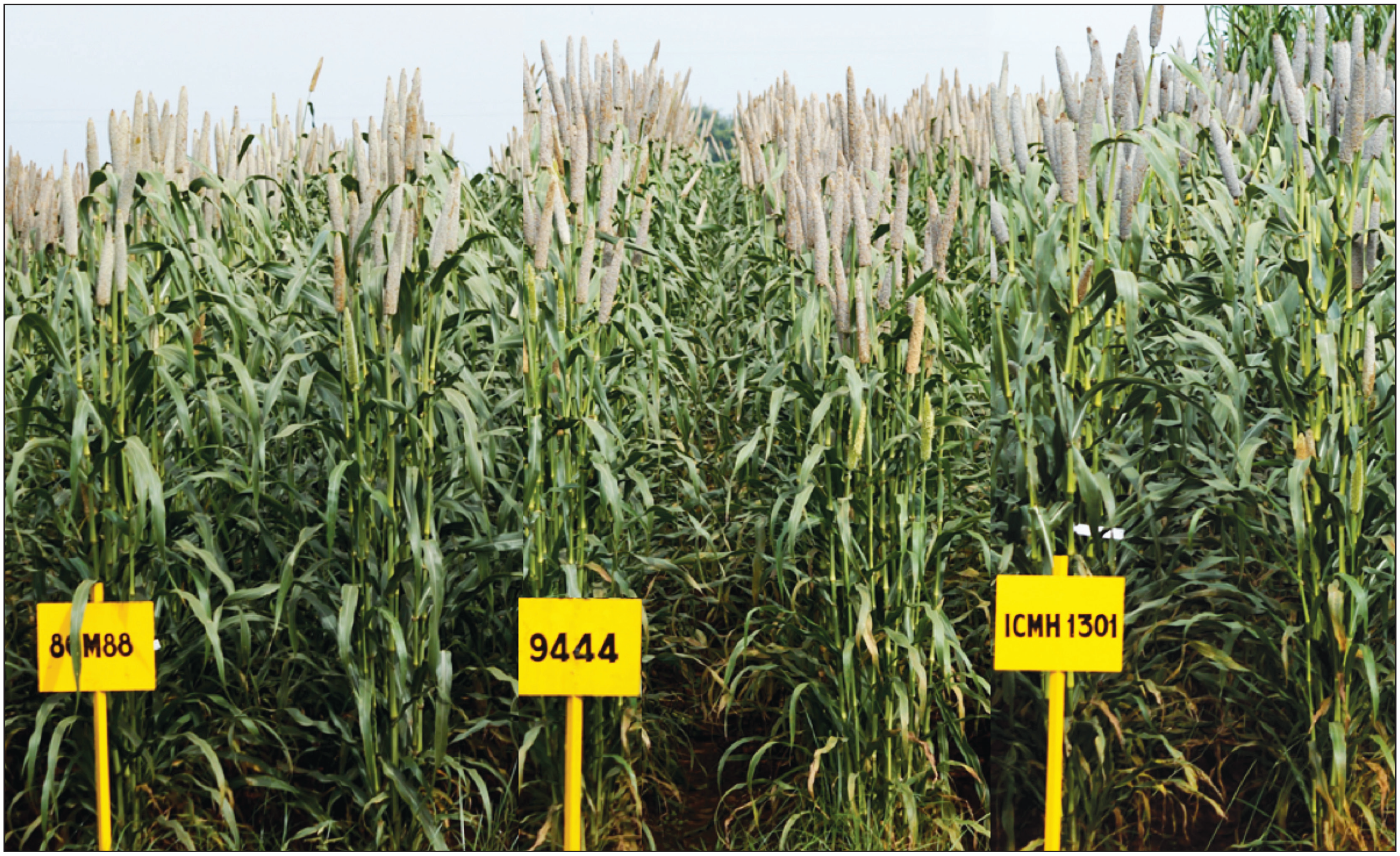
Does Soil Micronutrient Variability in Test Locations Influence Performance of Biofortified Pearl Millet in India?

By M. Govindaraj, A. Kanatti, K.N. Rai, and T. Satyanarayana



Field performance of biofortified pearl millet hybrid (ICMH 1301) compared to commercial hybrids (86M86 and Pro Agro 9444) in precision field at ICRISAT.

