International Journal of Plant & Soil Science

33(16): 36-48, 2021; Article no.IJPSS.66060
ISSN: 2320-7035

Promotor Anchored - RAPD Analysis of Foxtail Millet (*Setaria italica* L.) Accessions Selected For High Iron and Zinc Content

Dhiraj Gangtire[1], Nakul D. Magar[2], Vaibhav Khelurkar[1], Mangesh P. Moharil[1*], P. V. Jadhav[1], R. N. Katkar[3], R. B. Ghorade[4] and P. Suprasanna[5]

[1]Biotechnology Centre, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola 444 104 (M.S), India.
[2]ICAR-Indian Institute of Rice Research, Hyderabad 500 030 (T.S), India.
[3]Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola 444 104 (M.S), India.
[4]Sorghum Research Unit, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola 444 104 (M.S), India.
[5]Plant Stress Physiology and Biotechnology Section, Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai 400 085, India.

Authors' contributions

This work was carried out in collaboration among all authors. Authors DG and NDM performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author DG and Author MPM managed the analyses of the study. Author VK managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2021/v33i1630521
Editor(s):
(1) Prof. Bengi Uslu, Ankara University, Turkey.
(2) Dr. Hon H. Ho, State University of New York, USA.
Reviewers:
(1) Hani Meriem, Ferhat Abbas Sétif University, Algeria.
(2) Aouadj Sid Ahmed, University of Abou Bakr Belkaïd, Algeria.
Complete Peer review History: http://www.sdiarticle4.com/review-history/66060

Original Research Article

Received 01 March 2021
Accepted 01 May 2021
Published 03 July 2021

ABSTRACT

Foxtail millet (*Setaria italica* L.) is nutri-cereal crop having it is rich in β-carotene, vitamin B-complex and micronutrients like minerals. In the present research, we have studied biochemical properties and molecular profiling to identify the core set of foxtail millet (*Setaria italic* L.) accessions for high Iron (Fe) and Zinc (Zn) content. Total seventy-nine accessions and selected mutants variety PS4 of foxtail millet were used. The biochemical investigation revealed that accessions M_2-106, IC120407, M_3-61/HB-13, and IC120255 consist of high iron and zinc content. The genetic variability among the genotypes was revealed by 28 Promoter Anchored Amplified Polymorphic (PAAP-RAPD) primers of

**Corresponding author: E-mail: mpmoharil@gmail.com*

which OPE9+GC1, OPE9+CA1, UBC001+CA1, UBC001+TA1, UBC693+G1 showed 100% polymorphism, whereas UBC693+GC1 and OPE7+G1 showed 88% and 80% polymorphism, respectively, with an average of 45.95 % polymorphism. Total alleles per locus were 3.31, whereas, the average number of monomorphic and polymorphic alleles were 1.72 and 1.56, respectively. The extent of polymorphic information content (PIC) of PAAP-RAPD loci ranged from 0.5 to 0.87 with an average value of 0.41. For PAAP-RAPD, the maximum PIC value was observed in marker UBC693+GC1 (0.87 %) and the minimum were OPE5+CA1 and OPE9+CA1 (0.5 %). Molecular characterizations result showing highest similarity (0.932) between accessions Shrilakshmi and Prasad, whereas, the lowest similarity coefficient was observed between IC120255 and M_3-75/AM-1 (0.697) with PS4.UPGMA dendrogram grouped the foxtail millet accessions in five clusters which marked high diversity in M_3-61/HB-13 and M_3-75/AM-1. It implies that PAAP-RAPD markers are significantly screened in the foxtail millet accessions and have enumerated high genetic diversity.

Keywords: Promoteranchored; Setaria italica L; Iron; Zinc; PAAP-RAPD; etc.

1. INTRODUCTION

Cereals are the major staple diet source for the worldwide population. Majorly millets including sorghum and maize are growing in arid and semiarid regions of the world such as Asia and Central Africa [1]. Generally, people from arid or semi-arid regions of Asia are dependent on cereal as a major diet, and hence often suffer from nutrient and minerals deficiency (White and Broadley, 2005) [2,3]. In the context of increasing nutritional disorders and to fulfill the needs of over increasing population there is an immediate need to characterize the known high yielding accessions of the crop for its high nutritional content, which in turn will prove to help minimize the nutritional disorders and meet the needs of an ever-growing population.

Foxtail millet (*Setaria italica* L.) is one such crop having several benefits over other crops in terms of nutritional contents. Foxtail millet is a good source of β-caroten e, a precursor of vitamin A [4]. Itis named Nutri-cereal since it is rich in micronutrients like minerals and B-complex vitamins. Phytochemical screening revealed the presence of potent antioxidants in this millet such as flavonoids, phenolics, tannins along with other components. High antioxidant activity was observed in the extracts from bran-rich fraction compared to whole flour, suggesting the presence of antioxidant components in the bran rich layer [5]. Although, Itpossesses superior quality only pearl millet has been mainly focused on iron biofortification. Hence, the minor millet potential is still not exploited completely for biofortification. There are two strategies followed for biofortifying the millet one is the enhanced nutrient accumulation in milled grains and the other is the reduction of anti-nutritional factors which increases the bioavailability of minerals. The molecular characterization will prove to be an important step in fetching important accessions useful in building the interest of farmers in the cultivation of foxtail millet on large scales which in turn will benefit the consumers. The present study was conducted with a view of the Identification of high iron and zinc-containing foxtail millet accession. This gives the status of important nutrients at which in turn helps select genotypes or crops which satisfy the nutritional needs of the consumers.

