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Micronutrient Rich Pearl Millet for Nutritionally Secure India

2021

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PREFACE

Pearl millet [Pennisetum glaucum (L.) R. Br.] is the most important food crop subsequent to rice, wheat, maize and sorghum. It is staple food for 90 million poor people and widely grown on 30 million ha in the arid and semi-arid tropical regions of Asia and Africa and accounts for almost half of the global millet production. In India, pearl millet is the fourth most widely cultivated food crop after rice, wheat and maize. It is critically important for food and nutritional security of arid and semi-arid lands as it is early maturing, drought tolerant, requiring minimal external inputs and mostly free from biotic and abiotic stresses. Its inherent ability to endure high temperatures up to 42°C during reproductive phase makes it suitable for cultivation in adverse conditions making it a *climate resilient* crop. Due to its excellent nutritional properties, pearl millet is designated as *nutri-cereal* (Gazette of India, No. 133 dtd 13th April, 2018) for production, consumption, trade and was included in Public distribution system.

Pearl millet is the first crop in India to develop a biofortified variety "Dhanshakti" in 2013. Pearl millet is the first crop in the world to introduce bench mark levels for Fe (42ppm) and Zn (32 ppm) in cultivar promotion and release since 2018 ensuring nutritional security in the country falling in line with the vision of nutritionally secure India. Development of micronutrient rich pearl millet hybrids and varieties with enhanced levels of Fe and Zn is taken up as a priority leading to mainstreaming of biofortification happened in pearl millet and now is a routine affair.

This publication provides the first hand information on how breeding for micronutrient rich pearl millet in India evolved along with the national and international partners involved in Pearl millet improvement.

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FOREWORD

Pearl millet is the sixth most important crop globally, and is an important crop for Indian agriculture after rice, wheat and maize in terms of total cropped area. Pearl millet is capable of growing in some of the most marginal and poor agro-ecologies where other cereals cannot grow to give profitable returns. It is often regarded as a climate resilient crop. Pearl millet is a crop of high nutritious value in terms of micronutrient density, starch profile, protein content and quality. It produces high biomass within a short span of 70-90 days providing good grain yield, nutritious forage, stover and feed. It requires lesser inputs and is the most preferred crops in the dryland agricultural systems providing livelihood support to the poor and marginal farmers.

"Dhanshakti" variety of Pearl millet is the first biofortified variety to be released in India in 2013. Pearl millet is the first crop in the world to introduce bench mark levels for Fe (42ppm) and Zn (32 ppm) in cultivar promotion and release policy from 2018 ensuring nutritional security to the country and rural community in the dry land agricultural systems where it is consumed. For this, I would like to congratulate Dr. C. Tara Satyavati, Project Coordinator for this seminal work. Since 2018, a number of hybrids and varieties were developed with enhanced micronutrient content. At present breeding for enhanced micronutrient content has become a routine affair in pearl millet breeding in India.

I appreciate the efforts of the authors in bringing out this important publication on time and congratulate them for this work which would be of great significance to the readers.

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Micronutrient Rich Pearl Millet for Nutritionally Secure India

Food is the major source of energy and important for growth and development. Cereals are the important staple foods and the main source of daily dietary energy across the world. But, sole dependence on cereals and low dietary diversity is leading to nutrient deprivation and malnutrition especially in developing countries. Micronutrient malnutrition, primarily the result of diets poor in bioavailable vitamins and minerals, affects more than half of the world's population, especially women and preschool children. The costs of these deficiencies in terms of lives lost, adverse effect on economic growth and poor quality of life are staggering in developing countries, including India. Micronutrient malnutrition arising from dietary deficiency of one or more essential micronutrients affects two-third of world's population (White and Broadley, 2009; Stein, 2010). On a global scale, over three billion people suffer from micronutrient deficiencies (MNDs) of essential minerals and vitamins (Chasapis et al., 2012). The mineral elements most commonly lacking in human diets are iron (Fe) and zinc (Zn), which rank fifth and sixth, respectively, among the top ten risk factors contributing to burden of disease, especially in the developing countries (WHO, 2002). Though the level of hunger and undernutrition worldwide fell to 20.9, down from 29.2 in the year 2000, as per the global hunger index, India was ranked 94th out of 107 qualifying countries (Global Hunger Index, 2020) and as per the most recent available data, roughly 40% of children under five are stunted and 21% of children under five are severely undernourished. Considerable efforts are underway to improve the health of poor people by breeding staple food crops enriched with essential micronutrients, a process referred to as biofortification at global and national fronts.

