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Genotype x Environment interactions for higher Iron and Zinc in selected bean varieties

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Common bean is the most consumed pulse globally and a very important crop in tropical Africa, especially in the Central, East and southern Africa, both for its nutritional value and its market potential. Unfortunately, genotype by environment interactions has an important effect on the breeding of better varieties for beans nutritional traits especially iron and zinc. These therefore suggest the need to understand and estimate the magnitude of Genotype and environment interactions for high iron and zinc content in beans and to identify and select genotypes which are widely adapted and can withstand unpredictable environmental fluctuations. This study was carried out to determine the magnitude of G X E for high iron and zinc in sixteen selected varieties, identify and select among them which are consistent for high iron and zinc across environments to be recommended to farmers and for breeding purpose. The experiment was set in Kachwekano and Kawanda Agricultural Research Institutes in a lattice design with 2 replications in plot of 2 x 2m during the second season of 2011 and the first season of 2012. Seed iron and zinc content were analysed using X-Ray Fluorescence (XFR) at Rwanda agriculture Board. However, the results of this study revealed a strong genotype by environment effects on iron and zinc content at 0.001 probabilities. Despite these effects, random error effects contributed more on iron content followed by G X E effects and lastly by genotype effects at 38%, 32 % and 30 % respectively. In contrast the largest contribution on zinc content is due to genotype effects followed by random error effects and G X E effects at 54%, 24 and 22 % respectively. This study suggest that in the selection for stability, zinc content should come first then the iron content next since it is proved that in addition to large random error effects , the large variability of iron content make it unstable when compared to zinc content.

Keywords: malnutrition, iron, zinc, beans, stability.

INTRODUCTION

Genotype by environment interactions (G X E) is important for breeding programmes because it brings about the differences in the performance of a test material in several locations. Genotype by environment interactions makes it difficult to demonstrate superiority of any variety (Dabholkar, 1992). According to Frey (1964) a variety having wide or good adaptability is one which consistently gives superior performance over several environments. Nchimbi-Msolla and Tryphone (2010), reported that both genotypes and environment influence the concentration of iron and zinc in bean seed. Significant G X E for both iron and zinc content in beans were also reported by Beebe in 2000. However, because the difference between environments is large, a given genotype may perform very differently in each environment due to an interaction between genes and the environment.

According to Dabholkar (1992), the environment is the sum total of physical, chemical and biological factors that influence the development of an organism. The term environment relates to the set of fluctuating growing conditions at a given location (Gebeyehu and Assefa, 2003). The performance of genotypes grown in different environments relative to each other may be inconsistent and these inconsistencies result in changes to the ordering of genotypes from one environment to the next. Changes in the relative performance of genotypes across environments are referred to as genotype x environment interactions (Dabholkar, 1992). The presence of genotype x environment interactions automatically implies that the behaviour of genotypes depends upon the particular environment in which they are evaluated. If

this interaction is severe enough, an important trait required may not be revealed in the particular environment, which can result in the selection of poorly adapted cultivars (Murphy *et al.*, 2007). Therefore, information on genotype x environment interactions is important to plant breeders for the development, selection and recommendation of cultivars that are suitable for growth in different environments. Therefore, the objective of this study was to estimate the magnitude of Genotype and environment interactions for high iron and zinc content in beans and to identify and select genotypes which are widely adapted and can withstand unpredictable environmental fluctuations for iron and zinc content.

MATERIALS AND METHODS

Research sites

The study was carried out in Kachwekano and Kawanda Agricultural Research Institutes. The former is located 2200metres above sea level in a cool environment while the latter is located about 1200metres above sea level in a warm, humid environment. A lattice design with 2 replications was employed. Each plot was 2 x 2m. The experiment was conducted during the second season of 2011 (October to February) and the first season of 2012 (April to August). During this period Kachwekano had high relative humidity, high rainfall and low temperatures compared to Kawanda. The weather parameters for the two locations are presented in the table 1 below.

Table 1: Climatic conditions of the experimental sites during the two seasons in which the study was carried out.

