Research Article

Ekin
Journal of Crop Breeding and Genetics

10(1):36-51, 2024

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

Diversity Analysis for Drought Tolerance in Pearl Millet Inbred Lines using SSR Markers

Jagdeep SINGH[1*] Ashok K CHHABRA[2] Rishi K Behl[3] Akshay K VATS[4] Pooja MALIK[5]

[1,3,4,5] Department of Agriculture Maharishi Markandeshwar University Mullana-Ambala, Haryana, India
[2] Department of Genetics and Plant Breeding, CCSHAU Hisar, Haryana, India

[*] Corresponding author e-mail: jagdeep.singh@mmumullana.org

Citation:
Singh J., Chhabra AK., Behl RK., Vats AK., Malik P., 2024. Diversity Analysis for Drought Tolerance in Pearl Millet Inbred Lines using SSR Markers. Ekin J. 10(1):36-51.

Received: 24.09.2023 ***Accepted: 15.11.2023*** ***Published Online: 31.01.2024*** ***Printed: 31.01.2024***

ABSTRACT

50 pearl millet genotypes (inbred lines) were grown in RBD design at two contrasting locations in Haryana, India including one at CCSHAU Hisar and another at RRS, Bawal. Genomic DNA was isolated from leaves of 2-3 weeks old plants using CTAB extraction method. The SSR diversity data was used to determine the genetic relationship among the fifty genotypes. A similarity matrix was first made using SIMQUAL subprogram of software. The dendrogram was then constructed based on the simple matching coefficient using SAHN sub-program. The SAHN sub-program uses UPGMA algorithm to perform cluster analysis. Out of 50 SSRs used for identification of single marker analysis 9 SSRs were found polymorphic for further checking their behaviour on known drought tolerant (HTP 93-37, HTP 03/13-901-1) and drought-sensitive inbreeds (HM S33B, HMS 42B) and were used for amplification of DNA. SSRs showed amplification for all genotypes and thus confirmed in other genotypes for the study of drought in pearl millet genotypes. Primers amplified a total of 237 alleles which varied from 2 to 8 with a mean of 4.54 alleles per locus. The overall size of PCR-amplified products ranged from 140 bp (*PSMP 2271*) to 810 bp (*ICMP 10*). Polymorphic information content (PIC) value ranged from 0.326 (*PSMP2201*) to 0.89 (*XCUMP 009*) with an average of 0.579. 50 Inbred lines based on SSR marker polymorphism data were resolved into 11 diverse clusters two genotypes HTP 9337 and HMS 43B were failed to fall into any cluster. Based on SSR markers and morphophysiological data two inbred lines (HTP 93-37, HTP 03/13-901-1) appeared drought tolerant which may be used for hybrid development program.

Keywords: Inbred, drought tolerant, polymorphism, genotypes, SSR markers

Introduction

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is the fourth most important Nutri cereal crop in India, after rice, wheat and sorghum. It is grown in the arid ecology of Indian states like Rajasthan, Maharashtra, Gujarat and Haryana as well as many other sub-Saharan African countries. It occupies an area of 6.93 million ha with an average production of 8.61 million tons and productivity of 1,243 kg ha^{-1} (Directorate of Millets Development, 2020). The productivity of pearl millet is influenced by the genotypes of plants, its growing environment and genotype x environment interaction (Arya and Yadav, 2009). There is considerable variability was found in pearl millet for adaptation to different environments including water-stress environments leading to drought. This genetic variability can be accessed at morphophysiological and molecular levels. Assessment of genetic variability at morphophysiological is confronted with an unknown degree of G X E interaction influencing character expressions, hence it is less reliable. On the other hand, genetic variability at the molecular level is precise as the DNA is independent of environmental conditions and its genes by itself, thus genetic variability at the molecular level represents true variability that can be transmitted (Satyavathi et al., 2013; Bairwa et al., 2023). Several molecular markers have been used to characterize different crops and their varieties for their molecular diversity including RAPD,

ISSR, SSR SNPS (Jaiswal et al., 2007; Priya et al., 2022; Singh et al., 2023; Bairwa et al., 2023). SSR method is found to be the most appropriate method to study the molecular diversity among the pearl millet genotypes (Colagar et al., 2016).

Pearl millet is multi useful crop as food feed and biofuel which has high protein and minerals. Being gluten-free is an important crop for the wellness of human health particularly for challenging people with diabetes and cardiovascular diseases (Gagan et al. 2023). Although pearl millet is adapted to water stress conditions there are considerable variability for genotype-dependent drought tolerances is there among its genotypes. Therefore, development of pearl millet hybrids possessing high production potential coupled with high tolerance is the most important concern of pearl millet breeders for food security in arid and semi-arid regions (Abhay Bikash 2013; Arya et al., 2014). Screening of pearl millet inbreds plants for drought tolerance plants and their molecular characterization is their fore imperative to identify pearl millet inbred lines possessing desirable traits and diversity at the molecular level. Current study deals with the determination of molecular diversity among pearl millet inbred lines using molecular markers.

Materials and Methods

Materials

The experimental materials comprised fifty pearl millet inbred lines procured from Chaudhary Charan Singh Haryana Agricultural University (CCSHAU) Hisar listed in Table 1. The experimental material was raised at two contrasting locations in Haryana the first location was at CCSHAU, Hisar is situated in the semi-arid climate at 29° 17′ N latitude and 75° 47′E longitude at an altitude of 215.2 meters above mean sea level in the subtropical climatic zone of India. The second location at Regional Research Station (RRS), Bawal, CCSHAU, Hisar is situated at a latitude of 28^{0}08'N, longitude of 76^{0}58'E and altitude of 266 m above sea level in the semi-tropical region of the western zone of India. Chemicals used for preparing DNA extraction buffer, PCR amplification and gel electrophoresis were obtained from G-Biosciences, USA and Sigma Chemicals Co. USA. All other chemicals used were of molecular biology grade or analytical grade and procured from Sigma Chemicals Co., USA, G-Biosciences, USA, Sisco Research Laboratories Pvt. Ltd., India and Affymetrix Inc., USA. Glassware of Borosilicate quality and plastic-ware used throughout the investigation were obtained from Borosil India Ltd. and Tarsons Products Pvt. Ltd. respectively. Specifically, design 50 SSR primers were selected for studying molecular diversity among genotypes. These were synthesized on order from Imperial Life Sciences, USA, the primer pairs included 10 PSMP, 6 ICMP, 5 UMP, 5 CT M and 4 PGIRD series of markers (Allouis et al., 2001; Qi, 2004; Budak et al., 2003; Mariac et al., 2006). The sequence information of forward and reverse primers used for genotyping pearl millet SSR loci is given in Table 2.

Methods

Fifty pearl millet genotypes (inbred lines) were obtained from diverse sources and were grown at Chaudhary Charan Singh Haryana Agricultural University (CCSHAU), Hisar in Randomized Block Design (RBD) at CCSHAU Hisar (normal environment) and at Bawal (drought stress environment). Leaves samples were drawn from each of the 50 inbred lines to extract DNA using CTAB (Cetyl Trimethyl Ammonium Bromide) extraction method given by Murray and Thompson (1980) and modified by Saghai-Maroof et al. (1984). The extracted DNA was purified by removing RNA through the RNase enzyme. DNA samples were treated with 2 µl of RNase A solution (5 mg/ml) per 50 µl DNA sample to remove RNA contamination. The samples were incubated in water bath at 37°C for 4-5 h. After incubation samples were again checked for any RNA left. The purified DNA was analyzed for qualitatively and quantitatively. Quality and Quantity of the isolated genomic DNA was estimated by UV spectrophotometer and agarose gel electrophoresis. Absorbance at 260 nm and 280 nm wavelength was noted using UV Spectrophotometer, the ratio of two wavelengths was calculated and samples with a ratio of 1.7 to 1.8 was considered to be of good quality.

