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Characterization of iron and zinc rich pearl millet hybrids and varieties using molecular markers

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Abstract

The most extensively grown millet, pearl millet [*Pennisetum glaucum* (L). R Br.] has been a staple food crop for the poor people in Africa and the Indian subcontinent from prehistoric times. It is an excellent dryland crop which is well adapted for survival in the regions with harsh climatic conditions such as drought, high temperature, high salinity, low pH and poor soil fertility. In comparison to the other cereals like maize, wheat, rice and sorghum, it is rich in various nutrients, primarily iron and zinc and thus it is very important to eradicate malnutrition as well as ensure nutritional security. Hence, it is crucial to identify and validate genotypes rich in high iron and zinc in order to develop more cultivars rich in micronutrients. In this investigation, 103 markers were utilized for molecular characterization, screening and validation of 18 pearl millet hybrids and varieties that were rich in Fe and Zn. Out of these 103 SSRs, 84 primers amplified products with sizes ranging from 90 to 750 bp and 74 primers (71.8%) were reported to be polymorphic while 10 (9.7%) were monomorphic. A total of 246 alleles were reported in the present study and the number of alleles per locus ranged between 2 to 6 with an average of 3.32 alleles**.** The average Polymorphic Information Content (PIC) score was 0.59 with a range of 0.34 to 0.79. This research will be helpful in developing high Fe and Zn cultivars which may then further be utilized to confiscate malnutrition and hidden hunger.

Keywords: Biofortification, malnutrition, micronutrients, molecular markers, pearl millet, SSRs, validation

Introduction

Pearl millet (*Pennisetum glaucum* [L.] R. Br.) is a very nutritious and multipurpose C_4 cereal crop. It is mainly cultivated in India and Africa and is a chief staple food source for more than 90 million people living in these areas. It has high photosynthetic efficiency and exhibits high tolerance to heat, drought, salinity and acidic soils and thus produces high yield in low-rainfall areas of arid regions where other crops are not able to survive in such harsh conditions (Satyavathi *et al*. 2021a, b) [24, 26]. It is also rich in proteins (8-19%), carbohydrates, dietary fibers (1.2 g/100 g), fat (3-8%) ash, antioxidants, vitamins, essential amino acids and various minerals (296 mg/100 g P; 307 mg/100 g K; 42 mg/100 g Ca; 137 mg/100 g Mg; 75 mg/kg Fe and 40 mg/kg Zn) and can deliver about 80-90% of calories to poor people (Kumar *et al*., 2020a; Weckwerth *et al*., 2020) [15, 35]. Thus, it is not only considered as a chief staple food in the semi-arid tropics of India and Africa, but also used as the cheapest source of household nutrition as it can provide 361 Kcal/100 g of energy, which is similar to other major cereals like wheat (346 Kcal/100 g), rice (345 Kcal/100 g) maize (125 Kcal/100 g) and sorghum (349 Kcal/100 g) as per the Nutritive value of Indian foods (NIN, 2003) [20]. As it is rich in Fe and Zn, it can provide 19 to 63% of total iron (Fe) intake and 16 to 56% of the total zinc (Zn) intake from all food sources and thus is a more cheaper and economical source of Fe and Zn in comparison to other cereals and vegetables (Satyavathi *et al*., 2022) [27]. Nutritional security is a major challenge these days to meet the demands of growing population across the world as people are mainly dependent on micronutrient deficient cereal based diet. Diets deficient in Fe and Zn leads to malnutrition which is a big public health issue as it can effect >2 billion people worldwide (WHO, 2002) [36]. Iron and zinc deficiency are believed to be main micronutrient deficiencies (MNDs) leading to ill health, higher mortality, reduced work productivity, learning disabilities in children and poor economic

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growth of any nation (Bailey *et al*., 2015) [7]. Thus, breeding of grain crop varieties rich in these minerals is highly required to eradicate Fe and Zn deficiency. Since, pearl millet is a high-iron crop with a fairly high Zn content, but commercially available cultivars have lower Fe and Zn content and thus it needs focused breeding for these micronutrients along with high yield.

