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RESEARCH ARTICLE

Assessment of genetic diversity and population structure of barnyard millet [*Echinochloa frumentacea* (Roxb.) Link.] genotypes for yield and quality traits

Aditi Singh[$], Subhash C. Kashyap[*], Gyanendra Kumar Rai[1], Bupesh Kumar and Radheshyam Kumawat[&]

Abstract

Barnyard millet is an important minor millet crop that is valued for its superior nutritional qualities and short duration. The studies on genetic diversity aimed at exploring diversity within genotypes through morphological, molecular, and nutritional profiles (Fe and Zn) were undertaken to assess significant variation among 30 barnyard millet genotypes. The genotypes were categorized for qualitative and quantitative traits based on the Shannon diversity index and D^2 analysis, respectively. Thirty genotypes were grouped into four clusters, wherein Cluster I and Cluster IV comprised 19 and 9 genotypes, respectively; Cluster II and Cluster III each had a single genotype only. The genetic diversity analysis using 14 SSR markers on 12 genotypes of barnyard millet showed polymorphism. The highest number of alleles per locus was generated by BMESSR 16, BMESSR 19, and BMESSR 28. The higher PIC value (0.63) was found to be of the BMESSR 19 marker. The cluster analysis for both molecular and morphological analyses grouped the genotypes into two clusters. The first Cluster comprised 20 genotypes, and the other comprised 10 from the Jammu region, suggesting the presence of diversity among the genotypes. All the genotypes were stratified into 2 groups (K=2) in population structure analysis. The results of micronutrient content analysis showed the highest amount of iron content in IEC-217 (26.24 mg/100g) and the highest zinc content in BAR 1012 (6.35 mg/100g). The study highlighted the presence of genetic diversity in diverse barnyard millet genotypes for conservation and further use in crop improvement.

Keywords: Barnyard, millet, SSRs, D^2, micronutrient, diversity

Introduction

Barnyard millet is an excellent minor millet crop valued for both food and feed purposesand is rich in essential nutrients, making it a potential contributor to food and nutritional security under changing climatic conditions. Millets are commonly known as "orphan crops" but are now referred to as "nutri-cereals" due to their high nutritional value (Bhinda et al. 2023; Nagaraja et al. 2024). The genus has two major cultivated species: Japanese barnyard millet (*E. esculenta* (A. Braun) H. Scholz) and Indian barnyard millet (*E. frumentacea* Link). It is primarily grown in South and Eastern Asian countries, *viz*. Japan, Korea, North Eastern China, India, Pakistan, and Nepal (Yabuno *et al.* 1987; Sood *et al.* 2015). Its grains contain carbohydrate (65%), crude fiber (13.6%), protein (11%) and fat (3.9%) and most notably Iron (Fe), Zinc (Zn) and antioxidant compounds (Hadimani *et al.* 1993; Watanabe et al. 1999; Chandel et al. 2014; Nandini *et al.* 2020). It has a short cooking time, enriched with high nutrition and itsconsumption has been reported to have a number of health benefits, including protection against diabetes, cardiovascular disease, obesity, skin disorders and celiac disease (Bhatt et al. 2023). The total dietary fibre content of barnyard millet is very high (12.6%), including soluble (4.2%) and insoluble (8.4%) fractions, indicating that it is beneficial for type-II diabetics (Ugare et al. 2014). It is a valuable crop for marginal farmers due to its drought tolerance, rapid growth, and remarkable nutritional characteristics (Durairajan *et al.* 2024). It is also resistant to a variety of biotic and abiotic stresses, making it an excellent supplementary crop for subsistence farming as well as an alternative crop during monsoon crop failure.

