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GENETIC DIVERGENCE ANALYSIS IN BARNYARD MILLET (*ECHINOCHLOA FRUMENTACEA* ROXB.) GERMPLASM USING MORPHOLOGICAL AND MOLECULAR MARKERS

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ABSTRACT

Germplasm collection of 145 accessions of barnyard millet were evaluated for fifteen quantitative traits to study the genetic divergence. Among them, high yield along with earliness was seen in GB 6, GB 10 and GB 12. PCGB 41 was high yielding with earliness coupled with high Fe and Zn. GB 30, GB 35, PCGB 2 showed significant performance for high yield and Zn content. GB 41, GB 46, PCGB 1, PCGB 3, PCGB 5, PCGB 6, PCGB 9, PCGB 13 and PCGB 16 showed high Fe content and grain yield. D^2 Cluster analysis suggested composition of 145 genotypes into 24 clusters. Cluster I had a maximum of ninety six genotypes, followed by Cluster XVIII with nine genotypes, Cluster XVI with seven genotypes and Cluster XI, XX, XXIII with five genotypes each. The inter cluster distances varied from 15.46(between cluster II and V) to 246.21(between cluster XII and XX). Inter crossing between the accessions of cluster VII, X, XI, XX and XIX in all possible combinations would exhibit high heterosis and also generate a broad spectrum of variability for effective selection in the segregating generations for development of high yielding cultivars with increased Fe and Zn content in the grain. Out of 25 SSR markers used in this study 24 markers produced clear, scorable and polymorphic marker profile and were used for the further analysis. The PIC values ranged from 0.50 (p88) to 0.95 (b 126) with an average of 0.70. SSR markers used in this study were highly informative and polymorphic.

Keywords **:** Barnyard millet, Diversity analysis, Clustering, D^2.

Introduction

Domestication of *Echinochloa* spp. dates back to early Neolith era (11-8.6 kyBP). The *Echinochloa* spp. consists of two domesticated species, *E. Frumentacea* Roxb. (Indian lineage) and *E. colonum* (Japanese lineage). Other forms are used extensively in Sub Saharan Africa and S E Asia for human consumption as well as fodder purposes (Upadhyaya *et al*., 2014).

Indian Barnyard millet *E. frumentacea*, is the second most important small millet after finger millet with a production and productivity of 87 kilo tonnes and 857 kg/ha respectively (Padulosi *et al*., 2009). *E .frumentacea* consists of four races *Stolonifera*, *Intermedia*, *Robusta, Laxa*(Gupta *et al*., 2009). Valued for its drought tolerance, barnyard millet gives moderate yield in 90–100 days. Barnyard millet is considered a nutricereal with higher iron and fiber content, low glycemic index. The grain being colourless, odourless and bland in taste can aptly fit in Indian cuisine (Veena *et al.*, 2004). Wide variation in iron and zinc composition has been reported by several workers. Diversity in barnyard millet is being fast eroded due to considerable reduction in acreage and changing socio-cultural and economic dimensions of the farming community in India (Maikhuri *et al*., 2001). The crop is still considered as a minor food and feed crop of poor tribal people, has not attracted research efforts like other major crop plants and very limited work has been carried out for its improvement. In India, barnyard millet breeding is carried out mainly in the states of Uttarakhand and Tamil Nadu. So far, more than 20 improved cultivars have been developed and released for different barnyard millet growing regions of the country.

Selection of superior lines would be effective only when genetic variability exists in the material chosen for improvement. Once the core accessions are selected, the next logical step is to understand the level of genetic diversity in the core collection and identification of sources for traits of economic importance including resistance to biotic and abiotic stresses, yield and related traits for further use in breeding.

Materials and Method

The present investigation comprising of 145 barnyard millet (*Echinochloa frumentacea* Roxb.) germplasm

accessions was carried out at the Millet Breeding Station, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. Fifteen traits were studied to understand the genetic divergence following the procedures given in *Echinochloa* millet descriptors (IBPGRI,1983). The quantitative characters studied were days to first flowering, days to 50 *per cent* flowering, plant height (cm), number of productive tillers, flag leaf length (cm), flag leaf width (cm), peduncle length (cm), panicle exertion (cm), inflorescence length (cm), lower raceme length (cm), days to maturity, thousand grain weight (g), Zn content (mg 100^{-1}), Fe content (mg 100^{-1}) and grain yield per plant (g). Grain Fe and Zn content was determined by diacid mixture method (Piper, 1966). The digests were used for Fe and Zn determination using Atomic Absorption Spectrophotometer (AAS). The mean content of iron and zinc was calculated as milli gram per hundred grams (mg/100g). The mean values of the sample from each replication were measured as the replication data and they were subjected to statistical analysis. Mahalanobis' D^2 statistic was used for estimating the genetic divergence among the 145 genotypes.For determining the group constellations, a relatively simple criterion suggested by Tocher (Rao, 1952) was followed. Ranking of individual D^2 values contributed by each character was worked out for fifteen characters by using the principle that the highest contribution of a particular character is indicated by its lower rank total and vice versa. Ranking of individual D^2 values contributed by each character was worked out for fifteen characters by using the principle that the highest contribution of a particular character is indicated by its lower rank total and vice versa (Murty *et al.*, 1965).

