13th International Conference on Development of Drylands

Converting Dryland Areas from Grey into Green

February 11-14, 2019, Jodhpur, India

Proceedings

Editors

Adel El-Beltagy, M.C. Saxena, O.P. Yadav and N.R. Panwar

**International
Dryland Development Commission
2019**

Executive Board, International Dryland Development Commission (IDDC)

Chairman:

Adel El-Beltagy, Chair, the Food and Agriculture Council/Board Member, Egyptian Academy of Scientific Research & Technology; Emeritus Professor, Arid Land Agricultural Graduate Studies & Research Institute (ALARI), Ain Shams University Cairo, Egypt; Former Chair, Global Forum on Agricultural Research (GFAR)

Executive Secretary:

Mohan C. Saxena, Adjunct Professor, Indian Agricultural Research Institute, New Delhi; Fellow National Academy of Agricultural Sciences, India

Board Members:

Aly Abousabaa, Director General, International Center for Agricultural Research in the Dry Areas (ICARDA), Egypt

Atsushi Tsunekawa, Professor**,** Former Director, Arid Land Research Center (ALRC), Tottori University, Tottori, Japan

Ayman Abou-Hadid, Emeritus Professor, Arid Land Agricultural Graduate Studies and Research Institute (ALARI), Ain Shams University; Former President, Agriculture Research Centre, Egypt

Ch. Srinivasa Rao, Director, ICAR-National Academy of Agriculture Research Management (NAARM), Rajendranagar, Hyderabad, India

Djamin Akimaliev, General Director of Kyrgyz Agricultural Research Institute and Former President of Kyrgyz Agrarian Academy, Kyrgyzstan

Donald Slack, Professor of Agricultural and Biosystems Engineering & Professor of Watershed Management and Eco-Hydrology, Cecil H. Miller, Jr., & Cecil H. Miller, Sr., Families Dean's Chair for Excellence in Agriculture and Life Sciences, University of Arizona, Arizona, USA

J.A. (Hans) van Ginkel, Honorary Professor, Human Geography and Planning**,** Faculty of Geosciences, Universiteit Utrecht**,** The Netherlands

Magdi Madkour, Emeritus Professor of Biochemistry, Arid Land Agricultural Graduate Studies and Research Institute (ALARI), Ain Shams University, Cairo, Egypt

Tao Wang, President, Lanzhou Branch of Chinese Academy of Sciences (CAS), Director General, Northwest Institute of Eco-environment and Resources, CAS; Director and Professor, Key Laboratory for Desert and Desertification, CAS, China

Zafar Adeel, Executive Director, Pacific Water Research Centre at Simon Fraser University, Vancouver, Canada

Citation: El-Beltagy, A., Mohan C. Saxena, O.P. Yadav and N.R. Panwar (eds.). 2019. Proceedings. 13th International Conference on Development of Drylands: Converting Dryland Areas from Grey into Green, February 11-14, 2019, Jodhpur, India: International Dryland Development Commission'

Performance of biofortified pearl millet hybrids for grain yield in northern India

M. Govindaraj[1*], A. Kanatti[1], L.D. Sharma[2], D. Yadav[3] and H. Shivade[1]

[1]*International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, INDIA*
[2] *Rajasthan Agricultural Research Institute, Durgapura, Rajasthan, INDIA*
[3]*CCS Haryana Agricultural University, Hisar, Haryana, INDIA*
email: m.govindaraj@cgiar.org

Abstract

Pearl millet is a dryland resilient crop of the semi-arid tropics in India and Africa. It plays a major role in food and nutritional security in these regions. Dry-zone of northern India lags behind in pearl millet genetic improvement and adoption of hybrids. Aim of this study was to test and identify superior high-iron and high yielding hybrids for adaptation. Two trials with 17 (trial 1, 2016) and 14 (trial 2, 2017) hybrids were conducted in 3 sites each for grain yield, and iron (Fe) and zinc (Zn) density in grains. Analysis of variance showed highly significant variation for grain yield, flowering, Fe and Zn density in both the trials and across environments. Hybrids significantly contributed to total variability for all traits, while the G×E interaction contributed much lower in both the trials across environments. Further, high heritability for all traits suggests relatively low environmental influence on these traits. Trial 1 hybrids had 59-104 mg kg^{-1} Fe density, 35-52 mg kg^{-1} Zn density and 2.2-3.5 t ha^{-1} grain yield. Five hybrids were identified with 19-45% higher yield and >40% higher iron than control. Similar trend was observed in trial 2. Three commercial hybrids had 43-58 mg/kg mean Fe and 29-37 mg kg^{-1} mean Zn with an average yield of 1.9-2.5 t ha^{-1}. Although identified hybrids flowered a week later than controls, they yielded well in rainfed conditions without affecting Fe/Zn density. This is preliminary indication of the potential of ICRISAT high-Fe/Zn parents, and their utilization in hybrid breeding, for development of high iron and zinc hybrids without compromising the grain yield in northern India.