Biofortification of cereals is an effective strategy to address malnutrition and enhancing nutrients in major food crops. Biofortification Priority Index (BPI) and biofortification research in pearl millet has revealed that pearl millet posses high variability for Fe and Zn content and thus has good prospects for developing cultivars with high levels of these micronutrients and thus is a major target crop for iron and zinc biofortification. (Satyavathi *et al*., 2021c) [25]. One meal of biofortified high-iron variety of pearl millet can meet approximately 50-100% of the daily allowance of iron helping to combat iron deficiency in women, men and children (Kodkany *et al.*, 2013) [14]. Pearl millet has huge genetic variation for minerals like iron and zinc with majorly additive gene action and better parent heterosis in comparison to other cereal crops. This vast genetic variation (30-140 mg/kg Fe and 20-90 mg/kg Zn) can be proficiently utilized to develop high yielding cultivars along with high Fe and Zn densities. Thus, efforts are being put up to a large extent by India in order to develop biofortified crops with enhanced nutrients and several promising donors have been identified and breeding material is being generated combining high nutrient contents and yield (Kanatti *et al*., 2014; Kodkany *et al.*, 2013) [12, 14]. Screening of cultivars rich in micronutrients like Fe and Zn and breeding them for their introgression into locally adapted, high-yielding cultivars will be extremely beneficial to develop such cultivars and eliminate Fe and Zn deficiencies. Hence, there is a high need to encourage and support the high-iron testing and development research programs based on biofortification of pearl millet. In this context, ICAR-AICRP on Pearl Millet, Jodhpur has already included minimum standard for micronutrient (Fe = 42 ppm; Zn = 32 ppm) in the promotion criteria. Data collected from AICRP trials indicates that the average amount of grain iron content varied from 46 ppm to 65 ppm during Kharif, 2017 to Kharif, 2020 in different trials which were found to be above the benchmark level of iron content (42 ppm) while the average grain zinc content varied from 29 ppm to 79 ppm during Kharif, 2017 to Kharif, 2019 in different trials (Satyavathi *et al*., 2021c) [25]. Here, we screened pearl millet genotypes which are rich in iron and zinc content and characterized and validated those using molecular markers which can be further helpful for breeding programs targeting development of high Fe and Zn hybrids and varieties.

Materials and Methods

Plant Material

A total of 18 iron and zinc rich pearl millet hybrids and varieties were used in this study (Table 1). Screening, molecular characterization and validation of markers were carried out at Project Coordinating Unit, Indian Council of Agricultural Research-All India Coordinated Research Program on Pearl Millet, Jodhpur, India during 2020-21.

Genomic DNA isolation and quantification

Young and fresh leaves were collected from 1-12 days old plantlets of 18 pearl millet hybrids and varieties and genomic DNA was extracted by cetyl trimethyl ammonium bromide (CTAB) method with some modifications as accounted by Ambawat *et al.* (2020a) [5] and checked on 0.8% agarose gel.

Molecular characterization and PCR analysis

DNA was diluted further to a final concentration of 10 ng/µl and used for PCR reactions. A total of 103 SSR primers including 6 primers (Ppmsb1 to Ppmsb 6) which were observed to be specific for Fe and Zn characterization (Singhal *et al*., 2021, Ambawat *et al*., 2022) [32, 2] were used for PCR amplification and validation of the 18 pearl millet hybrids and varieties. PCR reactions were performed in a 96-well thermal cycler (Agilent Technologies) in 200µl PCR tubes containing 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 mM each dNTP, 0.4 µM 10-mer primer, 1 unit Taq DNA polymerase (GeNei, India) and 10 ng of DNA as template in a volume of 10 µl. Thermal cycler was programmed to 1 cycle of 5 mins at 94 °C for initial denaturation followed by 35 cycles of 30s at 94 °C for denaturation, 30 s at 58 °C for annealing and 1 min at 72 °C for primer extension. Finally, 1 cycle at 72 °C for 10 mins was used for final extension followed by hold at 4 °C (Ambawat *et al.* 2020b) [3]. The PCR products were checked on 2.5% agarose gel.

Results and Discussion

Molecular characterization and SSR analysis

A large number of genetic material needs to be screened to develop micronutrient rich cultivars through conventional breeding techniques. In addition, phenotyping for iron and zinc uses lot of breeding resources and destructive techniques are required for analysis. All these strategies are very time consuming and hence nowadays biotechnological approaches and molecular tools are preferred to overcome these limitations (Bollam *et al.*, 2018; Ambawat *et al.*, 2020c) [8, 6]. In the current study, a total of 103 markers were utilized for molecular characterization, screening and validation of 18 pearl millet hybrids and varieties. Among the 103 SSRs, 84 primers amplified products with sizes ranging from 90 to 750 bp and 74 primers (71.8%) were reported to be polymorphic while 10 (9.7%) were monomorphic indicating a good amount of polymorphic markers which will be helpful for pearl millet improvement (Fig. 1, 2). A total of 246 alleles were produced and the number of alleles per locus ranged between 2 to 6 with an average of 3.32 alleles**.** Similar types of findings were also observed by other researchers (Senthilvel *et al.*, 2008; Shaikh, 2015; Ambawat *et al.*, 2022, 2023) [28, 29, 2, 1]. But, these values were comparatively lower in comparison to Kumar *et al.*, (2020b) [16] with 4.62 alleles per primer (Mariac *et al.* 2006) [18] with 2-18 alleles (6.8 alleles per locus) and (Oumar *et al.*, 2008) [21] with (12.5 alleles per locus) which might be possible due to the diverse world collection of germplasm used by them. The average Polymorphic Information Content (PIC) score was 0.59 with a range of 0.34 to 0.79 (Table 2). The highest PIC values were recorded for the markers Ppmsb5 & PSMP2261 (0.79), PSMP2202 (0.78), Ppmsb1, PSMP2229 & PSMP2236 (0.77) while it was lowest for the markers PSMP2224 & ICMP3048 (0.34), PSMP2248 & PSMP2090 (0.35), ICMP 3063 (0.36), indicating that Ppmsb 5 &PSMP2261 were best and most informative markers for these genotypes while PSMP2224 & ICMP3048 were least influential markers.