The identified promising accessions were further used for the molecular characterization using genic marker PAAP-RAPD. This could be used as a functional marker targeted desired genes and QTLs used for genetic diversity assessment. Diverse genotypes can be incorporated further in the crop improvement program. For the development of biofortified (high iron and zinc content) in foxtail millet varieties which could be released in the nutrient-deficient areas to overcome the problem of nutritional disorder because of iron and zinc.

2. MATERIALS AND METHODOLOGY

2.1 Plant Material

A total of 79 mutants and accessions of foxtail millet (*Setaria italica* L.) provide by the National Bureau of Plant Genetic Resources (NBPGR), Akola. (Table 1). Mutants were generated from var. PS4 by using EMS and gamma radiation. Mutants understudy were all in M4/M5 generation. Three standard checks were used, namely, Prasad, PS4, and Shrilakshmi.

Table 1. Accessions used for study

Sr.No	Accessions	Sr.No	Accession
1.	M3-74/RT-11	41.	M2-32
2.	M3-34/LP-4	42.	M2-22
3.	M3-41/LP-11	43.	M2-165
4.	M3-61/HB-13	44.	M2-98
5.	M3-91/AM-17	45.	M2-108
6.	M3-93/AM-19	46.	M2-113
7.	M3-29/MP-22	47.	M2-106
8.	M3-36/LP-6	48.	M2-167
9.	M3-75/AM-1	49.	M2-48
10.	M3-49/HB-1	50.	M2-106
11.	M3-11/MP-4	51.	M2-30
12.	M3-88/AM-14	52.	M2-168
13.	M3-83/AM-9-1	53.	M2-17
14.	M3-17/MP-10-11	54.	M2-50
15.	M3-9/MP-2	55.	M2-31
16.	M3-59/HB-11	56.	M2-151
17.	M4-49/HB-6	57.	M2-152
18.	M4-50/HB-3-13	58.	M2-153
19.	M4-27/MP-20-15	59.	M2-154
20.	M4-52/HB-4-15	60.	M2-155
21.	M4-53/ HB-5-1	61.	IC120239
22.	M4-54/ HB-6-1	62.	IC120235
23.	M4-61/ HB-13-15	63.	IC120355
24.	M4-61/ HB-13-1	64.	IC120150
25.	M4-60/ HB-12-3	65.	IC120407
26.	M4-67/RT-4-9	66.	IC120251
27.	M4-68/ RT-5-11	67.	IC344225
28.	M4-69/ RT-6-14	68.	IC97179
29.	M4-12/ RT-12-7	69.	IC120175
30.	M4-82/AM-8-12	70.	IC120207
31.	M2-06	71.	IC120213
32.	M2-103	72.	IC97111
33.	M2-49	73.	IC480117
34.	M2-88	74.	IC372606
35.	M2-156	75.	IC120255
36.	M2-158	76.	IC120408
37.	M2-89	77.	Prasad (c)
38.	M2-106	78.	Ps4 (c)
39.	M2-164	79.	Shrilakshmi(c)
40.	M2-18		

2.2 Experimental Design

Total 79 accessions were grown in the plot of spacing single row with 45 X 10 cm (row to row and plant to plant distance). As flowering initiated, 2-3 irrigations were provided as per requirement. Harvesting was done as per the maturity of the genotypes.

2.3 Estimation of Iron and Zinc from Foxtail Millet Accessions

Estimation of iron and zinc from foxtail millet accessions Grain Fe and Zn densities were analysed in the laboratory of Soil science and soil chemistry (AICRP) on micronutrients at Dr. PDKV, Akola. Samples were digested according to di-acid digestion described by Wheal et al., 2011. Grain samples were finely ground and oven dried at 60°C for 48 h. Ground sample (0.2 g) with 7.0ml of 69 per cent conc. nitric acid (HNO_3) and 3ml of 30 per cent Perchloric acid ($HClO_4$) was digested by adding in 200ml digestion tubes. Temperature adjusted in three steps 80°C for 1 hour, 120°C for 2 hours, 200°C for 2 hours and 300°C for 1 hour. Final volume of digest is made to 50 ml by adding double distilled

water and filtered through Whatman number 1 filter paper. Micronutrient densities were analyzed using Atomic Absorption Spectrometer (AAS) (Model- Varian Spectra AA 220). Micronutrient standard solutions of (2mg/kg, 4mg/kg, 6mg/kg, and 8mg/kg) for Fe and Zn, were used as reference. Iron is very sensitive element so, care was taken to avoid any contamination with dust particles or any extraneous matter (Stangoulis and Sison, 2008).