Dietary deficiency of essential micronutrients such as Fe and Zn has widely been reported to be a food-related global health problem, affecting more than 2 billion people across the world (WHO, 2012). This problem is particularly serious in the under developed and developing countries of Africa and Asia, where large segments of malnourished people depend on cereal and legume-based diet that comes from marginal and low fertility lands (Sandstead, 1991; Gibson, 1994). As a result, the cereal-based diets produced from such lands are generally low in micronutrients and proteins. Farmers in the past cultivated pearl millet varieties, which are potential sources of micronutrients and vitamins. However, in the last few decades, the varieties of pearl millet are replaced with high-yielding hybrids and are cultivated with inadequate use of NPK fertilizers, neglecting the application of micronutrients. This created an imbalance in the nutrition ecosystem and led to multi-micronutrient deficiencies in soil, which further resulted in production of less nutritious food for humans (Kumar et al., 2016).

In India, pearl millet is grown in an area of 8 million (M) ha with an annual production of 8 M t. The growing environment of pearl millet is challenged with low fertility soils, erratic rainfall and adverse climate, not conducive to

SUMMARY

Testing of biofortified hybrids across varying pearl millet-growing regions of India indicated the need for maintaining sufficient Fe and Zn levels in soil to express the crops's full genetic potential and ensure successful loading of micronutrients in the grain. The study suggested the need for practicing balanced fertilization while growing biofortified hybrids to increase grain yield and micronutrient accumulation in grains.

KEYWORDS:

biofortification; human health; enriched fertilizers

ABBREVIATIONS AND NOTES:

P = phosphorus; K = potassium; S = sulfur; B = boron; Fe = iron; Zn = zinc; OC = organic carbon

Table 1. Variability of different soil properties in pearl millet testing locations in India.

	pH		EC, dS/m		OC, %		Avail-P, mg/kg		Exch-K, mg/kg		Avail-B, mg/kg		Avail-S, mg/kg		Avail-Fe, mg/kg		Avail-Zn, mg/kg	
Location	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Durgapura	7.68	-	0.08	0.27	0.21	0.23	16.56	15.00	71	85	0.38	0.49	2.45	7.99	4.07	4.04	1.86	2.37
Hisar	7.82	7.83	0.20	0.14	0.35	0.32	8.08	9.13	115	145	0.86	0.79	9.62	9.95	7.69	6.25	4.00	2.02
Jamnagar	7.76		1.14	-	0.46	-	7.15	-	91	-	2.51	-	131.54	-	5.49	-	1.22	-
Ahmedabad	7.94		0.16	-	0.31	-	10.58	-	76	-	0.86	-	6.57	-	4.42	-	0.76	-
Nagour	8.26	-	1.84	-	0.30	-	10.96	-	105	-	3.32	-	88.40	-	2.90	-	0.71	-
Hisar	-	7.72	-	1.05	-	0.59	-	17.09	-	240	-	1.13	-	277.51	-	2.29	-	4.71
Akola	-	7.11	-	0.70	-	0.40	-	13.33	-	188	-	0.52	-	6.16	-	16.45	-	2.04
Aurangabad	8.68	8.28	0.27	0.40	0.36	0.57	4.04	8.16	211	488	1.45	1.77	6.33	15.85	1.60	7.11	0.92	1.02
Aurangabad	8.20		0.24	0.17	0.43	0.40	10.69	8.00	133	323	0.56	0.52	8.51	5.83	4.11	7.99	0.32	0.63
Aurangabad	7.97	8.23	0.20	0.47	0.52	0.48	8.87	13.77	254	398	1.46	1.89	8.83	49.54	12.00	4.35	0.55	0.51
Medchal	-	7.60	-	0.18	-	0.56	-	46.88	-	236	-	0.94	-	8.41	-	8.51	-	2.31
Aurangabad	-	8.36	-	0.22	-	0.31	-	6.38	-	318	-	1.05	-	5.39	-	4.20	-	0.61
Patancheru	7.84	7.40	0.17	0.26	0.31	0.32	10.60	14.02	121	158	0.65	0.81	5.62	27.72	12.47	5.22	1.20	1.25
SE	0.10	0.16	0.20	0.10	0.03	0.04	1.14	4.13	20.71	43.86	0.33	0.17	15.59	29.73	1.29	1.40	0.37	0.45
Min	7.68	7.11	0.08	0.14	0.21	0.23	4.04	6.38	70.89	85.13	0.38	0.49	2.45	5.39	1.60	2.29	0.32	0.51
Max	8.68	8.36	1.84	1.05	0.52	0.59	16.56	46.88	254.26	487.67	3.32	1.89	131.54	277.51	12.47	16.45	4.00	4.71

support sustainable production. About 50% soils in India are deficient in available Zn and 12% in available Fe, with largest deficiency reported in the millet-growing states of Haryana (26%) and Uttar Pradesh (10%) (Singh, 2009). Thus, Fe deficiency in soil is only second in importance after Zn (Singh, 2009; Nayyar et al., 2001), while in human nutrition, Fe is considered predominantly deficient (NFHS, 2016). This highlights the need to enrich our native food crops, especially pearl millet, with Fe and Zn for sustaining plant and human nutrition.