Environment	Environmental Parameter							
	Total rainfall (mm)	daily	Daily temp (°C)	max.	Daily temp (°C)	min.	Daily RH (% at 9:00am)	Daily RH (% at 12:00 noon)
Kawanda 2011B	141		29		16		80	62
Kachwekano 2011B	297		25		13		90	67
Kawanda 2012A	153		27		17		87	
Kachwekano 2012A	451		23		12		92	54

Experimental soils sites were tested each season. Results showed that soils varied between seasons and locations. Kachwekano had high values for most of the soil parameters tested. The table 2 below shows the results of soil analysis for each experiment and each location for the seasons 2011B and 2012A.

Table 2: Soil results of the experimental sites (2011 and 2012)

Season	Location	pH	OM	N	P	Ca	Mg	K	Fe	Zn
			-----%-----			-----ppm-----				
October to February 2011	Kawanda	5.3	9.8	0.40	5.9	1716.78	361.82	240.44	147.7	4.9
	Kachwekano	7	4.5	0.24	16.0	1824.4	452.94	460.48	127.4	1.0
April to August 2012	Kawanda	5.1	5.3	0.25	4.75	1888.18	522.02	214.3	80.6	4.0
	Kachwekano	5.9	8.1	0.36	43.16	3736.48	942.66	711.62	160.1	4.6
	Critical values	5.2	3.0	0.20	5.0	350.0	100.0	150.0		
	Sufficient levels	5.2-7.0	6.0	0.30	20.0	2000.0	600.0	500	50	20

Plant material

The most promising varieties for high iron and zinc content from CIAT and Rwanda Agriculture Board were used. These include fourteen varieties high in

iron and zinc and a low iron and zinc checks (CAL 96 and DOR 500). The table 3 below describes the material used in this study.

Table .3 Genetic materials used in G X E study

Genotype name	Origin	Average Fe	Average Zn
CAL96	CIAT	58	30
MIB465	CIAT	102/72	43
NGWIN x CAB2/2/3/1/1	Rwanda	97	28
RWV 3316 (=CAB 2 * LAS 400)	Rwanda	93	31
KAB06F2.8-27	CIAT	85	43
RWV 2359	Rwanda	84	38
NUA 99	CIAT	82	33
Garukurare	Rwanda	82	34
Kivuzo	Rwanda	82	34
RWV 1129	Rwanda	81	34
Ndimirakaguja Volubile	Rwanda	81	38
Nyiramagorori 2	Rwanda	80	34
MBC 32	CIAT	79	34
Icyana	Rwanda	79	31
NUA 69	CIAT	78	40
DOR500	CIAT	64	36

Seed sampling

Before the main harvest, approximately 30 well-filled pods from the middle parts of plants of each germplasm and free from soil were randomly. These were hand threshed under conditions that kept the seed as free of dirt and dust as much as possible. For each germplasm, a seed sample weighing about 200 grams was taken (Stangouilis and Sison, 2008). Three sub-samples from each plot were used. Samples were sent to the Rwanda Agriculture Board for iron and zinc analysis using XRF.

identified and tagged. At maturity, these pods were harvested and put in clean new paper envelopes (to avoid contamination with dust and dirt while uprooting plants and threshing in bulk).

Field data collection

Apart from iron and zinc content, phenological, agronomic and some data on reaction to diseases were collected for each genotype in each environment following CIAT's new Ontology (2012). For fungal diseases, the AUDPC value for each genotype was calculated by trapezoidal integration following the protocol developed by Durham *et al.* (2011).

$AUDPC = \sum ((X_i + X_{i+1})/2(t_{i+1} - t_i))$ in which X_i and X_{i+1} is the disease severity for two consecutive assessments, $t_{i+1} - t_i$ is the interval between two consecutive assessments (Filho *et al.*, 1997).

Data analysis

Analysis of variance

Statistical analysis of variance was performed by the ANOVA procedure of Gen Stat computer program, Fourteenth Edition (GenStat 64-bit Release 14.1 (PC/Windows 7) 10 June 2012 12:59:13 Copyright 2011, VSN International Ltd. Makerere University).