Plant breeders face multiple global challenges that affect food security, productivity, accessibility, and nutritional quality. Despite multiple nutritional and economic advantages, barnyard millet has gained less scientific attention than major cereals in the past few decades. Germplasm collections possess the necessary genetic resources to further enhance barnyard millet's different agronomic and nutritional properties (Bhinda et al. 2023; Renganathan et al. 2020a). However, the use of these

Division of Plant Breeding and Genetics, Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu, Chatha 180 009, Jammu (J & K), India

[1]Institute of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu, Chatha 180 009, Jammu (J & K), India

[$]Present address: Department of Genetics and Plant Breeding, Sardar Vallabhbhai Patel University of Agriculture & Technology, Modipuram 250 110, Uttar Pradesh, India

[&]Present address: College of Post Graduate Studies in Agricultural Sciences, Central Agricultural University, (Imphal), Umiam, Meghalaya 793 103, India

***Corresponding Author:** Subhash C. Kashyap, Division of Plant Breeding and Genetics, Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu, Chatha 180 009, Jammu (J & K), India, E-Mail: sck2412@gmail.com

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genetic resources for real progress is typically hindered by the poor understanding of their genetic architecture. The assessment and utilization of this genetic variability are very much essential in plant breeding. The whole-genome sequencing (WGS) of millets threw light on the understanding of gene action, genome organization, and genomic-assisted millet improvement. This technique may lead to the discovery of genes that govern biotic and abiotic stress tolerance in millets. Growing consumer interest in healthy diets, coupled with the climate-resilient characteristics of minor millets; highlight the importance of focusing more research and development on these crops. Except for finger millet and foxtail millet, other small millets have received little scientific attention in terms of genetic and genomic resource development, as well as breeding for yield increase (Vetriventhan et al. 2020). Inadequate characterization of germplasm collections remains a major constraint in barnyard millet breeding, limiting the identification and use of trait-specific donors. Hence, genetic characterization is essential for the efficient utilization of barnyard millet germplasm in breeding programmes aimed at developing high-yielding, stress and disease-resistant cultivars (Elangovan and Venkatesh 2024). Molecular markers are ideal for understanding genetic links in crop species since they are free of environmental variables, dominant/co-dominant and enable accurate scoring techniques (Bjorklund et al. 2009). SSR markers are favoured over other marker systems because they are co-dominant, informative and highly repeatable, found in the majority of eukaryotes, and reflect a high allelic diversity (Mohan et al. 1997). Jayakodi et al. (2019) studied a number of barnyard accessions by using expressed sequence tag (EST) molecular markers and transcriptional changes, discovering genes related to drought and micronutrient content. Reports on genetic diversity using EST-SSR markers in barnyard millet genotypes are rare. With this prior knowledge, the objectives of the present investigation were aimed at the assessment of barnyard millet genotypes integrating morpho-agronomic, molecular, biochemical, iron and zinc and qualitative traits by doing diversity analysis for comprehensive characterization.

Materials and methods

The experimental material comprised of 30 genotypes of Barnyard millet, out of which 20 genotypes of diverse nature obtained from Indian Institute of Millets Research, Hyderabad and 10 selected superior genotypes (Table 1) collected from different locations of Jammu region of J&K. The genotypes were sown in Randomized Block Design with three replications at Research Farm of Plant Breeding and Genetics, Chatha, Jammu in the *kharif* season of 2021-22. Proper agronomic practices were followed throughout the cropping period.

Phenotyping

Keeping the DUS descriptors for barnyard millet in view, five randomly selected plants of each genotype in each replication for 14 days and five qualitative characters were recorded under study following the guidelines provided by the Protection of Plant Varieties and Farmers' Rights (PPV& FR) Authority, India. The data on other morphological and quality characteristics, such as days to 50% flowering, flag leaf length (cm), flag leaf width (cm), flag leaf area (cm^2), peduncle length (cm), panicle length (cm), number of productive tillers, days to maturity, plant height (cm), panicle weight (g), grain yield per plant (g), test weight (g), iron content (mg/100g) and zinc content (mg/100g) were recorded and means of all observations were calculated for further statistical analysis.

