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Research Article

EXPLOITING THE PEARL MILLET GENETIC DIVERSITY FOR IDENTIFICATION OF IRON AND ZINC DENSE SEED PARENTAL LINES

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Abstract: Micronutrient malnutrition has been recognized as massive health issue, affecting over two billion people, mostly women, children and infants worldwide especially in developing countries. Agricultural approaches including crop based food products and micronutrient (Fe and Zn) biofortified crop cultivars can provide the most cost effective and sustainable solution for this problem. Micronutrient bio-fortification in agricultural crops can be done with conventional breeding strategies, because traits for micronutrient enrichment exist within their genomes and can be used for considerable micronutrient levels in food grain crops without any significant negative impact on crop productivity. In the efforts to address this problem, pearl millet (*Pennisetum glaucum* [L.] R.Br.), field trials were conducted during *kharif* 2013 and 2014, to investigate the magnitude of variation in Fe and Zn contents. Composition of the genotypes tested was not affected to an appreciable extent by the growing season. During *kharif* 2013 and 2014, Fe content in 92 diverse genotypes (Inbreds and B-lines) varied from 23 to 123 mg/kg and 28 to 101 mg/ kg while Zn content varied from 26 to 72 mg/kg and 34 to 70 mg/kg. The correlation co-efficient between Fe and Zn content was positive and highly significant (r =0.523 to 0.702, p<0.01) during both the seasons, indicating likely usefulness of simultaneous improvement of both the micronutrients. Four promising parental lines (HMS 53B, HMS 40b HMS14B and DPHBL 11-123) with >85 mg/kg grain Fe and >55 mg/kg grain Zn content, have the potential to be used as seed parents for development of Fe and Zn biofortified pearl millet hybrids or composites.

Keywords: *Pearl Millet, Iron, Zinc, Biofortification, Seed Parents*

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Introduction

Dietary deficiency of mineral micronutrients such as iron (Fe) and zinc (Zn) has been documented as a worldwide human health problem, especially in the developing countries [1]. Dietary deficiency of these micronutrients affects more than two billion people worldwide [2,3] and iron deficiency is the leading cause of anemia [4,5]. Its prevalence is highest in resource-limited settings and among children and women of reproductive age [6] especially those heavily dependent on staple cereals as a major source of their energy and nutritional requirements in developing countries. Several approaches, such as medical supplementation, industrial food fortification, dietary diversification and crop bio-fortification have been suggested to address the problem of micronutrient malnutrition. Bio-fortification refers to the development of crop cultivars with higher level of micronutrients. This approach is of particular significance for predominantly agriculture based populations as it is a highly cost-effective and sustainable strategy and first targets those who need it the most. Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is an important coarse grain cereal and forage crop of the arid and semi-arid tropics of the Indian subcontinent and several African regions and is grown on 26 million ha in some of the most marginal arid and semi-arid tropical environments of Asia (11 million ha) and Africa (15 million ha), is a major source of dietary energy and nutritional security for a vast population in these regions [7]. It is drought resistant crop and grown mostly in marginal areas under agricultural conditions in which major cereals fail to give substantial yields [8]. For instance, a recent study showed that pearl millet accounts for 50% of the cereal consumption in some of the pearl millet growing areas of India, and it is the cheapest source of grain iron (Fe) and zinc (Zn) as compared to other cereals and vegetables [9].

Recently, ICARISAT Hyderabad has released four micronutrient biofortified pearl millet hybrids namely AHB 1200 Fe, HHB 299, RHB 233 and HHB 311 in collaboration with agricultural universities of Maharashtra (VNMKV), Haryana (CCSHAU), and Rajasthan (SKNAU) [10]. Has released four During 2013 also ICRISAT, Hyderabad has released Fe bio-fortified composite ICTP 8203 as ICTP 8203 Fe10-2 with a common name Dhanshakti. Consumption of 200g of pearl millet variety Dhanshakti (70 mg/kg Fe and 40 mg/kg Zn) can meet 82% of the recommended daily allowance (RDA) of Fe in adult man (17 mg) and 66% of the RDA of non-pregnant and non-lactating (NPNL) women (21 mg) in Indiabased on the assumption of 5% bioavailability of Fe and 25% bioavailability of Zn content [11]. Finkelstein et al [12] demonstrated that iron-biofortified pearl millet consumption significantly improved iron status in children by four months. Their findings suggested that feeding iron-biofortified pearl millet is an efficacious approach to improve iron status in secondary school–age children. Recently, pearl millet studies including inbred lines, hybrids, and composites reported a large variability for grain Fe and Zn content among the test entries [13, 14, 15, 16]. Keeping the view of the facts enumerated above, it was felt that micronutrient composition (Fe and Zn) of pearl millet might be altered to the advantage of its end users. Bio-fortification of this crop through conventional plant breeding approaches is the cheapest method for achieving this objective. For this it is first and foremost important thing to have micronutrient dense parental lines, therefore, the present investigation was carried out to identify the micronutrient dense pearl millet parental line to develop micronutrient (Fe and Zn) bio-fortified pearl millet hybrids or composites.