Analysis of G X E interaction and stability for higher iron and zinc

Different methods were used to analyze G X E interaction. Additive Main effects and Multiplicative Interactions (AMMI) model was used to test G X E effects on iron and zinc in beans and test their stability across environments (Sabaghpour *et al.*, 2012). The most stable genotype was selected based

Estimation of Broad Sense Heritability

Broad sense heritability was estimated using the variance methodology (Crossa, 1990) to determine the percentage of Fe and Zn due to genetic or environmental effects. Heritability was classified according to Johnson *et al.* (1955) as cited by

RESULTS

Genotype by environment interactions for high iron and content

Mean squares of genotypes by environment interactions from AMMI model of analysis revealed a

on the smaller principal components analysis (PCA) score (Bantayehu, 2009; Muhammed *et al.*, 2000). Conventional analysis of variance was also used. The analysis of variance of the combined data expresses the observed mean (Y_{ij}) of i^{th} genotype at the j^{th} environment as $Y_{ij} = \mu + G_i + E_j + GE_{ij} + e_{ij}$ where μ is the general mean, G_i , E_j and GE_{ij} represent the effects of the genotype, environment and the G X E respectively and e_{ij} is the average of random error associated with the i^{th} plot that received i^{th} genotype in the j^{th} environment (Crossa, 1990). Genotypes effects were assumed to be fixed while environmental (locations and seasons) effects were random. The ANOVA method for estimating variance components consist of equating mean squares to their expectations and solving the resulting set of simultaneous equations as shown in the table 4 below and is based on the model provided by Allard (1960). Satterthwaite's approximations were used to test the significance of genotypes (Allwood *et al.*, 2008)

Nadarajan and Gunasekaran (2005) as low when less than 30%, moderate when it is between 30-60% and high when it is more 60%. Different basis was used to estimate the broad sense heritability including individual plot basis, entry mean within environment basis and entry mean across environment basis.

strong significant G X E effect on iron content ($p \leq 0.001$) and moderately significant G X E effects on zinc content ($p \leq 0.01$). Highly significant differences ($p \leq 0.001$) were also observed among the genotypes (Table 4).

Table 4 Mean squares from the analysis of variance of iron and zinc content across environment using AMMI model

Source	DF	SS Iron content	MS Iron content	SS Zinc content	MS Zinc content
Total	127	13881.1	109.3	3952.24	31.12
Treatments	63	11573.1	183.7 ***	3687.39	58.53 ***
Genotypes	15	5436	362.4 ***	1629.9	108.66 ***
Environments	3	1977.9	659.3 ***	1644.27	548.09 ***
Block	4	264.8	66.2 ns	18.6	4.65 ns
Interactions	43	4158.1	96.7 ***	413.66	9.62 **
IPCA1	17	2483.7	146.1	264.35	15.55
IPCA2	15	1230	82	116.85	7.79
Residuals	11	441.1	40.1	32.34	2.94
Error	56	2044	36.5	246.4	4.4

ns, **,*** not significant, significant at 0.01, 0.001 probability respectively.

The conventional accumulated analysis of variance using unbalanced design (due to missing plots in either replication in 2011B making G x E degrees of freedom 43 instead of 45.) for iron and zinc content

showed again highly significant ($p \leq 0.001$) differences among genotypes under study and a strong G X E effect for both iron and zinc content in seed (Table 5).

Table 5 G X E Conventional accumulated analysis of variance in unbalanced design for iron and zinc content in four environments