Iron and Zinc estimation

Total Iron and zinc content were determined using the Atomic Absorption Spectrophotometer (Z-press 8000; Zeeman Atomic Absorption Spectrophotometer, Germany) (Lindsay *et al*. 1978). Seed sample (1.0 g) was taken in a 100 mL conical flask and digested in 10 mL of a diacid mixture, *i.e.,* HNO_3:$HClO_4$ in a 9:4 ratio for 24 hr at room temperature (33°C) and then at 250°C on a hot plate. The samples were filtered and shifted to a volumetric flask made to 50 mL with double-distilled water before analysis of micronutrients. The concentration of micronutrients was determined against a known concentration standard, prepared from a stock solution of 1000 mg/l (SRL chemicals, Mumbai, India), on

Table 1. A list of barnyard millet genotypes (Source: (Sl. No.1 to 20 from IIMR Hyderabad and 21 to 30 are local germplasm)

S. No.	Genotype
1.	VL-207
2.	VL-172
3.	VL-181
4.	C0-2
5.	PRJ-1
6.	IEC-217
7.	IIMR-BM-3-1920
8.	IIMR-BM-8-1920
9.	BAR-1452
10.	189-1(46)
11.	189-2(47)
12.	BAR 1456
13.	BAR 1446
14.	BAR1012
15.	BAR1396
16.	DHBM 93-3
17.	DHBM 93-2
18.	DHBM 23-3
19.	BM-2-17
20.	BM -29-17
21.	JBM-1
22.	JBM-2
23.	JBM-3
24.	JBM-4
25.	JBM-5
26.	JBM-6
27.	JBM-7
28.	JBM-8
29.	JBM-9
30.	JBM-10

Atomic Absorbance Spectroscopy at wavelengths of 248.3 nm and 213.9 nm for Fe and Zn, respectively.

Genotyping using the ESSR marker

The genomic DNA was isolated from 30 barnyard millet genotypes following the protocol of Zidani *et al.* 2005). The quality and quantity of DNA were checked by 0.8% agarose gel electrophoresis. The final concentration of all the samples was adjusted to 25 ng/µL. The 30 barnyard millet genotypes were genotyped with 14 EST-SSR markers obtained from Manimekalai et al. (2018) and Jayakodi et al. (2019). The EST-SSR markers amplification profiles were scored based on the presence (1) or absence (0) of bands across all individuals. Polymorphism information content (PIC) or expected heterozygosityscore for each EST-SSR marker was calculated based on the formula PIC =1-Σpi2 where Pi is the frequency for the i^{th} allele. The binary data scores were used to construct a dendrogram. The genetic distance between pairs of genotypes using EST-SSR markers was evaluated by calculating Jaccard's similarity coefficient for pairwise comparisons based on the proportions of shared bands produced by the primers. Similarity matrix was generated using the SIMQUAL programme of NTSYS-pc software, version 2.0. The similarity coefficients were used for cluster analysis and a dendrogram was constructed by the Unweighted Pair-group method with arithmetic means (UPGMA). The amplified products were categorized as monomorphic, polymorphic, and null alleles based on amplified bands. For all the barnyard millet genotypes, Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram and Principal Component Analysis (PCA) based on Jaccard's coefficient using DARwin6 (ver.) software was constructed. The genetic structure of 30 genotypes was inferred using the Bayesian algorithm-based STRUCTURE software (ver. 2.3.3).

Results and discussion

Agro-morphological characterization

Qualitative traits are significant with respect to the identification and characterization of genotypes as they are least influenced by the environment. In the table, the Shannon diversity index (H) for barnyard millet is shown together with the relative frequency (%) of several categories of minimum descriptors. The Shannon diversity index (H) ranged from 0.14 to 1.02 with a mean of 0.71. There was significant diversity among 30 genotypes except for inflorescence colour, in which only a single genotype, VL-207, had light purple inflorescence. The majority of genotypes possessed green inflorescence (96.67%), plant pigmentation absent (60.00%), cylindrical shape of inflorescence (60.00%), open panicle compactness (46.67%), and light grey grain colour (60.00%) (Table 2, Fig. 1).