Introduction

More than 2 billion people in the developing countries suffer from the nutritional deficiency of essential micronutrients. About 50% of children and women in India suffer from anemia whereas 52% of children are stunted (NFHS, 2016). Pearl millet [*Pennisetumglaucum* (L.) R. Br.] is staple cereal in drylands, accounting for 20-63% of total cereal consumption in major pearl millet growing states of India (Maharashtra, Gujarat, and Rajasthan). It contributes to higher intake of total Fe (19-63%) and Zn (16-56%) than other cereals (Parthasarathy Rao *et al.,* 2006). Genetic variability for Fe/Zn content and its genetic enhancement as ‘Proof of Concept’ were demonstrated by biofortification program at the International Crop Research Institute for Semi-Arid Topics (ICRISAT) that led to rapid development of mineral-dense cultivars with partners. Their adoption and utilization in food preparations is highly required to enable improved human nutrition in drylands.

Pearl millet is a major warm-season cereal grown on 28 mha for grain and fodder production in some of the most marginal areas of the arid and semi-arid tropical regions of Asia and Africa. In these regions, pearl millet is a major source of dietary energy and mineral micronutrients. Pearl millet is a highly cross-pollinated crop with open-pollinated varieties (OPVs) and hybrids as the two broad cultivar options. Hybrids are the most dominant cultivars in India, occupying >70% of area under improved pearl millet cultivars, with OPVs cultivated on limited scales. India is the largest producer of this crop with 7.5 mha area and 9.7 m tons of grain production (www.indiastat.com). Here, pearl millet growing area has been categorized into two zones: A-Zone (Dry Zone): and B-Zone (South central zone) (Yadav *et al.,* 2012). B-Zone includes Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu, with more than 600 mm annual rainfall, heavy soils and mild temperature conditions and it contributes 29% to the total pearl millet production from 26% of the total area in the country. A-Zone represents north-western states, Rajasthan, Haryana, Punjab, Delhi, Gujarat, Uttar Pradesh and Madhya Pradesh, with less than 600 mm annual rainfall. It has sandy to sandy-loam soils. This zone contributes about 71% to the total production from 74% of total area under pearl millet in the country. Within this zone, parts of Rajasthan, Haryana and Gujarat receiving <400 mm of rainfall are grouped into a sub zone i.e. A1 zone. This sub-zone is highly drought prone with average annual rainfall below 400 mm, light sandy soils, and high temperatures. Under this multi-location study performance of iron biofortified pipeline hybrids was evaluated to identify hybrids suitable for A-zone.

Materials and methods

Trial-2016 consisted of 21 entries (17 hybrids, 4 checks) and trial-2017 consisted of 18 entries (14 hybrids, 4 checks) (Table 1). The trials were laid out in randomized complete block design (RCBD) with two replications. Trials were evaluated across three locations during the rainy season of 2016 (Durgapura in Rajasthan and Bawal and Hisar in Haryana) and 2017 (Durgapura and Jaipur in Rajasthan and Hisar in Haryana) in the A-Zone of northern India. Grain yield (GY) and days to 50% flowering (BL) were recorded on plot basis. Grain Fe content (Fe) and Zn content (Zn) were analyzed using XRF at Pearl Millet Breeding XRF Laboratory, ICRISAT, Patancheru (Govindaraj *et al.,* 2016a; Paltridge *et al.*, 2012). The analysis of variance (ANOVA) of all the trials was done following Gomez and Gomez (1984). ANOVA for both individual environments and pooled data was carried out in using the PROC GLM procedure in SAS 14.1 software (SAS Institute Inc., 2015), considering environments factor fixed and genotypes as random factor. Phenotypic correlations were estimated among all the traits and tested for their significance (Snedecor and Cochran, 1989).