2.4 Molecular Characterization of Foxtail Millet Accessions Using PAAP-RAPD Marker

DNA extracted from young leaves of high minerals (iron and zinc) containing eleven foxtail millets accessions using [6] protocol standardized in our laboratory. Twenty-eight degenerate promoter-RAPD decamer primer combinations were used for PCR amplification. The details of primers and their sequences are given in (Table 3). The following PCR cycle was used (Eppendorf, Master Cycler Gradient, Germany): Initial denaturation at 98 °C for 3 min, followed by 35 cycles of 98°C for 30 Sec, annealing at 57 °C for 45 sec and an extension of 72 °C for 40 sec, with a final extension of 72 °C for 5 min. A 20 µL of PCR mixture contained; 1 µL of each primer (10 pmol), 10 µL of 2X master mix (NEB, England), 1 µL of DNA, and 7 µL of DNase free water. The amplified DNA was checked by PAGE (Polyacrylamide Gel Electrophoresis).

2.5 Data Analysis

The resolved PCR amplified bands of Foxtail millet genotypes with different PAAP-RAPD primers were scored manually as binary matrices for their presence (1) and absence (0) in the datasheet. To measure the informativeness of PAAP-RAPD markers polymorphism information content (PIC) for each PAAP-RAPD locus was calculated. Polymorphic PAAP-RAPD loci were scored as '1' for the presence and '0' for absence among the genotypes. Amplicons that were either diffused or those that were too difficult to score were considered as missing. This allowed estimating at each locus of the number of alleles present (NA) and the polymorphic information content (PIC) value. The PIC value of each primer was calculated by the online software (PIC Calculator) available at (https://www.liverpool.ac.uk/~kempsj/pic.html). Analyses were carried out using the computer packages NTSYSpc [7]. The similarity coefficients were estimated (Nei and Li, 1998) and used for clustering analysis SHAN module based on Unweighted Pair Group Method Using Arithmetic Averages (UPGMA) to construct a dendrogram [8]

2.6 Cluster Analysis

The basic aim of cluster analysis was to group individuals or objects based on the characteristics they possess, so that individuals with similar descriptions were mathematically gathered into a same clusters. The similarity matrix based on SSR profile was subjected to UPGMA (Un-weighted Pair Group Method for Arithmetic Mean) for cluster analysis and a dendrogram was generated as per the procedure given by [9]. These computations were performed using the program xlstat software (www.xlstat.com)

3. RESULTS

3.1 Identification of Core Set of Foxtail Millet Accession for High Iron and Zinc Content

`

Significant differences between the grain zinc and iron content were recorded (Table 2). The accession IC120407 (84.25 ppm), followed by M2-106 (71.8 ppm), and most of the accessions were found to have good iron content than standard checks. The accession M2-152 (12.22 ppm) is the lowest in iron content. Followed by IC120408 (10.56 ppm) as shown in (Table.2). Two accessions found promising zinc content than the standard checks PS4, Prasad and Shrilakshmi. The promising accessions for yield and zinc content were M3-61/HB-13 (28.07 ppm), IC120255 (25.07 ppm). The accession M3-75/AM-1 (10.09 ppm) was found to have the lowest zinc content. Followed by IC120175 (9.91 ppm) than the check PS4 (22.39), Prasad (19.48), Shrilakshmi (18.08), as shown in (Table. 2). Chandel et al. (2014) characterized some minor millets and find the iron content in whole grains of minor millets varied from 44.92ppm in Kodo millet to (46.20 ppm) in Barnyard millets. Barnyard millet showed the highest concentration of iron (40.2ppm) followed by Finger millet with 34.15ppm, Little millet with 32.71ppm, Kodo millet with (32.275ppm), and Foxtail millet with (27.19ppm) iron content in grains. The iron content in Kodo millet ranged from 25.86 ppm to

39.60ppm with the variety RK-92 showing the highest iron content of (39.60ppm). Surprisingly, our study indicates the range of grain iron content of foxtail millet (8.56ppm-84.25ppm) with variety IC120407 (84.25ppm) which is much high than the reported data.

Similarly, the Zinc (Zn) content ranged from 19.60 ppm in Kodo millet to 40.4 ppm in Foxtail millet reported by (Chandel et al. 2014). Foxtail millet showed the highest zinc content among all followed by Barnyard millet, Little millet, and Finger millet. Similar findings have been reported elsewhere where different minor millets varied considerably from one another in their zinc content (Iren, 2004). Their study showed 21.4 ppm sample zinc in Foxtail millet (highest among millets studied) and 15.0 ppm in Finger millet. The zinc content in Kodo millet ranged from 19.6 ppm to 24 ppm with variety RK-92 showing the highest zinc content of 24ppm. Among different genotypes of little millet, a narrow range. However, present investigation the range of (9 ppm to 28 ppm) for grain zinc content of seventy-nine accessions which is significantly high as compare to all reported data by various researchers.

3.2 Promoter-anchored RAPD Analysis of Foxtail Millet Accessions Selected for High Iron and Zinc Content

3.2.1 Molecular profiling using PAAP-RAPD marker

Molecular markers are predominantly used for characterization which helps us in analyzing the diversity at a genetic level.Development in functional genomics has generated considerable interest in identifying regulatory regions involved in gene expressions and networking of gene regulation. Pang et al. [10] developed four degenerated promoter primers based on conserved core promoter motif and integrated them with RAPD primer to identify polymorphism in the regulatory region of the genome.