A260 / A280 = 1.8 (pure DNA)

Quality of DNA was also checked by submerged horizontal electrophoresis. A 0.8% (w/v) agarose gel was prepared for this (Sambrook et al., 1989). Gel casting plate was washed air-dried and its ends were sealed with tape. Agarose was melted in 0.5 X TBE buffer and ethidium bromide (10 mg/ml) was added, 1 µl per 50 ml of the gel. Gel solution was then poured into gel casting plate inserted with an appropriate comb to get a 0.4-0.6 cm thick gel. After setting of gel, sealing tapes were removed from both the ends. Gel plate was placed in the electrophoresis chamber and submerged using 0.5 X TBE buffer, combs were removed gently. Samples were prepared by adding 1 µl 6X loading dye along with 8 µl sterile distilled water and pulse centrifuged for proper mixing. Samples were loaded in the wells and electrophoresis was carried out at constant voltage (3 V/cm of gel) until dye migrated to other end of the gel. Gel was then viewed under UV transilluminator and photographed using UV Gel

documentation system. For estimation of quantity of the DNA by UV Spectrophotometer, aliquot of each DNA sample was diluted to the appropriate concentration and absorbance was measured at 260 nm as well as at 280 nm wavelengths. Using the relationship of 1.0 O.D. at 260 nm equivalent to 50 µg DNA per ml, the quantity of DNA was estimated by using the following formula:

DNA (µg⁄ml)=A260×Dilution factor × 50 (µg⁄ml)

For estimation of quantity by 0.8% agarose gel electrophoresis, a lambda DNA of known concentration (50 ng/ µl) was run along with DNA samples.

Fifty specifically designed SSR markers were used to characterize diversity at the DNA level and to identify qualitative genes conferring drought tolerance. The PCR amplification reaction was carried out in G-Storm and Bio-Rad thermocyclers. The PCR reaction contained;

DNA template (50ng)	: 1.0 µl
DMSO	: 1.0 µl
PCR buffer (10 X)	: 2.0 µl
dNTPs mix (10 mM)	: 0.5 µl
F. primer (2.5 µM)	: 1.0 µl
R. primer (2.5 µM)	: 1.0 µl
Taq DNA Polymerase (5 U/µl)	: 0.5 µl
Sterile distilled water	: 12.5 µl
Total volume	: 20 µl

The PCR reaction (20 µl) was set up in 0.2 ml thin-walled PCR tubes with the following reaction conditions:

i. 94°C for 3 min (initial denaturation)
ii. 94°C for 45 s (denaturation)
iii 46-61°C for 1 min (primer annealing)
iv. 72°C for 45 s (primer extension)
Step ii to iv for 5 cycles
v. 94°C for 45 s (denaturation)
vi 44-59°C for 1 min (primer annealing)
vii. 72°C for 45 s (primer extension)
Step v to vii for 30 cycles
v. 72°C for 10 min (final primer extension)
The product was kept at 4°C till further use.

PCR-amplified products were first checked for amplification on 2.5% agarose gel electrophoresis. For this 2-3 randomly selected PCR amplified products for a particular SSR were resolved and viewed using UV transilluminator. The marker-positive samples were then finally resolved using Polyacrylamide Gel Electrophoresis. Bands for SSR analysis were scored based on the presence (taken as 1) or absence (taken as 0) of bands. The size (in nucleotides base pairs) of the most intensely amplified bands for each microsatellite marker was determined based on its migration relative to the standard DNA marker (20 or 100 bp DNA ladder). Multiple alleles were inferred whenever a given marker produced more than one cluster of bands. The polymorphism information content (PIC) for each SSR marker was calculated according to the formula given by Anderson et al. (1993).

Only 0/1 matrix of allele scoring was used to calculate the similarity genetic distance using 'SIMQUAL' sub-programme of NTSYS-pc (version 2.02e) software (Numerical Taxonomy and Multivariate Analysis System Programme, Rohlf, 2000). The dendrogram was constructed by using the distance matrix in SAHN sub-programme of NTSYS-pc by the Unweighted Pair-Group Method with Arithmetic Average (UPGMA) algorithm. Principal Component Analysis (PCA) was done to construct 2 and 3-dimensional diagrams**.** The PAGE was not run due to technical reasons; therefore, no information is available with regard to genetic diversity parameters.

Results

The quantity of DNA obtained from different plants ranged from 200-1000 µg/ml. A260/A280 ratio ranged from 1.75 to 1.85, indicating that the DNA was free from contaminants like polyphenols, polysaccharides, proteins and RNA, etc. A single band of high molecular weight, obtained on 0.8% agarose gel electrophoresis, confirmed that genomic DNA was intact and free from any mechanical or enzymatic degradation.

Variation in allelic profile for SSR markers

To check polymorphism among fifty genotypes, enlisted SSR markers were screened using 2.5% agarose gel electrophoresis for the resolution of bands. Agarose gels displaying allelic polymorphism among genotypes for SSR markers are shown in Fig. 1 to 8. Salient features of microsatellite marker analysis are as follows:

Primers amplified a total of 237 alleles which varied from 2 to 8 with a mean of 4.54 alleles per locus. The overall size of PCR-amplified products ranged from 140 bp (*PSMP 2271*) to 810 bp (*ICMP 10*). Polymorphic information content (PIC) value ranged from 0.326 (*PSMP2201*) to 0.84 (*ICMP 3056*) with an average of 0.579. The amplification range (bp), number of alleles per locus and polymorphic information content (PIC) value of PCR amplified product for individual primer is shown in Table 3.

Molecular marker-based genetic diversity analysis

The SSR diversity data was used to determine the genetic relationship among the fifty genotypes using NTSYS-pc software version 2.02e. A similarity matrix was first made using SIMQUAL subprogram of software. The dendrogram was then constructed based on the simple matching coefficient using the

SAHN sub-program. The SAHN sub-program uses UPGMA algorithm to perform cluster analysis. In this dendrogram (Fig. 9) fifty genotypes formed 11 clusters at a similarity coefficient value of 0.54 whereas two genotypes HTP 9337 and HMS 43B were failed to fall into any cluster. The cluster IV (HMS33B, HMS7B, H77/833-2-202, G73-107 and HBL0538), cluster VII(99HS-24, ARS 07114, MP 293/4, EMRT 11-112, RAJ 3 and HTP 92/80) and cluster IX (HMS 42B, HMS 39B, HMS 45B, H 77/833-2, HTP 93/4 and EMRT 11-116) consisted 6 lines flowed by cluster II (HMS 37B, H 90/4-5, H 77/29-2, MSS 833-22B and TCH 26-1) and cluster X (HMS 32B, HMS 41B, HFEL 10-163, HMS 38B and 78/11) consisted 5 lines each, cluster I(HMS 6B, HMS 22B, HMS 34B and HMS 49B), cluster III (HMS 50B, VCF 6862/98-1, AC O4/13 and EMRT 11-133) and cluster XI (S 97/120, H 94/46R and HTP 03/13-901-1) consisted 4 lines each, cluster V (HMS 40B, 1600 MT and EMRT 11-104), IV (HPT 94/54, H 96/4-5 x H 77/29 and EMRT 11-115), cluster VIII (HBL 11, HBL 056 and A5R10-119) consisted 3 lines each. Furthermore, the simple matching matrix was subjected to Principal component analysis (PCA) for the three principal components. The groupings of fifty genotypes using PCA analysis in 2-D (Fig. 10) and 3-D scaling (Fig. 11) followed the same pattern as depicted in the dendrogram with minor differences.