PIC values ranging from 0.02 to 0.97 were reported in several other findings (Yadav *et al.*, 2013; Tiwari *et al.*, 2015; Mahalingam *et al.*, 2016; Singh *et al.*, 2017; Kumar *et al.*, 2020b; Choudhary *et al.*, 2021a; Ambawat *et al.*, 2021, 2023) [37, 33, 17, 31, 16, 10, 4, 1]. An average PIC value of 0.59 observed in the current study is similar to 0.55, 0.56 and 0.58 as reported in other studies (Kumar *et al.*, 2020b; Kapila *et al.*, 2008; Ambawat *et al.*, 2021[4]) [16, 13, 4], respectively**.** However, it was lesser (average PIC value of 0.671) than the observation noted by Tiwari *et al.*, (2015) [33] while higher than (0.23 PIC value) observed by Choudhary *et al.*, 2021b [9] and 0.37, 0.41 and 0.43 as noticed by Salazar *et al.*, (2006) [23], Yadav *et al.*, (2013) [37] and Shrivastava *et al.*, (2015) [30], respectively. PIC values can vary between 0 and 0.5 because of bi-allelic nature of SNPs while it can go above 0.5 in case of SSRs due to their mutli-allelic nature (Singh *et al.*, 2017; Jangra *et al.*, 2019; Ambawat *et al.*, 2021, 2023) [31, 11, 4, 1]. Thus, SSR markers are the most desired and efficient markers due to their ability to detect multiallelic loci, easy, co-dominant, high reproducibility, high polymorphism with vast ability to differentiate the genotypes (Verma *et al.*, 2021) [34]. Similar kind of results for effectiveness of SSRs in molecular characterization were also reported in other studies (Senthilvel *et al.*, 2008; Narshimulu *et al.*, 2011; Singh *et al.*, 2017; Radhika Ramya *et al.*, 2017; Kumar *et al.*, 2020b; Ambawat *et al.*, 2020b, 2021, 2022; Choudhary *et al.*, 2021a) [28, 19, 31, 22, 16, 3, 4, 2, 10]. This research can be used to develop more high Fe and Zn cultivars which can be further helpful to eliminate malnutrition and hidden hunger.

Table 1: Pearl millet hybrids and varieties used for molecular characterization

S. No.	Name of Hybrid/ Variety	Year	Organization	Salient features	Fe (ppm)	Zn (ppm)
1.	Dhanshakti	2014	MPKV, Dhule	Early maturing variety containing high iron (76-91ppm) and zinc (39-48 ppm), bold, globular, shining slate grey coloured seed, cylindrical-lanceolate earhead, resistant to downy mildew	81	43
2.	HHB299	2018	CCS HAU, Hisar	Medium maturing, purple anther color, lanceolate shaped compact panicle, greyish hexagonal shape grains, resistant to major diseases and insect pests	73	41
3.	AHB1200	2018	NARP, Aurangabad	Medium maturing, high Fe content, long cylindrical panicle, resistant to downy mildew, resistant to stem borer, highly responsive to fertilizers	77	39
4.	AHB1269	2019	NARP, Aurangabad	Medium maturing, long cylindrical compact panicles, high Fe content, resistant to downy mildew and stem borer	91	43
5.	HHB311	2020	ICAR-AICRP on Pearl millet, CCS HAU, Hisar	Medium maturing, compact, conical, complete ear heads, grey, hexagonal grains, resistant to major diseases and pest	83	39
6.	RHB233	2019	ICAR-AICRP on Pearl millet, RARI, Jaipur	Medium maturing, yellow anther colour, complete excretion, greyish seed, resistant to major diseases & insect pest	83	46
7.	RHB234	2019	ICAR-AICRP on Pearl millet, RARI, Jaipur	Medium maturing, brown anther colour, complete excretion, greyish seed, resistant to major diseases and insect pests	84	41
8.	DHBH1397	2019	ICAR-AICRP on PM, BRS, MPKV, Dhule	Medium maturing, dual purpose hybrid, globular shaped grey colour seed, highly resistant to downy mildew and blast	59	36
9.	JKBH1326	2019	JK Agri Genetics, Hyderabad	Medium maturing, medium thick, very long, conical and compact head, clear exertion with small to medium size pale yellow grain, highly resistant to downy mildew, rust, smut, ergot and blast	48	33
10.	PB1852	2019	Bayer Bio Science, Hyderabad	Medium maturing, grey colour grain with bold size, lodging tolerant, responsive to fertilizers, resistant to DM and blast, tolerant to moisture stress	56	32
11.	Pusa 1201	2018	ICAR-IARI, New Delhi	Medium maturing, yellow anthers, cylindrical panicles, stay green trait, highly resistant to downy mildew, smut and rust, highly resistant to pests, highly responsive to fertilizers	55	48
12.	BHB1202	2018	ICAR-AICRP on Pearl millet, SKRAU, Bikaner	Early maturing, compact conical earhead, yellow brown globular shaped grains, yellow brown anther color, highly resistant to downy mildew, blast and major pests	47	42
13.	NBH4903	2018	Nuziveedu Seeds Pvt. Ltd., Hyderabad	Late maturing, medium plant height with long exerted compact panicles, medium bold grains, non lodging, non shattering, resistant to drought, tolerant to major diseases and pests	53	41
14.	RHB223	2018	ICAR-AICRP on Pearl millet, RARI, Jaipur	Early maturing, brown anthers, long brown bristles, resistant to DM, blast and smut, resistant to shoot fly, stem borer and grey weevil, tolerant to stress	46	40
15.	PB1705	2018	Bayer Bio Science Pvt. Ltd., Hyderabad	Medium maturing, grey color grain with bold size, resistant to DM, blast, rust, smut & ergot, tolerant to lodging, tolerant to shoot fly and stem borer	49	32
16.	ABV04	2019	ANGRAU, Ananthapuramu	Late maturing, panicles thick and compact with grey coloured bold size, highly resistant to downy mildew, smut and blast, resistant to major insect pests	70	63
17.	BHB1602	2020	ICAR-AICRP-PM, SKRAU, Bikaner	Early maturing, compact, conical ear heads, grey brown, globular grains, highly resistant to downy mildew, blast, insect pests and resistant to smut	55	37
18.	GHB1129	2020	Gujarat	Suitable for Kharif and summer seasons, Medium maturing, resistant to downy mildew and lodging, salt and water stress tolerant, good quality stover	72	43