Source of variation	DF	SS iron	MS iron	SS zinc	MS zinc
Environments	3	1919.55	639.85 *	1639.98	546.66 ***
Locations	1	927.28	927.28 *	1289	1289.00 ns
Seasons	1	990.09	990.09 *	308.701	308.701 ns
Locations*Seasons	1	2.17	2.17 ns	42.28	42.28 **
Environment/Replications	4	179.64	44.91 ns	6.48	1.62 ns
Genotypes	15	5109.45	340.63 **	1508.85	100.59 ***
Genotypes * Environments	43	4152.51	96.57 ***	17622.26	409.82 ***
Genotypes * Locations	15	497.55	33.17 ns	122.835	8.189 *
Genotypes * Seasons	15	2477.4	165.16 ns	252.75	16.85 ***
Genotypes * Locations * Seasons	13	1177.54	90.58 **	34.19	2.63 ns
Error	56	2045.12	36.52	246.4	4.4
Total	121	13406.8	110.80	3811.5	31.50
CV			8.78		6.08
Mean			68.79		34.50
LSD			10.56		4.83

ns, *, **,*** not significant, significant at 0.5, 0.01 and 0.001 probability respectively.

Though genotype effects on iron and zinc content in seeds of bean varieties was significantly high, G X E

effects were estimated by variance components at 32% for iron and 22 % for zinc (Table 6).

Table 6 G X E effects estimated by variance components

Trait	Source of variation	Variance components	Variance components %	BSH
Fe	Variety	29.11	30.03	0.30 (Individual plot)
	G X E	31.32	32.31	0.37 (Entry mean within environment basis)
	Pooled error	36.52	37.67	0.70 (Entry mean across environment basis)
	Total	96.95	100	
Zn	Variety	9.77	53.60	0.54 (Individual plot)
	G X E	4.06	22.28	0.61 (Entry mean within environment basis)
	Pooled error	4.40	24.11	0.86 (Entry mean across environment basis)
	Total	18.23	100	

Genotypic variation was observed among genotypes, between seasons and between locations.

where the missing plots are represented by the means of 3 sub-samples of one replication.

Means of iron and zinc content in each environment (location and season) are presented in table 7 and 8

Table 7 Means per environment and their respective ranks for iron content (ppm)

Code	Genotypes	Kawanda 2011B	Kachwekano 2011B	Kawanda 2012A	Kachwekano 2012A	Across environment
G1	KAB06F2.8-27	71.83 (6)	82.49 (4)	69.59 (5)	73.08 (4)	74.25 (4)
G2	NUA 99	67.92 (9)	64.08 (15)	53.52 (14)	63.98 (10)	62.38 (13)
G3	NUA 69	65.66 (12)	72.74 (8)	64.3 (8)	61.8 (12)	66.12 (10)
G4	DOR500	66.77 (10)	65.92 (13)	57.9 (11)	60.91 (14)	62.88 (12)
G5	CAL96(low check)	63.96 (13)	64.14 (14)	56.47 (13)	57.96 (16)	60.63 (15)
G6	MIB465(high check)	80.59 (2)	70.66 (11)	76.62 (1)	59.13 (15)	71.75 (6)
G7	Ngwin x CAB2/2/3/1/1	74.74 (5)	75.11 (7)	72.91 (4)	63.38 (11)	71.53 (7)
G8	RWV 3316	77.27 (4)	83.73 (3)	67.97 (6)	80.49 (2)	77.37 (2)
G9	RWV 2359	69.96 (8)	71.95 (9)	63.54 (9)	65.04 (8)	67.62 (9)
G10	Garukurare	78.4 (3)	80.45 (5)	75.54 (3)	70.04 (6)	76.11 (3)
G11	Kivuzo	62.91 (14)	71.39 (10)	57.34 (12)	64.86 (9)	64.12 (11)
G12	RWV 1129	58.75 (15)	93.11 (1)	60.08 (10)	84.56 (1)	74.12 (5)
G13	Ndimirakaguja volubile	83.36 (1)	84.85 (2)	76.37 (2)	78.42 (3)	80.75 (1)
G14	Nyiramagorori 2	70.74 (7)	58.85 (16)	42.21 (16)	71.2 (5)	60.75 (14)
G15	MBC 32	66.14 (11)	79.44 (6)	65.22 (7)	69.21 (7)	70 (8)
G16	Icyana	58.03 (16)	66.08 (12)	50.43 (15)	61.46 (13)	59(16)
Means		69.81	74.06	63.12	67.85	68.71

The number in parenthesis is the rank

High iron and zinc content were observed in Kachwekano than Kawanda based on means.

was high specifically at Kachwekano. MIB 465 (high check) was high specifically in Kawanda.