Cluster analysis based on morpho-biochemical traits

The phenotypic distance matrix among 30 genotypes using 14 morpho-nutritional traits was constructed following Mahalanobis D^2 statistics. Based on the Toucher clustering technique, these genotypes were grouped into 4 clusters. Among the 4 clusters, the Cluster I was the biggest comprising 19 genotypes followed by 9 in cluster IV, 1 in Cluster III and IV. The maximum intra cluster distance was observed in Cluster I (28.95) followed by cluster IV (19.01) (S4 Table). Intra cluster distance of 2 clusters namely Cluster II

Fig. 1. Inflorescence of diverse barnyard millet genotypes showed morphological diversity

Table 2. Distribution (%) of qualitative traits of barnyard millet genotypes

Character	No. of frequency	Relative frequency (%)	Shannon Diversity Index
Plant pigmentation			
Absent	18	60.00	0.67
Present	12	40.00	
Inflorescence colour			
Light purple	1	3.33	0.14
Green	29	96.67	
Shape of inflorescence			
Cylindrical	18	60.00	0.85
Pyramidical	10	33.33	
Globose	2	6.67	
Panicle compactness			
Open	14	46.67	1.02
Intermediate	11	36.66	
Compact	5	16.67	
Grain colour			
Straw white	3	10.00	0.89
Light grey	18	60.00	
Grey	9	30.00	
Mean			0.71

and III possess 0 as they have single genotype in each Cluster. The maximum inter cluster distance was observed between Cluster II and Cluster III (83.82) followed by Cluster II and IV (81.04), Cluster I and Cluster III (75.54), while minimum inter cluster distance was observed between cluster III and cluster IV (48.16) followed by Cluster I and cluster IV (52.44), Cluster I and cluster II (53.30) (Supplementary Tables S2 and S3). Previously also similar studies have been conducted for clustering of barnyard millet genotypes (Mehta *et al.* 2005; Trivedi *et al.* 2017; Dhanalakshmi *et al.* 2019; Vikram *et al.* 2020; Amasiddha *et al.* 2025; Singh *et al.* 2025) and drawn conclusions on cluster analysis.

Genotypes performance based on quality traits

Genetic variability in germplasm for the trait of interest is a prerequisite for the success of any breeding program. ANOVA showed significant difference among the 30 genotypes for all the characters studied suggesting availability of sufficient variability and hence selection of superior genotypes could be possible (Supplementary Table S1). The iron content variability ranged from 3.40 to 26.24 mg/100g for JBM-2 and IEC-217 respectively (Fig. 2). The general mean was found to be 14.36 mg/100g. In local germplasm collection, the genotype JBM-1 comprised highest iron content. The zinc content variability ranged from 0.75 to 6.35 mg/100g for JBM-3 and BAR 1012, respectively. The general mean

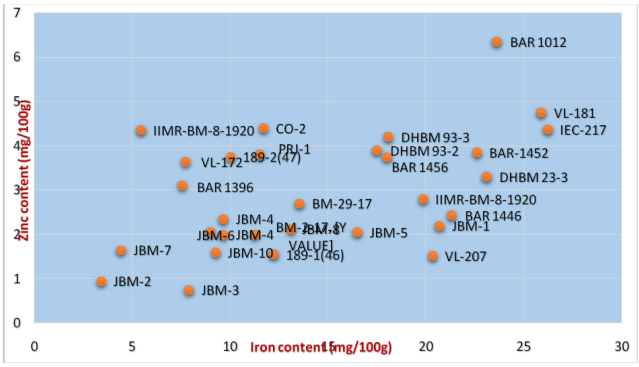

Fig. 2. Graphical representation of iron and zinc content of barnyard millet genotypes

was observed as 2.94 mg/100g, as similar magnitude of variability for micronutrient has also been reported earlier by the researchers (Renganathan et al. 2020b; Renganathan et al. 2021) in barnyard millet.

Contribution of each trait to total divergence

Contribution of each trait to the total divergence was estimated by ranking the individual character. The trait zinc content contributed maximum of 37.03% towards the genetic divergence followed by iron content (21.38%), test weight (20.70%), days to maturity (8.05%), peduncle length (3.22%), days to 50% flowering (2.30%), grain yield per plant (2.30%), number of productive tillers (1.15%), panicle weight (1.15%), flag leaf area (0.92%), plant height (0.68%), panicle length (0.45%), flag leaf width (0.45%), flag leaf length (0.22%). It has been observed that zinc content, iron content and test weight contributed maximum towards genetic divergence (Supplementary Fig.1). The overall contribution of these traits to genetic divergence was found to be 79.11%.