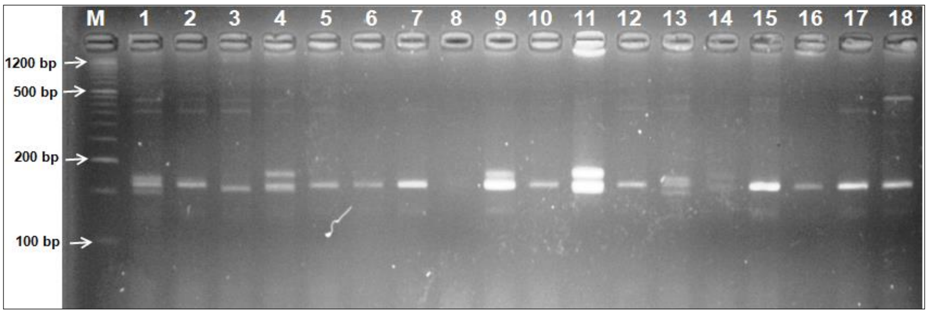

Fig 1: Amplification profile of pearl millet hybrids/varieties on 2.5% agarose gel with primer IPES 0153. Lane M-50 bp ladder, Lane 1-18 pearl millet hybrids/varieties

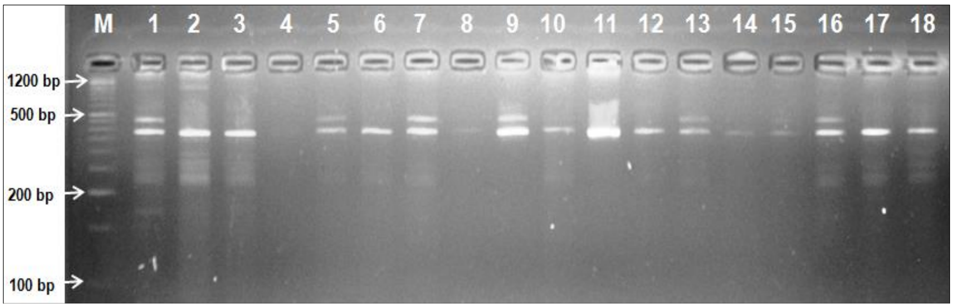

Fig 2: Amplification profile of pearl millet hybrids/varieties on 2.5% agarose gel with primer primer IPES 0205. Lane M-50 bp ladder, Lane 1-18 pearl millet hybrids/varieties