Ndimirakaguja, Garukurare and RWV 3316 were high in Fe in both locations and seasons while RWV 1129

Table 4 Means per environment and their respective ranks for zinc content (ppm)

Code	Genotypes	Kawanda 2011B	Kachwekano 2011B	Kawanda 2012A	Kachwekano 2012A	Across environment
G1	KAB06F2.8-27	32.68 (4)	44.65 (3)	30.35 (7)	38.82 (3)	36.62 (4)
G2	NUA 99	32.11 (6)	43.91 (4)	28.48 (12)	35.51 (8)	35 (7)
G3	NUA 69	31.68 (9)	36.69 (12)	29.25 (10)	31.88 (14)	32.37 (12)
G4	DOR500	31.85 (8)	41.57 (8)	30.67 (6)	38.41 (4)	35.62 (6)
G5	CAL96 (low check)	28.34 (15)	38.89 (9)	25.03 (15)	31.37 (15)	30.91 (15)
G6	MIB465 (high check)	42.44 (1)	47.03 (2)	37.57 (2)	37.46 (7)	41.12 (2)
G7	Ngwin x CAB2/2/3/1/1	30.22 (12)	35.64 (14)	29.84 (9)	34.83 (9)	32.63 (10)
G8	RWV 3316	30.71 (10)	35.99 (13)	29.98 (8)	34.51 (10)	32.8 (9)
G9	RWV 2359	30.64 (11)	37.44 (11)	28.85 (11)	33.58 (11)	32.62 (11)
G10	Garukurare	41.73 (2)	47.43 (1)	38.83 (1)	41.58 (1)	42.39 (1)
G11	Kivuzo	29.95 (13)	37.98 (10)	27.16 (14)	31.92 (13)	31.75 (14)
G12	RWV 1129	29.53 (14)	41.76 (7)	28.42 (13)	38.29 (6)	34.5 (8)
G13	Ndimirakaguja vol	36.45 (3)	42.07 (6)	34.96(3)	39.02 (2)	38.12 (3)
G14	Nyiramagorori 2	32.08 (7)	32.69 (16)	31.46 (4)	32.27 (12)	32.12 (13)
G15	MBC 32	32.25 (5)	42.12 (5)	30.78 (5)	38.36 (5)	35.88 (5)
G16	Icyana	26.67 (16)	33.15 (15)	24.86 (16)	29.32 (16)	28.5 (16)
Means		32.46	39.94	30.41	35.45	34.56

The number in parenthesis is the rank

Ngimirakaguja, Garukurare and MIB465 (high check) were high in Zn across locations and season while KAB06F2.8-27, DOR500 and MBC 32 were specifically high at Kachwekano.

