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Effects of soil fertility and cropping patterns on soil minerals (Fe/Zn) partitioning into bean seeds and their distribution within plant canopy

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In Rwanda, Common beans are grown under quite diverse conditions including soil fertility, rain fall, and cropping system. The objective of this study was to assess partitioning of soil minerals (Fe/Zn) into seeds and distribution of minerals within plant, effects of foliar Fe [Ferrous sulphate (2%)] application on seed iron and zinc content. Mineral concentrations in seeds were assessed at harvest using X-Ray Fluorescence Spectrometer (XRF). Genotypes were significantly different in their iron and zinc content with the means ranging between 51 – 126 ppm for iron and 28-45 ppm for zinc. Foliar iron application showed significant effect on some of the varieties in Rubona 2012A and Akanyirandoli 2012B and no significant effects on other varieties (bush and climbing) grown in Rubona and Akanyirandoli 2012A. Plant height has no significant effect on seed iron and zinc accumulation. Genetic and environmental interactions for bean seed concentration in iron and zinc was observed across sites at p<0.001.

Keywords: Soil fertility, Fe/Zn partitioning, plant canopy.

INTRODUCTION

Common bean (*Phaseolus Vulgaris* L.) is the most important grain legume for direct human consumption in Latin America and eastern Africa countries. In Rwanda, common bean constitutes the main source of vegetable protein, fibers, minerals (Ca, Cu, Fe, Mg, Mn, and Zn) and vitamins (folate). Per capita consumption of beans can be as high as 66kg/capita/year in Rwanda and parts of western Kenya (Broughton et al. 2003).

Micronutrient deficiencies are a major cause of malnutrition, and it was estimated that 2 billion people worldwide are iron deficient (Petry et al. 2013). Iron is

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an essential nutrient because it is a central part of haemoglobin, which carries oxygen in the blood. Diets deficient in Fe is often associated with Zn deficiency. In humans, iron is essential for preventing anaemia and for the proper functioning of many metabolic processes, while zinc is essential for adequate growth and sexual maturation and for resistance to gastro-enteric and respiratory infections, especially in children (Bouis, 2003). The main symptoms of Zn deficiency include pregnancy complications, low birth weight, maternal and infant mortality and reduction of growth in infancy and childhood (Frossard et al. 2000). Children and women of reproductive age are the most vulnerable. In Rwanda the state of food security and nutrition in 2012 shows that there is an increasing of food availability, acceptable food consumption, but still high rates of chronic malnutrition for children under 5 (2012 UN Rwanda report). Iron deficiency (anaemia) remains the most important public health problem in Rwanda. Strategies for fighting micro-nutrients deficiencies can be through supplementation of vulnerable groups with micronutrients, fortification of common foods with micronutrients, and biofortification or dietary crop improvement.

Legumes constitute a good source of many minerals including iron and zinc and other essential micronutrients that are found only in low amount in the cereals or root crops (Wang et al., 2003). Cultivars of Common beans show variability for seed mineral accumulation. In terms of biofortification, improvement of mineral content is advantageous precisely because the baseline grain iron content is high at 55 ppm (mg/kg) and variability of the trait is great, ranging up to 110 ppm (Beebe et al., 2000). The initial breeding attempt here is much more successful than in the cereals to increase in overall iron and zinc content. Biofortification of beans through breeding for higher micronutrient concentration is a potential sustainable solution to increase the intake of bioavailable iron.

The main objective of this study was to determine the effects of soil fertility and cropping patterns on soil minerals (Fe/Zn) partitioning into bean seeds and their distribution within plant canopy, while the specific objectives were as follows: (i)To assess the accumulation of soil minerals (Fe/Zn) into seeds (ii)To assess the distribution of minerals within plant, (iii) To determine Genetic and environmental interactions for bean seed concentration in iron and zinc content and (iv) to determine the effects of Foliar Fe application on seed iron and zinc content.

MATERIAL AND METHODS

Study location: The experiment was set in Rubona, Nyamagabe, Musanze, Rwerere, Rugarama, Rango, Gisagara and Ntyazo in 2012A and 2012B.

Germplasm:

Experiment1: Seven varieties including G2331, MAC28, MAC42, MAC44, MAC49, MAC9 and RWV1129 were planted only in Rugarama in 2012B in RCBD design in plots of 3 rows of 5m long.