Table 2: List of SSR primers showing polymorphism

S. No.	Oligo Name	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Product range	No. of alleles amplified	PIC
1	Ppmsb1	GGGAAGGTGTTGCTGACTTC	GGGCTGTTGTTCCATGACA	200-750	6	0.77
2	Ppmsb 3	GCGGAACTGAACACCATTTT	CTCGCCACCAGGATCGTC	90-700	6	0.76
3	Ppmsb 4	CTGCCAGCAGTGTACGTGTT	CTTTCTGGCCTCCTCGTTGT	90-110	2	0.50
4	Ppmsb 5	GCGGAACTGAACACCATTTT	CTCGCCACCAGGATCGTC	160-600	5	0.79
5	Ppmsb 6	CTGCCAGCAGTGTACGTGTT	CTTTCTGGCCTCCTCGTTGT	400-550	2	0.50
6	IPES0042	GATAGAAGCAGATGGGCCTG	CTCGTCATCATTCTCGCCAC	200-500	4	0.71
7	IPES0203	CCCTCGAAGAGATCGAAGTG	CTGAAACAACAGCCTGCAAA	240-400	4	0.74
8	IPES0079	GTTGGACAGGCGAACGATAC	AGCTCTCCTGCATTTTCGTG	200-400	2	0.44
9	IPES0197	GTGTTCTTCCGAATCCGTGT	CGCTTTGCATTGAACACAGT	270-300	3	0.65
10	IPES0126	CCAGCAGGGAAGTCTTTCAC	AAAGGCGCTTGCTGATTTT	100-150	2	0.50
11	IPES0009	TTGATCGATCGTCTACGGTT	TATACTCACTCACGGCAGCG	90-350	5	0.71
12	IPES0146	CATCAGAATACGGACGCCTT	CATCAGCTTTGGAGTCAGCA	150-350	3	0.47
13	IPES0103	CATGCCAAATCATCTCGATCT	CTGAACCCGGAATTGCATAC	90-275	4	0.75
14	IPES0226	CACCAAACAGCATCAAGCAG	AGGAGGGTAAACACACGCAC	180-500	3	0.67
15	IPES0045	CAGCACCATTAGTGGCAAAA	CGTAACTTTGGTCAGGCATACA	200-205	2	0.38
16	IPES0101	CCTGGAAGGAGGGAGAACAG	GATAGCCCAAAGGCAACAAA	300-700	4	0.75
17	IPES0127	TGTACAAATGATACTTGATATCCCAAA	TGCAGAATTACACTGCCCTG	200-275	2	0.48
18	IPES0007	ACACCTCGCTGCACCTCTA	GCAACACAGATGAGACTGGC	100-120	2	0.50
19	PSMP2227	ACACCAAACACCAACCATAAAG	TCGTCAGCAATCACTAATGACC	100-200	2	0.52
20	PSMP2249	CAGTCTCTAACAAACAAACACGGC	GACAGCAACCAACTCCAAACTCCA	100-150	3	0.66
21	PSMP2074	AGGACTGTAGGAGTGTGGACAAC	ACAACCAGACCTACCAGTGAATGAGA	110-275	3	0.60
22	PSMP2086	CGCTTGTTTTCCTTTCTTGCTGTT	CCTTCTCAGATCCTGTGCTTTCTT	100-140	2	0.50
23	PSMP2076	GGAATAGTATATTGGCAAAATGTG	ATACTACACACTGTAAGCATTGTC	90-160	3	0.65
24	PSMP2261	AATGAAAATCCATCCCATTTCGCC	CGAGGACGAGGAGGGCGATT	140-500	5	0.79
25	PSMP2229	CCACTACCTTCGTCTTCCTCCATTC	GTCCGTTCCGTTAGTTGTTGCC	100-250	5	0.77
26	PSMP2202	CTGCCTGTTGAGAATAAATGAG	GTTCCGAATATAGAGCCCAAG	90-300	6	0.78
27	PSMP2040	CATTACACGTTTCTTCAAACGC	TCTTCGGCCTAATAGCTCTAAC	140-150	2	0.48
28	PSMP2224	GGCGAAATTGGAATTCAGATTG	CGTAATCGTAGCGTCTCGTCTAA	150-160	2	0.34
29	PSMP2063	AAAGTGAATACGATACAGGAGCTGAG	CATTTCAGCCGTTAAGTGAGACAA	100-150	2	0.37
30	PSMP2231	TTGCCTGAAGACGTGCAATCGTCC	CTTAATGCGTCTAGAGAGTTAAGTTG	90-250	2	0.50
31	PSMP2206	AGAAGAAGAGGGGGTAAGAAGGAG	AGCAACATCCGTAGAGGTAGAAG	150-600	4	0.74
32	PSMP2232	TGTTGTTGGGAGAGGGTATGAG	CTCTCGCCATTCTTCAAGTTCA	225-250	3	0.66
33	PSMP2263	AAAGTGAATACGATACAGGAGCTGAG	CATTTCAGCCGTTAAGTGAGACAA	245-260	3	0.64
34	PSMP2266	CAAGGATGGCTGAAGGGCTATG	TTTCCAGCCCACACCAGTAATC	150-200	2	0.40
35	PSMP2248	TCTGTTTGTTTGGGTCAGGTCCTTC	CGAATACGTATGGAGAACTGCGCATC	160-165	2	0.35
36	PSMP2275	CCAGTGCCTGCATTCTTGGC	GCATCGAATACTTCATCTCA	190-500	4	0.72