Biofortification of cereals is one of the strategies to address malnutrition through the genetic enhancement of key food crops with enhanced nutrients. It is a multidisciplinary approach to bring the full potential of crop improvement and nutrition science to bear on the persistent problem of micronutrient malnutrition. Crop biofortification is a sustainable and cost-effective approach to address micronutrient malnutrition, especially in the developing world (Stein et al., 2007; Bouis et al., 2011). It has the potential to help alleviate the suffering, death, disability, and failures to achieve human potential, which result from micronutrient deficiency-related diseases. In comparison to other strategies (fortification, supplementation or dietary diversification), it provides a truly feasible means of reaching out to remote and rural areas to deliver naturally-fortified foods to population groups with limited access to diverse diets, supplements and commercially fortified foods (Bouis et al., 2011). Moreover, as the trace mineral requirements in human and plant nutrition are similar, biofortification could improve human nutrition as well as farm productivity (Ma, 2007).

Pearl millet is a highly nutritious cereal, largely grown under rainfed conditions, mostly in India and Sub-Saharan Africa. Pearl millet in India is largely consumed as staple food in Gujarat and Rajasthan,

with the highest per capita consumption in the rural population of western Rajasthan (92 kg year⁻¹) followed by dry areas of Gujarat (70 kg year⁻¹). The other pearl millet consuming regions include central, western and northern Maharashtra, Saurashtra region of Gujarat and north eastern Rajasthan. In these regions, pearl millet contributes to about 20-40% of the total energy and protein intake and 30 to 50% of the Fe and Zn intake. In spite of high consumption of pearl millet in Gujarat and Rajasthan, it is these two states that reported high prevalence of anaemia among children (66%) and severe anaemia (up to 34%) among adolescent girls.

Fe deficiency ranks 9th and Zn deficiency ranks 11th among the 26 major risk factors for the global burden of disease estimates (Ezzati et al., 2002). This problem is particularly serious in developing countries, especially in high risk groups such as pregnant women, infants and adolescent children. In India, about 80% of pregnant women, 52% of non-pregnant women and 74% of children in the age group of 6-35 months suffer from Fe deficiency-induced anaemia (Kramer and Allen, 2015). Zinc (Zn) deficiency affects 50% of the world population resulting in diarrhoea, impairment of physical growth and suppressed immune function (Gibson et al., 2008). This is probably due to lower bioavailability of Fe in pearl millet flour or inadequate quantity of Fe/Zn in the locally grown pearl millet cultivars to meet daily body requirement of Fe and Zn. Recent study showed that consuming biofortified pearl millet @250 g day⁻¹ can meet 84% of the RDA for iron and 100% of the RDA for zinc in non-pregnant-non-lactating (NPNL) women while ordinary pearl millet used in the study provided only 20% of their iron needs (Neeraja et al., 2017).

Pearl millet is a richer source of dietary protein, carbohydrate, fat, ash, calcium, phosphorus, iron, zinc and essential amino acids in comparison to other cereals such as maize, rice, sorghum and wheat (Table 1). The energy value of pearl millet grain is relatively higher compared to maize, wheat or sorghum. It is high in fibre (1.2g/100g) and in α -amylase activity when compared with other grains. Pearl millet is gluten free and retains its alkaline properties after being cooked which is ideal for gluten allergic people. It is a rich source of vitamins like thiamine, riboflavin and niacin and minerals (2.3mg/100g) like potassium, phosphorous, magnesium, iron, zinc, copper and manganese. Pearl millet is rich in fat content (5mg/100g) with better fat digestibility. It is rich source of unsaturated fatty acids (75%). It has high proportions of slowly digestible starch (SDS) and resistant starch (RS) which contribute to low glycemic index (GI) and is the need of the transforming diets, food habits and the food industry (Satyavathi, 2019). Due to the excellent nutritional properties and resilience to climate change, pearl millet along with other millets is renamed as *nutri-cereal* (Gazette of India, No. 133 dtd 13^{th} April, 2018) for production, consumption and trade and included in Public Distribution System (PDS).

Table 1. Nutritional comparison of pearl millet with sorghum, rice and wheat (in 100 grams grain)

Contont	Стор								
Contents	Pearl Millet	Sorghum	Rice	Wheat					
Carbohydrates (g)	61.8	67.7	78.2	64.7					
Protein (g)	10.9	09.9	07.9	10.6					
Fat (g)	5.43	1.73	0.52	1.47					
Energy (Kcal)	347	334	356	321					
Dietary fibre (g)	11.5	10.2	02.8	11.2					
Calcium (mg)	27.4	27.6	07.5	39.4					
Phosphorus (mg)	289	274	96	315					
Magnesium (mg)	124	133	19	125					
Zinc (mg)	2.7	1.9	1.2	2.8					
Fe (mg)	6.4	3.9	0.6	3.9					
Thiamine (mg)	0.25	0.35	0.05	0.46					
Riboflavin (mg)	0.20	0.14	0.05	0.15					
Niacin (mg)	0.9	2.1	1.7	2.7					
Folic acid (µg)	36.1	39.4	9.32	30.1					