Experiment2: Seven varieties including Gitanga, RWV3316, RWV 2887, RWV 3006, RWV 2361, RWV

3317 and G 2331 were planted in Musanze, Rwerere in plots 3 rows of 5m long spaced by 0.5 in 2012B season. Seed samples were collected from different levels of plant; at 50 cm, 100 cm and 150 cm.

Experiment 3.1: Eighteen varieties, including KAB06 F2-8-12, KAB06 F2-8-135, KAB06 F2-8-143, KAB06 F2-8-27, KAB06 F2-8-28, KAB06 F2-8-29, KAB06 F2-8-35, KAB06 F2-8-36, KAB06 F2-8-50, KAB07 F2-8-175, MAC 44, NUV 141, NUV 152, NUV 177, NUV 195, NUV 219-2, NUV149-2 and RW 1129 were planted in Rubona in 2012A in split plot design in 4 m^2 plot size (4 rows of 2m long spaced by 0.5m) in 3 replications. Nine (9) climbing bean varieties including MBC71, MCB72, MBC27, MBC12, MAC61, MBC32, MBC23, MAC44 and Vuninkingi were planted in Experiment on Foliar Fe application at the rate 2kg /ha in Rubona and Nyamagabe in 2012 A season in a split plot design in plots of 4 m^2 (4 rows of 2m long spaced by 0.5m) in 3 replications.

Experiment 3.2: Seven (7) bush bean varieties including ECAB0158, ECAB0266, MLB49-89A, ECAB0019, ECAB0086, ECAB0064 and ECAB0511 were planted in experiments on foliar Fe application at the rate 2kg /ha was set in Rubona and Nyamagabe in 2012 A season in a split plot design in plots of 4 m^2 (4 rows of 2m long spaced by 0.5m) in 3 replications.

Experiment 3.3: Nine climbing bean varieties including MAC44, MAC61, MBC12, MBC23, MBC27, MBC32, MBC71, MBC72, RWV1129 Were planted at Akanyirandori in 2012B in Split plot design in plots of 7.5 m^2 (3 rows of 5m spaced by 0.5 m) in 3 replications

Experiment 4: Ten climbing bean varieties including G 2331, GITANGA, MAC 42, MAC 44, RWV 1129, RWV 2361, RWV 2872, RWV 2887, RWV 3006 and RWV 3316 were planted in five environments of Rubona, Rango, Gisagara, Nyamagabe and Ntyazo in RCBD design in plots of 7.5 m^2 (3 rows of 5m spaced by 0.5 m) in 3 replications

Foliar iron application

Locally available foliar iron (Ferrous sulphate (2%)) was applied at rate of 2kg/ha once three weeks after emergence of bean seedlings after dissolution in water according to the recommendations from the manufacturer.

Seed sampling and Seed iron and zinc analysis

Seed sampling: Before the main harvest, 30 well-filled pods from the middle parts of plants of each germplasm and free from soil were randomly harvested and put in clean new paper envelopes (to avoid contamination with dust and dirt while uprooting plants and threshing in bulk). These were hand threshed under conditions that kept the seed as free of dirt and dust as much as possible. (HarvestPlus, 2008). For each genotype, a seed sample weighing about 200 grams was taken (Stangouilis and Sison, 2008), cleaned with distilled water, packed in new paper bags. The analysis was done at Rubona Agriculture Research Station

XRF analysis: The 200 gm seed samples were subdivided into smaller samples of 15-20 gm each and transferred to blue plastic cap tubes (HarvestPlus, 2008). Seed was further surface cleaned by rubbing between clean cloth dampened with distilled water for 60 seconds. A new piece of clean cloth was used for each sample and care was taken to thoroughly clean hands before conducting the activity (Paltridge, *et al*. (2011). Thereafter, each sample was oven-dried at 60ºC for at least 12 hours, and then ground using a Sunbeam Conical Burr Mill EM0480 Grinder. This was done by first grinding once with a coarse setting (20-25setting) and then grinding again on finer setting (0-5setting). Ground samples were stored in newly labelled paper bags for XRF analysis. Care was taken to clean the grinder between samples using a brush and vacuum (Stangoulis, 2010). The ground sample to be analyzed was then carefully transferred into small sample cups on the tray, positioned in the machine's tray and identified by labeling samples on the screen tray with the sample number. The amount of iron and zinc was determined by XRF spectrometry by scanning each sample for 100 seconds with spinning of sample cup to analyze Fe and Zn content and records intensities of emitted X-rays. After every 100 samples standard samples were run to standardize the machine so as to produce reliable results (Oxford Instruments, 2009)