37	PSMP2203	GAACTTGATGAGTGCCACTAGC	TTGTGTAGGGAGCAACCTTGAT	120-425	6	0.76
38	PSMP2236	ATAAGTGGGACCCACATGCAGCAC	CGAAAGACTAGCAAAATTGCGCCTTC	200-500	5	0.77
39	PSMP2090	AGCAGCCCAGTAATACCTCAGCTC	AGCCCTAGCGCACAACACAAACTC	200-230	2	0.35
40	PSMP2237	TGGCCTTGGCCTTTCCACGCTT	CAATCAGTCCGTAGTCCACACCCCA	225-230	2	0.50
41	PSMP2273	AACCCCACCAGTAAGTTGTGCTGC	GATGACGACAAGACCTTCTCTCC	90-150	3	0.59
42	PSMP2069	CCCATCTGAAATCTGGCTGAGAA	CCGTGTTCGTACATGGTTTTGC	210-215	2	0.39
43	ICMP3086	ACCAAACGTCCAAACCAGAG	ATATCTCTTCGCTGCGGTGT	150-700	2	0.49
44	ICMP3002	AAGATGGATGATGGATTGATGA	TACACACACATTGCCACACG	90-200	4	0.65
45	ICMP3058	CGGAGCTCCTATCATTCCAA	GCAAGCCACAAGCCTATCTC	150-450	3	0.56
46	ICMP3080	CAAACAGCATCAAGCAGGAG	GCGTAGACGGCGTAGATGAT	100-400	4	0.72
47	ICMP3063	TCCGGTAGAGACCGTAATGG	GGCACTCCCTAGCAAAATGA	175-200	2	0.36
48	ICMP3048	CGGAACTGCTGGAGTGAAAT	GCGACTTCGACCGACTTTT	200-250	2	0.34
49	ICMP3066	AGGCCCCAAGTACTTCCCTA	TGTCAGACACAGATGCCACA	180-215	4	0.72
50	ICMP3092	GTTGCTGTCATGTCGTCTGG	CATCATGCCTGTGAGCAATG	245-405	4	0.50
51	ICMP3056	ACGGAGCTACGGTTGGAATA	CACAAGGGACCCCACGATA	145-150	2	0.51
52	IPES0015	ATAACATGGCAACGCCTACC	CGAGGACGCAATAACACAGA	100-120	2	0.40
53	IPES0105	GGGGGCTCACAGAACAAGTA	CCGAAGTTCCCACAGAATGT	100-300	6	0.72
54	IPES0153	CTCTTTGGTCAGTGCGTCAA	CATCGAACACAGGGCATCTA	160-400	4	0.69
55	IPES0205	CGGAACTGCTGGAGTGAAAT	ATCAACGCTCCACACACAAC	100-450	5	0.76
56	IPES0195	GGGAATGATTGATGGGAGTG	GACTAGAGATGCCGGGCTTT	100-400	4	0.59
57	IPES0179	TGTACATGTCAGGATCGCGT	CTATCCTAGCCCGGTGTTCA	100-350	4	0.74
58	IPES0208	CGAAGGAGGAGTACGACGAG	TCCACAAGGTGACCTCACTG	110-500	5	0.76
59	IPES0174	TCTGGGAAGGAGGAGGATTT	TGCTGCTGCTCTCTGACTGT	250-255	2	0.39
60	IPES0129	GACCATGATTCGATTCTGCAT	CCTTTGTCCATCTCGTTTGA	150-160	2	0.47
61	PSMP2077	GCCAATATTATTCCCAAGTGAACA	CTCTTGGTTGCATATCTTTCTTTT	145-150	2	0.38
62	PSMP2059	GGGGAGATGAGAAAACACAATCAC	TCGAGAGAGGAACCTGATCCTAA	100-300	3	0.51
63	PSMP2089	TTCGCCGCTGCTACATACTT	TGTGCATGTTGCTGGTCATT	130-450	5	0.73
64	PSMP2214	CGCACAGTACGTGTGAGTGAAG	GATTGAGCAGCAAAAACCAGC	110-600	6	0.75
65	IPES0003	GTTCAGATGAACAGCGGGAT	AGTCCTCGGCAAGCCTTTAT	200-205	2	0.44
66	IPES0027	TGCTTGGGACAAAAGGCT	TAACTCAAGTGAGCGCAAGG	200-600	6	0.72
67	IPES0236	GGCCAGCTCGCCTAGAT	AGATCCACCGCCTAATGAAA	240-450	4	0.73
68	IPES0210	ATTCCTGTGATGCCGAAGAC	GCACCATGACCACAAAAATG	190-600	5	0.76
69	IPES0218	CCTGGGAACACAAAACCAGA	CCAGGTCCATGTCCTTGACT	90-240	2	0.50
70	IPES0118	AAGGTGCAGAAGTTCACGCT	TTTTACAATCACGGCACGAC	150-250	3	0.63
71	IPES0221	TAGCCAGCGCTTTCTCTAGC	TCAGGAAACCACCACCTAGC	300-350	2	0.58
72	IPES0161	GGATCCATCCATCATCACCT	TCAGGGGAACCAATTAACCA	160-500	3	0.60
73	IPES0095	GTCTTTGCCGTGATAACCGT	CAAGAGGACTGGCTGATTGC	110-500	3	0.66
74	IPES0166	CCCGCTGATAGATGACGAAT	CAGAAAGGCCTCACTTTTCG	240-350	3	0.63

Conclusion

There are many opportunities for micronutrients in staple food crops but they also present a number of challenges. To maximize the benefits of pearl millet, the establishment of nutrition missions, millet missions or other government-sponsored missions must collaborate with agricultural research and production organizations. Further, biofortification could be a key strategy for its advancement as it is feasible to developing high-Fe and high-yielding hybrids and the partners should be encouraged to breed for these micronutrient traits. The findings of this study will be helpful in developing hybrids and varieties rich in zinc and iron, which will help to remove the effects of hidden hunger and malnutrition. Therefore, as part of an advanced pearl millet breeding program, products with essential nutrition traits and their commercialization consortiums should be included. For the sake of human wellbeing, nutri-dense varieties and hybrids must guarantee value propositions in smart food products and their supply chain.