PAAP–RAPD may provide an important and focused link between DNA sequence polymorphism and traits. The sequence obtained from PAAP-RAPD could be used for designing more sequence-specific primers to develop promoter-specific markers. PAAP will provide a good stretch point to enhance the difference in regulatory sequence among genomes. Divergence genotype identified through PAAP-RAPD based system may be used in a further breeding programme to characterize the desired traits. Twenty-eight PAAP-RAPD primers were used to detect the genetic variability among the foxtail millet accession. Among Twenty eight primers twenty-two primers were amplified depicted in (plate-6 to plate-9). All 5 primers OPE9+GC1, OPE9+CA1, UBC001+CA1, UBC001+TA1, UBC693+G1 showed 100% polymorphism, UBC693+GC1 and OPE7+G1 showed 88% and 80% polymorphism with average of 45.95 % polymorphism. Total alleles per locus were 3.31, whereas, the average number of monomorphic and polymorphic alleles were 1.72 and 1.56, respectively. The extent of polymorphic information content (PIC) of PAAP-RAPD loci ranged from 0.5 to 0.87 with an average value of 0.41. For PAAP-RAPD the maximum PIC value was observed in marker UBC693+GC1 (0.87 %) and the minimum was OPE5+CA1 and OPE9+CA1 (0.5 %).Similar observations were reported by Mokate et al. [11] in soybean using the PAAP-RAPD marker.

A null allele frequency analysis of all PAAP-RAPD had null allele frequencies close to zero. From Table 3 it can be concluded that the primer UBC001+TA1 showed 100 % polymorphism and 0.59 PIC values respectively.

3.3 Genetic Diversity Analysis

PAAP-RAPD markers were used to analyze the genetic diversity of foxtail millet genotypes. The similarity matrix coefficient among the genotype understudy is mentioned ranged from 0.697 to 0.932 (Table 5). Among eleven genotypes, the lowest similarity coefficient of 0.697 was found between IC120255 and M_3-75/AM-1 to PS4indicating that both parents are less similar to each other with more genetic divergence and the highest similarity coefficient of 0.932 was found between, Prasad and Shrilakshmi showing less genetic diversity and more genotypic similarity depicted in Fig.1

Table 2. Iron (Fe) and Zinc (Zn) content (PPM) of foxtail millet mutants and accessions

S.N.	Accessions	Fe (ppm)	Zn (ppm)	S.N.	Accessions	Fe (ppm)	Zn (ppm)	S.N.	Accessions	Fe (ppm)	Zn (ppm)
1	M3-74/RT-11	19.19	13.56	28	M4-69/ RT-6-14	36.25	15.67	55	M2-31	53.35	16.93
2	M3-34/LP-4	19.07	14.56	29	M4-12/ RT-12-7	20.4	16.71	56	M2-151	38.51	15.49
3	M3-41/LP-11	17.12	11.66	30	M4-82/AM-8-12	15.4	19.77	57	M2-152	12.22	16.96
4	M3-61/HB-13	19.78	28.07	31	M2-06	20.8	11.26	58	M2-153	14.71	20.07
5	M3-91/AM-17	56.35	14.94	32	M2-103	28.82	15.02	59	M2-154	61.2	16.78
6	M3-93/AM-19	45.63	10.59	33	M2-49	24.6	15.64	60	M2-155	12.93	20.49
7	M3-29/MP-22	55.5	16.06	34	M2-88	16.8	16.05	61	IC120239	19.18	16.54
8	M3-36/LP-6	29.71	15.31	35	M2-156	16.74	15.49	62	IC120235	10.94	14.72
9	M3-75/AM-1	16.2	10.9	36	M2-158	30.71	15.51	63	IC120355	12.03	22.31
10	M3-49/HB-1	19.74	12.05	37	M2-89	25.03	17.74	64	IC120150	20.89	14.66
11	M3-11/MP-4	19.79	15.92	38	M2-106	60.5	23.49	65	IC120407	84.25	10.96
12	M3-88/AM-14	22.21	18.42	39	M2-164	35.15	17.98	66	IC120251	21.65	16.45
13	M3-83/AM-9-1	22.78	9.98	40	M2-18	34.84	10.22	67	IC344225	17.33	15.14
14	M3-17/MP-10-11	21.15	16.16	41	M2-32	16.62	15.45	68	IC97179	16.22	12.28
15	M3-9/MP-2	24.86	19.26	42	M2-22	17.57	19.14	69	IC120175	13.21	9.91
16	M3-59/HB-11	36.02	12.29	43	M2-165	16.35	20.93	70	IC120207	25.82	11.33
17	M4-49/HB-6	23.49	14.66	44	M2-98	17.71	15.74	71	IC120213	51.85	15.86
18	M4-50/HB-3-13	11.78	16.81	45	M2-108	27.44	16.96	72	IC97111	41.2	14.71
19	M4-27/MP-20-15	58.65	10.39	46	M2-113	17.99	15.63	73	IC480117	11.78	16.64
20	M4-52/HB-4-15	56.25	17.14	47	M2-106	19.38	15.56	74	IC372606	35.15	14.07
21	M4-53/ HB-5-1	30.37	15.06	48	M2-167	17.46	11.75	75	IC120255	36.75	25.07
22	M4-54/ HB-6-1	40.03	16.51	49	M2-48	24.69	15.26	76	IC120408	10.56	14.43
23	M4-61/ HB-13-15	16	20.05	50	M2-106	71.8	14.19	77	Prasad (c)	23.66	19.48
24	M4-61/ HB-13-1	27.76	16.35	51	M2-30	25.97	15.04	78	PS4 (c)	26.51	22.39
25	M4-60/ HB-12-3	51.7	13.68	52	M2-168	66.3	21.16	79	Shrilakshmi(c)	28.08	18.08
26	M4-67/RT-4-9	46.85	15.21	53	M2-17	22.18	16.13				
27	M4-68/ RT-5-11	12.75	17.76	54	M2-50	30.51	19.66				