Increasing grain Fe and Zn concentration in pearl millet through biofortification could significantly increase the dietary intake of Fe and Zn in the areas where millets are primarily grown for human consumption. Great possibilities are open upfront for biofortification by exploring the effectiveness of micronutrient fertilization to improve crop productivity and nutritional quality, while enriching human nutrition. Therefore, the current study was established with an objective to determine the extent of variability of available soil micronutrients and determine its potential influence on grain micronutrient accumulation in the biofortified hybrids of pearl millet.

The study was initiated at 19 pearl millet-growing locations spread over five states, representing two different growing environments with respect to rainfall and temperature in 2014 and 2015. About 20 biofortified hybrids were evaluated for grain micronutrient accumulation in varying soil types represented by Vertisols (43%), Inceptisols (42%), Alfisols (10%), and Ultisols (5%). Soil samples were collected before planting from each location and analyzed for available nutrients. Each hybrid was planted in four rows of 4 m long with 3 replications at all the locations. Diammonium phosphate (100 kg/ha) was applied at field preparation and urea (100 kg/ha) was applied as side-dressing after thinning. Recommended agronomic practices were followed to ensure a good crop stand. Grain samples were collected from all the locations and analyzed for grain Fe and Zn concentration using Energy Dispersive X-ray Fluorescence (ED-XRF), a non-destructive quantitative method (Paltridge et al., 2012).

Performance of biofortified hybrids was assessed based on the grain yield, as well as grain Fe and Zn concentrations in comparison to commercial non-biofortified hybrids.

Results

The soils at the experimental sites were neutral to alkaline (pH 7.1 to 8.7) and non-saline to saline (EC 0.08 to 1.84) in nature (**Table 1**). Organic C was low (< 0.5%) in all locations except three sites (Aurangabad, Medchal, and Hisar), which reported medium organic C (0.5 to 0.75%). Available P and K were medium to high at the majority of the locations. Available B ranged from 0.4 to 3.3 mg/kg, while available S varied from 2.5 to 278 mg/kg. Available Fe and Zn in the experimental fields varied largely across locations. Available Fe varied from 1.6 mg/kg in Aurangabad to 12.5 mg/kg in Patancheru during 2014, while in 2015, it varied from 2.3 mg/kg in Hisar to 16.5 mg/kg in Akola, respectively. Aurangabad, Nagour, and Hisar reported available Fe less than 3.7 mg/kg, indicating deficiency of Fe in the soil at these locations (**Table 1**). Available Zn varied from 0.32 mg/kg in Aurangabad to 4.0 mg/kg in Hisar during 2014, while in 2015, it varied from 0.5 mg/kg in Aurangabad to

Table 2. Performance of biofortified hybrids at different testing locations in India.

	2014 trial data from 9 locations					
	Grain Fe, mg/kg		Grain Zn, mg/kg		Grain yield, t/ha	
Hybrid	Range	Average	Range	Average	Range	Average
1	66-104	86	27-56	41	1.68-3.81	2.84
2	61-107	84	24-57	38	1.94-4.17	2.85
3	66-116	91	26-54	39	1.85-4.05	3.04
4	53-101	80	19-69	39	1.98-4.28	3.25
5	45-90	68	21-53	37	1.73-4.05	3.19
6	60-93	70	23-43	32	1.95-4.01	2.86
7	61-100	78	24-59	40	2.07-4.19	2.87
8	72-110	89	28-74	46	1.73-4.53	3.26
9	43-80	66	17-48	34	1.58-4.34	3.01
10	61-104	80	20-59	38	1.96-4.11	3.03
11	63-87	73	27-50	39	1.70-3.88	2.83
12	57-85	68	26-46	37	1.77-3.46	2.80
13	60-97	77	24-48	38	2.18-4.07	3.16
14	56-92	70	14-51	35	1.70-4.91	3.15
15	56-88	72	21-49	36	1.43-4.56	3.25
16	57-89	72	22-51	36	2.08-4.45	3.18
17	59-105	82	26-60	41	1.90-4.29	3.07
18	57-93	74	24-51	35	1.89-4.32	3.26
19	63-109	80	23-53	36	1.97-4.81	3.28
20	70-105	84	24-56	38	2.28-4.00	2.77
21	47-78	61	19-43	34	2.38-5.36	3.54
22	67-95	83	26-55	39	2.22-4.28	3.09
23	41-76	56	23-45	36	1.50-4.56	2.82
24	35-60	48	19-35	29	1.53-5.08	3.00
25	39-63	54	19-44	33	0.68-2.46	1.70
LSD		3.8		2.6		0.16
Min		48		29		1.70
Max		91		46		3.54