(Source: NIN, Hyderabad, 2018)

Biofortification research in Pearl millet, initially at ICRISAT supported by the Harvest Plus conducted in partnership with public and private sector research organizations, has shown large variability for Fe and Zn content with good prospects of developing cultivars with higher levels of these micronutrients. Biofortification Priority Index (BPI) indicates pearl millet is a major target crop for iron and zinc biofortification. Since pearl millet is a highly cross-pollinated crop, open-pollinated varieties (OPVs) and hybrids are the two distinct cultivar options that can be developed and are grown by farmers. Pearl millet as such is a high-iron crop with a fairly high Zn content; however, commercially available cultivars have lower Fe and Zn content. The thrust of the pearl millet biofortification research targeted for India is on the development of high-yielding and high-Fe/Zn hybrids since the entire efforts in public and private sector are geared towards hybrid development. Targeting the development of biofortified crops

with enhanced nutrients, ICAR is supporting a Consortia Research Platform (CRP) of rice, wheat, maize, sorghum, pearl millet and small millets since 2014. Several promising donors have been identified and breeding material is being generated combining high nutrient content and yield. Pearl millet is found to have very large variability for grain Fe (31-125 ppm) and Zn (35-82 ppm) among advanced breeding lines, population progenies, hybrid parents and hybrids released and commercialized (Table 2). The large genetic variability (30-140 mg/kg Fe and 20-90 mg/kg Zn) available in this crop can be effectively utilized to develop high-yielding cultivars with high iron and zinc densities (Kanatti et al., 2014).

Table 2. Variability for Iron (Fe) and Zinc (Zn) densities in pearl millet

Material	Entry		Perce	nt entry	in micr	onutrie	nt class	(mg kg ⁻¹)	
					Fe	density			
		≤ 45	46-55	56-65	66-75	76-85	86-95	96-105	>105
Mainstream hybrid-parents	290	24	31	18	10	7	7	3	1
Commercial cultivars	140	56	35	9	1	0	0	0	0
Germplasm accessions	406	11	19	16	19	17	12	5	1
Biofortified breeding lines	514	0	0	0	2	11	22	27	38
					Zn	density			
		≤ 35	36-45	46-55	56-65	66-75	76-85	86-95	>95
Mainstream hybrid-parents	290	5	47	34	11	2	0	0	0
Commercial cultivars	140	8	76	16	0	0	0	0	0
Germplasm accessions	406	2	16	31	32	17	2	0	0
Biofortified breeding lines	514	8	45	40	7	0	0	0	0

(Source: Govindaraj et al., 2019)

In pearl millet biofortification program, the initial strategy was to utilize the existing high-Fe hybrid parents and advanced breeding lines identified from the mainstream breeding program to serve the immediate objective of developing high-yielding and high-Fe hybrids (Figure 1).

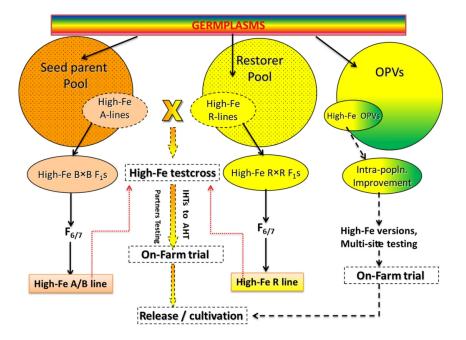


Figure 1. Current breeding approaches followed for developing biofortified cultivars in Pearl millet (Source: Govindaraj et al., 2019)