(PPM-Parts Per Million, c - Control)

Table 3. PAAP-RAPD primers used in the study and their PIC values

RAPD primers degenerate promoter primers		No. of amplicon	Monomorphic amplicon	Polymorphic Amplicon	Polymorphism (%)	PIC value
(5'-3') ⟶	(5'-3') ⟶					
OPE 1	G1 (GCCACSTGTC)	2	2	0	0	0.37
(CCCAAGGTCC)	GC1 (NNNGGGCGGN)	2	1	1	50	0.37
	CA1 (YRRCCAATWSR)	2	2	0	0	0.5
	TA1(CTATAWAWASM)	-	-	-	-	-
OPE 5	G1 (GCCACSTGTC)	1	1	0	0	0
(TCAGGGAGGT)	GC1 (NNNGGGCGGN)	5	4	1	0	70
	CA1 (YRRCCAATWSR)	2	1	1	50	0.5
	TA1(CTATAWAWASM)	-	-	-	-	-
OPE 7	G1 (GCCACSTGTC)	5	1	4	80	0.59
(AGATGCAGCC)	GC1 (NNNGGGCGGN)	6	6	0	0	0.81
	CA1 (YRRCCAATWSR)	2	1	1	50	0.00
	TA1(CTATAWAWASM)	-	-	-	-	-
OPE 9	G1 (GCCACSTGTC)	2	2	0	0	0.37
(CTTCACCCGA)	GC1 (NNNGGGCGGN)	1	0	1	100	0
	CA1 (YRRCCAATWSR)	2	0	2	100	0.5
	TA1(CTATAWAWASM)	-	-	-	-	-
UBC001	G1 (GCCACSTGTC)	3	1	2	66	0.37
(CGTGGGCTTC)	GC1 (NNNGGGCGGN)	5	3	2	40	0.64
	CA1 (YRRCCAATWSR)	2	0	2	100	0
	TA1(CTATAWAWASM)	3	0	3	100	0.59
UBC002	G1 (GCCACSTGTC)	1	1	0	0	0
(CCTGGGCTTG)	GC1 (NNNGGGCGGN)	5	5	0	0	0.76
	CA1 (YRRCCAATWSR)	8	3	5	62	0.83
	TA1(CTATAWAWASM)	-	-	-	-	-
UBC693	G1 (GCCACSTGTC)	1	0	1	100	0
(GACGAGACGG)	GC1 (NNNGGGCGGN)	9	1	8	88	0.87
	CA1 (YRRCCAATWSR)	4	3	1	25	0.36
	TA1(CTATAWAWASM)	-	-	-	-	-
	Total	**73**	**38**	**35**	**1011**	**9.13**
	Average	**3.31**	**1.72**	**1.59**	**45.95**	**0.41**

(RAPD-Random Amplified Polymorphic DNA, PIC-Polymorphic Information Content)

Table 4. Cluster analysis derived for PAAP-RAPD analysis

Group	Cluster	Genotypes
1	C1	M3-61 HB-13
2	C2	IC120175, IC120255, M2-106, IC120407, M2-152, IC120408
3	C3	M3-75 AM-1
4	C4	PRASAD, SHRILAKSMI
5	C5	PS4

Table 5. Similarity matrix based on PAAP-RAPD profiling of foxtail millet

	M3-61/HB-13	IC120175	IC120255	M3-75/AM-1	M2-106	IC120407	M2-152	IC120408	PRASAD	SHRI LAKSHMI	PS4
M3-61/HB-13	1										
IC120175	0.828	1									
IC120255	0.791	0.915	1								
M3-75/AM-1	0.765	0.852	0.871	1							
M2-106	0.818	0.915	0.902	0.841	1						
IC120407	0.809	0.902	0.919	0.859	0.919	1					
M2-152	0.771	0.857	0.875	0.846	0.905	0.892	1				
IC120408	0.833	0.900	0.887	0.857	0.887	0.846	0.921	1			
PRASAD	0.765	0.852	0.841	0.841	0.841	0.831	0.905	0.918	1		
SHRILAKSMI	0.788	0.850	0.810	0.810	0.839	0.828	0.844	0.855	0.932	1	
PS4	0.731	0.730	0.697	0.697	0.750	0.742	0.731	0.738	0.778	0.803	1