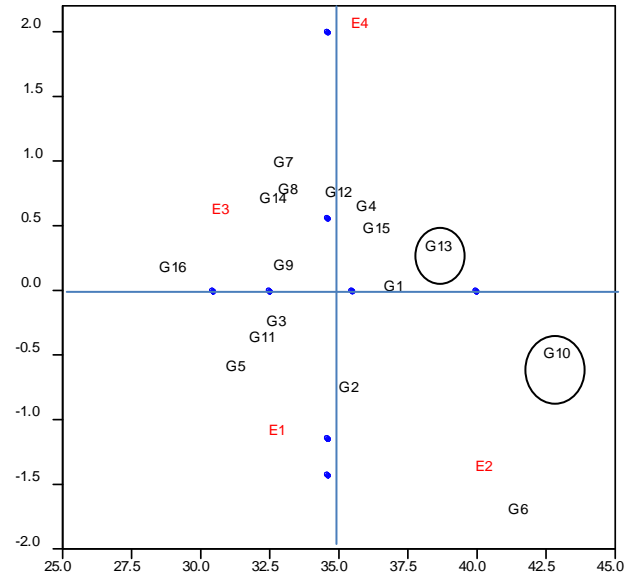
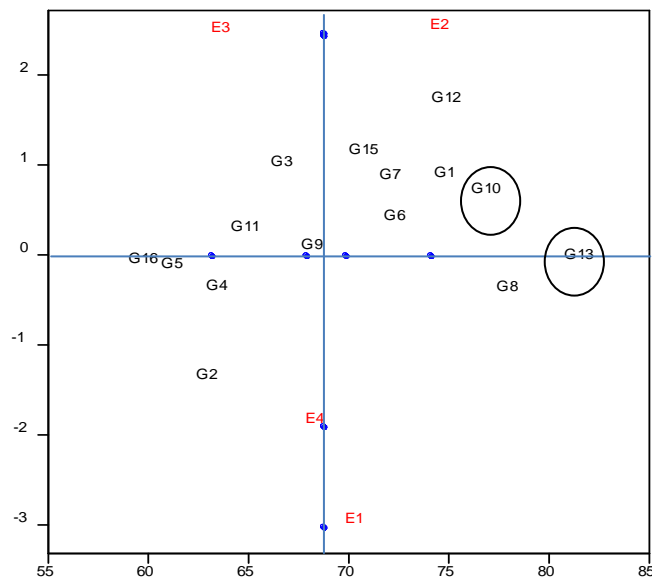
Performance consistency analysis for iron and zinc content across environments

Performance consistency analysis for iron and zinc content across environments revealed that different

Relationship between iron and zinc in genotypes under investigation.

Based on regression analysis, iron and zinc were strongly positively correlated ($r=0.59$)

genotypes performed differently for iron and zinc with a high variation for iron content than zinc as shown in the figure 1 below.



Genotype & Environment means for iron content

Genotype & Environment means for zinc content

Note: The horizontal blue line is a stability line while the vertical blue line is the genotype mean across environment

Figure 1 Performance consistency analysis for iron and zinc content across environments.

The smaller the IPCA score the more stable the genotype over all environments. Entries 13 and 10 representing Ndimirakaguja Volubile and Garukurare were found to be the most stable genotypes since they performed consistently better across environments for iron and zinc content. Their iron and zinc contents were above the grand mean.

Genotype 8 representing RWV 3316 was consistent for iron content, genotypes 1 representing KAB06F2.8-27 was consistent for zinc but not for iron content. Genotype 12 representing RWV1129 had specific adaptation for iron and zinc in Kachwekano while genotype 6 representing MIB4659 (high Fe check) had specific adaptation for iron and zinc in Kawanda respectively.

Agronomic, phenological and biotic MS across environment and broad sense heritability estimates along iron and zinc content

A significant difference was observed among genotypes and among environments for different traits (Appendix 2, 3, 4, and 5). A strong G X E effect was observed for most of the traits (Appendix 2, 3, 4 and 5) while correlations between the different traits also varied across environments (Appendix 9). Variance components and broad sense heritability (BSH) were estimated (Appendix 6,7 and 8). Apart from Ascochyta resistance, pods per plant and yield per plant, BSH was high for other diseases, agronomic and phenologic traits. Iron and zinc content means,

agronomic, phenological traits data and biotic stress mean scores across environments are presented in Appendix 1.

DISCUSSION

The results of this study revealed a strong genotype by environmental effects on both iron and zinc content in seed. Despite these effects, random error effects contributed more on iron content followed by G X E effects and lastly by genotype effects at 38%, 32 % and 30 % respectively. In contrast the largest contribution on zinc content is due to genotype effects

followed by random error effects and G X E effects at 54%, 24 and 22%, respectively. The significance of mean square for genotypes x location suggested that the regions for which genotypes were being bred for iron and zinc comprises of a number of special environments (see soil results and weather data). These results suggest that iron and zinc content is conditioned by environment effects, genotype effects as well as G X E effects just as Nchimbi-Msolla and Tryphone (2010) had reported. This study suggests that in the selection for stability, tough high Zn and Fe content are both important, the selection of genotypes with both minerals should start by zinc followed by iron since they are correlated and it is proved that in addition to large random error effects, the large variability of iron content make it unstable when compared to zinc content.