Discussion

The analysis of genetic variation in breeding materials is of fundamental interest to plant breeders, as it contributes to selection, monitoring of germplasm and prediction of potential genetic gain (Chakravarthi and Naravaneni, 2006). Traditionally, breeders have relied on visible traits to select for improvement of varieties which is less reliable. With the advent of molecular markers, diversity analysis is being conducted using various markers including SSR markers (Koli and Arya, 2022). SSRs markers show polymorphism between species and within species in wheat (Plaschke and Röder 1995) and can help breeders to assess genetic diversity and select genotypes carrying gene(s) of interest. The high reproducibility of SSRs makes them ideal for genome mapping and landmarks for map-based cloning of genes, therefore, molecular maps based on these markers provide the breeders powerful tools for MAS that may optimize time and resources (Plaschke et al., 1995, Korzun et al., 1998, Song et al., 2005). SSRs associated with QTLs have been reported for many important traits. After a linkage between a QTL and a molecular marker has been determined, the QTL can be transferred into any genetic background by marker-assisted selection.

In the present investigation, out of 50 SSRs used for identification of single marker analysis 9 SSRs were found polymorphic for further checking their behaviour on known drought tolerant (HTP 93-37, HTP 03/13-901-1) and drought-sensitive inbreeds (HMS 33B, HMS 42B) and were used for amplification of DNA. SSRs showed amplification for all genotypes and thus confirmed in other genotypes for the study of drought in pearl millet genotypes. Primers amplified a total of 237 alleles which varied from 2 to 8 with a mean of 4.54 alleles per locus. The overall size of PCR-amplified products ranged from 140 bp (*PSMP 2271*) to 810 bp (*ICMP 10*). The molecular size difference between the smallest and the largest allele at an SSR locus varied from 47 bp (*XCUMP 001*) to 880 bp (*ICMP 10*). Polymorphic information content (PIC) value ranged from 0.326 (*PSMP2201*) to 0.89 (*XCUMP 009*) with an average of 0.579 which is near to 0.582 (Kapila et al., 2008), 0.58 (Nepolean et al., 2012) and higher than 0.44 (Singh et al., 2013). The molecular analysis was conducted from pre-selected fifty inbred lines for drought-tolerant traits the current study therefore focused on discerning differences among inbred lines for diversity and not on drought-tolerance traits. It would have been useful to use GenAlEx Software for determining differences among various diversity groups however non-availability of software precluded its use.

Thus, in the study, it could be concluded drought tolerance caused due to rainfed leads to a reduction in the mean performance of the varieties for almost all economic traits. However, this reduction can be avoided to some extent by using drought-tolerant varieties. Breeding for such genotypes/varieties can be eased by identifying markers using molecular marker-assisted selection.

SSR markers exhibited significant variability and divergence among the pearl millet genotypes. Further considering the importance of these molecular tools in the present study drought tolerant genotypes of pearl millet i.e. HTP 93-37, HTP03/13-901-1 were identified. The genetic relationship presented among these genotypes is quite more useful for further hybridization as both these genotypes belong to genetically diverse clusters. Therefore, the study can be helpful in marker-assisted breeding for genetic enhancement of pearl millet genotypes for drought tolerance.

Acknowledgements

The authors wish to acknowledge CCSHAU, Hisar pearl millet section and Regional Research Station (RRS), Bawal, for their support for conducting this experiment.

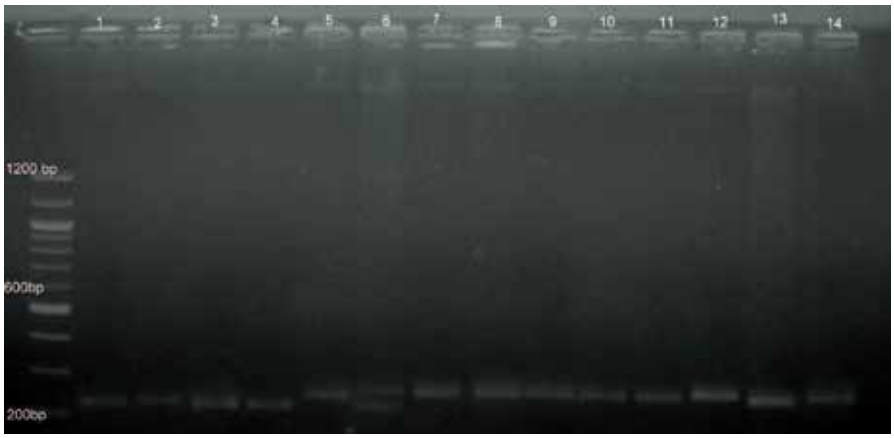

Figure 1. Agarose gel showing allelic polymorphism among pearl millet genotypes at PSMP 2263 locus. Lane L1=100bp ladder, 1 to 14 represents HMS 6B(1), HMS 22B(2), HMS 34(3), BHMS 37B(4), HMS 32B(5), HMS 33B(6), HMS 7B(7), HMS 38B(8), HMS 40B(9), HMS 41B(10), HMS 42B(11), HMS 39B(12), HMS 50B(13), and HMS 49B(14).

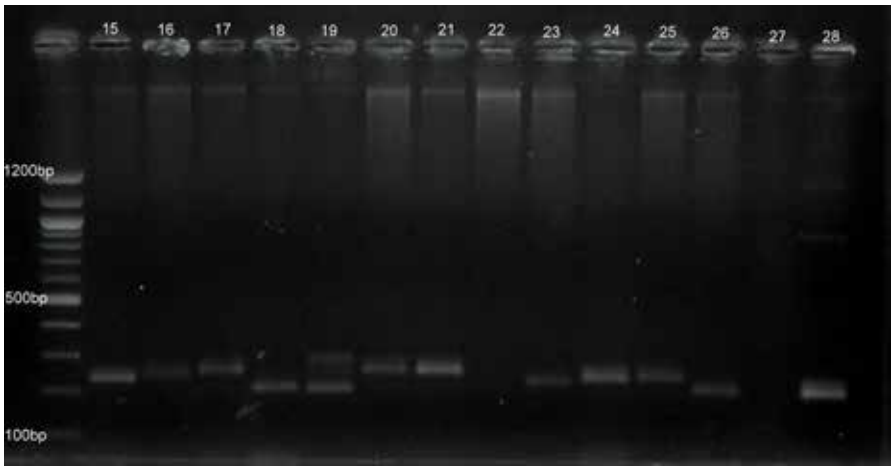

Figure 2. Agarose gel showing allelic polymorphism among pearl millet genotypes at PSMP 2263 locus. Lane L1=100 bp ladder, 15 to 28 represents HMS 45B(15), HMS 43B(16), MS 833-22B(17), HTP 94/54(18), H 77/29-2(19), H 77/833-2(20), H 77/833-2-202(21), G 73-107(22), H 90/4-5(23), HBL 11(24), TCH-26-1(25), HBL 0565(26), VCF6 862/98-1(27), and HBL 0538(28).

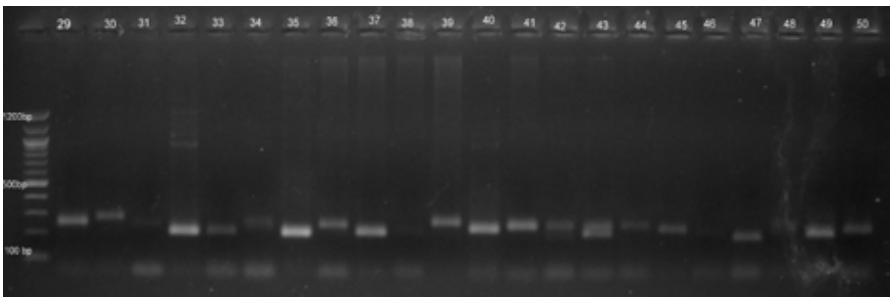

Figure 3. Agarose gel showing allelic polymorphism among pearl millet genotypes at PSMP 2263 locus. Lane L1=100 bp ladder, 29 to 50 represents HTP 93/4(29), AC O4/13(30), EMRT 11-104(31), 78/711(32), (H 96/4-5 x H 77/29-2)(33), A5R 10-119(34), 1660 (MT)(35), EMRT 11-115(36),99 HS-24(37), RAJ 3(38), S 97/120(39), ARS 07114(40), MP 293-4(41), EMRT 11-133(42), HTP 92/80(43), EMRT 11-112(44), HTP 93-37(45), EMRT 11-137(46), EMRT 11-116(47), HTP 03/13-901-1(48), HFeL 10-163(49), and H 94/46R(50).