Table 1. *Pedigree details of entries in 2016 and 2017 multi-location*

Geno	Seed parent		Pollinator Pedigree
			2016 trial
1	ICMA1 98222	×	MRC S1-155-4-3-B-B-B-B-1-B-B-1
2	ICMA4 02333	×	MRC S1-155-4-3-B-B-B-B-1-B-B-1
3	ICMA1 04999	×	(EERC-HS-6)-B-12-1-1-3-B
4	ICMA4 02333	×	(EERC-HS-6)-B-12-1-1-3-B
5	ICMA1 1502	×	MRC HS-225-3-5-2-B-B-B-1
6	ICMA1 98222	×	MRC HS-225-3-5-2-B-B-B-1
7	ICMA4 03999	×	AIMP 92901 S1-296-2-1-1-3-B-1-3-4-1
8	ICMA4 03999	×	AIMP 92901 S1-15-1-2-3-B-3-B-9-2-1
9	ICMA1 1502	×	AIMP 92901 S1-296-2-1-1-3-1-B-3-B-1
10	ICMA1 04999	×	ICMV 221 S1 -123-B-B-B-B-B-P2
11	ICMA1 00111	×	[(IPC 1617×SDMV 90031-S1-84-1-1-1-1)×AIMP 92901 S1-296-2-1-1-3-B-1]-4-4-5-3-2
12	ICMA4 04222	×	MRC S1-416-2-1-2-3-B-B-B-1-B-B
13	ICMA1 98222	×	MRC S1-97-3-4-B-B-1-B-1-B
14	ICMA1 04999	×	(EERC-HS-6)-B-12-1-1-2-B-P12
15	ICMA1 94333	×	Jakhrana × ESRC II S2-11-B-1-2-1-1-B
16	ICMA1 97444	×	[[(IPC 1617×SDMV 90031-S1-84-1-1-1-1)×AIMP 92901 S1-296-2-1-1-3-B-1]-4-4-2-1 X ICMV 96490-S1-15-4-1-1-2]-29-3-3
17	ICMA1 97444	×	ICTP 8203 S1-121-12-2
18	Check		RHB 177
19	Check		ProAgroTejas
20	Check		HHB 67 Improved
21	Check		Dhanashakti
			2017 trial
1	ICMA1 04999	×	(EERC-HS-6)-B-12-1-1-2-B-P12
2	ICMA4 99444	×	(EERC-HS-6)-B-12-1-1-2-B-P12
3	ICMA1 97222	×	ICMR 12555
4	ICMA1 97444	×	ICMR 12555
5	ICMA1 97444	×	ICTP 8203 S1-121-12-2
6	ICMA1 04999	×	(MC 94 C2-S1-3-2-2-2-1-3-B-B x AIMP 92901 S1-488-2-1-1-4-B-B)-B-2-2-3
7	ICMA1 1502	×	MRC HS-225-3-5-2-B-B-B-1
8	ICMA1 1505	×	[ICMV 96490-S1-15-1-4-3-1 X MRC HS-130-2-2-1-B-B-3-B-B-B-1-3-1]-98-2-2
9	ICMA1 94333	×	(ICMB 08666 x (ICTP 8203 x 40258-B-1)) F3-77-3-1
10	ICMA1 98222	×	MRC S1-97-3-4-B-B-1-B-1-B
11	ICMA1 98222	×	(ICMS 7704-S1-127-5-1 × RCB-2 Tall)-B-19-3-2-1-4-B-1
12	ICMA4 99444	×	ICTP 8203 S1-386-B-B-B-B
13	ICMA1 04999	×	ICMR 12555
14	ICMA1 04999	×	(MC 94 C2-S1-3-2-2-2-1-3-B-B x ICMR 312 S1-3-2-3-2-1-1-B-B)-B-34-1-1
15	Check		RHB 177
16	Check		ProAgroTejas
17	Check		HHB 67 Improved
18	Check		Dhanashakti

Results and discussion

Genotypes performance across environments

In trial-2016, Fe density varied from 59-104 mg kg^{-1} with an average of 78 mg kg^{-1}; Zn density varied from 35-52 mg kg^{-1} with an average of 42 mg kg^{-1} (Table 2). While the grain yield varied from 2.16 to 3.53 t ha^{-1} with an average of 2.73 t ha^{-1}, and days to 50% flower varied from 45 to 56 days with an average of 50 days. In trial-2017, Fe density varied from

60 to 110 mg kg^{-1} with an average of 81 mg kg^{-1}, Zn density varied from 29 to 57 mg kg^{-1} with an average of 40 mg kg^{-1}, and grain yield varied from 1.73 to 2.75 t ha^{-1} with an average of 2.19 t ha^{-1}, and days to 50% flower varied from 46 to 53 days with an average of 50 days. Earlier studies also reported larger variability for both micronutrients and agronomic traits in biofortified hybrids and their parents and segregating progenies (Velu *et al.,* 2008a, 2008b; Gupta *et al.,* 2009; Rai *et al.,* 2012; Govindaraj *et al.,* 2012; Govindaraj *et al.,* 2013; Kanatti *et al.*, 2016b).

Table 2*. Mean performance for Fe, Zn, GY and BL in 2016 and 2017 multi-location trials*

2016					**2017**				
Entry	**Fe**	**Zn**	**GY**	**BL**	**Entry**	**Fe**	**Zn**	**GY**	**BL**
1	59	36	2323	46	1	60	29	2753	51
2	61	36	2724	45	2	74	33	2053	52
3	75	43	2897	51	3	81	47	2269	50
4	87	39	2702	46	4	76	36	2020	51
5	86	35	2687	48	5	77	36	2283	51
6	76	37	2161	49	6	83	46	2159	51
7	95	47	2637	54	7	90	43	2040	46
8	94	44	2944	56	8	101	44	2225	46
9	104	43	2790	53	9	80	42	2189	52
10	85	50	3532	53	10	64	31	2491	50
11	80	44	2515	56	11	79	39	2388	49
12	70	41	2821	48	12	110	57	1737	49
13	65	37	2936	50	13	80	43	1821	52
14	59	39	3257	51	14	73	39	2246	53
15	77	45	2460	47					
16	78	52	2537	54					
17	69	41	2509	51					
RHB 177	42	30	2444	46	RHB 177	43	27	2567	46
ProAgroTejas	53	33	2734	43	ProAgroTejas	63	31	1655	44
HHB 67 Improved	54	40	2034	44	HHB 67 Improved	53	33	1733	44
Dhanashakti	90	49	2057	48	Dhanashakti	99	52	1504	49
CV (%)	7	13	11	2.7		8	10	14	2.1
Mean	78	42	2731	50		81	40	2191	50
Min	59	35	2161	45		60	29	1737	46
Max	104	52	3532	56		110	57	2753	53