References

1. Ambawat S, Meena RC, Satyavathi CT, Meena R, Khandelwal V, Singh S, *et al*. Molecular Characterization of drought tolerant pearl millet [*Pennisetum glaucum* (L.) R. Br.] lines using SSR markers. Frontiers in Crop Improvement. 2023;11(Special Issue-6):2691-2696.
2. Ambawat S, Satyavathi CT, Meena R, Khandelwal V, Meena RC, Singh S, *et al*. Characterization and validation of markers for Fe and Zn rich pearl millet hybrids and varieties. Frontiers in Crop Improvement. 2022;10(Special Issue-7):3494-3499
3. Ambawat S, Satyavathi CT, Meena R, Khandelwal V, Meena RC. Molecular characterization and genetic diversity analysis of released hybrids and varieties of pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Curr J Appl Sci Tech. 2020b;39(31):92-104.
4. Ambawat S, Satyavathi CT, Meena RC, Meena R, Gurjar NR, Khandelwal V. Molecular characterization of drought tolerant genotypes of pearl millet [*Pennisetum glaucum* (L.) R. Br.] for A_1 Zone. International Journal of Environment and Climate Change. 2021;11(11):292-301.
5. Ambawat S, Singh S, Satyavathi CT, Meena R, Meena RC, Khandelwal V. Development of a high quality, rapid, efficient and economical DNA extraction protocol from climate resilient pearl millet crop without liquid nitrogen. International Journal of Environment and Climate Change. 2020a;10(12):85-94.
6. Ambawat S, Singh S, Shobhit, Meena RC, Satyavathi CT. Biotechnological applications for improvement of the pearl millet crop. In: Gahlawat SK, Punia S, Siroha AK, Sandhu KS, Kaur M (eds) Pearl millet: properties, functionality and its applications. Taylor &

Francis (CRC Press), Boca Raton, Florida 2020c. p. 115-138. doi.10.1201/9780429331732-7