Cluster analysis based on the EST-SSR markers and Population Structure

A total of 14 EST-SSR primers were used for the estimation of genetic diversity among 30 barnyard millet genotypes. Out of 14 primers, amplification was in all the primers (100%) but polymorphism was observed in 12 primers (85%). The range of number of alleles per locus amplified by 12 polymorphic primers varied from 1-4. A total of 37 alleles were generated by 14 SSRs marker for 30 barnyard millet genotypes showed that high genetic variability (Table 3) is present among the experimental material. Highest allele per locus (4) was generated by marker BMESSR 16, BMESSR 19 and BMESSR 28. The polymorphic information content (PIC) was employed for each locus to analyse the information of each marker and its discriminatory power, also, it is an indication of allele diversity and frequency among genotypes. The range of PIC value varied from 0.38- 0.63. The highest PIC value (0.63) was observed for BMESSR 19.

On the basis of dendrogram, the Cluster was divided in two clusters at 0.21 similarities co-efficient *viz.,* Cluster I comprising of all the genotypes obtained from IIMR, Hyderabad and Cluster II comprising of all the collection of Jammu region. Cluster I further divided into 5 clusters *viz.*, A, B, C, D, E comprising 3, 7, 1, 7 and 2 genotypes, respectively (Supplementary Fig. 2). Cluster C comprising of PRJ-1 and IEC-217 genotype was found to be the most diverse genotypes in Cluster I. Cluster II further divided into 3 sub-groups at similarity co-efficient of 0.65 *viz.*, a, b and c comprising 3, 4 and 3 genotypes respectively. Similar studies were been carried out earlier (Padha et al. 2025; Renganathan et al. 2021; Babu et al. 2018) to explore the potential utility of molecular markers which generated high percentage of unique alleles.

Table 3. List of SSR markers used for screening of 30 genotypes of barnyard millet

S. No.	Marker	Annealing temperature (°C)	Product size (bp)	No. of alleles per locus	PIC
1	BMESSR 2	54.0	155	3	0.53
2	BMESSR 5	54.4	129	2	0.50
3	BMESSR 8	54.0	142	2	0.38
4	BMESSR 16	56.0	171	4	0.46
5	BMESSR 19	56.0	197	4	0.63
6	BMESSR 22	56.0	150	3	0.53
7	BMESSR 26	55.0	184	2	0.50
8	BMESSR 27	56.0	103	3	0.45
9	BMESSR 28	54.4	105	4	0.52
10	BMESSR 30	55.0	115	2	0.46
11	BMESSR 33	54.4	167	1	-
12	BMESSR 34	57.4	152	1	-
13	BMESSR 37	56.0	120	3	0.49
14	BMESSR 39	56.8	190	3	0.53

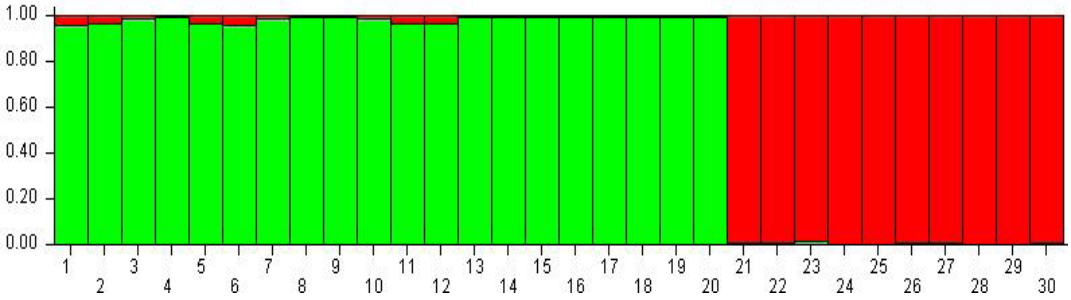

Fig. 3. Gene pool introgression based on population structure analysis at K = 2

A varied genetic similarity ranged from 0.08 to 1.00 has been observed. Highest similarity was observed between genotype IIMR-BM-8-1920 and BAR-1452, DHBM 93-3 and DHBM 23-3, BM-2-17 and BM-29-17, JBM-1 and JBM-2 & JBM-9 and 10 whereas, lowest similarity was observed between CO-2 and JBM-5, CO-2 and JBM-6 & CO-2 and JBM-7 (Supplementary Table S4).