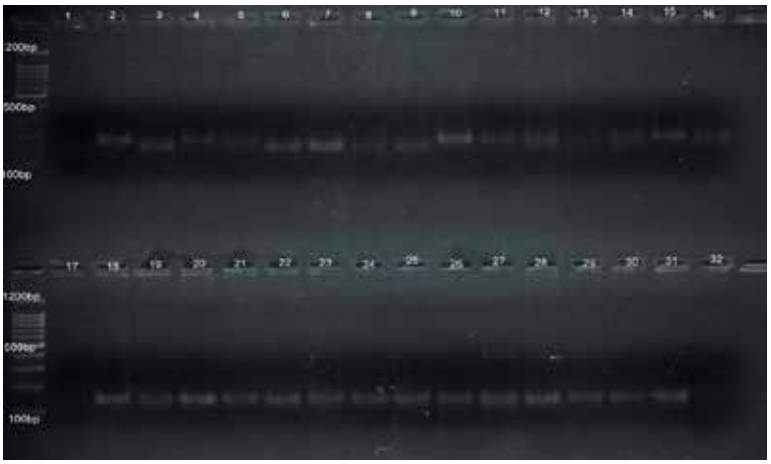

Figure 4. Agarose gel showing allelic polymorphism among pearl millet genotypes at ICMP 3050 locus. Lane L1=100 bp ladder, 1 to 32 represents HMS 6B(1), HMS 22B(2), HMS 34(3), BHMS 37B(4), HMS 32B(5), HMS 33B(6), HMS 7B(7), HMS 38B(8), HMS 40B(9), HMS 41B(10), HMS 42B(11), HMS 39B(12), HMS 50B(13), HMS 49B(14), HMS 45B(15), HMS 43B(16), MS 833-22B(17), HTP 94/54(18), H 77/29-2(19), H 77/833-2(20), H 77/833-2-202(21), G 73-107(22), H 90/4-5(23), HBL 11(24), TCH-26-1(25), HBL 0565(26), VCF6 862/98-1(27), HBL 0538(28), HTP 93/4(29), AC O4/13(30), EMRT 11-104(31), and 78/711(32).

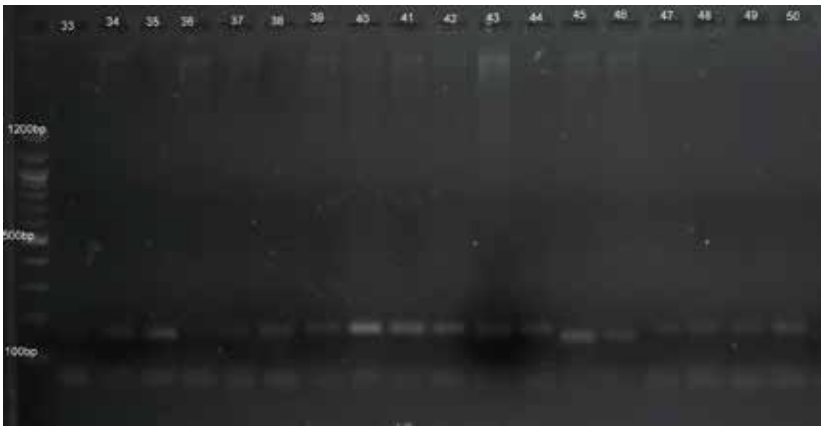

Figure 5. Agarose gel showing allelic polymorphism among pearl millet genotypes at ICMP 3050 locus. Lane L1=100 bp ladder, 33 to 50 represents (H 96/4-5 x H 77/29-2)(33), A5R 10-119(34), 1660 (MT)(35), EMRT 11-115(36), 99 HS-24(37), RAJ 3(38), S 97/120(39), ARS 07114(40), MP 293-4(41), EMRT 11-133(42), HTP 92/80(43), EMRT 11-112(44), HTP 93-37(45), EMRT 11-137(46), EMRT 11-116(47), HTP 03/13-901-1(48), HFeL 10-163(49), and H 94/46R(50).

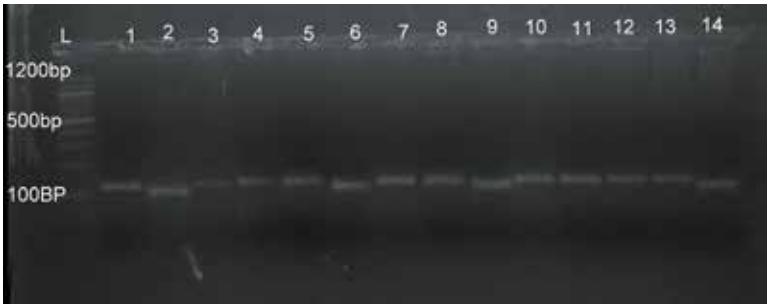

Figure 6. Agarose gel showing allelic polymorphism among pearl millet genotypes at ICMP 3088 locus. Lane L1=100 bp ladder, 1 to 14 HMS 6B(1), HMS 22B(2), HMS 34(3), BHMS 37B(4), HMS 32B(5), HMS 33B(6), HMS 7B(7), HMS 38B(8), HMS 40B(9), HMS 41B(10), HMS 42B(11), HMS 39B(12), HMS 50B(13), and HMS 49B(14)

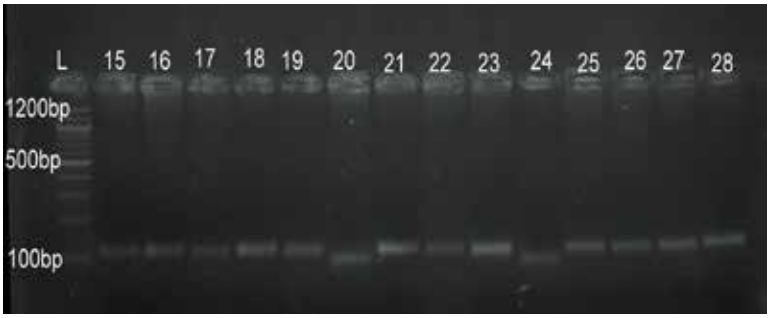

Figure 7. Agarose gel showing allelic polymorphism among pearl millet genotypes at ICMP 3088 locus. Lane L1=100 bp ladder, 15 to 28 represents HMS 45B(15), HMS 43B(16), MS 833-22B(17), HTP 94/54(18), H 77/29-2(19), H 77/833-2(20), H 77/833-2-202(21), G 73-107(22), H 90/4-5(23), HBL 11(24), TCH-26-1(25), HBL 0565(26), VCF6 862/98-1(27), and HBL 0538(28)

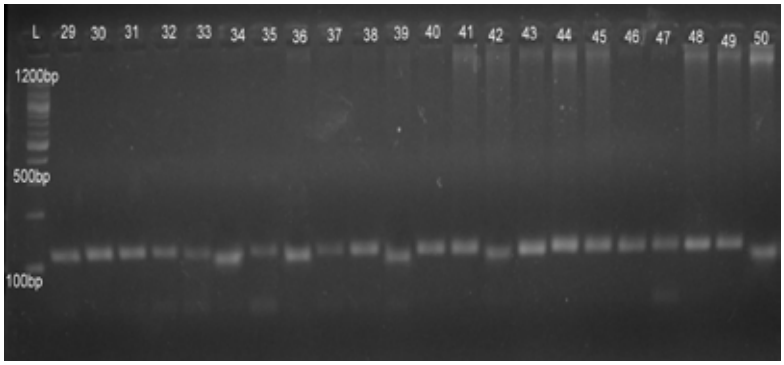

Figure 8. Agarose gel showing allelic polymorphism among pearl millet genotypes at ICMP 3088 locus. Lane L1=100 bp ladder, 29 to 50 represents HTP 93/4(29), AC O4/13(30), EMRT 11-104(31), 78/711(32), (H 96/4-5 x H 77/29-2)(33), A5R 10-119(34), 1660 (MT)(35), EMRT 11-115(36), 99 HS-24(37), RAJ 3(38), S 97/120(39), ARS 07114(40), MP 293-4(41), EMRT 11-133(42), HTP 92/80(43), EMRT 11-112(44), HTP 93-37(45), EMRT 11-137(46), EMRT 11-116(47), HTP 03/13-901-1(48), HFeL 10-163(49), and H 94/46R(50)