Molecular diversity analysis

Leaf samples of 145 germplasm accessions were collected at two leaf stage seedlings grown in germination paper. The DNA was extracted as per Mace *et al*. (2003) and stored for further analysis. There were no SSR markers publicly available in barnyard millet during the time of start of this work and hence a set of 25 genomic SSR markers of foxtail millet which were already reported for polymorphism in foxtail millet were selected. Foxtail millet SSR markers were used due to their high cross transferability with barnyard millet reported by Muthamilarasan *et al*. (2014). The list of markers used are presented in Table 4.

Clear and unambiguous bands of the amplified products were scored for their presence or absence with the score 1 indicating their presence and 0 indicating their absence for each primer genotype combination. The data entry was done in binary data matrix as discrete variables. Jaccard's coefficient of similarity was calculated and a dendrogram based on similarity coefficient was generated using Unweighted Pair Group Method based on Arithmetic Mean (UPGMA) through the computer package NTSYS-PC 2.02i.

Polymorphism information content (PIC) values were calculated for SSR markers, in order to characterize the capacity of each primer to reveal or detect polymorphic loci among the genotypes. It is the sum total of the polymorphism information content values of all the markers produced by a particular primer. PIC value was calculated using the formula $PIC = 1-\sum pi^2$, where pi is the frequency of the i^{th} allele (Smith *et al*., 1997).

Results and Discussion

The genetic divergence both within the genotypes and the characters was tested by Wilk's criterion and was found to be significant. Thus, the analysis of genetic divergence among the genotypes taken for the study was considered to be relevant.

Cluster analysis

Genetic diversity was analysed among 145 barnyard millet genotypes on the basis of fifteen characters using Mahalanobis' D^2 statistic. The mean values of all 145 genotypes were transformed into standardized uncorrelated mean values. The D^2 values were computed for all possible combinations. By using clustering technique as suggested by Tocher, all the 145 genotypes were grouped into 24 clusters. The constituents of different clusters are given in Table 1. Based on this, dendrogram is made and depicted in Figure 1. Percentage contribution of fifteen characters to genetic divergence is given in Table 5 and Figure 2. Cluster I had a maximum of ninety six genotypes, followed by Cluster XVIII with nine genotypes, Cluster XVI had seven genotypes and Cluster XI, XX, XXIII with five genotypes each. All the other clusters were solitary and comprised of only one genotype each. The nature of selection forces operating under one eco-geographical region seemed to be similar to that of other regions since the accessions from different locations were grouped together in all twenty four clusters. Nevertheless the accessions from one eco-geographical region were grouped in different clusters indicating substantial variability within them. Gupta *et al.,*(2009) supported this in his findings.

The cluster mean values are furnished in the Table 2. The cluster mean values of the characters indicated that Cluster I, II, IV, IX, X, XI, XXIII, XVI exhibited moderate mean values for almost all characters. Plant height was highest for Cluster VII (149.1 cm) and lowest for cluster XXII (66.7 cm). Number of tillers was highest in cluster XXII (7.75) and lowest in VII (2). Flag leaf length was longest in cluster VII (30.3 cm) and lowest in cluster XXII (11.5 cm) whereas flag leaf width varied from 1 cm (cluster XIV and XV) to 3.3 cm (cluster VII). Peduncle length exhibited high range of 22.33 cm (cluster XXII) to 42.13 cm (cluster VI). Inflorescence length which directly is responsible for grain yield was high in cluster VII (23.9 cm) and least in cluster XXII (10.5 cm). Higher percentage of panicle exertion was exhibited by cluster XIV (7.6 cm) whereas it was least in cluster (1.5 cm). Longest lower raceme character was present in cluster XXIV (8.25 cm) but lower raceme length was shortest for cluster XV (2 cm). Earliest flowering cluster was XIV (32 days for first flowering and 37 days to reach 50% flowering) and late flowering cluster was (61.5 days for first flowering and 64.5 days to reach 50% flowering). Cluster XXII (CGB 41) took

119 days to mature (late maturing), whereas cluster XIV (GB 10) was the most early maturing type (82.5 days) in the whole germplasm. Thousand grain weights varied from 2.5 g (in cluster XXI) to 3.8 g (in cluster VII). Zinc content showed a great variation of 0.21 mg g^{-1} (cluster XII) to 4.13 mg g^{-1} (cluster XX), whereas Fe content showed least amount of 0.81 mg g^{-1} in cluster XVII to 25.1 mg^{-1} in cluster XIX. Highest grain yield was recorded for cluster VIII (28.43 g) whereas yield was least in cluster V (6.94 g). Among all the clusters cluster VII (FLL, FLW), VIII (TGW, GY) XIX (Fe), XX (Zn) had desirable characters which could be well utilised for future crop improvement.

The knowledge of characters influencing divergence is an important aspect for a breeder. Information on the nature and degree of genetic divergence would help the plant breeder to choose right parents for breeding programmes (Vivekanandan and Subramanian, 1990). Among the multivariate procedures, Mahalanobis (1936) generalized distance (D^2) has been used extensively.