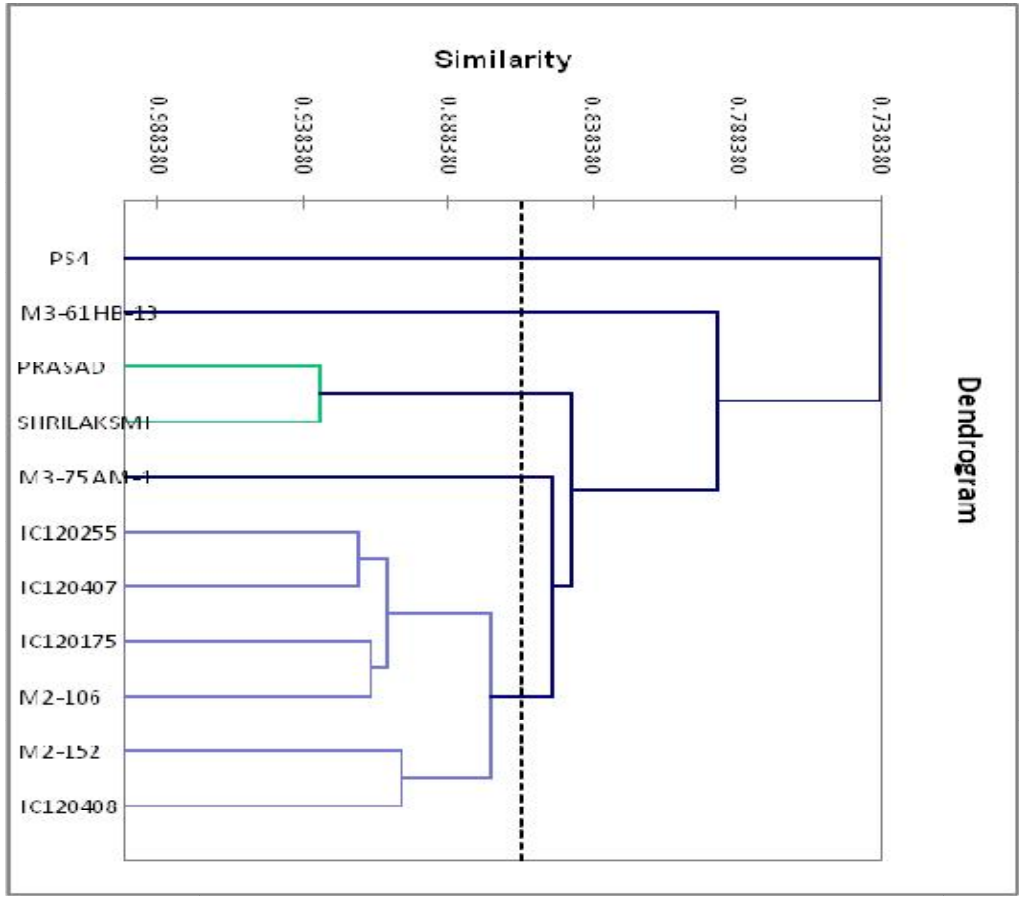

Fig. 1. UPGMA dendrogram of foxtail millet genotypes based on the Jaccard's similarity co-efficientusing PAAP-RAPD primer