G×E interaction

Significant variability was observed for all the traits (GY, BL, Fe, Zn) in both the trials (Table 3). G×E interaction was significant for all the trails in both the trials. The proportion of G×E variability component, relative to variability due to hybrid component, was in the sequence: 50% flower (14%) <Fe (33%) <Zn(54%) <GY(71%) in trial-2016, and was in the sequence: Fe (21%) <50% flower (33%) <Zn(37%) <GY(86%) in trial-2017. This implies that Fe had lower G×E interaction than that of Zn. Likewise, for GY, G×E interaction component was higher in magnitude and also larger than both the micronutrients and BL. Earlier studies in pearl millet reported significant G×E interaction across the seasons (Gupta

et al., 2009; Velu *et al.,* 2011; Govindaraj *et al.,* 2013; Kanatti *et al.,* 2014a, 2016a). Further, multi-location evaluation of two sets of pearl millet hybrids by Kanatti *et al.* (2014b), reported higher G×E interaction relative to those due to differences among the hybrids denser in Zn than Fe content. Similar results of higher G×E interaction for Zn content than Fe content have been reported in maize (Prasanna *et al.,* 2011). This may apparently imply greater sensitivity and differential response of hybrids for Zn than Fe content to changes in the soil and climatic conditions. And this could also be due to proportionately larger differences among the hybrids for Fe content (59-104 mg kg^{-1} in 2016 and 60-110 in 2017) than for Zn content (35-52 mg kg^{-1} in 2016 and 29-57 mg kg^{-1} in 2017).

Table 3. *Mean square for Fe, Zn, GY and BL in 2016 and 2017 multilocation trials*

		Mean square							
		2016							
Source of variation	**df**	**Fe**		**Zn**		**GY**		**BL**	
Environments (E)	2	12816	**	228	**	39227229	**	702	**
Replications /E	3	132	**	176	**	112573		0.8	
Hybrids (G)	20	1438	**	172	**	794091	**	85	**
G×E	40	237	**	46		281505	**	6	**
Error	60	30		29		83499		1.8	
CV%		7		13		11		3	
		2017							
Source of variation	**df**	**Fe**		**Zn**		**GY**		**BL**	
Environments (E)	2	592	**	444	**	1657465	**	216	**
Replications /E	3	104	*	83	**	14534		14.5	**
Hybrids (G)	17	1611	**	390	**	701417	**	49	**
G×E	34	166	**	72	**	300862	**	8	**
Error	51	34		16		94493		1	
CV%		8		10		14		2	

Table 4. *Genetic parameters and heritability for Fe, Zn, GY and BL in 2016 and 2017 multi-location trials*

Variance components	**2016**				**2017**			
	Fe	Zn	GY	BL	Fe	Zn	GY	BL
Vg	217	22	101932	13	252	58	83957	7.5
Vp	257	30	148849	14	279	70	134100	8.8
Vg×e	103	8	99003	2	66	28	103185	3.5
Ve	30	29	83499	2	34	16	94493	1.1
H2 (bs)	0.85	0.74	0.68	0.93	0.90	0.83	0.63	0.85

Heritability of traits

High heritability was observed in both the trials (2016 and 2017) for Fe (85%, 93%) and Zn (74%, 83%) densities and also BL (93%, 85%) (Table 4). Previous studies on pearl millet found that broad sense heritability (h^2bs) varied from 65 to 86% for Fe density and 65 to 84% for Zn density in S1 genotypes of open-pollinated varieties (Gupta *et al.,* 2009; Kanatti *et al.,* 2015) and narrow sense heritability (h^2ns) varied from 45 to 80% for Fe and 45 to 86% for Zn (Velu, 2006; Govindaraj *et al.,* 2016b; Kanatti *et al.*, 2016b). Seasons had significant impact on heritability estimates for grain Fe and Zn: high h^2bs for Fe (81%) and Zn (70%) in rainy season but moderate in summer (Fe 52% and Zn 44%) season crops (Velu, 2006), whereas variances due to interaction of additive gene effects with the environment (A×E) were much smaller than those arising from interaction of dominance effects with the environment (D×E) (Kanatti *et al.*, 2016b). While the grain yield had lower magnitude of heritability compared to micronutrients and BL, it was 68% in trial-2016 and 63% trial-2017, respectively. Earlier studies in pearl millet reported high heritability (Govindaraj *et al.,* 2010; Sumathi *et al.,* 2010) as well as low heritability (Subi and Idris, 2013) for grain yield.