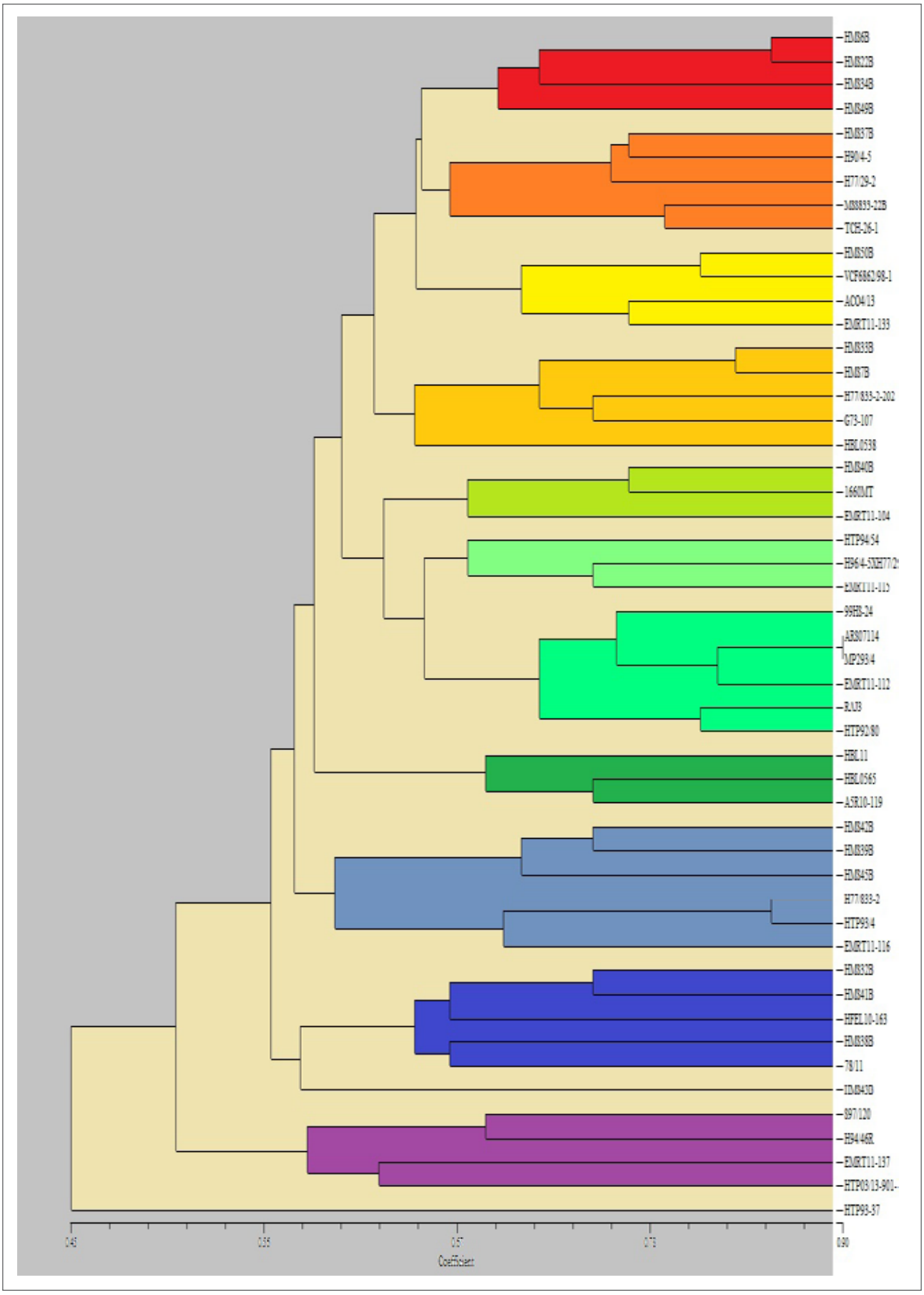

Figure 9. Dendrogram showing relationship among fifty pearl millet genotypes based on similarity matrix data using 50 SSR markers

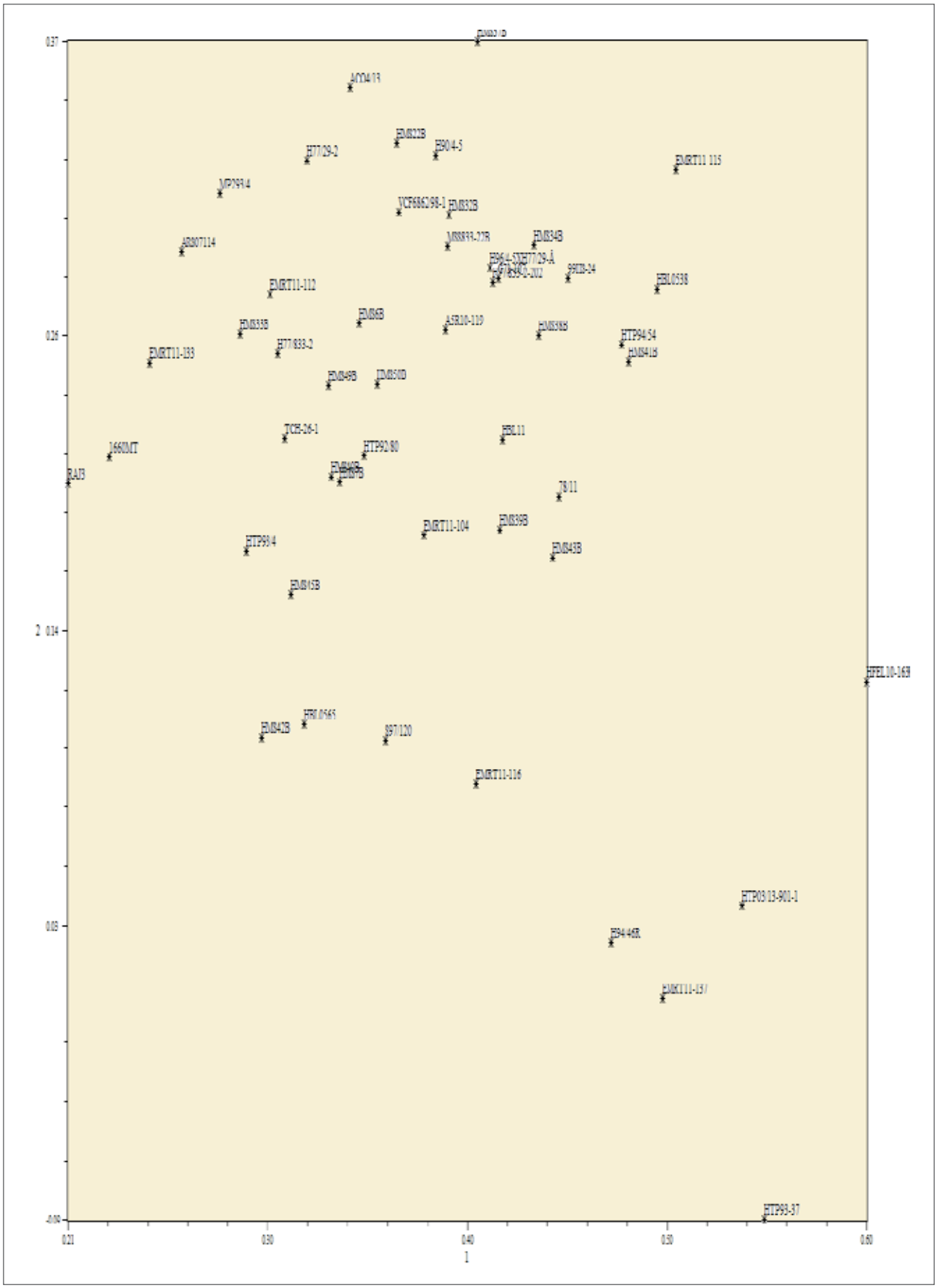

Figure 10. Two dimensional PCA (Principal component analysis) scaling of fifty pearl millet genotypes using similarity matrix data of 50 SSR markers