	2015 trial data from 8 locations					
	Grain Fe, mg/kg		Grain Zn, mg/kg		Grain yield, t/ha	
Hybrid	Range	Average	Range	Average	Range	Average
1	93-108	101	37-76	51	2.04-4.58	3.26
2	67-84	75	31-58	42	1.56-4.66	3.42
3	64-92	76	30-68	44	1.67-4.61	3.50
4	70-109	84	28-49	37	1.28-4.35	3.53
5	73-122	87	32-61	45	1.59-4.16	3.28
6	77-134	99	34-71	46	1.78-4.3	3.37
7	85-123	100	36-81	51	2.09-4.41	3.26
8	77-112	91	36-68	46	1.79-4.17	2.99
9	68-111	86	35-60	44	1.81-4.61	3.35
10	59-83	74	29-52	39	1.65-4.09	3.32
11	71-98	86	35-54	45	1.63-4.28	3.17
12	79-106	92	34-60	43	1.78-3.67	3.05
13	75-96	84	35-54	45	1.78-4.02	3.13
14	65-108	86	32-60	41	1.25-4.27	3.23
15	85-131	101	37-68	48	1.71-4.22	3.14
16	71-109	92	38-69	46	2.25-4.51	3.36
17	42-61	48	26-43	33	1.6-4.98	3.84
18	60-83	68	32-54	41	2.11-5.07	3.82
19	81-113	92	34-65	44	2.27-4.45	3.56
20	48-78	60	29-58	41	1.29-4.25	2.98
21	49-69	60	27-55	38	1.65-5.02	3.94
22	91-119	102	36-72	52	1.24-4.02	2.48
LSD		4.4		2.9		0.21
Min		48		33		2.48
Max		102		52		3.94

4.7 mg/kg in Hisar. All locations had sufficient Zn except two locations in Aurangabad, which exhibited Zn deficiency. Results of soil analysis in biofortification program helps to ensure ample levels of micronutrients at the test sites, so that the soils are not challenged with micronutrient deficiency while planting higher micronutrient-dense hybrids.

Yield and Grain Concentration of Fe and Zn in Pearl Millet

The grain yield of pearl millet in the biofortified hybrids varied from 0.7 to 5.4 t/ha, with an average of 3.0 t/ha in 2014, whereas, during 2015, the yield varied from 1.2 to 5.1 t/ha, with an average of 3.3 t/ha, indicating that the productivity of pearl millet varied across the growing environments similar to that of variability recorded in the available soil micronutrient status (**Table 2**). The grain Fe concentration varied from 35 to 116 mg/kg, with an average of 74 mg/kg in 2014, while in 2015, the corresponding grain Fe ranged from 42 to 134 mg/kg, with an average of 84 mg/kg, respectively. The grain Zn concentration varied from 14 to 69 mg/kg with an average of 37 mg/kg in 2014, while in 2015, it varied from 26 to 81 mg/kg, with an average of 44 mg/kg, respectively. The difference among the biofortified pearl millet hybrids for grain yield and accumulation of Fe and Zn in grain was significant in both the years (**Table 2**), indicating the need for evaluating biofortified hybrids for potential accumulation of Fe and Zn in the grain. The hybrids evaluated in 2015 had higher mean Fe (14%) and Zn (18%) content in the grain, and 11% higher mean grain yield over the entries tested in 2014, which could be due to higher availability of macro and micronutrients in test locations selected during 2015 (**Table 1**). In 2015, available P, K, S, Fe, and Zn levels of the test locations were 56, 97, 39, 9, and 36% higher than the locations that were selected