Data analysis

Genotype effects of zinc and iron were subjected to analysis of variance (ANOVA) statistical procedure of Gen Stat 14th Edition. Differences between genotypes were analyzed with the Least Significant Difference (LSD) test. Stability analysis was tested by AMMI of Genstat 14th edition.

RESULTS

Partitioning of soil minerals (Fe/Zn) into seeds.

A significant difference at P<0.001 was observed among varieties planted only in Rugarama in 2012B and tested for iron and zinc content. Table below (Table1) shows the analysis of variance of the varieties planted in Rugarama in 2012B

Table1. Analysis of variance for Partitioning of soil minerals (Fe/Zn) into seeds

Source of variation	DF	MS/ Fe content	MS/ Zn content
Replication	2	8.26	42.8
Variety	6	1277.60 ***	90.81 ***
Residual	12	53.56	10.29
GM		84.30	36.06
CV (%)		8.68	8.89

LSD	13.02	5.71

Based on the results in this study RWV1129, MAC 42, MAC 28 and MAC 44 performed well for both iron and zinc content (>80pp and 35pp, of iron and zinc respectively)

Table 2: Mean of seed Fe and Zn content and their respective rank in Rugarama

Variety	Iron Mean	Rank		Zinc Mean	Rank
RWV1129	126.89	a	RWV1129	45.33	A
MAC42	88	b	MAC42	40.11	Ab
MAC28	86.11	b	MAC28	38.44	B
MAC44	80.11	bc	MAC44	34.67	Bc
MAC9	73	cd	G2331	32.22	C
MAC49	72.89	cd	MAC9	31.89	C
G2331	63.11	d	MAC49	29.78	C

Distribution of minerals within plant

Significant differences were observed among varieties tested in different environments (p<0.01) and a strong effects of genotype by environment interactions on iron accumulation were observed at p<0.001. The levels (plant height) of plant have no significant effects on seed iron accumulation.

Table 3: Analysis of variance of iron content in Musanze, Rwerere and across environment

Rwerere			Musanze			Across environment		
Source of variation	DF	MS	Source of variation	DF	MS	Source of variation	DF	MS
Replication	2	362.23 ***	Replication	2	71.77 ns	Site	1	215.88 ns
Variety	6	2010.03 ***	Variety	6	2373.47 ***	Site/Replication	4	217.00 ***
Level	2	88.28 *	Level	2	22.55 ns	Variety	6	4003.94 **
Variety/Level	12	20.89 ns	Variety/Level	12	21.45 ns	Variety/Level	14	18.03 ns
Residual	34	21.3	Residual	39	31.68	Site * Variety	6	382.63 ***
						Residual	87	27.8
CV	5.58		CV	6.64		CV	6.29	

Mean	82.75	Mean	84.82	Mean	83.79
LSD	4.69	LSD	5.69	LSD	5.24

Table 4: Means of Iron content in ppm

Variety	Rwerere	Musanze	Mean across environment
GITANGA	117.94	103.07	110.51
RWV3316	91	104.49	97.74
RWV 2887	85.67	92.47	89.07
RWV 3006	76.11	88.32	82.22
RWV 2361	74.04	72.79	73.41
RWV 3317	67.27	67.78	67.53
G 2331	67.26	64.84	66.05
Means	82.75	84.82	83.79

Variability of iron and zinc content across environments.

The analysis of variance for for iron and zinc across five environments revealed that there is significant difference between environment means at p<0.001. The environments have a significant impact on the performance of tested genotypes. A significant difference among varieties means at p<0.01 was observed. Tested varieties are different from each other where RWV 1129, RWV 2887 and RWV 3316 are the best. Significant and strong G XE effects on iron accumulation at p<0.001 was observed implying that varieties are not stable across environments and it is not easy to select a variety suitable for all these environments.