Cluster XVIII comprised of nine accessions (GB 29, CGB 13, PCGB 20, CGB 12, GB 30, PCGB 12, GB 27, CGB 15, GB 13) and showed maximum intra cluster distance. The maximum inter cluster distance was between XII and XX. However, the mean performance of grain yield per plant and yield attributes were low in cluster XII. The mean performance of grain yield and yield attributes were very high in cluster VIII, cluster X, cluster XI with desirable plant height (118–128 cm). The *per se* performance of the accessions belonging to the cluster XIX had highest for Fe content and cluster XX for Zn content. Collectively, inter-crossing between the accessions of cluster VII, X, XI, XX and XIX in all possible combinations would exhibit high heterosis and also generate a broad spectrum of variability for effective selection in the segregating generations for development of high yielding cultivars with increased Fe and Zn content in the grain. Cluster XIV (GB 10) had early flowering with fair grain yield which can be used in breeding with genotypes of the above clusters for improved yield fortified with Fe and Zn and early maturity.

The nearest and farthest clusters from each cluster are presented in Table 3. Cluster XVIII showed the maximum intra cluster distance of 46.67 and was followed by cluster XX, cluster XI, cluster XXIII, cluster XVI and cluster I with their intra cluster distances of 46.04, 40.58, 40.06, 39.72 and 36.69 respectively. Other clusters possess no intra cluster distances due to presence of single genotype in each cluster. The inter cluster distances varied from 15.46(between cluster II and V) to 246.21(between cluster XII and XX). All other distances lie between these values.

Cluster distance was high between cluster XX (GB 35, GB 55, PCGB 41, CGB 6, CGB 25)and other clusters. These accessions were genetically more diverse. However, distance was narrow between cluster II (PCGB 50) and cluster V (GB 44) indicating closeness and similar response for the expression of all the metric traits. These findings were in accordance with findings of Selvarani and Chandirasekaran (2000), Mehta *et al*. (2005), Mehta *et al*. (2007), Gupta *et al*. (2009), Patro*et al*. (2014) and Upadhyaya *et al*. (2014).

SSR marker derived phylogenetic analysis

On considering molecular diversity, 25 markers were used to screen the 145 germplasm accession. Out of 25 SSR markers in this study 24 markers produced clear, scorable and polymorphic marker profile and were used for the further analysis..Based on the Jaccard's similarity coefficient the germplasm has grouped in to 23 clusters in which nine are solitary ones. Cluster XX was the largest with 91 accessions followed by XX1 (9) and XIX (5) Table 4.

The difference in SSR allelic richness can be explained by several factors like diversity range of the germplasm, number of accessions used, number of SSR loci and SSR repeat type (Yang *et al*., 2010). A larger number of SSR loci and the use of dinucleotide repeat SSRs rather than tri- or higher may lead to a higher number of alleles and higher genetic diversity (Yang *et al*., 2010). The SSR markers used in this study are di-nucleotides that might be one of the reasons for higher allelic diversity. Moreover, the higher number of alleles may also be attributed to the material used in this study.

The PIC value is a reflection of allele diversity and the informativeness of each marker. Out of 25 markers, 24 markers were highly polymorphic with PIC values more than 0.50. The PIC values ranged between 0.50 to 0.95. The marker with highest and lowest PIC value was b 126 and p88 respectively .The PIC values ranged from 0.50 (p88) to 0.95 (b 126) with an average of 0.70. This was as per the findings of Nirmalakumari and Vetriventhan (2010) in foxtail millet, higher than that reported in sweet sorghum (0.54, Wang *et al*., 2009) and rice (0.603, Pervaiz *et al*., 2009; 0.42, Jin *et al*., 2010), but lower than that reported in chickpea (0.854, Upadhyaya *et al*., 2008). SSR markers used in this study were highly informative and polymorphic. Thus, validating the polymorphic markers would nourish accurate genetic information for future breeding by characterizing the germplasm projecting genetic diversity.

Conclusion

The absence of relationship between genetic diversity and geographic diversity suggests that forces other than geographic origin, such as exchange of breeding material, genetic drift, variation, natural and artificial selection are responsible for diversity. Selection of parents should be based on genetic diversity and not geographic diversity. The present study suggests that the assessment of genotypes on the basis yield attributing traits along with SSR alleles seems to be more reliable strategies for selection of parents in hybridization. These results in the numbers of divergent clusters with variability for yield, earliness and Fe and Zn content will serves as the donor to develop improved cultivars with high yield and rich nutritional profile.