Model-based population structure analysis (STRUCTURE) depicts homogeneous mixture and provides understanding about the introgression in the present population within gene pool, thereby elucidate grouping better than dendrogram. The differentiations at K = 2 revealed almost consistency in pedigree of genotypes with introgression in few genotypes and this mix is quite obvious as breeding genotypes is a continuous process through recombination and out-crossing events (Fig.3). Several studies on population structure have been conducted in foxtail millet (Reddy et al. 2025), porso millet (Vetriventhan et al. 2024; Zargar et al. 2023); broomcorn millet (Li et al. 2021) and finger millet (Ramakrishnan *et al*. 2016) but limited molecular research is conducted in barnyard millet.

It was observed that a good range of diversity was reflected from grouping pattern of barnyard millet genotypes. The result of the present study demonstrated a considerable genetic diversity among barnyard millet genotypes based on both morphological and molecular markers. The grouping of genotypes based on SSR markers was consistent with the conventional grouping with some minor changes based on morphological traits. The utilization of more SSR markers will support to identify novel genes that can provide platform for DNA fingerprinting, genome mapping and gene pyramiding.

Barnyard millet is well-known for having a high nutritional content and exceptional production. Despite its significance, barnyard millet has received little research attention, and the genetic resources of the crop that have been conserved in gene banks have not yet been used for breeding purposes. This is because characterization and assessment of data are not readily available and are not easily accessible for wider applications. The current study's characterization and evaluation of barnyard millet yields valuable information on diversity in both morphological and molecular level. To sum up, this research provided us with a visual representation of the diversity present among the genotypes present in Jammu region and recognized genotypes. Based on this finding, crossing germplasms from several groups is highly recommended.

Authors' contributions

Conceptualization of research (SCK, GKR, BK); Designing of the experiments (SCK, GKR); Contribution of experimental materials (SCK); Execution of field/lab experiments and data collection (AS, RK) Analysis of data and interpretation: (AS, BK, GKR); Preparation of the manuscript (AS, SCK, RK).

Supplementary materials

Supplementary Tables S1 to S4 and supplementary Figures 1 and 2 can be accessed at www.isgpb.org

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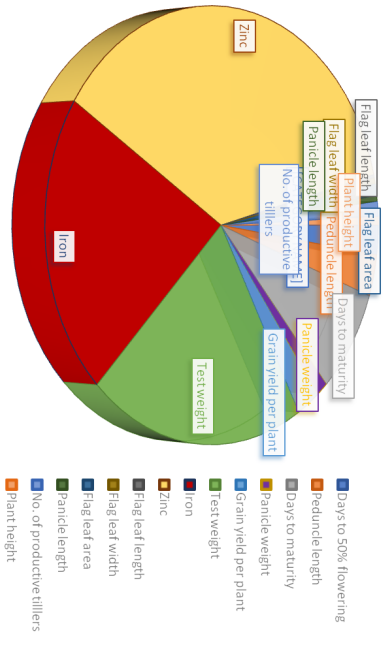
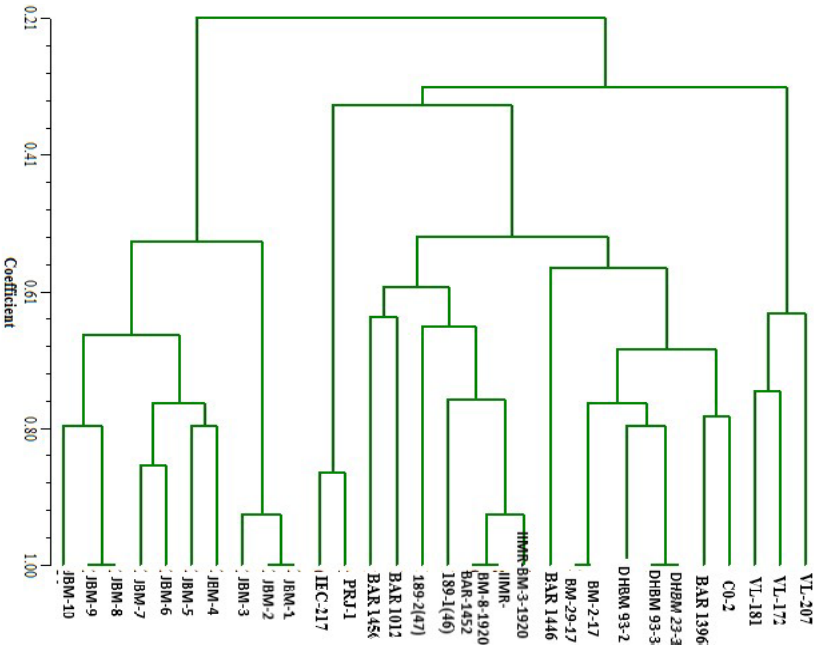
Supplementary Table S1. Analysis of variance for agro-morphological and quality traits in barnyard millet genotypes