Table 5. Summary of analysis of variance for iron and zinc across five environments: Rubona, Rango Gisagara, Nyamagabe and Ntyazo

		Iron content		Zinc content	
Source of variation	DF	MS	LSD	MS	LSD
Environment	4	1070.77 ***	2.22	149.13 ***	1.28
environment/rep	10	7.42 ns	2.58	2.47 ns	2.05
Variety	9	813.62 ***	10.16	51.74 *	3.38
G x E (3 mv)	33	187.17 ***	4.72	20.66 ***	3.74
Residual	83	8.43		5.29	
Total	139	133.5		15.89	
GM		75.81		31.97	
Cv (%)		3.83		7.20	

Considering the means, 3 varieties RWV 1129, RWV 2887 and RWV 3316 are better than other and can be selected for these particular environments in general. For each environment, RWV 1129 can be selected specifically for Rubona, Rango and Gisagara, RWV 2887 for Nyamagabe and Ntyazo. Gisagara influenced high iron content accumulation than other sites.

For zinc content accumulation across five environments, There is a significant difference between environment means at p<0.001. The environments have a significant impact on the performance of tested genotypes. Replications within environment are not significantly different. The performance of genotypes is almost the same in 3 replications of each environment. There is a significant difference between variety means at an alpha level of 0.05. Tested varieties are different from each other where RWV 3006 is the best. There is a significant and strong G XE effects on the zinc content accumulation at an alpha level of 0.001 so the tested varieties are not stable across environments. So it is not easy to select a variety suitable for these different environments. Considering the zinc content means, the variety RWV 3006 is better than others and can be selected for these particular environments in general for high zinc content. For each environment, RWV 2361 and RWV 3006 can be especially for Gisagara MAC 44 for Rubona and Nyamagabe, RWV 3006 at Ntyazo and Rango. Rubona and Ntyazo influences high accumulation of zinc content than other experimental sites tested.

Table 6: Means of Iron content in each environment and across environment

Site	G 2331	GITANGA	MAC 42	MAC 44	RWV 1129	RWV 2361	RWV 2872	RWV 2887	RWV 3006	RWV 3316	Envi mean	Rank (LSD 4.7)
	Variety											
Gisagara	66.33	77.33	101.67	87	124.33	74	76	83.67	79.67	90.33	86.03	a
Ntyazo	60.33	73.67	71.33	73	74.67	79.67	64.67	93.67	70.67	80.33	74.20	b
Nyamagabe	64	71.67	63.67	72.33	80.67	75	63.67	86.33	74	80.33	73.17	b
Rango	56.33	77.67		74	78	60	61.67	77.33	71	75.33	70.15	c
Rubona		77.78		71.67	80.44	64.56	70.33	78.11	76	78.78	74.71	b
Variety mean	61.75	75.62	78.89	75.60	87.62	70.65	67.27	83.82	74.27	81.02	75.81	b
Rank (LSD 10.1)	D	bc	bc	Bc	a	cd	Cd	ab	bc	ab		

Gisagara was significantly different from other sited with 86pp while Rango environment was influenced low iron content in investigated genotypes

Table 7: Means of Zinc content in each environment and across environment

Site	G 2331	GITANGA	MAC 42	MAC 44	RWV 1129	RWV 2361	RWV 2872	RWV 2887	RWV 3006	RWV 3316	Environment mean	Rank LSD (3.6)
	Variety											
Gisagara	29.33	24.67	32.33	24.67	32	32.67	28.33	28	32.67	25.67	29.034	C
Ntyazo	31.67	36.67	35.67	31	32.67	36	29	34.33	41	34	34.201	A
Nyamagabe	28	29.33	33	38	32	30	26.33	29.67	33.33	32	31.166	B
Rango	29.33	32.33		32	32.67	27.33	29	29.67	33.67	32	30.88889	b
Rubona		36.78		30.56	32.89	33.11	33	36	39	35	34.5425	A
	29.58	31.96	33.67	31.25	32.45	31.82	29.13	31.53	35.93	31.73	31.97	
Rank (LSD 3.4)	C	Bc	ab	Bc	Bc	bc	c	bc	a	bc		

Stability analysis revealed that RWV 3316 is the most stable genotypes across the environments for Fe and MAC 42 for Zn