Table 1 : Distribution of 145 genotypes into 24 clusters based on fifteen characters

Cluster	No. of genotypes	Accession number
I	96	GB2, GB 3, GB 4, GB 5, GB 6, GB 7, GB 8, GB 9, GB 11, GB 12, GB 14, GB 15, GB 16, GB 18, GB 20, GB 21, GB 24, GB 25, GB 26, GB 28, GB 31,, GB 38, GB 39, GB 40, GB 43, GB 45, GB 46, GB 48, GB 49, GB 50, GB 51, GB 52,GB 53, GB 58, GB 59, GB 60, PCGB 1, PCGB 5, PCGB 6, PCGB 7, PCGB 9, PCGB 10, PCGB 11, PCGB 13, PCGB 14, PCGB 15, PCGB 16, PCGB 19, PCGB 21, PCGB 22, PCGB 23, PCGB 24, PCGB 27, PCGB 28, PCGB 29, PCGB 30, PCGB 31, PCGB 32, PCGB 33, PCGB 34, PCGB 35, PCGB 3, PCGB 38, PCGB 39, PCGB 40, PCGB 42, PCGB 43, PCGB 44, PCGB 45, PCGB 46, PCGB 47, PCGB 48, CGB 1, CGB 2, CGB 3, CGB 4, CGB 7, CGB 8, CGB 11, CGB 16, CGB 17, CGB 18, CGB 19, CGB 20, CGB 21, CGB 22, CGB 23, CGB 24, CGB 26, CGB 27,CGB 28, CGB 29, CGB 30, CGB 32, CGB 33, CGB 34
II	1	PCGB 50
III	1	PCGB 26
IV	1	GB 22
V	1	GB 44
VI	1	PCGB 25
VII	1	GB 36
VIII	1	GB 23
IX	1	GB 62
X	1	GB 17
XI	5	GB 63, PCGB 3, GB 41, GB 42, CGB 10
XII	1	GB 32
XIII	1	PCGB 8
XIV	1	GB 10
XV	1	PCGB 4
XVI	7	GB 57, CGB 14, PCGB 49, CGB 5, PCGB 2, GB 33, GB 34
XVII	1	GB 19
XVIII	9	GB 29, CGB 13, PCGB 20, CGB 12, GB 30, PCGB 12, GB 27, CGB 15, GB 13
XIX	1	CGB 9
XX	5	GB 35, GB 55, PCGB 41, CGB 6, CGB 25
XXI	1	PCGB 17
XXII	1	CGB 31
XXIII	5	GB 1, PCGB 18, PCGB 36, GB 47, GB 61
XXIV	1	GB 37

Table 2 : Cluster mean values for fifteen characters in barnyard millet

Cluster	Character														
	PH	**PT**	**FLL**	**FLW**	**PL**	**IL**	**PE**	**LRL**	**DF**	**DFF**	**DTM**	**TGW**	**Zn**	**Fe**	**GY**
I	104.3	3.74	24.98	2.1	33.53	18.6	4.06	3.45	45.1	50.04	101.45	3.42	0.35	11.11	15.22
II	102.6	4.9	26.48	2.0	34.8	18.25	6.55	4.35	39	46	102.5	2.87	0.3	15.01	9.36
III	95.3	4.0	24.9	1.8	32.35	17.35	6.75	2.35	28.5	32	83.5	3.45	**0.21**	12.05	11.37
IV	128.9	3.85	24.5	2.1	32.6	20.75	1.9	3.95	54	56.5	95.0	3.36	0.3	4.8	19.11
V	85.7	3.63	20	1.93	31.88	15	7.22	3.16	41	47.5	87.0	3.2	0.32	14.0	**6.94**
VI	101.2	3.0	26	2.27	**42.13**	20.35	7.1	5.2	38.5	43.5	99.5	3.52	0.4	14.4	9.4
VII	**149.1**	**2.0**	**30.3**	**3.3**	34.05	**23.9**	2.88	4.95	54	61	112.5	3.35	0.32	2.9	19.28
VIII	128.8	4.2	29.5	2.25	36.2	20.25	4.85	3.85	53	57.5	110.5	**3.8**	0.34	8.8	**28.43**
IX	108.8	3.2	28.2	2.4	31.55	21.3	4.1	3.2	53.5	56.0	105.0	3.42	0.94	12.23	19.03
X	118.7	3.7	29.08	2.55	38.13	23.3	1.75	2.95	47.5	55.5	107.5	3.68	0.35	8.0	24.55
XI	120.6	4.51	26.06	2.35	32.88	19.37	4.05	3.66	51.2	54.4	107.4	3.3	0.47	9.39	27.67
XII	81.5	3.8	19.3	1.15	28.05	12.8	3.95	2.85	40.5	46.0	91.0	2.54	**0.21**	16.31	7.84
XIII	101.5	5.75	25.5	2.25	27.5	18.5	**1.5**	2.46	**61.5**	**64.5**	115.5	3.11	0.22	12.06	7.62
XIV	77.6	4.2	16.15	**1.0**	28.9	12.0	**7.6**	2.35	**32**	**37.0**	**82.5**	3.05	0.29	6.9	18.49
XV	75.6	6.5	13.3	**1.0**	27.2	10.8	2.75	**2.0**	40.5	45.5	98.5	2.72	0.43	11.5	3.99
XVI	96.96	4.39	24.9	1.93	30.21	17.0	4.38	2.97	46.9	51.64	103.9	3.39	3.27	11.23	11.41
XVII	107.7	4.1	21.5	1.45	33.3	16.15	4.9	2.9	49	55.5	106.0	2.58	0.29	**0.81**	10.24
XVIII	104.3	4.51	25.29	2.25	32.31	18.57	3.29	3.41	48.4	53.89	104.1	3.36	2.22	10.41	17.59
XIX	105.4	3.8	24.7	1.9	32.55	18.0	4.1	3.6	37.2	49.5	92.5	3.44	0.31	**25.1**	18.26
XX	112.4	2.86	25.7	2.34	33.27	20.24	3.84	3.16	44.3	50.7	106.0	3.29	**4.13**	13.13	18.39
XXI	80.3	5.0	23.38	1.52	32.45	14.43	3.88	3.22	37	40.5	101.5	**2.5**	0.86	15.91	14.72
XXII	**66.7**	**7.75**	**11.5**	1.18	**22.33**	**10.5**	3.0	4.0	36.5	41.0	**119.0**	3.73	0.34	13.1	9.65
XXIII	104.11	3.14	24.13	1.83	31.86	17.11	3.76	3.34	41.9	48.9	98.6	3.46	1.32	11.14	11.01
XXIV	134.1	3.2	27.1	2.05	33.1	22	1.65	**8.25**	58	61	107	3.3	0.24	6.5	17.35