Correlation among traits

Highly significant and positive correlation was observed between Fe and Zn in 2016 ($r=0.65$, $P<0.01$) and 2017 ($r=0.90$, $P<0.01$) trials (Fig. 1). Earlier pearl millet studies also reported the same (Velu *et al.,* 2008a, b; Gupta *et al.,* 2009; Rai *et al.,* 2012; Govindaraj *et al.,* 2012; Govindaraj *et al.,* 2013; Kanatti *et al.*, 2016b). Similarly, positive correlation between Fe and Zn was also observed in sorghum (Ashok Kumar *et al.,* 2010, 2013), maize (Oikeh *et al.,* 2003, 2004b), rice (Stangoulis *et al.,* 2007; Anandan *et al.,* 2011), wheat (Garvin *et al.,* 2006; Peleg *et al.*, 2009; Zhang *et al.,* 2010) and finger millet (Upadhyaya *et al.,* 2011). Genomic studies in wheat (Peleg *et al.,* 2009), rice (Stangoulis *et al.,* 2007), common bean (Blair *et al.,* 2009; Cichy *et al.,* 2009) and pearl millet (Kumar *et al.,* 2016) have identified common and overlapping Quantitative Trait Loci (QTL) for Fe and Zn densities. The existence of highly significant positive association and predominance of additive genetic control (Velu *et al.,* 2011; Govindaraj *et al.,* 2013; Kanatti *et al*., 2014a) for Fe and Zn densities would be helpful for simultaneous genetic improvement of both the traits. Both Fe and Zn showed significant positive correlation with BL (0.62, $P<0.01$; 0.64 $P<0.01$, respectively) in trial-2016 and both traits had non-significant correlation with BL in trial-2017 (Fig. 1). Previous studies in pearl millet reported significant negative (Velu *et al.,* 2008a), significant positive (Gupta *et al.*, 2009) and non-significant (Kanatti *et al.,* 2014b) correlation of Fe with BL and non-significant correlation between Zn and BL (Velu *et al.*, 2008a; Gupta *et al.,* 2009; Kanatti *et al.*, 2014). This implies that the relationship of Fe and Zn with BL varies with genetic material used in the studies.

Grain yield did not show significant correlation with Fe in both 2016 and 2017 trials (Fig. 1), whereas with Zn it had significant negative correlation ($r=-0.49$, $P<0.05$) only in trial 2017. Earlier studies in pearl millet (Rai *et al.,* 2012; Gupta *et al.,* 2009; Kanatti *et al.,* 2014 a, b) reported significant negative to non-significant correlation with grain yield and the direction and magnitude of correlation varied with type of genetic material and environment. Such associations might have resulted due to the involvement of *iniadi* germplasm as a common source of high Fe and Zn content in both male and female parents, thereby reducing the genetic diversity between the parental lines for traits associated with heterosis for grain yield. This relationship could also be due to natural negative association between genetic factors for these micronutrients and grain yield (Kanatti *et al.,* 2014b), the resolution of this issue merits further studies through selection experiments.

Per se performance of hybrids

In trial-2016, top five hybrids (H3, H8, H10, H13, H14) for *per se* grain yield had 19-45% higher grain yield than control (RHB 177) (Fig. 2). These hybrids had >40% higher Fe content than control and days to 50% flowering ranged from 50-56 days. In trial-2017, top five hybrids (H1, H3, H5, H10, H11) for grain yield *per se* had 3-12% higher yield than that of control (RHB 177) with >40% Fe density and 49-51 days to 50% flowering. These hybrids can be further included in advanced hybrid trials along with promising hybrids to reconfirm location specific performance.