The results of this study showed the importance of testing genotypes under different environmental conditions to identify varieties that are stable for iron and zinc content and are high yielding. This study showed the advantages of adding AMMI model for the analysis of performance consistency and G X E effects on iron and zinc content of beans. Similar advantages were observed by Bantayehu (2009) for grain yield in malting barley. The importance of G X E effects was also observed for other traits in other crops such as soybean (Asfaw *et al.*, 2009), wheat (Sakin *et al.*, 2011) and sweet potato (Tumwegamire, 2011). This suggests that the importance of G X E effects on a particular trait should be investigated carefully since its consequence can be major in a breeding program (Dabholkar, 1992).

Differences in iron and zinc content were observed in each genotype, within and between locations suggesting that there are differences in the uptake and loading of iron and zinc in common bean. This is in agreement with studies by De Arunjo *et al.* (2003) who reported that no single variety was stable for all characteristics studied under different environments. In this study, genotypes performed differently for iron and zinc in each season and in each location. Gregorio (2002) in his study, found that the same of genotypes he had used had a relatively low iron and zinc content, regardless of the environment while others had high Fe and zinc content regardless of the environment.

The cross environmental mean of 69 ppm for iron content and 35 ppm for zinc content observed in this study are slightly higher in iron content and the same for zinc content observed in Colombia by Blair *et al.* (2010a). They reported 55 ppm and 35 ppm for Fe and Zn respectively. These were higher than the means observed in Tanzania in 2010 by Nchimbi-Msolla and Tryphone (2010) (57 ppm and 3 ppm for Fe and Zn, respectively, and in Ethiopia (64 and 21 ppm for Fe and Zn respectively) by Shimelis and Rakshit (2005).

The within location means showed that genotypes in Kachwekano performed better than in Kawanda with 71 and 67 ppm respectively for iron and 38 and 31 ppm respectively for zinc. Differences of iron and zinc content in different environment might have been due to the soil characteristics of the sites (Blair *et al.*, 2010b) and also the weather differences. Therefore, variations in iron and zinc content are attributed to the genotype background and the environment in which they are grown.

Based on the results of this study, environmental factors can interact with the plant-gene expression to influence the amount of a micronutrient accumulated in a seed (Ortiz-Monasterio *et al.*, 2007). Despite low soil iron and zinc content in Kachwekano, high seed iron and zinc content observed in Kachwekano might have been due to high soil pH, cold weather conditions providing long time for vegetative growth and therefore more time to accumulate minerals. These suggest that conditions in Kachwekano favour solubility of these minerals since even if soil is the main source of nutrients including iron and zinc for plant growth, productivity and accumulation in the seed (Dwivedi *et al.*, 2012), iron availability is the function of solubility rather than of its abundance in the soil (Pirzadah *et al.*, 2010). The results of this study also showed that Kachwekano has high phosphorus, potassium, calcium, and magnesium contents compared to Kawanda. This may suggest

that these minerals facilitate iron and zinc absorption and accumulation. They may also interact with the environment to influence some physiological processes that facilitate increased absorption and accumulation of these minerals in bean seed. According to Borg *et al.* (2009) the ability of plants to translocate Fe and Zn is controlled by a homeostatic mechanism in the plant that regulates absorption, translocation and phloem sap loading-unloading rates of Fe and Zn. Hao *et al.* (2007) in their study reported that the application of Nitrogen increased the concentration of minerals, including Fe and Zn in rice as a result of the improved transport from roots to shoots.

More recently, the effects of nitrogen on improving seed Fe and Zinc were also reported for wheat (Cakmak *et al.*, 2010; Kutman *et al.*, 2010). In this study, however, high soil nitrogen were observed in Kawanda 2011 yet iron and zinc contents in the seed were low compared to the values recorded for Kachwekano. Wu *et al.* (2010) reported that genotypic differences exist in the allocation of micronutrient such as Zn and Fe to seed; and that Zn content is closely associated with the ability to translocate Zn from old tissue to new tissues at both early and growth stages of the rice crop with phloem remobilizing Zn from non-seed parts especially leaves and stems to seeds. Therefore continued Zn absorption requires genetic capacity to absorb it from soil (Alloway, 2009).