PH - Plant height (cm) **PT**- No. of productive tillers **FLL**- Flag leaf length (cm) **FLW**- Flag leaf width (cm)
PL- Peduncle length (cm) **IL**-Inflorescence length (cm) **PE**- Panicle exertion (cm) **LRL**-Lower raceme length(cm)
DF- Days to first flowering **DFF**- Days to 50% flowering **DM**- Days to maturity **TGW**- Thousand grain weight (g)
Zn - Zn content (mg/100g) **Fe**-Fe content (mg $100g^{-}$) **GY**- Grain yield per plant (g)

Table 3 : Nearest and farthest clusters based on Cluster's D^2 values for fifteen characters

Cluster	No. of genotypes	Nearest cluster	Farthest cluster
I	96	XXI (42.99)	XX (229.97)
II	1	V (15.46)	XX (239.74)
III	1	V (22.04)	XX (244.36)
IV	1	VII (17.59)	XX (227.81)
V	1	II (15.46)	XX (241.41)
VI	1	II (17.28)	XX (232.44)
VII	1	X (32.49)	XX (227)
VIII	1	X (27.42)	XX (226.2)
IX	1	XXI (29.2)	XX (193.04)
X	1	IV (27.2)	XX (223.99)
XI	5	VIII (28.2)	XX (220.99)
XII	1	II (24.79)	XX (246.21)
XIII	1	XV (29.31)	XX (241.52)
XIV	1	III (35.41)	XX (235.38)
XV	1	XXII (28)	XX (234.36)
XVI	7	XX (71.54)	XIX (191.78)
XVII	1	VII (40.95)	XX (231.31)
XVIII	9	XXIII (74.35)	XIX (137.35)
XIX	1	XII (46.43)	XX (241.65)
XX	5	XVI (71.54)	XII (246.21)
XXI	1	IX (29.2)	XX (202.24)
XXII	1	XV (28)	XX (242.08)
XXIII	5	IX (44.24)	XX (176.61)
XXIV	1	VII (32.74)	XX (235.67)

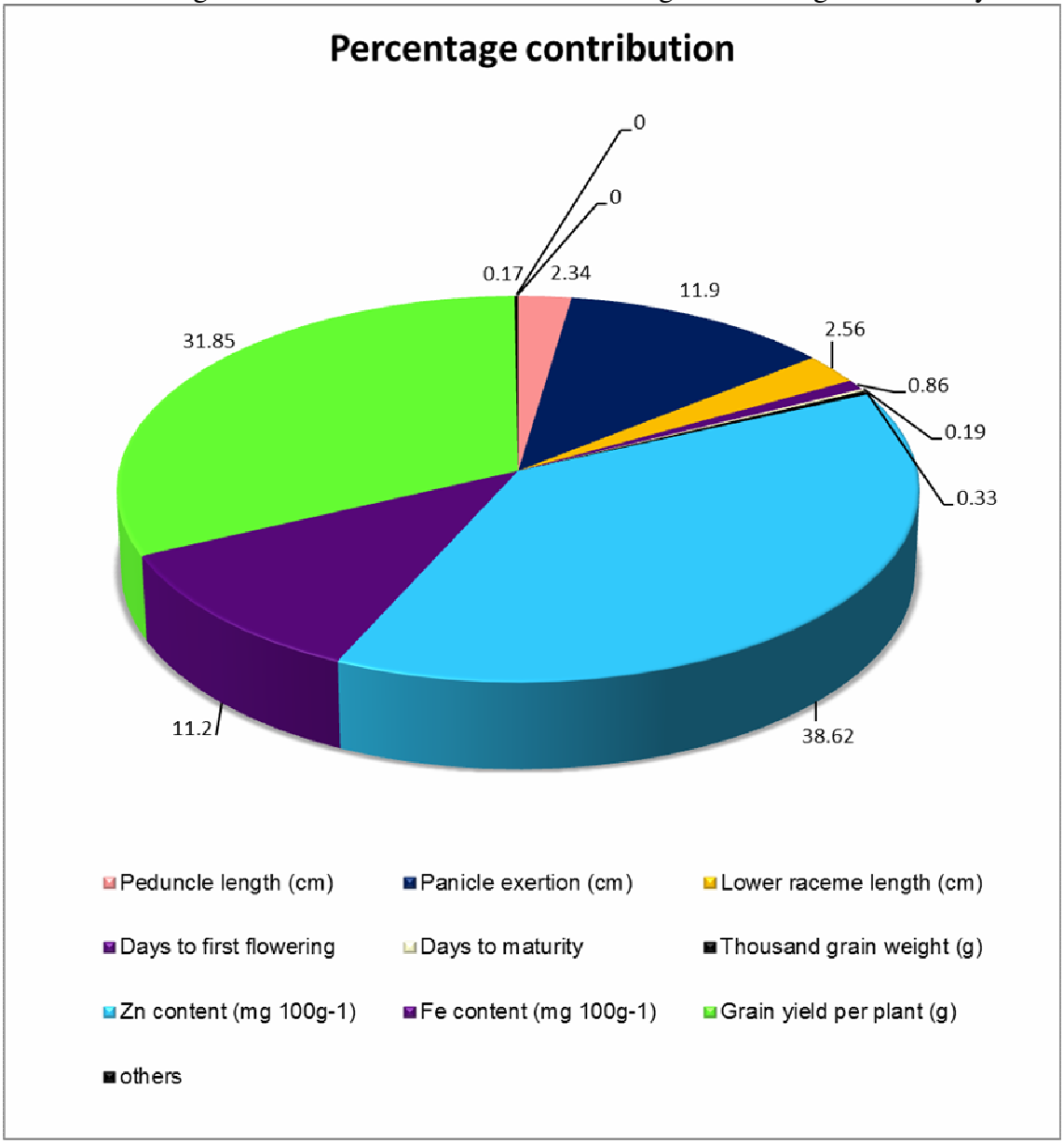
Ta**ble 4 :** Details of 25 foxtail millet SSR markers with forward and reverse primer sequences