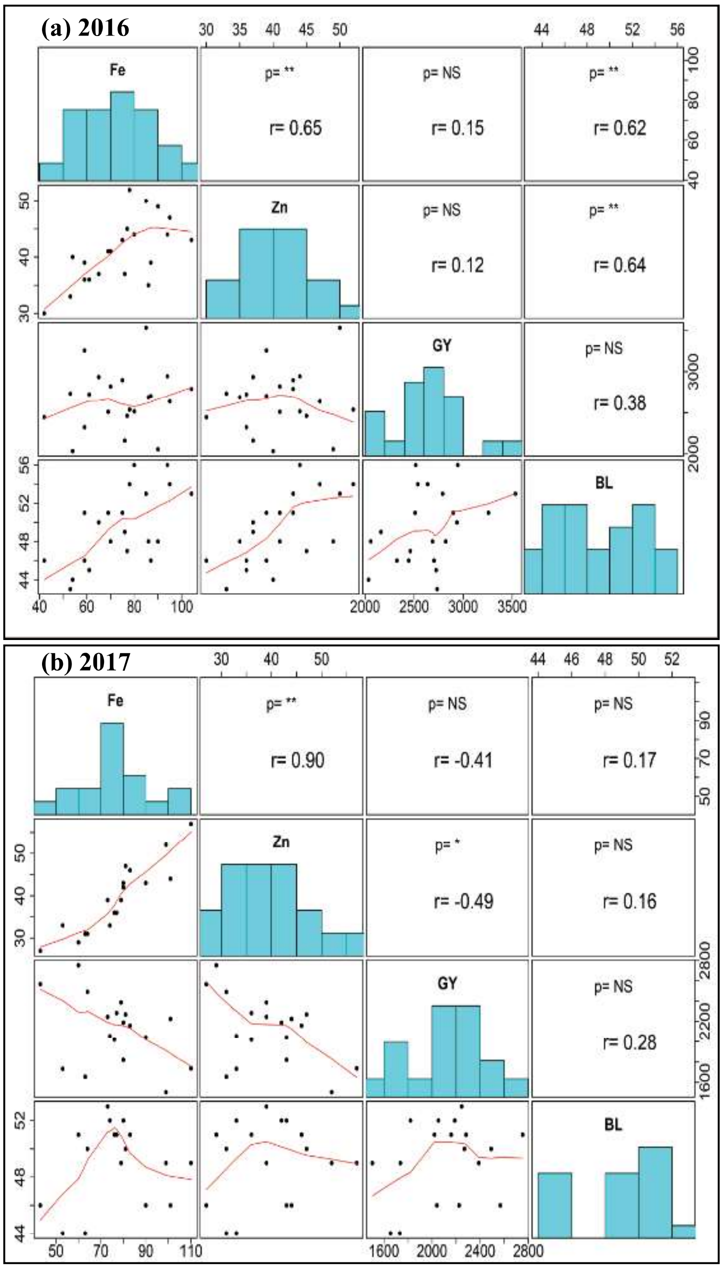

Figure 1. *Correlation among traits in 2016 and 2017 multi-location trials.*

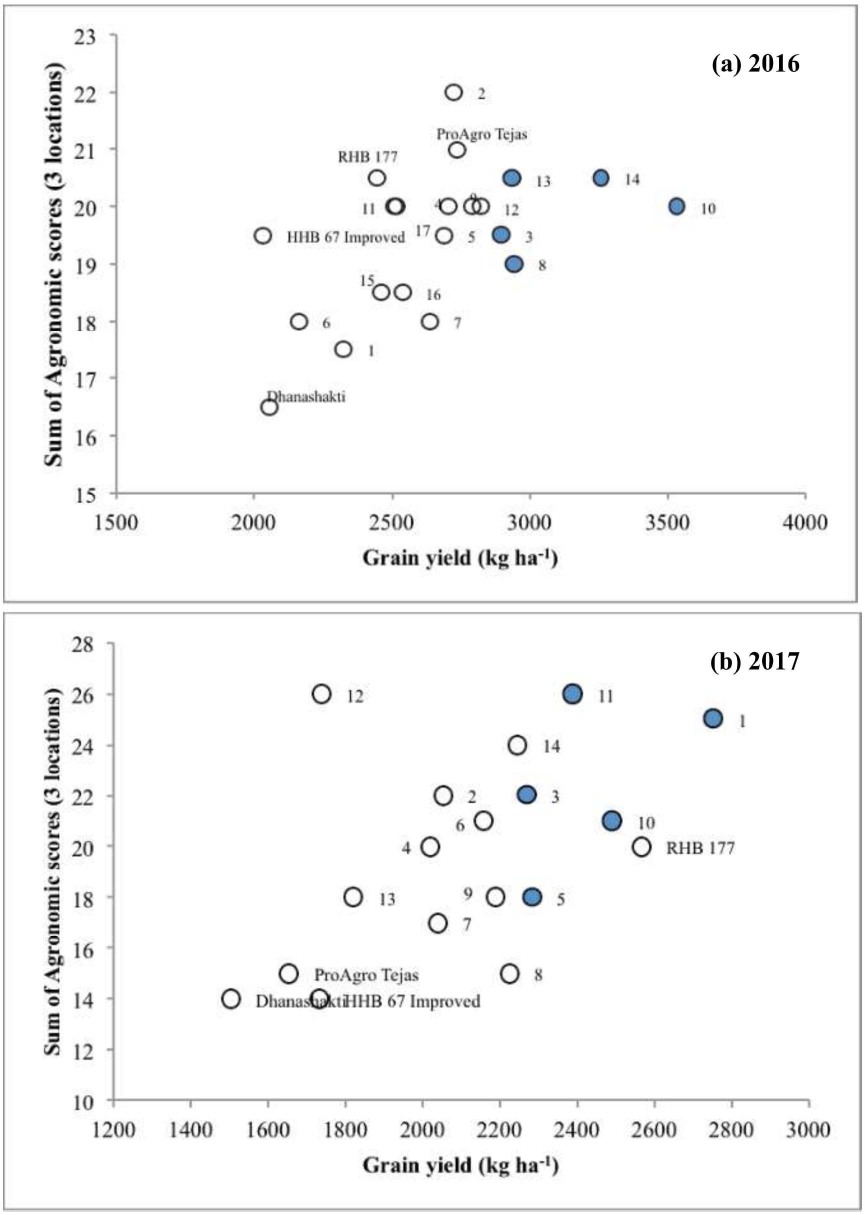

Figure 2. *Scatter plot for grain yield and agronomic score in 2016 and 2017 multi-location trials (solid circles highest top five high-yielding entries).*

Acknowledgement

This research was supported by funding from HarvestPlus Challenge Program of the CGIAR. It was carried as part of the CRP on GLDC and A4NH.

References

Anandan, A., G. Rajiv, R. Eswaran and M. Prakash. 2011. Genotypic variation and relationships between quality traits and trace elements in traditional and improved rice (*Oryza sativa* L.) genotypes. *Journal of Food Science* 76(4): H122-H130.