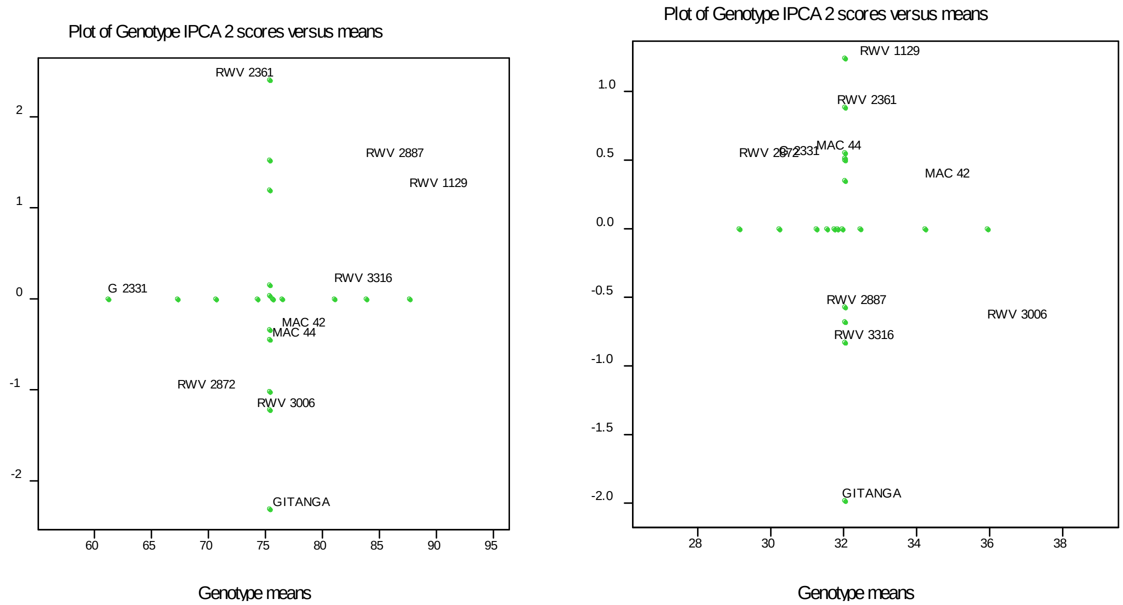

Figure 1: Stability analysis of iron and zinc across sites (left: suitability of seed Fe content, Right: stability of seed Zn content)

Effects of foliar iron application on seed iron and zinc content

Significant differences were observed among climbing bean varieties for seed zinc content (p<0.001), and seed iron content (p<0.5) while the foliar iron application has no significant effects on high iron and zinc content.

Table 8. ANOVA on effects of foliar iron application on seed iron and zinc content in climbing beans.

Source of variation	DF	Zn	Fe
Variety (Main Plot)	8	37.353 *	656.78 ***
Main Plot error	16	10.969	47.74
Fe_application (Subplot)	1	4.822 ns	2.21 ns
Variety.Fe application	8	8.13 ns	23.15 ns
Sub plot error	18	4.276	43.53

Three varieties, including MBC 23 MBC 32 and Vuninking performed well for both high iron and zinc content.

Table 9. General Means and rank of iron and zinc content

Variety	Mean Fe content	Rank	Variety	Mean Zn content	Rank
MBC23	80.46	A	MBC23	33.42	a
VUNINKINGI	77.83	ab	MBC32	32.83	ab
MBC32	77.79	ab	VUNINKINGI	31.63	abc

MAC61	76.12	abc	MBC27	31.46	abc
MBC71	74.33	bc	MAC61	30.38	bcd
MAC44	73	bc	MBC12	30.08	bcd
MBC27	70.33	C	MBC71	29.71	cd
MBC12	60.38	D	MAC44	29.33	cd
MCB72	60.25	D	MCB72	27.83	d

Based on Fe and Zinc content means of genotypes, a positive impact of foliar Fe application was observed only on MBC 32 for high iron content and on MAC 44, Vuninkingi and MBC 71 for high zinc content (Table below)

Table 10.Effects of foliar iron application on seed iron and zinc content of climbing beans

	Fe means		Zn means	
Genotype	Foliar Fe application	No foliar Fe application	Foliar Fe application	No foliar Fe application
MBC71	74.59	74.09	29.01	30.43
MCB72	62.26	58.26	28.26	27.43
MBC27	71.92	68.76	32.1	30.85
MBC12	59.94	60.44	30.4	29.85
MAC61	75.59	76.67	31.01	29.76
MBC32	75.17	80.42	33.43	32.26
MBC23	80.51	80.42	35.1	31.76
MAC44	74.26	71.76	28.6	30.1
VUNINKINGI	77.34	78.34	30.85	32.43

Significant differences were observed among bush bean varieties for both seed iron and zinc content at ($p<0.01$) while the foliar iron application has no significant effects on high iron and zinc content.