Materials and Methods

The experimental material consisted of 46 each of inbred lines and designated B line (counterpart of CMS lines) developed at CCS HAU, Hisar and ICRISAT, Hyderabad. The field trials were conducted during *kharif*-2013 and *kharif*-2014 as at Research Farm of CCS HAU, Hisar, India in randomized block design (RBD) with two replications. Each entry was grown in 2 rows of 4 m length with 10 cm intra-row and 45 cm inter-row spacing. All the recommended agronomic practices were followed. The Fe and Zn levels of soil were estimated at the time of planting. The available (DTPA extractable-Fe) soil Fe varied from 4.5 to 7 mg/kg and The DTPA extractable-Zn varied from 0.6 to 1.7 mg/kg. Grain samples from each entry, produced from harvests at physiological maturity, were analyzed for Fe and Zn contents in by Energy Dispersive X-rays fluorescence (EDXRF), at ICRISAT Patancheru, Hyderabad [17]. Analysis of variance for randomized block design was carried out for protein content according to the method described by Panse and Sukhatame [18].

Results

Inbred Lines

Statistically significant variation in Fe content of inbreds grown during *kharif*-2013 as well as *kharif*-2014 was observed [Table-1] and [Table-2]. Fe content of inbred lines grown during *kharif*-2013 varied from 28 to 83 mg/kg with an average value of 50 mg/kg. DPHBL 11-123 (83 mg/kg), LPBL 10/120 (82 mg/kg), HBL 0843-4 (77mg/kg), HBL 112/H12/1011 (74mg/kg), and H 1305 (72mg/kg) [Table-1], were identified as promising inbred for Fe content and were also grown during the next season (*kharif*-2014) and analyzed Fe content. Qualitatively similar results during *kharif*-2014 were observed in respect of Fe content of the raised in breds selected during preceding season. In *kharif*-2014 the selected line along with lines selected for other parameters showed a marked variation in the metal content *i.e.* 34 to 112 mg/kg with an average value of 63 mg/kg Fe. On the basis of mean Fe content recorded after testing in two season's *i.e. kharif*-2013 and *kharif*-2014, all the selected lines were superior. DPHBL-11-123 with grain Fe content of 83 and 112 mg/kg during *kharif*-2013 and *kharif*-2014 with an average of 98 mg/kg was found superior. Statistically significant variation was also observed in Zn content of in breds grown during *kharif*-2013 as well as *kharif*-2014 but to a lesser extent than that of Fe content [Table-1] and [Table-2].

Table-1 *Iron and Zinc contents (mg/kg) of pearl millet inbreds grown during kharif-2013*