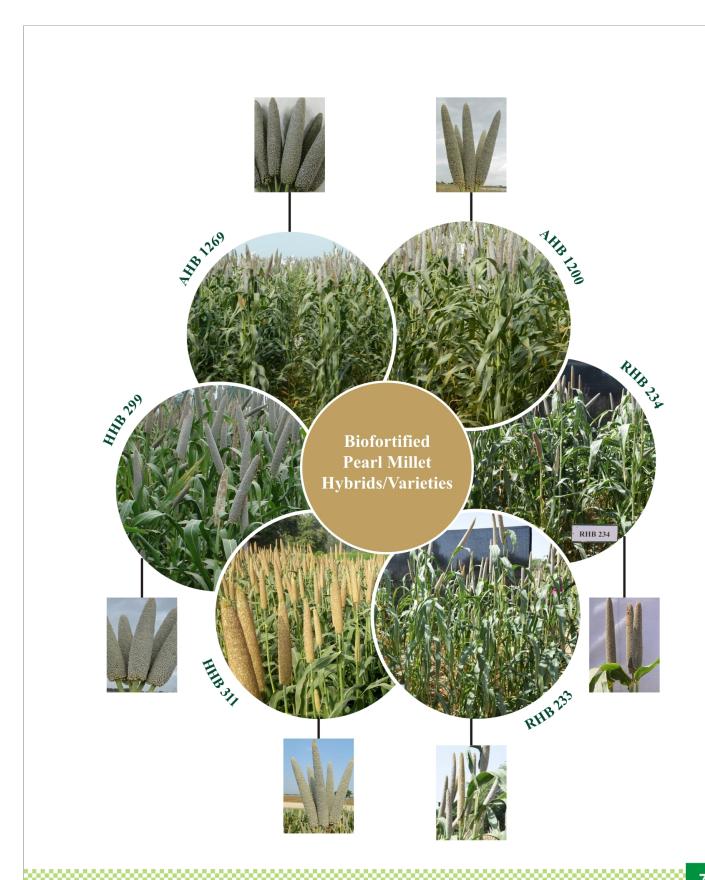
The bioavailability (absorption) of iron in pearl millet is high enough to provide more than 50% of the daily requirement for children or adult males. One meal of biofortified high-iron variety of pearl millet can meet approximately 50-100% of the daily allowance of iron helping to combat iron deficiency in women, men and children (Kodkany et al., 2013). The global baseline for iron pearl millet irrespective of cultivars (hybrid or OPVs) is set at 47 ppm and targets at 77 ppm (+30 ppm) to lessen iron deficiencies by providing up to 70% daily needs (www.harvetsplus.org) (Rai et al., 2013; Govindaraj et al., 2016). The gradual shift of biofortification research from detection of variability in 2008 through breeding high-Fe cultivars using existing high-Fe lines in 2011 to testing and delivery of biofortified varieties and hybrids is realized in India and became conscious breeding part in pearl millet at various centres of public and private partners. In order to strategize biofortification research at national level, All-India Coordinated Research Project on Pearl Millet (AICRP-PM) initiated special biofortified hybrid trial in 2014 to encourage NARS breeding programs for micronutrient along with yield traits. A first high-iron pearl millet variety 'Dhanashakti' was released for the state of Maharashtra in 2013. It was subsequently released and notified by Central Variety Releasing Committee in April 2014, for cultivation in all pearl millet growing states of India. From the joint coordinated biofortification research between AICRP on Pearl millet centres, ICRISAT and Harvest Plus one variety Dhanshakti from Dhule and two hybrids AHB 1200 and AHB 1269 from NARP, Aurangabad were released. Later, along with support from CRP on Biofortification project from ICAR, the AICRP on Pearl millet centres from CCSHAU, Hisar and

SKRAU, Durgapura along with ICRISAT and Harvest Plus ongoing programme developed another 4 hybrids namely HHB 299, HHB 311, RHB 233, and RHB 234 for release and cultivation in India under the biofortified pearl millet category through special trials for biofortified pearl millet in the All India Coordinated testing system of Pearl millet (Table 3).

Table 3. Biofortified pearl millet hybrids/varieties

S. No.	Name of Hybrid/ Variety	Year	Area of adoption	Salient features	Yield (kg/ha)	Fe (ppm)	Zn (ppm)
1.	Dhanshakti	2013	Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu, Rajasthan, Haryana, MP, Gujarat, UP, Punjab	Early maturing variety containing high iron(76-91ppm) and zinc (39-48ppm), bold, globular, shining slate grey coloured seed, cylindrical-lanceolate earhead, resistant to downy mildew	2199	81	43
2.	HHB 299 (MH 2076)	2018	Rajasthan, Haryana, Gujarat, Punjab, Delhi, Maharashtra and Tamil Nadu	Medium maturing, purple anther color, lanceolate shaped compact panicle, greyish hexagonal shape grains, resistant to major diseases and insect pests	3274	73	41
3.	AHB 1200 Fe (MH 2072 (AHB 1200)	2018	Rajasthan, Gujarat, Haryana, Punjab, Delhi, Maharashtra, Telangana, Andhra Pradesh and Tamil Nadu	Medium maturing, high Fe content, long cylindrical panicle, resistant to downy mildew, resistant to stem borer, highly responsive to fertilizers	3170	77	39
4.	AHB 1269Fe (MH 2185)	2019	Rajasthan, Gujarat, Haryana, Punjab, Delhi, Maharashtra and Tamil Nadu	nna, Punjab, content , Maharashtra		91	43
5.	RHB 234 (MH 2174)	2019	Rajasthan, Haryana, Gujarat, Punjab, Delhi Maharashtra and Tamil Nadu	Medium maturing, brown anther colour, complete excertion, greyish seed, resistant to major diseases & insect pest	3169	84	41
6.	RHB 233 (MH 2173)	2019	Rajasthan, Haryana, Gujarat, Punjab, Delhi, Maharashtra and Tamil Nadu	Medium maturing, yellow anther colour, complete excertion, greyish seed, resistant to major diseases & insect pest	3157	83	46
7.	ННВ 311	2020	Rajasthan, Haryana, Gujarat, MP, Punjab, Delhi, Maharashtra and Tamil Nadu,	Medium maturing, compact panicle having grey coloured hexagonal shaped grain, Highly resistant to downy mildew and other diseases.	3173	83	39