S.No	Name of SSR marker	Repeat motif	Forward Primer	Reverse Primer
1	b111	(GT)18(GA)23	AGGATGGTTTGTGTAGCCTG	TTAGTAGTTATGTGTATCGCCG
2	b126	(CT)18(CA)28	TCGCTCCTTATTAGCTTACCACA	ATGATTTGCATTTGCTTTGC
3	p100	(CA)20	AGTTGACACCACACATAACAA	AGAATACTCCTACCTGCCAC
4	p59	(AC)22	TAATTTTGTGGCGTGGGATG	GCACTGGTTTTGTTGAATGG
5	b190	(GA)25	GAAATTTCACAAGTGTTGGTG	TGATCGGAGCAGAGTGTTGA
6	p8	(AC)26	CGATCGAATGATCGATGAAC	CCCTTTGTCCGATCACGTC
7	b186	(GA)40	CCCGTATAAATGTCATCATCCC	GCACCTGGCTTCCCTTT
8	p16	(AC)16	TTTCTCCCTCTCTCGATTCC	AAATTGGCGTGCTAACAACC
9	b223	(GA)34	GGCATTAACTACATTGACAGTGG	AAAACCAACAGTTCCCTCGT
10	p61	(CA)17	CATCCGCGTCATCTGAATC	ACCTGCTGCTATCCATCACC
11	p34	(GT)17	GAGTCTCTTCCCCGTCTCTG	TTTGCCAAGCCTTCATAACC
12	b225	(GA)28	ACCAAGAACTGCCTGCAC	TGCTTAGAACCCACTTGATCG
13	b255	(GA)30	ACCAAGAACTGCCTGCAC	TGCTTAGAACCCACTTGATCG
14	b177	(GA)56	GCACCTTTCTCCTTGTTCCTG	TGTTACTCTCTCTCAACTTGCAG
15	b165	(CT)36	GCTTTGGTTTGGTTTGGTTGG	CCATTAGTCTCTGCCCTTGTT
16	b236	(CT)45	TCTGGACCAGCATTCTGTCTT	GGTAACTCTGCTTGGACGAG
17	p56	(CA)24	GATGTGTACGGGTTGCATTG	TGGGTTTCAGGGCTCTCTC
18	b163	(CT)23	CTCGGAAGCTCAGATTCTCC	CACTTCCTGCAGCTCTCACA
19	p88	(AC)5(GT)22	CAAGCCACCCAGTCTAGAGG	TTCATCAGAACTGCGCAAAC
20	p50	(AC)30	GGGGATACACCGAGATAGAGG	CCCCACATACCAGCAGTTG
21	b159	(CT)24	GCCAGTCCGAGATGGTTAAG	AGCTCTAGCAGTTGGGGACA
22	b234	(CT)26	GCCGCAACGAACAACCG	CCTGTCCCTATCCCTGTCG
23	b129	(CA)24	CACACTCTTCTCCCCTTTTCC	ACGGTAACGGAGGATGGCTA
24	b112	(CA)16(TA)6	CCACCCATTTCAGGTTCTGC	TTGTGGTCAGATTAGGTTGGTC
25	p58	(AC)19	CCTGAGCTCATCCACACAAC	CAGCCTGGAGGAAAGGAATAG