SN	Pedigree	Iron	Zinc	SN	Pedigree	Iron	Zinc
1	B08/2013	44	43	24	HBL-0854	49	50
2	Brs-10-2	39	30	25	HBL-0902-1	45	37
3	Brs-10-6	38	46	26	HBL-0902-5	60	51
4	Brs-10-7	37	43	27	HBL-0904-1	67	55
5	DPHBL-11-123	83	53	28	HBL-0904-2	37	33
6	EBLT-11-101	55	46	29	HBL-0906-2	54	32
7	EBLT-11-114	60	50	30	HBL-0906-3	45	39
8	HBL- 0703	47	49	31	HBL-1108	28	37
9	HBL-0508	67	43	32	HBL-112/H12/1011	74	47
10	HBL-0510-2	56	54	33	HBL-1120	67	37
11	HBL-0547	41	38	34	HBL-34	46	41
12	HBL-0561	51	42	35	HBL-72	64	43
13	HBL-0620	46	36	36	HBL-828-1	38	31
14	HBL-0706	46	37	37	ICMB-88006	35	41
15	HBL-0802	43	38	38	LPBL-10/112	52	41
16	HBL-0809	57	44	39	LPBL-10/120	82	49
17	HBL-0825-1	41	37	40	TPBL-11-109	41	32
18	H-1305	72	53	41	94/54-1	38	31
19	HBL-0828-2	46	46	42	G-73/107	42	26
20	HBL--0832	33	37	43	HTP-94/54	50	42
21	HBL-0843-2	59	45	44	HBL-11	28	30
22	HBL-0843-04	77	46	45	H-77/833-2-202	51	48
23	HBL-0847-3	38	35	46	78/711	34	32
	Mean					50	41
	C.D. (p <0.05)					3.88	2.89
	SE(d)					1.92	1.43
	C.V. (%)					3.87	3.48

Inbred lines varied for Zn content from 26 to 55 mg/kg with average value of 41mg/kg (*kharif*-2013) and from 34 to 70 mg/kg with an average value of 49 mg/kg (*kharif*-2014). Average Zn content of these genotypes varied from 33 to 61 mg/kg. H-1305 (61 mg/kg), DPHBL 11-123 (58 mg/kg), HBL 0843-04 (55 mg/kg) and HBL 112/H12/1011 (50 mg/kg) were selected as superior than the other in breds in respect to Zn content.

Table-2 *Iron and Zinc contents (mg/kg) of selected pearl millet inbreds grown during kharif-2013 and kharif-2014*

SN	Pedigree	Iron			Zinc		
		K-13	K-14	Mean	K-13	K-14	Mean
1	DPHBL-11-123	83	112	98	53	64	58
2	LPBL-10/112	52	64	58	41	41	41
3	LPBL-10/120	82	53	68	49	34	42
4	HBL-0843-04	77	64	71	46	63	55
5	HBL-112/H12/1011	74	64	69	47	52	50
6	H-1305	72	75	74	53	70	61
8	G-73/107	42	54	48	26	40	33
10	94/54-1	38	50	44	31	34	33
11	HBL-11	28	34	31	30	41	36
	Mean	-	63		-	49	
	C.D. (p <0.05)	3.88	3.86		2.89	3.04	
	SE(d)	1.92	2.05		1.43	1.46	
	C.V. (%)	3.87	4.28		3.48	3.26	

**K-13/K-14- kharif 2013/kaarif-2014*

Designated B-Lines

On the basis of statistically significant differences in grain Fe content the designated B-lines (CMS lines) were also distinguished from each other [Table-3] and [Table-4]. Variation between 23 to 123 mg/kg in Fe content of these lines was observed when their produce was analyzed after harvest (*kharif*-2013) with an average content equivalent to 58 mg/kg. HMS 53B (123 mg/kg), HMS 14B (111 mg/kg), HMS 40B (84 mg/kg), ICMB 89111 (83 mg/kg), HMS 59B (81 mg/kg), HMS 33B (78 mg/kg), HMS 32B (75 mg/kg), HMS 16B (73 mg/kg) and HMS 52B (70 mg/kg) were selected as promising lines for Fe content and were grown during the next season (*kharif*-2014) for observing any change in their performance [Table-4].

Table-3 *Iron and Zinc contents (mg/kg) of pearl millet designated B-lines (CMS lines) grown during kharif-2013*