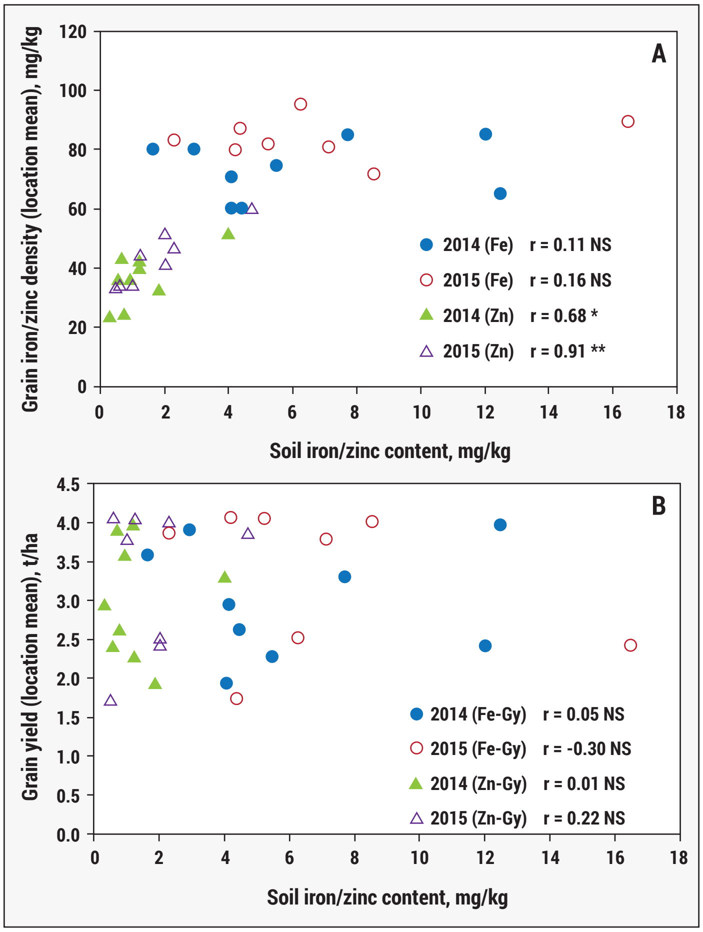


Figure 1. Relationship of soil micronutrient status with grain micronutrient content (A) and grain yield (B) of biofortified pearl millet hybrids.

in 2014, indicating the need for supplying adequate levels of macro and micronutrients in the soil for ensuring higher accumulation of essential micronutrients in the grain.

Relationship of Yield and Grain Micronutrient Concentration with Available Soil Micronutrients

The mean grain Fe content in the test entries was not significantly correlated with available Fe in both the years, whereas such relationship was significant between the grain and soil available Zn in both the years (**Figure 1**). Available Fe was high at 16 out of 19 locations, but its relationship with grain Fe was non-significant in both years. On the other hand, available Zn established a positive and significant relationship with grain Zn, in spite of being deficient in 12 out of 19 locations. The results even though failed to establish a positive linkage between the available soil and grain Fe content, however, indicated a significant relationship with the available Zn and grain Zn concentration, highlighting the need for ensuring adequate soil Zn levels for improving the Zn concentration in biofortified pearl millet hybrids. The mean grain yield also had no significant correlation with soil available Fe and Zn in both the years (**Figure 1**). This merit further strategic study in Fe/Zn deficient soils, which could give a better understanding of this relationship.

This will support in a way that the seed mineral-dense cultivars are reported to grow well and produce more grains when grown under micronutrient deficient soils (Ruel and Bouis, 1998; Graham et al., 2001).

Although it is well established that plants absorb micronutrients from soil, optimum crop growth and yield depends not only on the available micronutrient status but also on the growing environment of the study locations. Soil factors, such as pH, moisture, organic matter and temperature, governs the micronutrient availability to any crop or its variety to express its full genetic potential in any given environment. Thus, the growing environment, especially soil and climate, seems to have strong influence on grain mineral content, and makes it imperative to test the stability of grain Fe and Zn content in the biofortified varieties over different geographical regions to make the most valid comparisons of the genetically controlled variation.

While the relationship between the available Fe with that of grain Fe density and grain yield was non-significant (**Figure 1**), the correlation between grain yield and grain Fe was also non-significant (**Figure 2**). On the other hand, available Zn showed significant correlation with grain Zn, whereas, the grain yield was non-significantly correlated with available Zn (**Figure 1**) and grain Zn (**Figure 2**), respectively.