Ashok Kumar, A., B.V.S. Reddy, B. Ramaiah, K.L. Sahrawat and W.H. Pfeiffer. 2013. Gene effects and heterosis for grain iron and zinc concentration in sorghum [*Sorghum bicolor* (L.) Moench]. *Field Crops Research* 146: 86-95.

Ashok Kumar, A., B.V.S. Reddy, K.L. Sahrawat and B. Ramaiah. 2010. Combating micronutrient malnutrition: Identification of commercial sorghum cultivars with high grain iron and zinc. *Journal of SAT Agricultural Research* 8: 1-5.

Blair, M.W., C. Astudillo, M. Grusak, R. Graham and S. Beebe. 2009. Inheritance of grain iron and zinc content in common bean (*Phaseolus vulgaris* L.). *Molecular Breeding* 23: 197-207.

Cichy, K.A., S. Forster, K.F. Grafton and G.L. HosWeld. 2005. Inheritance of grain zinc accumulation in navy bean. *Crop Science* 45: 864-870.

Gomez, K.A. and A.A. Gomez. 1984. Statistical procedures for agricultural research. 2nd ed. John Wiley & Sons, New York.

Govindaraj, M., K.N. Rai, P. Shanmugasundaram, S.L. Dwivedi, K.L. Sahrawat, A.R. Muthaiah and A.S. Rao. 2013. Combining ability and heterosis for grain iron and zinc densities in pearl millet. *Crop Science* 53: 507-517.

Govindaraj, M., K.N. Rai and P. Shanmugasundaram. 2016b. Intra-population genetic variance for grain iron and zinc contents and agronomic traits in pearl millet. The *Crop Journal* 04(01): 48-54.

Govindaraj, M., K.N. Rai, P. Shanmugasundaram and A.S. Rao. 2012. Efficiency of single plant selection for grain iron and zinc density in pearl millet. *The European Journal of Plant Science and Biotechnology* 6(2): 114-117.

Govindaraj, M., K.N. Rai, W.H. Pfeiffer, A. Kanatti and H. Shivade. 2016a. Energy-dispersive X-ray fluorescence spectrometry for cost-effective and rapid screening of pearl millet germplasm and breeding lines for grain iron and zinc density. *Communications in Soil Science and Plant Analysis* 47(18): 2126-2134.

Govindaraj, M., P. Shanmugasundaram, and A.R. Muthiah. 2010. Estimates of genetic parameters for yield and yield attributes in elite lines and popular cultivars of India's pearl millet. *African Journal of Agricultural Research* 5(22): 3060-3064.

Gupta, S.K., G. Velu, K.N. Rai and K. Sumalini. 2009. Association of grain iron and zinc content with grain yield and other traits in pearl millet (*Pennisetum glaucum* (L.) R.Br.). *Crop Improvement* 36(2): 4-7.

Kanatti, A., K.N. Rai, K. Radhika and M. Govindaraj. 2016a. Tester effect on combining ability and its relationship with line performance *per se* for grain iron and zinc densities in pearl millet. *Crop Science* 56: 689-696.

Kanatti A., K.N. Rai, K. Radhika, M. Govindaraj and A.S. Rao. 2016b. Genetic architecture of open-pollinated varieties of pearl millet for grain iron and zinc densities. *Indian Journal of Genetics and Plant Breeding* 76(3): 299-303.

Kanatti, A., K.N. Rai, K. Radhika, M. Govindaraj, K.L. Sahrawat and A.S. Rao. 2014a. Grain iron and zinc density in pearl millet: combining ability, heterosis and association with grain yield and grain size. *SprigerPlus* 3: 763.

Kanatti, A., K.N. Rai, K. Radhika, M. Govindaraj, K.L. Sahrawat, K. Srinivasu and H. Shivade. 2014b. Relationship of grain iron and zinc content with grain yield in pearl millet hybrids. *Crop Improvement* 41(1): 91-96.

Kanatti, A., K. Radhika, V. Padma, P. Janila, M. Govindaraj and K.N. Rai. 2015. Intra-population variability for grain iron and zinc densities in commercial open-pollinated varieties of pearl millet. *International Journal of Economic Plants* 2(4): 171-174.

Kumar, S., C.T. Hash, N. Thirunavukkarasu, G. Singh, V. Rajaram, A. Rathore, S. Senapathy, M.D. Mahendrakar, R.S. Yadav and R.K. Srivastava. 2016. Mapping quantitative trait loci controlling high iron and zinc content in self and open pollinated grains of pearl millet [*Pennisetumglaucum* (L.) R. Br.]. *Frontiers in Plant Science* 7(1636): 01-16.

National Family Health Survey (NFHS). 2016. NFHS-4-fact sheets for key indicators. International Institute for Population Sciences, Mumbai, India.

Oikeh, S.O., A. Menkir, B. Maziya-Dixon, R. Welch and R.P. Glahn. 2003. Genotypic differences in concentration and bioavailability of kernel-iron in tropical maize varieties grown under field conditions. *Journal of Plant Nutrition* 26(10&11): 2307-2319.

Oikeh, S.O., A. Menkir, B. Maziya-Dixon, R. Welch and R.P. Glahn. 2004b. Assessment of iron bioavailability from twenty elite late-maturing tropical maize varieties using an in vitro digestion/Caco-2 cell model. *Journal of the Science of Food and Agriculture* 84: 1202-1206.