(Source: Satyavathi et al., 2020b)



Genesis of bench mark for Fe and Zn in AICRP testing of pearl millet

With great scale of demonstration of biofortification by strong partnership between ICRISAT, ICAR and CGIAR, pearl millet biofortification programme lined up for the mainstream breeding. In 2016, ICRISAT and AICRP-PM jointly published status of iron and zinc content among released cultivars in India and found baseline for Fe (42 ppm) and Zn (32 ppm) (Rai et al., 2016).

Though the biofortified pearl millet varieties and hybrids were ready for identification and release in 2017, there were certain issues left to be addressed -

- 1. A special premium price in the market for biofortified varieties/hybrids of pearl millet with enhanced levels of micronutrients is not available.
- 2. Lack of segregation for biofortified and non-biofortified material in the markets
- 3. Lack of easily detectable established markers for biofortified pearl millet for identification during purchase of seed by the farmers and grain by the consumers
- 4. The sole purpose of developing biofortified products meant for addressing nutritional security is lost-if premium price is to be fixed for biofortified pearl millet.

Looking into the above issues in 2017, the Varietal Identification Committee discussed the sustainability of special biofortification trial and possibility of fixing baseline for iron and zinc for the cultivars that will be included in the national testing trials. Since then, ICAR-All India Coordinated Research Project on Pearl Millet has decided to alleviate malnutrition in the country through developing pearl millet hybrids and varieties with enhanced levels of iron and zinc that would be within the reach of common man. Hence, a conscious and landmark decision was taken during the 52nd Annual Group Meeting of All India Coordinated Research Project on Pearl millet during 28-30th April, 2017 held at PAU, Ludhiana to include bench mark levels of minimum 42 ppm of iron and 32 ppm of zinc in the promotion criteria of the pearl millet test entries in the coordinated trials from 2017 onwards in the presence of researchers from national, international, public and private partners and stakeholders (Satyavathi, 2017 in Proceedings of 52nd AGM).

Inclusion of nutritional quality traits in the varietal promotion criteria is first of its kind in any of the food crop and the world too. This was recognized and an appreciation certificate <u>was awarded to ICAR-AICRP on Pearl millet stating that establishing global minimum standard for micronutrients in the cultivar release policy that has a significant and lasting positive impact on the mission for improving human nutrition (Figure 2).</u>



Figure 2. An appreciation certificate received by ICAR-AICRP on Pearl millet for establishing global minimum standard for micronutrients in the cultivar release policy

Since then, a total of 25 hybrids/varieties (Table 4) were developed as micronutrient rich pearl millet cultivars including the six biofortified cultivars identified from the special biofortification trial given in Table 3.

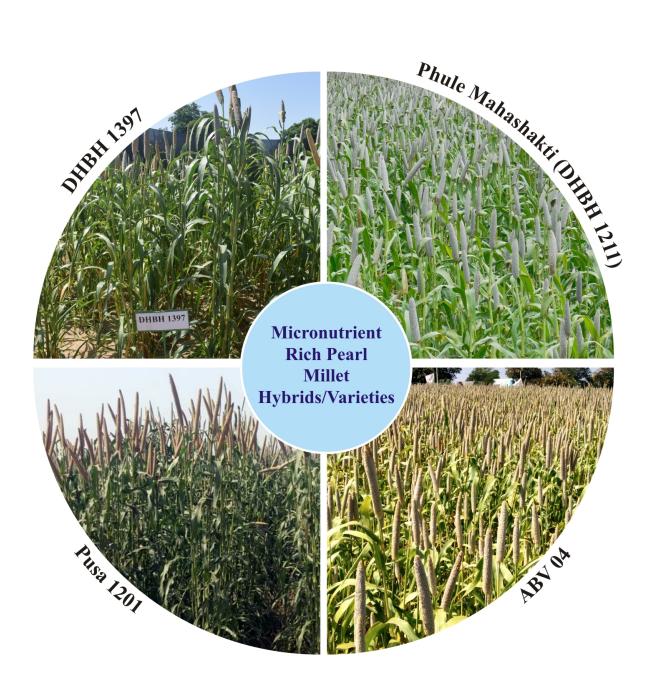
Table 4. Micronutrient rich pearl millet hybrids/varieties released during 2017-2020