SN	Pedigree	Iron	Zinc	SN	Pedigree	Iron	Zinc
1	HMS 7B	37	32	24	HMS 42B	52	48
2	HMS 7B-1	50	35	25	HMS 44B	56	48
3	HMS 13B	64	65	26	HMS 45B	54	39
4	HMS 14B	111	69	27	HMS 46B	62	34
5	HMS 16B	73	69	28	HMS 49B	64	48
6	HMS 18B	52	46	29	HMS 50B	54	44
7	HMS 20B	67	62	30	HMS 51B	59	29
8	HMS 21B	56	39	31	HMS 52B	70	55
9	HMS 22B	37	32	32	HMS 53B	123	61
10	HMS 23B	33	31	33	HMS 55B	67	33
11	HMS 26B	23	38	34	HMS 56B	63	46
12	HMS 28B	43	36	35	HMS 57B	59	46
13	HMS 29B	34	35	36	HMS 58B	57	33
14	HMS 30B	44	34	37	HMS 59B	81	49
15	HMS 32B	75	72	38	HMS 60B	60	38
16	HMS 33B	78	50	39	HMS 61B	64	48
17	HMS 34B	65	61	40	HMS-18A	48	44
18	HMS 36B	41	47	41	ICMB-89111	83	53
19	HMS 37B	40	27	42	ICMB-95222	62	43
20	HMS 38B	57	53	43	ICMB-97111	52	35
21	HMS 39B	43	49	44	ICMB-94555	55	52
22	HMS 40B	84	56	45	ICMB-94222	43	36
23	HMS 41B	57	48	46	ICMB-843-22	66	49
	Mean					58	45
	C.D.(p <0.05)					4.55	3.18
	SE(d)					2.25	1.58
	C.V. (%)					3.90	3.51

A marked variation in Fe content ranging from 39 to 101mg/kg with an average value of 67mg/kg Fe was observed in grains of selected lines produced during *kharif*-2014. Average grain Fe content of HMS 53B (111 mg/kg), HMS 14B (106 mg/kg) and HMS 40B (88 mg/kg) recorded during *kharif*-2014 indicated the promising nature of these designated B-lines.

Other lines possessing equal to or more than 70 mg/kg Fe content after testing for two years were: HMS 59B (76 mg/kg), ICMB 89111 (75 mg/kg), HMS 32B (75 mg/kg) and HMS 52B (71 mg/kg).Statistically significant variation was also observed in Zn content of the designated B-lines (CMS lines) grown during *kharif*-2013 as well as *kharif*-2014 but to a lesser extent than that of Fe content [Table-3] and[Table-4]. Designated B-lines differed from each other in respect to Zn content from 27 to 72 mg/kg with an average value of 45 mg/kg (*kharif*-2013) and from 33 to 74 mg/kg with an average value of 52 mg/kg *(kharif*-2014) and Zn content of these lines varied from 34 to 65 mg/kg. On the basis of mean performance of selected lines (*kharif*-2013 and *kharif*-2014), HMS 32B (65 mg/kg) HMS 40B (64 mg/kg), HMS 14B (62 mg/kg) and HMS 53B (61 mg/kg) were selected as superior than the other designated B-lines in respect of Zn content. Other designated B-lines possessing equal to or more than 55 mg/kg Zn content after testing for two years were; HMS 16B (59 mg/kg), HMS 52B (58 mg/kg), HMS 13B (58 mg/kg), HMS 38 (58 mg/kg) and ICMB 89111 (56 mg/kg).

Table-4 *Iron and Zinc contents (mg/kg) of selected pearl millet designated B-lines grown during kharif-2013 and kharif-2014*

SN	Pedigree	Iron			Zinc		
		K-13	K-14	Mean	K-13	K-14	Mean
1	HMS 53B	123	99	111	59	62	61
2	HMS 14B	111	101	106	67	56	62
3	HMS 40B	84	92	88	54	74	64
4	ICMA89111	83	67	75	51	61	56
5	HMS 59B	81	70	76	48	51	50
6	HMS 33B	78	56	67	49	51	50
7	HMS 32B	75	73	74	70	59	65
8	HMS 16B	73	65	69	67	51	59
9	HMS 52B	70	72	71	53	62	58
10	HMS 13B	65	73	69	63	53	58
11	HMS 38B	58	59	59	52	63	58
12	HMS 21B	57	57	57	38	40	39
13	HMS 18B	52	52	52	46	33	40
14	HMS 7B-1	51	48	50	34	34	34
15	HMS 39B	44	62	53	48	57	53
16	HMS 36B	42	51	47	45	39	42
17	HMS 26B	23	39	31	37	44	41
	Mean	-	67		-	52	
	C.D. (p <0.05)	4.55	3.86		3.18	3.04	
	SE(d)	2.25	2.05		1.58	1.46	
	C.V. (%)	3.90	4.28		3.51	3.26	