Oikeh, S.O., A. Menkir, B. Maziya-Dixon, R. Welch, R.P. Glahn and G. Jr. Gauch. 2004a. Environmental stability of iron and zinc concentrations in grain of elite early-maturing tropical maize genotypes grown under field conditions. *Journal of Agricultural Science* 142: 543-551.

Paltridge, N.G., L.J. Palmer, P.J. Milham, G.E. Guild and J.C.R. Stangoulis. 2012. Energy-dispersive X-ray fluorescence analysis of zinc and iron concentration in rice and pearl millet grain. *Plant and Soil* 361: 251-260.

Peleg, Z., I. Cakmak, L. Ozturk, A. Yazici, Y. Jun, H. Budak, A.B. Korol, T. Fahima and Y. Saranga. 2009. Quantitative trait loci conferring grain mineral nutrient concentrations in durum wheat × wild emmer wheat RIL population. *Theoretical and Applied Genetics* 119: 353-369.

Parthasarathy Rao, P., P.S. Birthal, B.V.S. Reddy, K.N. Rai, and S. Ramesh. 2006. Diagnostics of sorghum and pearl millet grains-based nutrition in India. *Inernational Sorghum and Millets Newsletter* 46: 93-96.

Prasanna, B.M., S. Mazumdar, M. Chakraborti, F. Hossain, K.M. Manjaiah, P.K. Agrawal, S.K. Guleria and H.S. Gupta. 2011. Genetic variability and genotype × environment interactions for kernel iron and zinc concentrations in maize (*Zea mays*) genotypes. *Indian Journal of Agriculture Sciences* 81(8): 704-711.

Rai, K.N., M. Govindaraj and A.S. Rao. 2012. Genetic enhancement of grain iron and zinc content in pearl millet. *Quality Assurance and Safety of Crops & Foods* 4: 119-125.

SAS Institute Inc. 2015.SAS/STAT® 14.1 User's Guide. Cary, NC: SAS Institute Inc.

Snedecor, G.W. and W.G. Cochran. 1989. Statistical Methods, Eighth Edition, Iowa State University Press.

Stangoulis, J.C.R., B.L. Huynh, R.M. Welch, E.Y. Choi and R.D. Graham. 2007. Quantitative trait loci for phytate in rice grain and their relationship with grain micronutrient content. *Euphytica* 154: 289-294.

Subi, M.I.M. and A.E. Idris. 2013. Genetic variability, heritability and genetic advance in pearl millet (*Penisetum glaucum* [L.] R. Br.) genotypes. *British Biotechnology Journal* 3(1): 54-65.

Sumathi, P., S. Madineni and P. Veerabadhiran. 2010. Genetic variability for different biometrical traits in pearl millet genotypes (*Pennisetum glaucum* LR BR.). *Electronic Journal of Plant Breeding* 1(4): 437-440.

Upadhyaya, H.D., S. Ramesh, S. Shivali, S.K. Singh, S.K. Varshney, N.D.R.K. Sarma, C.R. Ravishankar, Y. Narasimhudu, V.G. Reddy, K.L. Sahrawat, T.N. Dhanalakshmi, M.A. Mgonja, H.K. Parzies, C.L.L. Gowda and S. Singh. 2011. Genetic diversity for grain nutrients contents in a core collection of finger millet (*Eleusine coracana* (L.) Gaertn.) germplasm. *Field Crops Research* 121: 42-52.

Velu, G. 2006. Genetic variability, stability and inheritance of grain iron and zinc content in pearl millet (*Pennisetum glaucum* (L.) R.Br.). Ph.D., Thesis, Tamil Nadu Agricultural University, Coimbatore, India.

Velu, G., K.N. Rai and K.L. Sahrawat. 2008a. Variability for grain iron and zinc content in a diverse range of pearl millet populations. *Crop Improvement* 35(2): 186-191.

Velu, G., K.N. Rai., K.L. Sahrawat and K. Sumalini. 2008b. Variability for grain iron and zinc contents in pearl millet hybrids. *Journal of SAT Agricultural Research* 6.

Velu, G., K.N. Rai, V. Muralidharan, T. Longvah and J. Crossa. 2011. Gene effects and heterosis for grain iron and zinc density in pearl millet (Pennisetumglaucum (L.) R. Br). *Euphytica* 180: 251-259.

Yadav, O.P, K.N. Rai, B.S. Rajpurohit, C.T. Hash, R.S. Mahala, S.K. Gupta, H.S. Shetty, H.R. Bishnoi, M.S. Rathore, A. Kumar, S. Sehgal and K.L. Raghvani. 2012. Twenty-five Years of Pearl Millet Improvement in India. All India Coordinated Pearl Millet Improvement Project, Jodhpur, India

Zhang, Y., Q. Song, J. Yan, J. Tang, R. Zhao, Y. Zhang, Z. He, C. Zou and I. Ortiz-Monasterio. 2010. Mineral element concentrations in grains of Chinese wheat cultivars. *Euphytica* 174: 303-313.