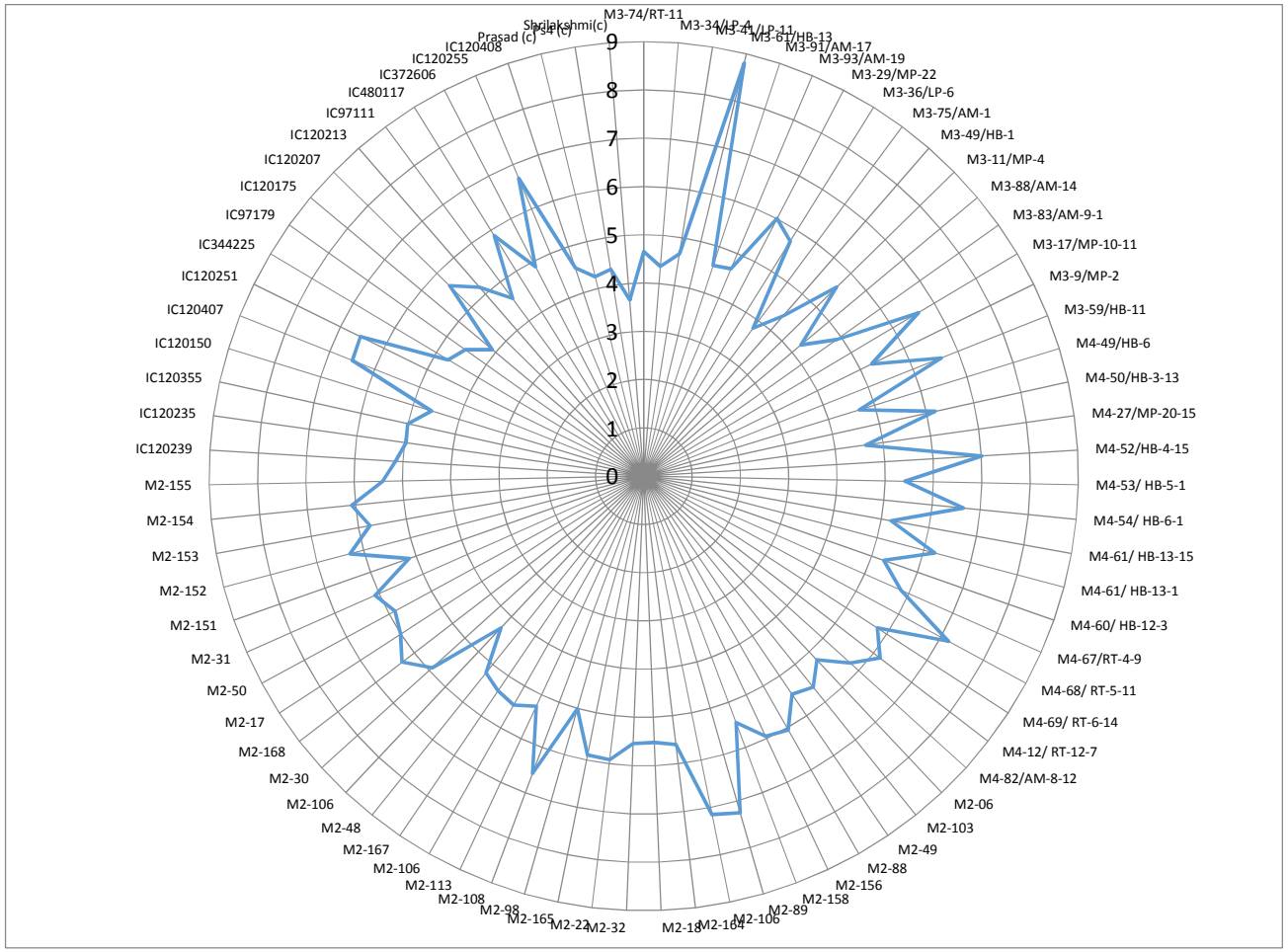

Fig. 2. Graph showing Zn content (PPM) in foxtail millet accesions

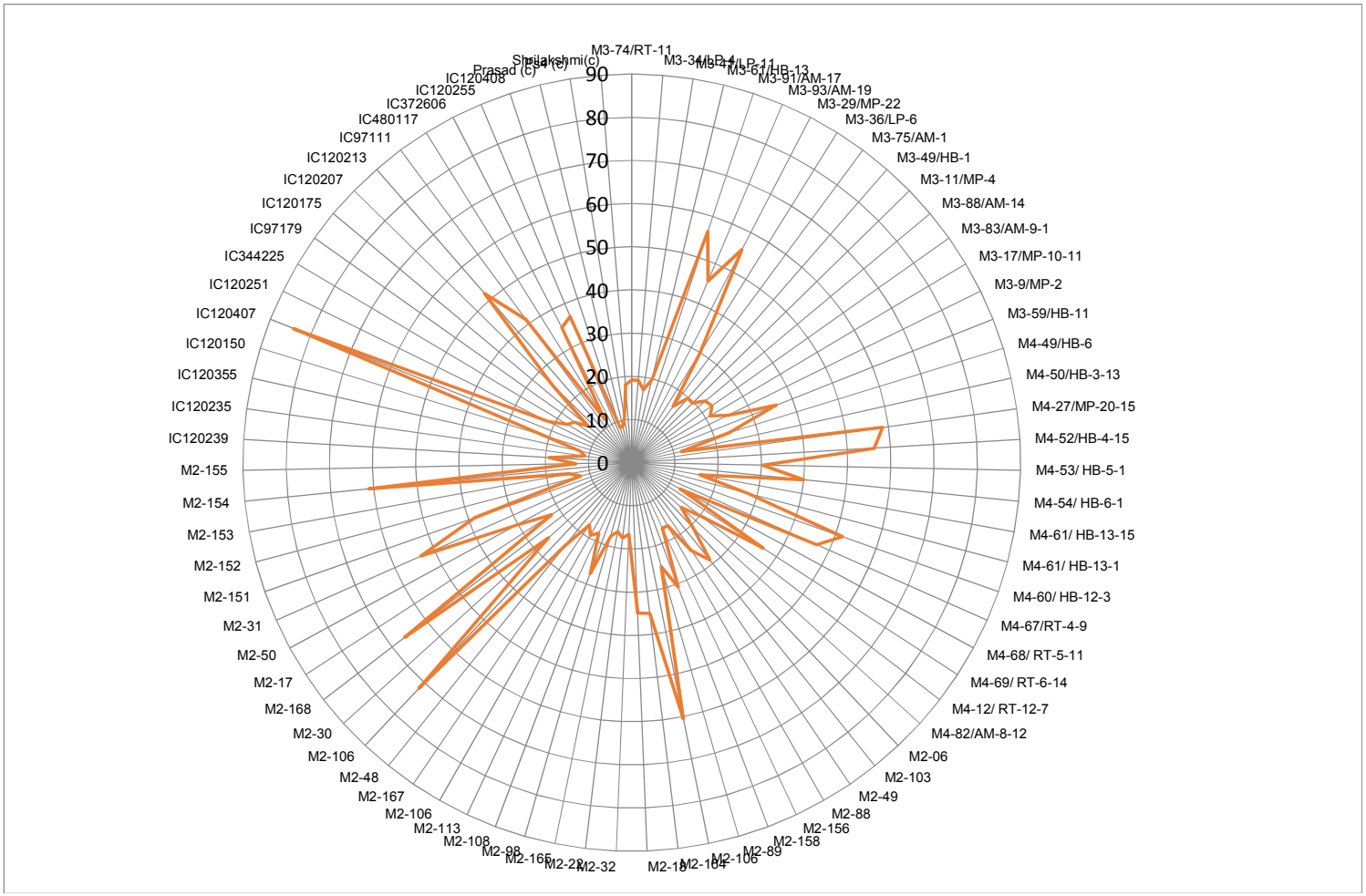

Fig. 3. Graph showing Fe content (PPM) in foxtail millet accesions

The study of genetic diversity within closely related crop germplasm is essential for the rational use of genetic resources. Above and beyond, analysis of genetic variation among breeding materials is of fundamental interest to plant breeders, as it contributes immensely to the selection, monitoring of germplasm, and also to prediction of genetic gain (Chakravarthy and Rambabu, 2006). Similarity matrixes and dendrogram analysis of selected eleven genotypes were classified into five clusters as represented in (Table. 5).

Cluster - I: It includes one accession, M_3-61 HB-13. The accessions were scored for the biochemical attributes. The accessions were found superior in high zinc content M_3-61/HB-13 98.807ppm).

Cluster – II: This cluster represented six accessions namely IC120175, IC120255, M_2-106, IC120407, M_2-152, and IC120408. Among these six genotypes, IC120175 showed the lowest zinc content in a plant seed and IC120255 had the highest zinc content in a plant seed. But M_2-106 and IC120407 genotypes were found superior in high iron content in plant seed. The accession M_2-152 and IC120408 had the lowest iron content in plant seed.

Cluster – III: It consists of a single genotype M_3-75 AM-1and found the lowest zinc content in plant seed.

Cluster – IV: It represents two genotypes namely PRASAD and SHRILAKSMI have control of this biomolecular observation.

Cluster – V: It represents a single genotype namely PS4 It represents the control PS4 plant variety.

4. CONCLUSION

Due to richness of important micronutrients like iron and zinc along with previously discovered health benefits, Foxtail millets can be one of the magic cereal foods to combat micronutrient malnutrition. Based on the finding of this study, it can be concluded that foxtail millet is superior cereal grain with good nutrient profile and hence will be worthy addition to one's diet to minimize the malnutrition effects in the tribal areas. Enhancing micronutrient in the diet