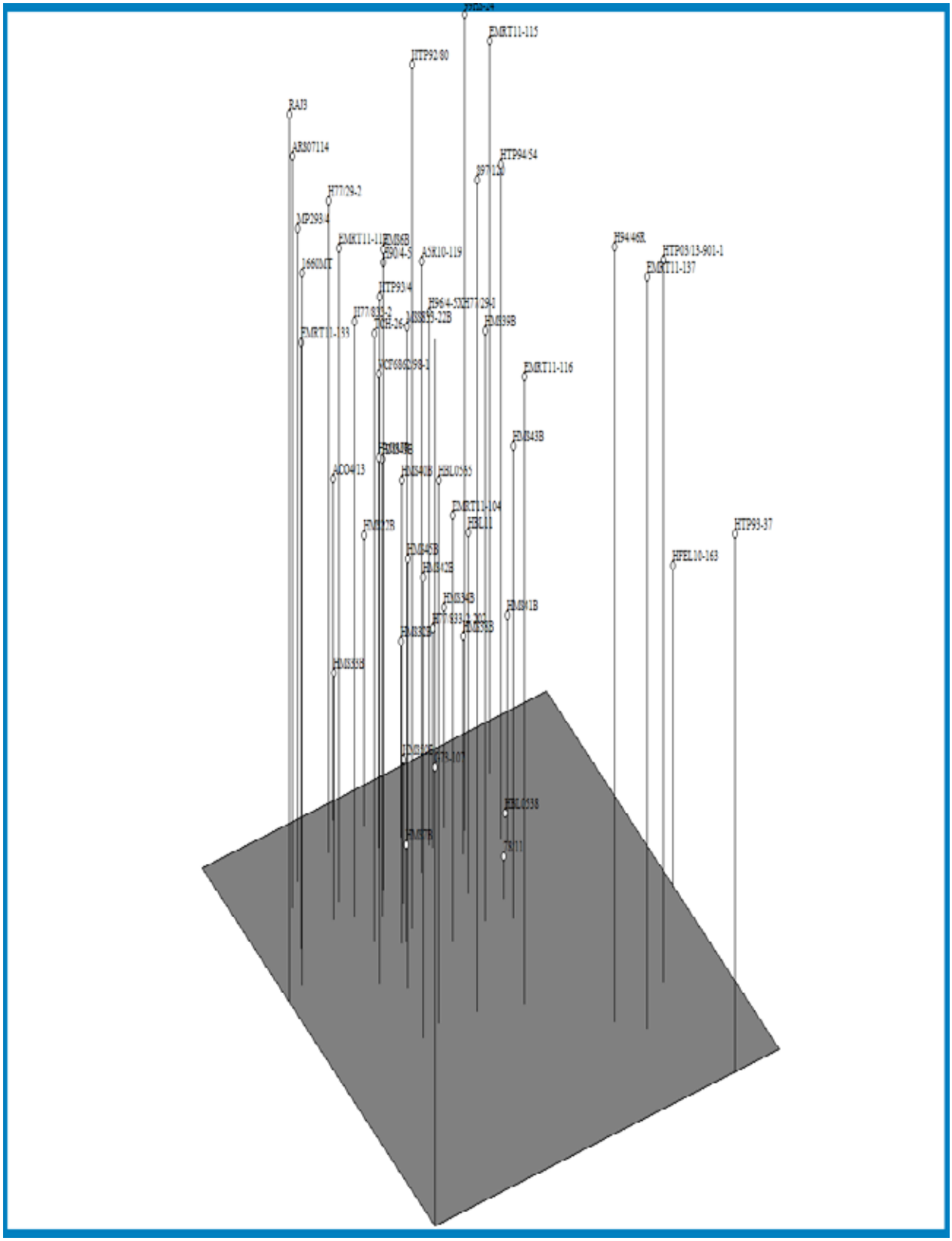

Figure 11. Three dimensional PCA (Principal component analysis) scaling of fifty pearl millet genotypes based on 50 SSR markers

Table 1. List of pearl millet genotypes used in the present study.

S. No.	Genotype	Source	S. No.	Genotype	Source
1	HMS 6B	CCSHAU, Hisar	26	HBL 0565	CCSHAU, Hisar
2	HMS 22B	CCSHAU, Hisar	27	VCF6 862/98-1	CCSHAU, Hisar
3	HMS 34B	CCSHAU, Hisar	28	HBL 0538	CCSHAU, Hisar
4	HMS 37B	CCSHAU, Hisar	29	HTP 93/4	CCSHAU, Hisar
5	HMS 32B	CCSHAU, Hisar	30	AC O4/13	CCSHAU, Hisar
6	HMS 33B	CCSHAU, Hisar	31	EMRT 11-104	CCSHAU, Hisar
7	HMS 7B	CCSHAU, Hisar	32	78/711	CCSHAU, Hisar
8	HMS 38B	CCSHAU, Hisar	33	(H96/4-5xH 77/29-2)	CCSHAU, Hisar
9	HMS 40B	CCSHAU, Hisar	34	A5R 10-119	CCSHAU, Hisar
10	HMS 41B	CCSHAU, Hisar	35	1660 (MT)	CCSHAU, Hisar
11	HMS 42B	CCSHAU, Hisar	36	EMRT 11-115	CCSHAU, Hisar
12	HMS 39B	CCSHAU, Hisar	37	99 HS-24	CCSHAU, Hisar
13	HMS 50B	CCSHAU, Hisar	38	RAJ 3	CCSHAU, Hisar
14	HMS 49B	CCSHAU, Hisar	39	S 97/120	CCSHAU, Hisar
15	HMS 45B	CCSHAU, Hisar	40	ARS 07114	CCSHAU, Hisar
16	HMS 43B	CCSHAU, Hisar	41	MP 293-4	CCSHAU, Hisar
17	MS 833-22B	CCSHAU, Hisar	42	EMRT 11-133	CCSHAU, Hisar
18	HTP 94/54	CCSHAU, Hisar	43	HTP 92/80	CCSHAU, Hisar
19	H 77/29-2	CCSHAU, Hisar	44	EMRT 11-112	CCSHAU, Hisar
20	H 77/833-2	CCSHAU, Hisar	45	HTP 93-37	CCSHAU, Hisar
21	H 77/833-2-202	CCSHAU, Hisar	46	EMRT 11-137	CCSHAU, Hisar
22	G 73-107	CCSHAU, Hisar	47	EMRT 11-116	CCSHAU, Hisar
23	H 90/4-5	CCSHAU, Hisar	48	HTP 03/13-901-1	CCSHAU, Hisar
24	HBL 11	CCSHAU, Hisar	49	HFeL 10-163	CCSHAU, Hisar
25	TCH-26-1	CCSHAU, Hisar	50	H 94/46R	CCSHAU, Hisar

Table 2. List of 50 SSR markers used for studying polymorphism in fifty pearl millet genotypes.