The present study failed to explain the relationship of grain micronutrient concentration in the biofortified hybrids with soil micronutrient status at different pearl millet-growing locations in India. This gives an indication that the potential of biofortification could be explored not just by mere planting of biofortified entries across different locations but through ensuring adequate plant nutrition. The observations further gains support from the visual differences, which showed improved growth performance of biofortified hybrids over commercial hybrids recorded in the precision fields with high soil fertility (**See comparison in photo above**). Dwivedi et al. (2009) reported substantial yield responses to application of micronutrients in pearl millet and suggested that balanced fertilizer use in pearl millet no longer meant the application of NP or NPK alone, but should include all nutrients that are deficient at a particular site. Pearl millet hybrids, being exhaustive in nature, demand application of right rates of macro, secondary and micronutrients to optimize yields and profits. However, the nutrient management practice followed at each study location considered application of only N and P fertilizer, neglecting the application of deficient nutrients. Thus, the testing of biofortified entries need to be integrated with proper agronomic management with focus on balanced nutrient management.

The results of the present study suggested that significant micronutrient enhancement in pearl millet can be achieved through biofortification breeding. However, ensuring sustainable pearl millet yields and nutrient accumulation of grains through biofortification merits future research investigations on agronomic management, with special emphasis on balanced nutrient management in the study locations. Success of agronomic biofortification largely depends on the bioavailability of micronutrients in the soil-plant-human health continuum. This can be achieved through application of micronutrient-enriched fertilizers along with NPK through integrated soil fertility management. Genetic biofortification may thus be more cost-effective in the long run with the complementarity of agronomic and soil fertility management practices. **BCSA**

Dr. Govindaraj is IPNI Scholar award recipient and currently Senior Scientist and Crop Leader-Millet Biofortification (e-mail: m.govindaraj@cgiar.org), Dr. Kanatti is visiting scientist, Dr. Rai is former Principal Scientist and India Director of Biofortification, with International Crop Research Institute for Semi-Arid Tropics (ICRSI-AT) and Dr. Satyanarayana is Director, IPNI South Asia Program.

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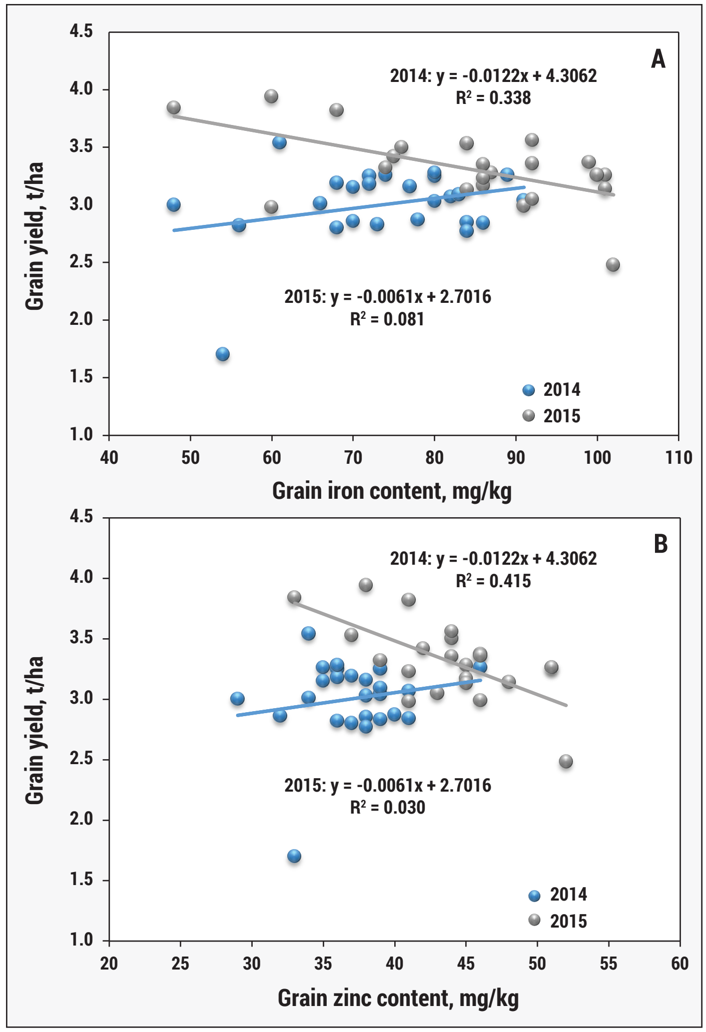

Figure 2. Relationship between grain yield and grain iron content (A) and grain zinc content (B) across locations in 2014 and 2015.