S. No.	Name of Hybrid/ Variety	Year	Area of adoption	Salient features	Yield (Kg/ha)	Fe (ppm)	Zn (ppm)
1.	Proagro Marutej (XMT 1358)	2020	Rajasthan	Early maturing, tolerant to moisture stress and lodging, tolerant to downy mildew, rust, blast, shoot fly and stem borer	2175	43	35
2.	Moti Shakti (GHB 1225)	2020	Gujarat	Late maturing, resistant to downy mildew, blast, smut, rust and ergor, salt and water stress tolerant, good quality stover	3023	76	46
3.	Jam Shakti (GHB 1129)	2020	Gujarat	Medium maturing, resistant to downy mildew and lodging, salt and water stress tolerant, good quality stover	2957	72	43

4.	Central Pearl Millet Hybrid BHB 1602 (MH 2192)	2020	Rajasthan, Gujarat and Haryana	Early maturing, compact, conical ear heads, grey brown, globular grains, highly resistant to downy mildew, blast, insect pests and resistant to smut	2529	55	37
5.	PB 1852 (MH 2224)	2019	Rajasthan, Gujarat, Haryana, Madhya Pradesh, Uttar Pradesh, Punjab, Delhi	Medium maturing, grey colour grain with bold size, lodging tolerant, responsive to fertilizers, resistant to DM and blast, tolerant to moisture stress	3383	56	32
6.	JKBH 1326 (MH 2228)	2019	Rajasthan, Gujarat, Haryana, Madhya Pradesh, Uttar Pradesh and Punjab	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	3292	48	33
7.	DHBH 1397 (MH 2114)	2019	Rajasthan, Gujarat, Haryana, Madhya Pradesh, Uttar Pradesh, Punjab and Delhi	Medium maturing, dual purpose hybrid, globular shaped grey colour seed, highly resistant to downy mildew and blast	3390	59	36
8.	PROAGRO 9450 (Bayer 9450)	2019	Uttar Pradesh	Late maturing, dual purpose hybrid, tall, long compact conical panicles, tolerant to lodging, tolerant to downy mildew and blast diseases	3861	71	58
9.	ABV 04 (MP 552)	2019	Maharashtra, Karnataka, Andhra Pradesh, Telangana and Tamil Nadu	Late maturing, panicles thick and compact with grey coloured bold size, highly resistant to downy mildew, smut and blast, resistant to major insect pests	2863	70	63
10.	Phule Mahashakti	2019	Maharashtra	Late maturing, medium tall, cylindrical shape earhead, resistant to downy mildew	2581	85	37
11.	Pusa 1201 (MH 1849)	2018	National Capital Territory of 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	2810	55	48

12.	BHB 1202 (Bikaner Hybrid Bajra1202) (MH 1831)	2018	Rajasthan	Early maturing, compact conical earhead, yellow brown globular shaped grains, yellow brown anther color, highly resistant to downy mildew, blast and major pests	1776	47	42
13.	Balwan (NBH 4903) (MH 1743)	2018	Rajasthan	Late maturing, medium plant height with long exerted compact panicles, medium bold grains, tolerant to major diseases and pests, non lodging, non shattering, resistant to drought	3103	53	41
14.	GK 1116 (MH 1974)	2018	Rajasthan	Medium maturing, medium plant height, very compact ear head cylindrical spike shape, tolerant to downy mildew, rust, smut and ergot, tolerant to stem borer, highly responsive to fertilizers	3644	46	27
15.	Central Pearl Millet Hybrid RHB 223 (MH 1998)	2018	Rajasthan, Gujarat and Haryana	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	2969	46	40
16.	PB 1705 (MH 2008)	2018	Rajasthan, Gujarat, Haryana, Punjab, Delhi, Madhya Pradesh and Uttar Pradesh	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	3640	49	32
17.	NBH 4903 (MH 2035)	2018	Maharashtra, Karnataka, Telangana, Andhra Pradesh and Tamil Nadu	Late maturing, medium plant height with long exerted compact panicles, medium bold grains, non lodging,non shattering, resistant to drought	4444	70	63
18.	Mahabeej 1005 (MH 1852)	2017	Maharashtra	Medium maturing, yellow anther, complete spike exertion, conical, long and thick panicle, tolerant to stress	2994	62	37
19.	PBH 306 (MH 1962)	2017	Maharashtra, Karnataka, Telangana, Andhra Pradesh and Tamil Nadu	Late maturing, medium plant height, long compact panicles, medium bold grains, resistant to drought, tolerant to major diseases and pests	3828	54	34