**K-13/K-14- kharif 2013/kaarif-2014*

Discussions

The screened pearl millet genotypes varied significantly in respect of contents of both the micronutrients (Fe and Zn) raised during both the seasons but composition of the genotypes tested was not affected to an appreciable extent by the growing season. Iron content varied from 23 mg/kg to 123 mg/kg and Zn content ranged from 21 mg/kg to 74 mg/kg. On the basis of average value of *kharif*-2013 and *kharif*-2014, HMS 53B (111mg/kg Fe and 61mg/kg Zn), HMS 14B (106mg/kg Fe and 62 mg/kg Zn) and HMS 40B (88mg/kg Fe and 64 mg/kg Zn) from designated B-lines and DPHBL 11-123 (98mg/kg Fe and 58mg/kg Zn), and H 1305 (74mg/kg Fe and 61mg/kg Zn) were the superior parental lines in respect of Fe and Zn content. A strong positive correlation was observed between Fe and Zn contents with correlation coefficient of 0.523 to 0.702, P <0.01. These results are comparable with the earlier reports. Velu *et al*. [7, 14, 15], reported a large variability for grain Fe and Zn content in pearl millet, involving inbred lines, improved open pollinated varieties and germplasm. The mean Fe content in 68 diverse pearl millet populations ranged from 42.0 to 79.9 mg/kg and Zn content ranged from 27.2 to 50.2 mg/kg, under intra-population variability some progenies having >120 mg/kg Fe and >80 mg/kg Zn contents and showed positive and significant correlation co-efficient (r = 0.66 to 0.85; P<0.01) for Fe and Zn content. Nithya *et al* [13] reported 86 mg/kg and 88mg/kg Iron and 52 mg/kg and 41.2 mg/kg zinc content in traditional (Co7) and hybrid (Cohcu-8) pearl millet respectively. Jambunathan and Subramanian [19] and Abdella *et al* [13] also reported 10 to 66 mg/kg and 53 to 70 mg/kg Zn in pearl millet varieties. Eighteen OPVs, released during 1982-2009 were analyzes at seven locations for their Fe and Zn content under AICPMIP during 2010-11and reported that Fe content (average of seven location) varied from 40 to 73 mg/kg and Zn 37 to 54 mg/kg. Variety ICTP 8203 had highest level of both micronutrients (73 mg/kg Fe and 54 mg/kg Zn) followed by ICMV 221 with 61 mg/kg Fe and 44 mg/kg Zn content [11]. Recently, Fe bio-fortified composite ICTP 8203 has been released as ICTP 8203 Fe10-2 with a common name Dhanshakti by ICRISAT [11]). Improved bioavailability of Fe as well as Zn on consuming the biofortified pearl millet has also been demonstrated (NIN, ICRISAT and others).The four promising lines identified above with > 85 mg/kg Fe and > 55 mg/kg Zn, will add to the existing pool of the few already identified germplasm lines (either for Fe or Zn content). Use of HMS 53B, HMS 40B, HMS 14B and DPHBL 11-123 might prove to be more fruitful as these are rich in both micronutrients.

Conclusion

Among the all inbreds and designated B-lines analyzed for Fe and Zn content, DPHBL 11-123 (98 mg/kg Fe and 58 mg/kg Zn), and H-1305 (74 mg/kg Fe and 61 mg/kg Zn) from inbreds and HMS 53B (111 mg/kg Fe and 61 mg/kg Zn), HMS 14B (106 mg/kg Fe and 62 mg/kg Zn), HMS 40B (88 mg/kg Fe and 64 mg/kg Zn) and HMS 32B (74 mg/kg Fe and 65 mg/kg Zn), was superior than that of other inbreds and designated B-lines analyzed. These selected pearl millet lines dense with both the micronutrients provide the vast scope for development of bio-fortified pearl millet hybrids/composite which can cope with the micronutrient deficiency in rural poor area where pearl millet is used as staple food. The positive correlation between grain Fe and Zn content providing more advantages of simultaneous improvement of both the micronutrients. Some hybrids have been developed with these selected lines and are already under trial.

Application of research: The output of this particular research is very useful in development of micronutrient bio-fortified pearl millet hybrids or composite varieties which will be helpful in eradication of Fe and Zn deficiency in rural poor population

Research Category: Micronutrient bio-fortification.

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Study area / Sample Collection: CCS HAU, Hisar and ICRISAT, Hyderabad

Cultivar / Variety name: Pearl millet - *Pennisetum glaucum* L. R.Br.

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