by using minor millet cereal foods in the diet to ensure adequate attainment of iron and zinc seems to be most suitable strategy to combat micronutrient malnutrition. These found promising mutant and accessions can be further use in the future breeding program to develop the nutria-rich genotype in foxtail millet. The diverse genotype found in the present work may be helpful while selection of more diverse genotype in foxtail improvement program.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Saleh AS, Zhang Q, Chen J, Shen Q. Millet grains: nutritional quality, processing, and potential health benefits. Comprehensive Reviews in Food Science and Food Safety. 2013;12(3):281-295.
2. Hirschi KD. Nutrient biofortification of food crops. Ann. Rev. Nutr. 2009;29:401–421. DOI: 10.1146/annu. rev-nutr-080508-141143
3. Zhao FJ, McGrath SP. Biofortification and phytoremediation. Current Opinion in Plant Biology. 2009;12(3):373-380.
4. Murugan R, Nirmalakumari A. Genetic divergence in foxtail millet *Setaria italica* (L.) Beauv. Indian Journal of Genetics. 2006;66(4):339-340.
5. Suma PF, Urooj A. Antioxidant activity of extracts from foxtail millet (*Setaria italica*). Journal of Food Science and Technology. 2012;49(4):500-504.
6. Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RWL. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proceedings of the National Academy of Sciences. 1984;81(24):8014-8018.
7. Rohlf FJ. NTSYSpc numerical taxonomy and multivariate analysis system version 2.0 user guide; 1998.
8. Sneath PH, Sokal RR. Numerical taxonomy. The principles and practice of numerical classification; 1973.
9. Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences. 1979; 76(10):5269-5273.
10. Pang M, Percy RG, Hughs E, Zhang J. Promoter anchored amplified

polymorphism based on random amplified polymorphic DNA (PAAP-RAPD) in cotton. Euphytica. 2009;167(3):281-291.

11. Mokate YS, Chimote VP, Deshmukh MP, Gawai K, Kale AA, Jadhav AS. Use of promoter-anchored RAPD analysis for divergence assessment in soybean as against conventionally used ISSR assay. Applied Biological Research. 2017;19(1), 1-9.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/66060