(Source: Satyavathi et al., 2020b)



Mainstreaming of micronutrients (Fe & Zn) in pearl millet breeding

Breeding for micronutrient dense cultivars needs screening large number of genetic material, such as germplasm collections, elite lines, segregating populations, hybrids etc. and phenotyping for iron and zinc though destructive techniques involves high analytical cost and breeding resources. The success of Pearl millet biofortification programme lies in the high throughput precision phenotyping for estimation of grain micronutrient content. Atomic Absorption Spectrophotometry (AAS) and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) techniques (both are destructive method) are highly used by researchers and its results are reproducible for grain Fe and Zn densities. High-throughput, non-destructive and low-cost quantitative technique XRF is now being used in several laboratories for initial screening of large genetic and breeding material. For final validation of those top-ranking pipeline entries based on XRF value can be further re-evaluated using ICP to obtain precision estimates for wider reporting and documentation. In case of Pearl millet, it was possible through use of X-ray Fluorescence Spectrometry (XRF) (Figure 3) for phenotyping.

An XRF machine with high throughput was installed at ICRISAT for rapid and cost-effective screening of a large number of breeding materials. ICAR-AICRP on Pearl millet is getting the pearl millet samples estimated for iron and zinc content using this facility on payment basis for data generation of the test entries in the coordinated trials. From 2017 onwards, ICAR-AICRP on Pearl millet has tested 7707 samples for promotion of test entries in the All India Coordinated trials of Pearl millet as mentioned in Table 5.

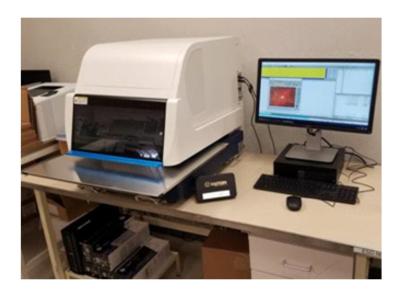


Figure 3. X-ray Fluorescence Spectrometry (XRF) machine for Fe-Zn estimation

Table 5. Pearl millet grain samples tested during *kharif* 2017-2019

Trial Name	2017	2018	2019	Total
Initial Hybrid Trial (Early) IHT (E)A ₁	108	234	395	737
Initial Hybrid Trial (Medium) IHT(M) A	175	215	479	869
Initial Hybrid Trial (Medium) IHT (M) B	210	144	384	738
Initial Hybrid Trial (Late) IHT (L) A	252	264	666	1182
Initial Hybrid Trial (Late) IHT(L) B	210	147	313	670
Advanced Hybrid and Population Trial (Early) AHPT-(E)	269	90	126	485
Advanced Hybrid Trial (Medium) AHT(M) A	384	225	-	609
Advanced Hybrid Trial (Medium)AHT (M) B	126	-	-	126
Advanced Hybrid Trial (Late) AHT (L) A	123	162	105	390
Advanced Hybrid Trial (Late) AHT (L) B	126	-	72	198
Population Trial-PT A	349	198	351	898
Population Trial-PT B	195	144	287	626
Summer Hybrid Trial -SHT	-	-	179	179
Total	2527	1823	3357	7707

The micronutrient (iron and zinc) contents of the test entries in different coordinated trials of ICAR-AICRP on Pearl millet over years is given in Tables 6 and 7.

Table 6. Iron content in the hybrid and population test entries in the multilocation All India Coordinated trials of Pearl millet from kharif, 2017 to kharif, 2019

Name of the trial	2017			2018			2019		
	Min (ppm)	Max (ppm)	Average (ppm)	Min (ppm)	Max (ppm)	Average (ppm)	Min (ppm)	Max (ppm)	Average (ppm)
Zone A ₁									
IHT (E)	27	100	51	29	91	52.2	30	108	46
AHPT (E)	50	89	50	34	83	53	35	83	47
Zone A									
IHT (M)	31	69	50	25	79	53	26	85	51.4
IHT (L)	32	89	50	30	76	46	28	85	52
AHT(M)	28	86	54.2	26	73	48	-	-	-
AHT (L)	32	79	53.3	19	84	47	32	70	45