Table 5 : Percentage contribution of fifteen characters to genetic divergence in barnyard millet

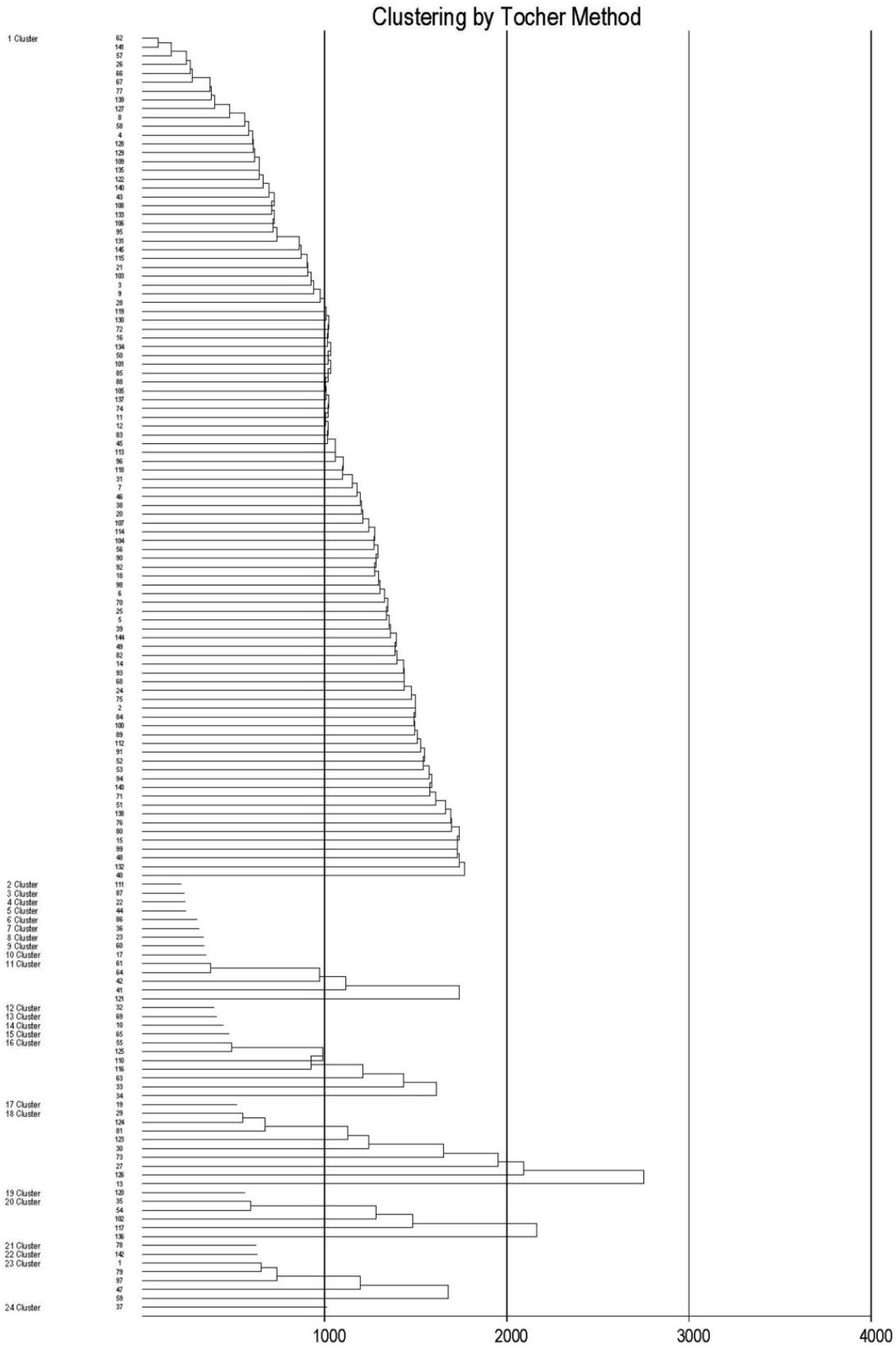
Character	Times Ranked 1st	Contribution %
Plant height (cm)	0	0.00%
Number of productive tillers	5	0.05%
Flag leaf length (cm)	0	0.00%
Flag leaf width (cm)	5	0.05%
Peduncle length (cm)	244	2.34%
Inflorescence length (cm)	7	0.07%
Panicle exertion (cm)	1242	11.90%
Lower raceme length (cm)	267	2.56%
Days to first flowering	90	0.86%
Days to 50% flowering	0	0.00%
Days to maturity	20	0.19%
Thousand grain weight (g)	34	0.33%
Zn content (mg $100g^{-1}$)	4032	38.62%
Fe content (mg $100g^{-1}$)	1169	11.20%
Grain yield per plant (g)	3325	31.85%

Figure 2 : Percentage contribution of fifteen characters to genetic divergence in barnyard millet



*Others includes the 3 characters *viz.*, number of productive tillers, flag leaf width and inflorescence length

Figure 1. Dendrogram based on D^2 values for fifteen characters in barnyard millet



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References

Gupta, A.; Mahajan, V. and Kumar, M. (2009). Biodiversity in the barnyard millet (*Echinochloa frumentacea* Link, Poaceae) germplasm in India. *Genetic Resource and Crop Evolution* 56: 883–889.