Table 11. Analysis of variance on foliar Fe application on seed iron and zinc content in bush beans

Source of variation	DF	Fe MS	Zn MS
Variety (Main Plot)	6	303.21 **	75.33 **
Main Plot error	12	20.87	11.02
Fe_application (Subplot)	1	0.43 ns	6.57 ns
Variety.Fe_application	6	26.29 ns	4.87 ns
Sub plot error	14	13.98	13.92

ECAB 00158 performed well for seed high iron content while ECAB 0266, ECAB 0019 and ECAB 0086 performed well for high zinc content.

Table 12. General Means and rank of iron and zinc content in bush beans

	Iron content			Zinc content	
Variety	Means of Fe content	Rank	Variety	Means of Zn content	Rank
ECAB0158	69.75	a	ECAB0266	36.79	A
ECAB0266	65.42	b	ECAB0019	35.79	A
MLB49-89A	63.58	bc	ECAB0086	35.5	ab
ECAB0019	61.21	cd	ECAB0158	34.21	abc
ECAB0086	60.88	cd	MLB49-89A	32.62	bcd
ECAB0064	58.71	d	ECAB0511	31.58	cd
ECAB0511	53.96	e	ECAB0064	29.88	D

Foliar Fe application showed an increase of iron accumulation on ECAB 0511 which is very low than the mean. Foliar Fe application showed negative impact on the seed iron content of ECAB 0086 and ECAB 0064 and on seed zinc content of ECAB 0158

Table 13. Effects of foliar iron application on seed iron and zinc accumulation

	Fe means		Zn means	
Genotype	Foliar Fe application	No foliar Fe application	Foliar Fe application	No foliar Fe application
ECAB0019	62.25	60.17	35.92	35.67
ECAB0158	70.83	68.67	32.75	35.67
ECAB0064	57.5	59.92	29.42	30.33
ECAB0266	65.17	65.67	36.42	37.17
ECAB0511	56.08	51.83	31.75	31.42
MLB49-89A	63.5	63.67	32.17	33.08
ECAB0086	58.67	63.08	36	35

A Significant difference was observed among varieties ($p<0.001$) for both iron and zinc content but the foliar iron application showed no significant effects in increasing seed iron and zinc content in Rubona

Table 14. ANOVA of foliar iron application in Rubona

Source of variation	DF	Fe MS	Zn MS
Variety (Main Plot)	17	206.37 ***	31.999 ***
Main Plot error	34	9.3	6.272
Fe-application (Subplot)	1	1.29 ns	12.266 ns
Variety .Fe-application	17	35.51 *	5.289 ns
Sub plot error	32	14.15	3.456

KAB06 F2-8-36, RW 1129, KAB07 F2-8-175 and KAB06 F2-8-135 performed better in accumulating

high iron content than the rest of genotypes while KAB06 F2-8-35 and KAB06 F2-8-36 performed better in accumulating high Zinc content.