Source of variation	*DF*	*Mean Sum of Squares*													
		DTF	*FLL*	*FLW*	*FLA*	*PEL*	*PAL*	*NPT*	*DTM*	*PH*	*PW*	*GYP*	*TW*	*FE*	*ZN*
Replication	2	4.44	7.08	0.18	4.79	1.06	0.85	0.79	1.91	0.40	2.75	57.48	0.01	0.12	0.01
Treatments	29	516.37**	115.82**	1.74**	1026.60**	50.57**	20.03**	21.83**	108.60**	335.68**	19.27**	339.20**	2.92**	132.53**	5.02**
Error	58	0.88	2.18	0.01	8.08	0.34	0.45	0.82	0.63	8.95	0.29	16.32	0.01	0.20	0.01

Where, ** denotes significant) at 1% leveland
DF= Degree of Freedom, DTF= Days to 50% flowering, FLL= Flag leaf length, FLW= Flag leaf width FLA= Flag leaf area, PEL= Peduncle length, PAL= Panicle length, NPT = Number of productive tillers DTM= Days to maturity, PH= Plant height, PW= Panicle weight, GYP= Grain yield per plant, TW= Test weight, FE= Iron content, ZN=Zinc content



Supplementary Fig. 1. Graphical presentation of individual trait towards genetic divergence



Supplementary Fig. 2. Dendrogram of 30 barnyard millet genotypes showing clusters-based similarity

Supplementary Table S2. Genotypic grouping in different clusters

+ Cluster No.	*No. of genotypes*	*Genotypes*
Cluster I	19	VL-207, VL-172, VL-181, CO-2, IEC-217, IIMR-BM-3-1920, IIMR-BM-8-1920, BAR-1452, 189-1(46), 189-2(47), BAR 1456, BAR 1446, BAR1012, BAR 1396, DHBM 93-3, DHBM 93- 2, DHBM 23-3, BM-2-17, BM-29-17
Cluster II	1	PRJ-1
Cluster III	1	JBM-1
Cluster IV	9	JBM-2, JBM-3, JBM-4, JBM-5, JBM-6, JBM-7, JBM-8, JBM-9, JBM-10

Supplementary Table S3. Intra (diagonal) and inter cluster distance

	Cluster) 1	*Cluster) 2*	*Cluster) 3*	*Cluster) 4*
Cluster8 1	28.95	53.30	75.54	52.44
Cluster! 2		0.00	83.82	81.04
Cluster! 3			0.00	48.16
Cluster! 4				19.01

Supplementary Table S4. Similarity matrix based on similarity coefficient value obtained from SSR analysis