IBPGR (1983) *Echinochloa* millet descriptors, Rome, Italy: IPGRI, pp. 18

Jin, L.; Lu, Y.; Xiao, P.; Sun, M.; Corke, H. and Bao, J. (2010) Genetic diversity and population structure of a diverse set of rice germplasm for association mapping. *Theoretical and Applied Genetics,* 121: 475-487.

Mace, E.S.; Buhariwalla, H.K. and Crouch, J.H. (2003). A high-throughput DNA extraction protocol for tropical molecular breeding programs. *Plant Molecular Biology Reporter,* 21: 459a-459h.

Mahalanobis, P.C. (1936). On the generalised distance in statistics. *Proceedings of the National Institute of Sciences*. Calcutta: pp. 49-55

Maikhuri, R.K.; Rao, K.S. and Semwal, R.L. (2001) Changing scenario of Himalayan agro ecosystem: loss of agro-biodiversity an indicator of environment change in central Himalaya, India*. Environmentalist,* 21:23–29.

Mehta, H.; Tyagi, P.C. and Mohapatra, K.P. (2005) Genetic diversity in barnyard millet (*Echinochloa frumentacea*). *Indian Journal of Genetics and Plant Breeding,* 65: 293-295.

Mehta, H.; Tyagi, P.C. and Mohapatra, K.P. (2007) Genetic analysis of morpho physiological and yield component traits in barnyard millet. *Crop Improvement,* 34: 82-85.

Murthy, B.R.; Mathur, J.B.L. and Arunachalam, V. (1965). Self incompatibility and genetic divergence in *Brassica campestris* var. brown sarson. *Sankhya-Series* B(27): 271-278.

Muthamilarasan, M.; Bonthala, V.S.; Pandey, G.; Kumari, K.; Parida, S. and Prasad, M. (2013). Development of 5123 Intron-Length Polymorphic Markers for Large-Scale Genotyping Applications in Foxtail Millet. *DNA Research,* 21: 41-52.

Nirmalakumari, A. and Vetriventhan, M. (2010). Characterization of foxtail millet germplasm collections for yield contributing traits. *Electronic Journal of Plant Breeding,* 1: 140-147.

Padulosi, S.; Mal, B.; Bala, R.S.; Gowda, J.; Gowda, K.T.K.; Shanthakumar, G.; Yenagi, N. and Dutta, M. (2009). Food security and climate change: role of plant genetic resources of minor millets. *Indian Journal of Plant Genetic Resources,* 22: 1-16.

Patro, T.S.S.K.; Neearja, B.; Rani, S.Y.; Keerthi, S. and Jyothsna, S. (2014). Identification of resistant sources for banded blight in Proso millet(*Panicum miliaceum*). *Plant Disease Research,* 29: 14-18.

Perviaz, Z.H.; Rabbani, M.A.; Pearce, S.R. and Malik, S.A. (2009). Determination of genetic variability of Asian rice varieties using microsatellite markers. *African Journal of Biotechnology,* 8: 5641-5651.

Piper, J. (1966). Soil and plant analysis: a laboratory manual of methods for the examination of soils and the determination of the inorganic constituents of plants. Hans publishers Mumbai, pp. 368.

Rao, C.R. (1952). Advanced Statistical Methods in Biometric Research. John Willey and Sons, New York, pp. 390.

Selvarani, M. and Chandirasekaran, V.M. (2000). Genetics divergence and variability in barnyard millet (*Echinochloa frumentacea*). *Annals of Agricultural Research,* 21: 212-215.

Smith, J.M. and Szathmar, E. (1997). The major transition in evolution. Oxford University Press.

Upadhyaya, H.; Dwivedi, S. and Singh, S. (2014). Forming Core Collections in Barnyard, Kodo, and Little Millets Using Morpho-Agronomic Descriptors. *Crop Science* 54: 1-10.

Veena, B.; Chimmad, B.V.; Naik, R.K. and Shantakumar, G. (2004). Development of barnyard millet based traditional foods*. Karnataka Journal of Agricultural. Science* 17: 522-527.

Vivekanandhan, P. and Subramanian, S. (1990) Genetic divergence in rainfed rice. *Oryza*, 30: 60-62.

Wang, M.L.; Zhu, C.; Barkley, N.A.; Chen, Z.; Erpelding, J.E.; Murray, S.C.; Tuinstra, M.R.; Tesso, T.; Pederson, G.A. and Yu, J. (2009). Genetic diversity and population structure analysis of accessions in the Us historic sweet sorghum. *Theoretical and Applied Genetics,* 120: 13-23.

Yang, X.; Yan, J.; Shah, T.; Warburton, M.L.; Li, Q.; Li, L.; Gao, Y.; Chai, Y.; Fu, Z.; Zhou, Y.; Xu, S.; Bai, G.; Meng, Y.; Zheng, Y. and Li, J. (2010). Genetic analysis and characterization of a new maize association mapping panel for quantitative trait loci dissection. *Theoretical and Applied Genetics,* 121: 417–431.