Table 15 General Means and rank of seed iron and zinc content in Rubona

Entry	Fe Mean	Rank	Entry	Zn Mean	Rank
KAB06 F2-8-36	70.17	A	KAB06 F2-8-35	37.17	A
RW 1129	70.08	A	KAB06 F2-8-36	37	A
KAB07 F2-8-175	69.83	A	KAB06 F2-8-143	36.5	ab
KAB06 F2-8-135	69.5	A	KAB07 F2-8-175	36.5	ab
MAC 44	68.08	Ab	RW 1129	36.17	abc
KAB06 F2-8-143	65.83	Bc	MAC 44	36.08	abc
KAB06 F2-8-27	65.17	Bc	NUV 177	35.33	abc
KAB06 F2-8-35	64.42	C	NUV 195	35.33	abc
KAB06 F2-8-12	60.67	D	KAB06 F2-8-135	34.83	abcd
NUV 219-2	60.17	De	KAB06 F2-8-27	34.33	abcd
KAB06 F2-8-28	59.95	De	NUV 141	33.92	bcde
KAB06 F2-8-50	59	Def	KAB06 F2-8-50	33.67	bcdef
NUV 152	57.75	Def	NUV149-2	33.25	cdef
NUV 177	57.58	Def	NUV 152	32	defg
KAB06 F2-8-29	56.67	Ef	KAB06 F2-8-29	31.33	efg
NUV 141	56.33	F	KAB06 F2-8-12	30.83	Fg
NUV149-2	56.17	F	KAB06 F2-8-28	30.77	Fg
NUV 195	51.17	G	NUV 219-2	30.25	G

The variety KAB06 F2-8-27 increased its iron and zinc accumulation after foliar iron application.

The variety NUV 149-2 unfortunately showed negative effects of foliar Fe application on both high seed iron and zinc content, while KAB06 F2-8-135 showed negative effects of foliar Fe application only on high seed iron content and KAB06 F2-8-29 showed negative effects of foliar Fe application only on high seed zinc content.

Table 16. Mean of iron and zinc content with/ without foliar iron application in Rubona

	Iron means		Zinc means	
Entry	Foliar Fe application	No foliar Fe application	Foliar Fe application	No foliar Fe application
KAB06 F2-8-12	58.17	63.17	30.5	31.17
KAB06 F2-8-135	64.33	74.67	34.17	35.5
KAB06 F2-8-143	66.17	65.5	37.5	35.5
KAB06 F2-8-27	70	60.33	36.33	32.33
KAB06 F2-8-28	62.17	57.73	32.5	29.03
KAB06 F2-8-29	54.83	58.5	29.83	32.83
KAB06 F2-8-35	66.67	62.17	38	36.33
KAB06 F2-8-36	71.83	68.5	38	36
KAB06 F2-8-50	59.5	58.5	33.67	33.67
KAB07 F2-8-175	72	67.67	38	35
MAC 44	69.67	66.5	36	36.17
NUV 141	55	57.67	34	33.83
NUV 152	59.5	56	32.67	31.33
NUV 177	58.5	56.67	35.33	35.33
NUV 195	51.67	50.67	34.67	36
NUV 219-2	59.67	60.67	31	29.5
NUV149-2	53.5	58.83	32.5	34
RW 1129	67.33	72.83	36.67	35.67

Significant differences were observed among varieties grown in Akanyirandori in 2012B and the effect of foliar Fe application was significant for both Fe and Zn at $P<0.001$ and $p<0.01$ respectively

Table 17. Analysis of variance on effect of foliar iron application on seed Fe and Zn in climbing beans grown at Akanyirandoli in 2012B

Source of variation	DF	Fe	Zn
Variety (Main Plot)	8	455.282 ***	27.16 ***
Main Plot error	16	0.775	1.45
Fe_application (Subplot)	1	42.66 ***	13.5 **
Variety.Fe_application	8	68.792 ***	20.75 ***
Sub plot error	18	1.278	1.11

The varieties MAC 44 and MBC 27 showed significant effect of foliar Fe application on Seed Fe and Zn respectively (Table below)

Table 18. Means of Fe and Zn whit and / or without foliar Fe application at Akanyirandoli

	Fe means		Zn means	
Variety	Fe application	No Fe application	Fe application	No Fe application
MAC44	73	66.33	32.67	31.67
MAC61	64.33	61.67	32.67	30.67
MBC12	59.67	63	28	33.33
MBC23	72.33	78.33	34.33	33
MBC27	70.67	68.67	35.67	28
MBC32	61.33	67	30.67	34
MBC71	67	62	30.33	28
MBC72	63.33	48	28.67	25.67
RWV1129	85	85.67	34	33.67

RESULTS AND DISCUSSION

The partitioning of soil minerals into seed is significantly different between varieties tested for iron and zinc content. Based on the results in this study RWV1129, MAC 42, MAC 28 and MAC 44 performed well for both iron and zinc content (>80pp and 35pp, respectively) in Rugarama. Levels or plant height has no significant effect on seed iron and zinc accumulation implying that climbing beans can accumulate more iron and zinc content as well as bus bean varieties. Significant differences were observed among varieties tested in different environments and a strong effect of genotype by environment interactions on iron accumulation were observed. Andrade et al,. (2012) in their study of interaction of genotype by season and its influence on the identification of beans with high content of zinc and iron, reported that mineral contents in the common bean seed are influenced in addition to genetic variation by environmental crop conditions especially the soil type, chemical composition and genotype by environment interactions.