Sr. No.	Primer	Forward Sequence	Reverse Sequence	Temp (°C)
1	PSMP 2008	GATCATGTTGTCATGAATCACC	ACACTACACCTACATACGCTCC	55
2	PSMP 2013	GTAACCCACTAACCCTTACC	GTAACCCACTAACCCTTACC	54
3	PSMP 2027	AGCAATCCGATAACAAGGAC	AGCTTTGGAAAAGGTGATCC	50
4	PSMP 0020	CATTACACGTTTCTTCAAACGC	TCTTCGGCCTAATAGCTCTAAC	53
5	PSMP 2059	GGGGAGATGAGAAAACACAATCAC	TCGAGAGAGGAACCTGATCCTAA	56
6	PSMP 2084	AATCTAGTGATCTAGTGTGCTTCC	GGTTAGTTTGTTTGAGGCAAATGC	54
7	PSMP 2087	GGAACAGACTCCATACCTGAAA	TACCTGCCTGTGCTGTTAGT	53
8	PSMP 2090	AGCAGCCCAGTAATACCTCAGCTC	AGCCCTAGCGCACAACACAAACTC	59
9	PSMP 2201	CCC GAC GTT ATG CGT TAA GTT	TCCATCCATCCATTAATCCACA	52
10	PSMP 2224	GGCGAAATTGGAATTCAGATTG	CGTAATCGTAGCGTCTCGTCTAA	55
11	PSMP 2227	ACACCAAACACCAACCATAAA	TCGTCAGCAATCACTAATGACC	53
12	PSMP 2229	CCACTACCTTCGTCTTCCTCCATTC	GTCCGTTCCGTTAGTTGTTGCC	59
13	PSMP 2232	TGTTGTTGGGAGAGGGTATGAG	CTCTCGCCATTCTTCAAGTTCA	55
14	PSMP 2233	TGTTTTCTCCTCTTAGGCTTCGTTC	ACCTTCTCCGCCACTAAACAACT	56
15	PSMP 2237	TGGCCTTGGCCTTTCCACGCTT	CAATCAGTCCGTATCCACACCCCA	61
16	PSMP 2246	CGGATGCTAAATTAACCGAAGC	CCAGCTTGCTTCTGTTCGGTTC	57
17	PSMP 0022	TCTGTTTGTTTGGGTCAGGTCCTTC	CGAATACGTATGGAGAACTGCGCATC	60
18	PSMP 2263	AAAGTGAATACGATACAGGAGCTGAG	CATTTCAGCCGTTAAGTGAGACAA	56
19	PSMP 2270	AACCAGAGAAGTACATGGCCCG	CGACGAACAAATTAAGGCTCTC	57
20	PSMP 2271	CCTTATATTGGACCGACTGCTGAC	CTCCCCCATACACGAGCGAGAA	59
21	PSMP 2273	AACCCCACCAGTAAGTTGTGCTGC	GATGACGACCAAGACTTCTCTCC	59
22	PSMP 2274	CACCTAGACTCTACACAATGCAAC	AATATCAAGTGATCCACCTCCCAA	56
23	ICMP 3016	GTCAACCATTTGGGCTCACT	GGGAGAAATGTGGGGAGAGA	52
24	ICMP 3017	CACCAAACAGCATCAAGCAG	AGGTAGCCGAGGAAGGTGAG	56
25	ICMP 3018	ACGAGGACAAGCTCTTGGAA	ACGGCGCATACTCGATCATA	52

Continuing Table 2

Sr. No.	Primer	Forward Sequence	Reverse Sequence	Temp (°C)
26	ICMP 3019	GCGCACCACCTGTGTCTAT	CATGCAGAGAAAAATCAAGCA	53
27	ICMP 3020	GTTCCATGGAGCTGGAAGC	GCTAGAACAGGGCCGTTACA	54
28	ICMP 3029	ATCGATCTGTTCCACCCAGT	GGACTGGTACTGCTGCTGCT	56
29	ICMP 3050	ATGTCCAGTGTTGACGGTGA	CGGGGAAGAGACAGGCTACT	56
30	ICMP 3056	ACGGAGCTACGGTTGGAATA	CACAAGGGACCCCACGATA	53
31	ICMP 3088	TCAGGTGGAGATCGATGTTG	TTACGGGAGGATGAGGATG	54
32	ICMP 10	ATCCCCTACAGCATCAGCAC	CGGCGGAGAGATCTTATTCA	54
33	XCUMP 001	GCACGAGGCTTATCTGTGTTTC	CAACTCTTGCCTTTCTTGGCCT	55
34	XCUMP 005	GCACGAGGGCCAGATTCTAGAA	CACGGTGATGACACGACATGGT	57
35	XCUMP 006	GAAATCGGCAGAGGGCAT	CAATGAGTATGTGCACGCTGCA	55
36	XCUMP 009	ATCTGATCGTGAGGCCTCAAC	GCCGACCAAGAACTTCATACAAT	54
37	XCUMP 0011	TGATGGGAACCGAGAGCATGA	TAGCACAGCAATAACATGGCATC	54
38	XCUMP 0012	TGTGATCTGTGGTCTCAGGC	CGTGAAAGCTCTCCAGGACT	54
39	XCUMP 0016	CATTTCTCTCGCCAGTGCTC	ATCTCCAGAACCGAGCGCA	54
40	XCUMP 0017	TGCTTTCTTCCCAACCAGTGG	TGCTGAGTGGGGTGCTGCT	54
41	XCUMP 0018	TGCTTTCTTCCCAACCAGTGG	TGCTGAGTGGGGTGCTGCT	55
42	XCUMP 0019	GGCCTAACTCTCTGTTCTTCTTC	GAGAAGCTAACATTTGGGGCCTA	55
43.	CTM 8	GCTGCATCGGAGATAGGGAA	CTCAGCAAGCACGCTGCTCT	56
44	CTM 10	GAGGCAAAAGTGGAAGACAG	TTGATTCCCGGTTCTATCGA	52
45.	CTM 21	ATGCCTCCCACCCCACGTCG	CGTCGCACTAGCCACAGTCA	60
46.	CTM 25	GCGAAGTAGAACACCGCGCT	GCACTTCCTCCTCGCCGTCA	58
47	CTM26	GCAAGTGATCCATGACATTACGA	ACTTGCTAGCTGCTGCTCTTG	54
48	CTM 27	GTTGCAAGCAGGAGTAGATCGA	CGCTCTGTAGGTTGAACTCCTT	55
49	CTM 55	CGTCTTCTACCACGTCCT	CATAATCCCACTCAACAATCC	50
50	CTM 56	GCGTTGTTTCGGTGACCAC	GCGTATCTTTAAATTGCCTTTGTT	53

a 2°C lesser T_m was used for step wise of PCR amplification

Table 3. Amplification results of 50 SSR markers.