7. Bailey RL, West KP Jr, Black RE. The epidemiology of global micronutrient deficiencies. Annals of Nutrition and Metabolism. 2015;66:22-33 Doi: 10.1159/ 000371618
8. Bollam S, Pujarula V, Srivastava RK, Gupta R. Genomic approaches to enhance stress tolerance for productivity improvements in pearl millet: genomic approaches in Biotechnologies of Crop Improvement Vol. 3, eds. S. S. Gosal and S. H. Wan., 2018;239-264. Springer International Publishing AG, part of Springer Nature
9. Choudhary ML, Tripathi MK, Gupta N, Tiwari S, Tripathi N, Parihar P, *et al*. Morpho-physiological and molecular characterization of pearl millet [*Pennisetum glaucum* (L.) R. Br.] germplasm lines for drought tolerance. In book: New Visions in Science and Technology. 2021b;5:39-60 DOI: 10.9734/bpi/nvst/v5/2541E
10. Choudhary ML, Tripathi MK, Tiwari S, Pandya RK, Gupta N, Tripathi N, *et al*. Screening of pearl millet [*Pennisetum glaucum* (L.) R. Br.] germplam lines for drought tolerance based on morpho-physiological traits and SSR markers. Current Journal of Applied Science and Technology. 2021a;40(5):46-63.
11. Jangra S, Rani A, Yadav RC, Yadav NR, Yadav D. Introgression of terminal drought stress tolerance in advance lines of popular pearl millet hybrid through molecular breeding. Plant Physiol. Rep. 2019. https://doi.org/10.1007/s40502-019-00464-w
12. Kanatti A, Rai KN, Radhika K, Govindaraj M, Sahrawat KL, Srinivasu K, *et al*. Relationship of grain iron and zinc content with grain yield in pearl millet hybrids. Crop Improvement. 2014;41:91-96.
13. Kapila RK, Yadav RS, Plaha P, Rai KN, Yadav OP, Hash CT *et al*. Genetic diversity among pearl millet maintainers using microsatellite markers. Plant Breed. 2008;127:33-37.
14. Kodkany BS, Bellad RM, Mahantshetti NS, Westcott JE, Krebs NF, Kemp JF, *et al*. Biofortification of pearl millet with iron and zinc in a randomized controlled trial increases absorption of these minerals above physiologic requirements in young children. The Journal of Nutrition. 2013;143:1489-1493. Doi.10.3945/jn.113.176677.
15. Kumar RR, Rai GK, Goswami S, Singh SP, Satyavathi CT, Praveen S. Millets the orphan crop for uncertain future. Scientific International Pvt. Ltd., 2020a;194p.
16. Kumar S, Hash CT, Singh G, Basava RK, Srivastava RK. Identification of polymorphic SSR markers in elite genotypes of pearl millet and diversity analysis. Ecological Genetics and Genomics. 2020b;14:100051. 28.
17. Mahalingam A, Sarawathi R, Ramalingam J. SSR marker diversity analysis for WA cytoplasm based fertility restorer genes of hybrid rice (*Oryza sativa* L.). J Innov Agric. 2016;3(1):19-27.
18. Mariac C, Luong IV, Kapran A, Mamadou F, Sagnard M, Deu J *et al*. Diversity of wild and cultivated pearl millet accessions (*Pennisetum glaucum* [L.] R. Br.) in Niger assessed by microsatellite markers. Theor. Appl. Genet. 2006;114:49-58.
19. Narshimulu G, Jamaloddin M, Vemireddy LR, Anuradha G, Siddiq E. Potentiality of evenly distributed hyper-variable microsatellite markers in marker-assisted breeding of rice. Plant Breed. 2011;130:314-320. DOI: 10.1111/j.1439-0523.2010.01834.x
20. NIN. Nutritive value of Indian foods. In: Gopalan C, Deosthale YG (eds) Nutritive value of Indian foods. National Institute of Nutrition, Hyderabad. 2003
21. Oumar I, Mariac C, Pham JL, Vigouroux Y. Phylogeny and origin of pearl millet (*Pennisetum glaucum* [L.] R. Br) as revealed by microsatellite loci. Theor. Appl. Genet. 2008;117:489-497.
22. Radhika Ramya A, Ahamed ML, Srivastava RK. Genetic diversity analysis among inbred lines of pearl millet [*Pennisetum glaucum* (L.) R. Br.] based on grain yield and yield component characters. Int. J Curr. Microbiol. App. Sci. 2017;6(6):2240-2250
23. Salazar B, Laurentin H, Davila M, Castillo MA. Reliability of the RAPD technique for germplasm analysis of sesame (*Sesamum indicum* L.) from Venezuela. Interciencia 2006;31:456-460.
24. Satyavathi CT, Ambawat S, Khandelwal V, Srivastava RK. Pearl millet: A climate-resilient nutricereal for mitigating hidden hunger and provide nutritional security. Frontiers in Plant Science. 2021a;12:659938. doi: 10.3389/fpls.2021.659938.
25. Satyavathi CT, Ambawat S, Khandelwal V, Neeraja CN, Govindaraj M. Micronutrient Rich Pearl millet for Nutritionally Secure India. ICAR-All India Coordinated Research Project on Pearl Millet, Mandor, Jodhpur, India; 2021 c. p. 20.
26. Satyavathi CT, Ambawat S, Sehgal D, Lata C, Tiwari S, Srivastava RK, *et al*. Genomic designing for abiotic stress tolerance in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. In: Kole C. (eds) Genomic designing for abiotic stress resistant cereal crops. Springer, Cham; 2021, p. 223-253. https://doi.org/10.1007/978-3-030-75875-2_6
27. Satyavathi CT, Tomar RS, Ambawat S, Kheni J, Padhiyar SM, Desai H, *et al*. Stage specific comparative transcriptomic analysis to reveal gene networks regulating iron and zinc content in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Scientific Reports. 2022;12:1-13. Doi: 10.1038/s41598-021-04388-0.
28. Senthilvel S, Jayashree B, Mahalakshmi V, Kumar PS, Nakka S, Nepolean T, *et al*. Development and mapping of simple sequence repeat markers for pearl millet from data mining of expressed sequence tags. BMC Plant Biology. 2008;8:119.
29. Shaikh TR. Assessment of hybrid purity in sorghum by using molecular markers. Thesis submitted to Vasantrao Naik Marathwada Krishi Vidyapeeth Parbhani-431402 (M.S.) India; 2015.
30. Shrivastava A, Mishra D, Koutu G, Singh S, Prakash V, Sohgaura N. Assessment of genetic diversity of restorer and maintainer lines of rice using SSR markers. The Ecoscan. 2015;9(1-2):265-269.
31. Singh S, Singh VV, Supriya A, Dubey M, Singh D. Screening and estimation of allelic differentiation in Indian mustard (*Brassica juncea* L.) genotypes based on SSR Markers for background selection. Int. J. Curr. Microbiol. App. Sci. 2017;6(9):2506-2516.
32. Singhal, T, Satyavathi CT, Singh SP, Kumar A, Sankar SM, Bhardwaj C, *et al*. Multi-Environment Quantitative

TraitLoci Mapping for Grain Iron and Zinc Content Using Bi-parental Recombinant Inbred Line Mapping Population in Pearl Millet. Frontiers in Plant Science. 2021;12:659789. doi: 10.3389/fpls.2021.65978.

33. Tiwari KK, Singh A, Pattnaik S, Sandhu M, Kaur S, Jain S, *et al*. Identification of a diverse mini-core panel of Indian rice germplasm based on genotyping using microsatellite markers. Plant Breed. 2015;134(2):164-171.
34. Verma R, Tripathi MK, Tiwari S, Pandya RK, Tripathi N, Parihar P. Screening of pearl millet [*Pennisetum glaucum* (L.) R. Br.] genotypes against blast disease on the basis of disease indexing and gene-specific SSR markers. Int J Curr Microbiol Appl Sci. 2021;10(2):1108-1117.
35. Weckwerth W, Ghatak A, Bellaire A, Chaturvedi P, Varshney RK. PANOMICS meets germplasm. Plant Biotechnol. J. 2020;18:1507-1525. Doi: 10.1111/pbi.13372.
36. WHO. Reducing risks and promoting healthy life. The World Health Report. Geneva: WHO; 2002.
37. Yadav S, Singh AK, Singh MR, Goel N, Vinod KK, Mohapatra T. Assessment of genetic diversity in Indian rice germplasm (*Oryza sativa* L.): Use of random versus trait-linked microsatellite markers. J Genet. 2013;92:545-557.