile of 30 foxtail millet genotypes using primer **UBC-829**

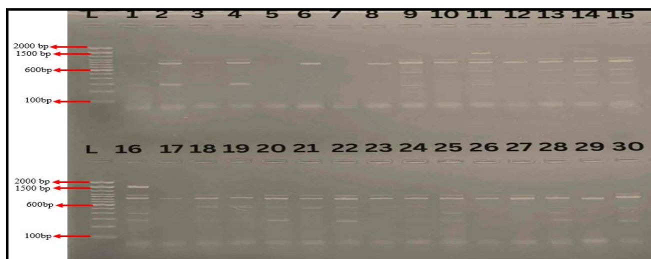

Fig. 3(e): ISSR profile of 30 foxtail millet genotypes using primer **UBC-816**

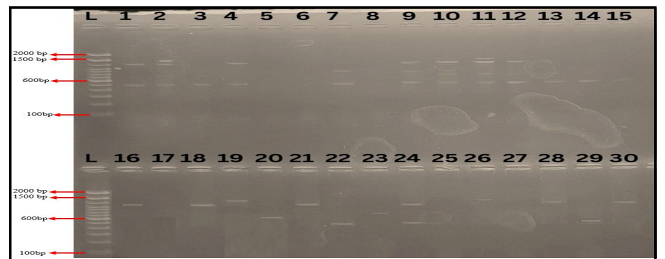

Fig. 3(j): ISSR profile of 30 foxtail millet genotypes using primer **UBC-830**

Fig. 3: ISSR Profiling using 10 different markers.

by Karvar *et al.,* (2021), Mohan *et al.,* (2019), Sachan (2017). Medium broad sense heritability with low genetic advance over percent mean which indicated high influence of environmental effect and selection would not be effective. This finding was closely similar to the finding of Chidambaram and Palanisamy (1995) and Yadav *et al.,* (2024) for moderate broad sense heritability; Johar (2015) and Bhakuni *et al.,* (2021) for low genetic advance over percent mean.

Molecular Diversity Analysis

The amplification results obtained using ISSR markers were scored as presence (1) or absence (0) of amplicons. This binary matrix was then utilized for statistical analysis to assess genetic similarity and infer evolutionary relationships among the foxtail millet genotypes. The dendrogram representing the relationships between several germplasms is depicted. In a dendrogram, the germplasms that are closer together are more similar to one another than those that are far apart and also shows the relative magnitude of resemblance among different clusters. Cluster I had maximum number of genotypes (28) which indicated that these genotypes had high genetic similarity then those genotypes present in cluster II and Cluster II had only 2 genotypes that indicated less genetic diversity then Cluster I.

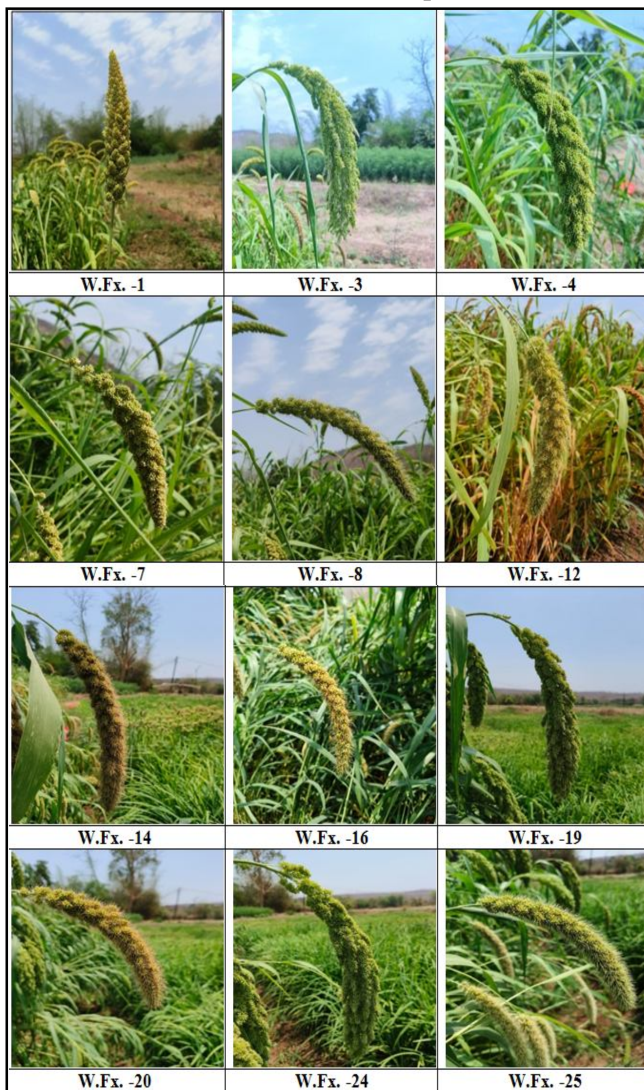

Fig. 4: Morphological Diversity in Foxtail Millet Germplasm.

Conclusion

Evaluation and characterization of germplasm accessions generate valuable information on genetic variability, which is important for the enhancement of yield-contributing traits and other agronomically important characteristics. In the present study, the analysis of variance revealed that the mean sum of square due to genotypes were found highly significant for all the ten characters under study indicating the existence of considerable amount of genetic variability in the experimental material. PCV was observed slightly higher than genotypic coefficient of variation for all the characters studied indicating a negligible influence of environmental variation for their expression. Panicle weight, grain yield per plant and straw yield per plant reported high value for GCV and PCV, which indicated good potential for improvement through selection. Productive tillers per plant, panicle weight, grain yield per plant and straw yield per plant reported higher broad sense heritability coupled with high genetic advance over percent mean, so these traits can be effectively improved by direct phenotypic selection in breeding programme.

The ISSR analysis with selected 10 primers, of which maximum MI (76.17) was found in UBC -810 and PIC (0.922) values were recorded in UBC-830, whereas the maximum RPI (7.02) value was recorded in UBC-818. So, the primer UBC-810, UBC-818, UBC-830 were found to be most efficient for the diversity analysis in foxtail millet genotypes. The cluster pattern divided 30 genotypes into two major clusters. Cluster I containing 28 genotypes indicating high genetic similarity and Cluster II contain 2 genotype which suggested that Cluster II had less genetic diversity then Cluster I.

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Data Availability

The datasets generated and/or analyzed in this study are available from the corresponding author upon reasonable request.

Author's contribution

Conceptualization and designing of the research work (SAM, VPA, VGD, CBN, MKS, NYV); Execution of field/lab experiments and data collection (SAM, VPA, VGD, NKP); Analysis of data and interpretation (SAM, VPA, VGD, NKP); Preparation of manuscript (SAM, VPA, VGD, NKP, CBN, MKS, NYV).

Conflict of interest: The author declares no conflict of interest.

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