Seed iron and zinc concentrations were again evaluated on a set of 10 varieties during the season of 2012 in five different environments. The ranges of seed iron and zinc concentrations were 61-88ppm and 29-36ppm respectively. Based on the performance of the entries across environments, three varieties, including RWV1129, RWV 2887 and RWV 3316 were identified as highly promising for seed Fe content. Similarly for seed zinc content RWV 3006 were identified as highly promising. No significant relationship was found between seed Fe and Zn content, indicating the need for independent selection for enhancing the concentration for these traits. Analysis of variance revealed significant role of environment and GX E interactions in accumulating the levels of seed Fe and Zn. Therefore, it is not easy to select a variety suitable for all environments since the tested varieties are not stable across environments. The study also identified RWV 3316 as the most stable genotypes across the environments for Fe and MAC 42 for Zn. Gisagara was significantly different from other sited with 86ppm while Rango environment was influenced by low iron content in investigated genotypes. The effects of genotypes, environment and G X E interactions were also observed in bean genotypes (Nchimbi-Msolla and Tryphone (2010), in maize genotypes (Oihek. et al,. 2004, Prasanna et al 2011), as well as in rice where genotype by environment interactions are sufficiently moderate in a way that breeding for high iron and zinc content is considered worthwhile (Gregorio et al 2000)

The results in this study revealed that the foliar iron application has no effect on seed iron and zinc accumulation. The variability among genotypes was larger for seed iron than zinc content with the means ranging between 52 – 72ppm of Fe for bush and 60 to 81 ppm of Fe for climbers, 29-38 ppm of Zn for bush and 28-35ppm of Zn for climbers where foliar Fe was applied, while 51-75ppm of Fe for bush and 58-80ppm of Zn for climbers and 29-37ppm of Zn for bush and 27-32ppm of Zn for climbers were observed where foliar Fe was not applied. The results of the present study revealed that the foliar iron application has no significant effects on high iron and zinc content of the genotypes in different experiments despite significant differences among climbing and bush bean varieties for seed Zn and Fe content. Based on Fe and Zinc content means, different varieties performed differently in accumulating iron and zinc content. Though the effects of foliar iron application is not significant, seed iron/zinc was decreased on some of the varieties like NUV 149-2 , ECAB 0086 , ECAB 0064, ECAB 0158 which were introduced like biofortified varieties and increased on others like KAB06 F2-8-27 implying that the accumulation of these micronutrients into seeds is due mostly to the ability of the genotype to absorb and accumulate these micronutrients than the availability of these micronutrients in the soil or from the other source. The positive effects of foliar Fe application was observed in some beans varieties grown in Rubona in 2012A and Akanyirandori in 2012B especially on Mac 44 and MBC 27 for Fe and Zn respectively. The positive effects of foliar iron and zinc application was observed on Wheat yield and quality in low sandy soil fertility, (Zeidan et al.,2010). Genetic biofortification may be more suitable for increasing seed Fe and zinc content than agronomic strategy like foliar applications which is reported to be more effective for zinc and other micronutrients (Cakmak, 2008) since the distribution of Fe into seed is a genotypic trait (Moraghan and Grafton, 2002) and these traits (seed Fe and Zn content) are genetically inherited (Blair et al,. 2009).

General conclusions and recommendations

The capacity of accumulating high seed iron and zinc content is different from genotype to genotype and is affected by genotype, environments and genotype by environment interactions. Foliar iron application has a significant effect on some varieties and no effects on others. Climbing bean varieties can accumulate more seed iron and zinc content as well as bush bean varieties since plant height has no significant effect on seed iron and zinc accumulation. More studies on effects of foliar iron application on seed iron and zinc content using different varieties should be carried out to confirm the results in this study. More work on genetic biofortification is to be encouraged since it can contribute more than agronomic biofortification in increasing the amount of these micronutrients in bean seed.

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