Sr. No.	Primer	Amp. Range (bp)	Allele No.	PIC	Sr. No.	Primer	Amp. Range (bp)	Allele No.	PIC
1	PSMP 2008	170 - 500	5	0.49	26	ICMP 3019	250 - 700	4	0.57
2	PSMP 2013	250 - 600	3	0.50	27	ICMP 3020	220 - 310	6	0.62
3	PSMP 2027	210 - 550	4	0.50	28	ICMP 3029	170 - 450	2	0.45
4	PSMP 20	300 - 810	2	0.51	29	ICMP 3050	310 - 700	5	0.57
5	PSMP 2059	180 - 600	4	0.32	30	ICMP 3056	250 - 600	8	0.84
6	PSMP 2084	250 - 700	4	0.60	31	ICMP 3088	210 - 550	3	0.45
7	PSMP 2087	190 - 650	7	0.64	32	ICMP 10	300 - 810	5	0.65
8	PSMP 2090	200 - 650	2	0.10	33	XCUMP 001	148 - 195	4	0.72
9	PSMP 2201	145 - 570	3	0.09	34	XCUMP 005	145 - 570	2	0.62
10	PSMP 2224	260 - 710	5	0.47	35	XCUMP 006	180 - 500	3	0.41
11	PSMP 2227	200 - 580	4	0.66	36	XCUMP 009	216 - 250	8	0.89
12	PSMP 2229	210 - 225	8	0.74	37	XCUMP 0011	170 - 500	3	0.51
13	PSMP 2232	190 - 400	4	0.56	38	XCUMP 0012	250 - 700	4	0.56
14	PSMP 2233	216 - 250	3	0.52	39	XCUMP 0016	190 - 260	7	0.84
15	PSMP 2237	148 - 195	7	0.75	40	XCUMP 0017	170 - 500	2	0.45
16	PSMP 2246	145 - 570	5	0.64	41	XCUMP 0018	250 -700	5	0.65
17	PSMP 22	180 - 500	2	0.56	42	XCUMP 0019	170 - 500	8	0.82
18	PSMP 2263	210 - 320	8	0.75	43.	CTM 8	180 -500	6	0.61
19	PSMP 2270	200 - 580	5	0.54	44	CTM 10	210 - 320	7	0.84
20	PSMP 2271	140 - 410	4	0.65	45.	CTM 21	200 -580	2	0.49
21	PSMP 2273	200 - 620	5	0.54	46.	CTM 25	140 - 410	3	0.54
22	PSMP 2274	250 - 450	4	0.62	47	CTM26	200 - 650	8	0.85
23	ICMP 3016	170 - 500	2	0.32	48	CTM 27	145 -570	5	0.62
24	ICMP 3017	250 -700	3	0.58	49	CTM 55	260 – 710	4	0.55
25	ICMP 3018	190 - 260	5	0.62	50	CTM 56	190 - 650	5	0.56

References

Abhay Bikash, Yadav IS, Arya RK and Lamba RAS, (2013). Genotype x environment interaction and stability for grain yield and its attributes in pearl millet. Forage Research, 39(2):53-58.

Allouis S, Qi X, Lindup S, Gale MD and Devos KM, (2001). Construction of a BAC library of pearl millet, Pennisetum glaucum. Theoretical and Applied Genetics 102:1200-1205.

Anderson, JA, Churchill GA, Autrique JE, Tanksley SD and Sorrells ME, (1993). Optimizing parental selection for genetic linkage maps. *Genome* 36:181-186.

Arya RK and Yadav HP, (2009). Stability of grain yield and its contributing traits in white and grey grain hybrids in Bajra. Indian Journal of Agricultural Sciences. 79(11):941-944.

Arya RK, Singh MK, Yadav AK, Kumar A and Kumar S, (2014). Advances in pearl millet to mitigate adverse environmental conditions emerged due to global warming. Forage Research, 40(2):57-70.

Bairwa RK, Yadav MC, Subbaiyan GK, Kushwaha AK, Joshi MA, (2023). Morphological and molecular analyses of grain traits in aromatic rice landrace accessions from Indo-Gangetic plain region of India. Indian J Plant Genetic Resources. 36(2):290-300.

Budak H, Pedraza F, Cregan PB, Baenziger PS and Dweikat I, (2003). Development and utilization of SSRs to estimate the degree of genetic relationships in a collection of pearl millet germplasm. Crop Science. 43:2284-2290.

Chakravarthi BK and Naravaneni R, (2006). SSR marker-based DNA fingerprinting and diversity study in rice (*Oryza sativa*. L). African Journal of Biotechnology. 8:684-688

Colagar AH, Haghighatnia MJ, Amiri Z, Mohadjerani M and Tafrihi M, (2016). Microsatellite (SSR) amplification by PCR usually led to polymorphic bands: Evidence which shows replication slippage occurs in extend or nascent DNA strands. Mol Biol Res Commun. 5(3):167–174.

Directorate of Millets Development, (2020). Available online at: http://millets.dacfw.nic.in

Gagan T, Kumar J, Patel Harsh P, Borah A, Nath D, Das H, Bansal S, Singh N, Singh Bal Veer, (2023) A review on nutritional and health benefits of millets. International Journal of Plant & Soil Science, 35(19):1736-1743.

Jaiswal SK, Parsad LC, Kumar U, Tygi K, Verma RPS, Joshi AK, (2007). Characterization of genetic diversity in vulgare (*Hordeum vulgare* L.) through DNA fingerprinting. Indian J. Genet. 67(2):169-170.

Kapila RK, Yadav RS, Plaha P, Rai KN, Yadav OP, Hash CT and Howarth CJ, (2008). Genetic diversity among pearl millet maintainers using microsatellite markers. Plant Breeding 127:33-37.

Koli GK, Arya RK, (2022). DUS Characterization of the most promising high root yielding genotype HWS 8-18 of Ashwagandha (*Withania somnirefa*). Ekin Journal of Crop Breeding and Genetics, 8(1):70-74.

Korzun V, Ganal MW and Röder MS, (1998). Microsatellites as markers for genetic mapping in wheat (*Triticum aestivum* L.). Current topics in plant cytogenetics related to plant improvement. P. 243-249. In international symposium on current topics in plant cytogenetics related to plant improvement, Feb 21-22, 1997 Tulln, Australia.

Mariac C, Loung V, Kapran I, Mamadou A, Sagnard F, Deu M, Chantereau J, Gerard B, Ndjeunga J, Bezancon G, Pham J and Vigouroux Y, (2006). Diversity of wild and cultivated pearl millet accessions [*Pennisetum glaucam* (L.) R. Br.] in Niger assessed by microsatellite markers. Theoretical and Applied Genetics 114:49-58.

Murray HG and Thompson WF. (1980). Rapid isolation of high molecular weight DNA. Nucleic Acids Research 8:4321-4325.

Nepolean T, Gupta SK, Dwivedi SL, Bhattacharjee R, Rai KN and Hash CT, (2012). Genetic diversity in maintainer and restorer lines of pearl millet. Crop Science 52:255-2563.

Plaschke J, Ganal WM, Roder MS, (1995). Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theory of Applied Genetics. 91:1001-1007.

Priya BN, Reddy PS, Ramana JV, Jacob J, Sreekanth B and Nepolean T, (2022). Assessment of molecular diversity in pearl millet using SSR markers. The Pharma Innovation Journal; SP-11(8):2235-2240.

Qi X, Pittaway TS, Lindup S, Liu H, Waterman E, Padi FK, Hash CT, Zhu J, Gale MD and Devos KM, (2004). An integrated genetic map and a new

set of simple sequence repeat markers for pearl millet (*Pennisetum glaucam).* Theoretical and Applied Genetics 109:1485-1493.

Saghai-Maroof MA, Soliman KM, Jorgensen RA and Allard RW, (1984). Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. Proceedings of the National Academy of Sciences, USA. 81:8014-8019.

Sambrook J, Fritsch EF and Maniatis T, (1989). Molecular cloning: a laboratory manual. Cold spring Harbor Laboratory Press, Cold Spring, Harbour, New York, USA.

Satyavathi CT, Tiwari S, Bharadwaj C, Rao AR, Bhat J, Singh P, (2013). Genetic diversity analysis in a novel set of restorer lines of pearl millet [*Pennisetum glaucum* (L.) R. Br] using SSR markers. Vegetos. 26(1):72-82.

Song QJ, Shi JR, Singh S, Fickus EW, Costa JM, Lewis J, Gill BS, Ward R and Cregan PB, (2005). Development and mapping of microsatellite (SSR) markers in wheat. Theory of Applied Genetics. 110(3):550-560.

Singh J, Ranwah BR, Chaudhary L, Lal C, Dagla MC and Kumar V, (2013). Evaluation for genetic variability, correlation and path coefficient in mutant population of forage sorghum (*Sorghum bicolar* Moench). The Bioscan. 8(4):1471-1476.

Singh SK, Deo S, Chaudhary SB, Singh K, Rana MK, (2023). Genetic relationships and population structure among maize (*Zea mays* L.) landraces as revealed by simple sequence repeat markers. Indian J. Plant Genetic Resources. 36(2):216-226.

Rohlf FJ, (2000) NTSYS-pc: Numerical taxonomy and multivariate analysis system version 2.1. Exeter Publishing Setauket, New York.