PT	31	104	54	28	102	54	37	78	54.4	
EDV							34	77	46	
Zone B										
IHT (M)	25	73	48	31	64	49	25	87	51	
IHT (L)	24	80	50	32	96	60	26	71	46	
PT	31	112	53	44	116	65	24	92	47	
AHT(M)	32	88	54.2	-	-	-	-	-	-	
AHT(L)	31	73	52	-	-	-	31	81	54	

(Source: Satyavathi et al. 2018, 2019, 2020a)

Table 7. Zinc content in the hybrid and population test entries in the multilocation All India Coordinated trials of Pearl millet from *kharif*, 2017 to *kharif*, 2019

		2017			2018		2019			
Trial	Min (ppm)	Max (ppm)	Average (ppm)	Min (ppm)	Max (ppm)	Average (ppm)	Min (ppm)	Max (ppm)	Average (ppm)	
Zone A ₁										
IHT (E)	23	74	42	13	65	34	15	63	29	
AHPT (E)	13	70	33	20	50	38	21	72	33	
Zone A										
IHT (M)	23	67	39	11	56	29	18	57	34.1	
IHT (L)	12	57	33	8	59	30	19	47	33	
AHT(M)	16	53	33.1	10	54	33	-	-	-	
AHT (L)	26	57	38	9	50	26	14	55	30.4	
PT	17	76	37	24	75	42	15	79	37	
EDV	-	-	-	-	-	-	13	55	34	
Zone B				,		,				
IHT (M)	9	48	32	8	48	31	13	42	27	
IHT (L)	12	54	35	13	61	39	17	50	33	
PT	22	59	34	16	51	34	12	54	31	
AHT(M)	18	43	29	-	-	-	-	-	-	
AHT(L)	22	50	35	-	-	-	21	43	35	

(Source: Satyavathi et al. 2018, 2019, 2020a)

Enhancing the bench mark levels for Fe and Zn

As the awareness about biofortification is increasing among the researchers, administrators and public, concerns about the bioavailability of the enhanced nutrients in the humans and animals surface up. Along with the concerns, suggestions also pour in to enhance the benchmark levels for the micronutrient content in the testing of the entries.

As shown in Table 6, the average amount of grain iron Fe content varied from 45 ppm to 65 ppm during *kharif*, 2017 to *kharif*, 2019 in different trials which are above the benchmark level of iron content (42 ppm). A closer observation of the minimum values show that there are still certain genotypes with grain iron content as low as 24 ppm and certain genotypes with as high as 116 ppm. As there is a difference of nearly 92 ppm between minimum and maximum values, it will take some time for the breeders to develop the potential parents and breeding material with increased iron content.

As shown in Table 7, the average grain zinc content varied from 26 ppm to 42 ppm during *kharif*, 2017 to *kharif*, 2019 in different trials while the minimum value of Zn content was 9 ppm and maximum was 79 ppm. among the genotypes. Thus, the variation of 70 ppm between maximum and minimum values is observed in the test entries over 3 year period of multilocation testing. With such a large variation, it is expected and understood that the targets set should be achievable and also promote enthusiasm and competitiveness in breeding rather than suprressing the zeal of the breeders. Hence, a review of the bench mark values was done during February, 2020 and it was decided by the breeders from public and private sector to continue with the same levels of iron and zinc for two more years.

Conclusion

The challenges and opportunities are very high of micronutrients in staple food crops. Establishment of nutrition mission, millet mission or other mission supported by Govt. of India need to work together with agriculture research and production organization to increase befits to reach nutrition commitments. Biofortification journey is quite long and are being discussed in several forums but yet more efforts are required to take it into mainstream. Dissemination of the biofortified breeding lines and hybrid parents to and their utilization by user-research organizations (both public and private sector) on a continuing basis, as done so far for the non-biofortified materials, will make biofortified hybrid development a matter of routine and hence significantly contribute to improved nutrition. Further, demonstration of the feasibility of developing high-Fe and high-yielding hybrids, and encouraging the partners to breed for these micronutrient traits by means of mainstreaming the biofortification can be an important approach for its promotion. There is a high need to facilitate the focused screening of partners breeding materials for grain Fe and Zn content for their introgression of high Fe content into locally adapted, high-yielding and farmer-preferred cultivars. Promotion and strengthening of the pipeline of

high-iron and high-yielding partners-bred hybrids and testing of their grain samples for grain Fe and Zn content can be a useful step for enhancing its importance. In addition to this, bioavalablity is another aspect which needs to be focused. Many bioavailability studies are underway and same levels of bioavailability for Fe/Zn are expected in future cultivars as well.

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