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*29*	*30*
1	1.00																													
2	0.59	1.00																												
3	0.69	0.75	1.00																											
4	0.37	0.59	0.50	1.00																										
5	0.29	0.27	0.27	0.35	1.00																									
6	0.35	0.33	0.33	0.42	0.87	1.00																								
7	0.24	0.29	0.35	0.44	0.35	0.29	1.00																							
8	0.29	0.33	0.40	0.50	0.40	0.33	0.93	1.00																						
9	0.29	0.33	0.40	0.50	0.40	0.33	0.93	1.00	1.00																					
10	0.20	0.25	0.25	0.50	0.32	0.25	0.71	0.79	0.79	1.00																				
11	0.33	0.25	0.25	0.41	0.39	0.32	0.60	0.67	0.67	0.69	1.00																			
12	0.19	0.24	0.24	0.39	0.37	0.30	0.56	0.63	0.63	0.64	0.64	1.00																		
13	0.18	0.29	0.35	0.53	0.35	0.35	0.44	0.50	0.50	0.50	0.33	0.39	1.00																	
14	0.20	0.25	0.25	0.41	0.32	0.25	0.50	0.56	0.56	0.69	0.57	0.64	0.60	1.00																
15	0.25	0.44	0.37	0.79	0.44	0.37	0.56	0.63	0.63	0.64	0.53	0.50	0.56	0.53	1.00															
16	0.23	0.40	0.33	0.69	0.33	0.33	0.50	0.56	0.56	0.56	0.47	0.53	0.69	0.47	0.73	1.00														
17	0.24	0.42	0.35	0.63	0.35	0.29	0.53	0.59	0.59	0.60	0.50	0.56	0.53	0.50	0.79	0.80	1.00													
18	0.23	0.40	0.33	0.69	0.33	0.33	0.50	0.56	0.56	0.56	0.47	0.53	0.69	0.47	0.73	1.00	0.80	1.00												
19	0.23	0.40	0.33	0.59	0.33	0.27	0.50	0.56	0.56	0.56	0.56	0.63	0.50	0.56	0.73	0.75	0.80	0.75	1.00											
20	0.23	0.40	0.33	0.59	0.33	0.27	0.50	0.56	0.56	0.56	0.56	0.63	0.50	0.56	0.73	0.75	0.80	0.75	1.00	1.00										
21	0.29	0.22	0.22	0.23	0.27	0.33	0.23	0.27	0.27	0.32	0.39	0.37	0.17	0.25	0.18	0.22	0.17	0.22	0.22	0.22	1.00									
22	0.29	0.22	0.22	0.23	0.27	0.33	0.23	0.27	0.27	0.32	0.39	0.37	0.17	0.25	0.18	0.22	0.17	0.22	0.22	0.22	1.00	1.00								
23	0.30	0.23	0.23	0.24	0.29	0.35	0.18	0.23	0.23	0.26	0.33	0.32	0.18	0.26	0.19	0.17	0.13	0.17	0.17	0.17	0.93	0.93	1.00							
24	0.18	0.17	0.13	0.13	0.23	0.17	0.24	0.23	0.23	0.26	0.33	0.32	0.08	0.20	0.19	0.17	0.24	0.17	0.23	0.23	0.59	0.59	0.53	1.00						
25	0.17	0.12	0.12	0.08	0.22	0.17	0.23	0.22	0.22	0.25	0.32	0.30	0.08	0.19	0.13	0.12	0.17	0.12	0.17	0.17	0.65	0.65	0.59	0.80	1.00					
26	0.13	0.17	0.13	0.08	0.17	0.13	0.24	0.23	0.23	0.26	0.26	0.32	0.08	0.20	0.14	0.13	0.18	0.13	0.17	0.17	0.59	0.59	0.53	0.73	0.80	1.00				
27	0.18	0.17	0.13	0.08	0.23	0.17	0.24	0.23	0.23	0.26	0.33	0.32	0.08	0.20	0.14	0.13	0.18	0.13	0.17	0.17	0.59	0.59	0.53	0.73	0.80	0.86	1.00			
28	0.17	0.27	0.17	0.17	0.27	0.22	0.17	0.17	0.17	0.19	0.25	0.24	0.13	0.14	0.24	0.22	0.29	0.22	0.27	0.27	0.47	0.47	0.42	0.80	0.65	0.69	0.69	1.00		
29	0.17	0.27	0.17	0.17	0.27	0.22	0.17	0.17	0.17	0.19	0.25	0.24	0.13	0.14	0.24	0.22	0.29	0.22	0.27	0.27	0.47	0.47	0.42	0.80	0.65	0.69	0.69	1.00	1.00	
30	0.18	0.23	0.17	0.18	0.23	0.23	0.13	0.13	0.13	0.14	0.20	0.19	0.18	0.09	0.19	0.29	0.24	0.29	0.23	0.23	0.50	0.50	0.44	0.73	0.59	0.53	0.53	0.80	0.80	1.00