Stability analysis in this study allowed identification of promising varieties with wide and specific adaptation for accumulating iron and zinc in seed. The results of this study showed that genotypes performed differently for iron and zinc across environment, but the varieties Ndimirakaguja Volubile and Garukurare had consistently high values for these minerals. These varieties may be well buffered (Frey, 1964) and withstood unpredictable transient environmental fluctuations since they gave consistently superior

performance over four environments for iron and zinc. Performance consistency analysis by AMMI Model helped to characterize the response of different genotypes to changing environments on iron and zinc content. Mohammadi *et al.* (2008) reported that AMMI is one of the best estimator of grain yield performance and stability of spring safflower. Therefore, it could also be used for other traits.

In this study, iron and zinc were strongly and positively correlated ($r=0.59$). The correlation in this study was slightly higher than that reported by Tryphone and Nchimbi-Msolla (2010) ($r=0.42$). Unfortunately, strong positive correlation has been observed between iron content and susceptibility to rust ($r=0.48$) as well as zinc content and susceptibility to leaf rust ($r=0.50$). In this particular study, apart from Fe/Zn and rust susceptibility, there were no strong relationships between iron / zinc and other traits.

Zinc accumulation appeared to be more consistent than iron supporting results of Anuradha *et al.* (2012). This implies that in selections, the first step should be aimed at high Zn content. In this study, the correlation between seed contents of Zn and Fe were strong and Zn was less sensitive to the environment compared to Fe. It also exhibited much higher heritability compared to iron.

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Appendices

Appendix 1 Iron and zinc content means, agronomic , phonological data and biotic stress mean scores across environments.

Genotype	KAB06F2.8-27	NUA 99	NUA 69	DOR500	CAL96	MIB465	Ngwin x CAB2/2/3/1/1	RWV 3316	RWV 2359	Garukurare	Kivuzo	RWV 1129	Ndimrakaguja volubile	Nyiramagorori 2	MBC 32	Icyana
Trait																
Iron content	74.25	62.38	66.12	62.88	60.63	71.75	71.53	77.37	67.62	76.11	64.12	74.12	80.75	60.75	70.00	59.00
Zinc content	36.62	35.00	32.37	35.62	30.91	41.12	32.63	32.80	32.62	42.39	31.75	34.50	38.12	32.12	35.88	28.50
DM	97	92	93	100	92	97	113	109	106	106	114	100	98	104	108	104
Yield/plant (grs)	12	15	10	6	9	8	12	16	18	14	23	16	22	23	23	22
Yield(kg/ha)	1332	1139	1340	635	1015	370	745	1826	2189	1365	2195	2279	3270	2399	2673	2065
DF	49	43	45	56	43	53	59	57	55	57	56	54	55	56	58	56
BCMV	2	3	3	2	2	2	7	4	4	6	6	2	2	6	2	6
BCMNV	2	5	2	51	17	60	5	3	2	0	0	0	11	1	4	1
CBB	2	2	3	2	2	2	3	3	3	3	3	3	4	3	2	3
Vigor	3	4	4	4	4	5	7	5	4	5	5	3	3	5	4	5
100seed weight	51	43	43	17	45	17	45	46	47	47	48	56	32	38	47	40
ALS	4	4	3	5	6	4	3	3	4	4	3	4	4	4	3	3
RUST	2	1	1	1	2	1	2	1	1	4	1	3	3	2	2	1
ANTH	2	1	2	1	3	1	1	1	1	1	1	1	1	1	1	1
ASCO	4	3	3	3	4	3	2	3	2	2	2	2	3	3	2	2
Survivor %	72.3	81.5	74.7	39.8	52.9	24.6	71.6	77.3	66.5	74	66.1	71.2	79.1	65.3	64.5	75.2

DF= Days to Flowering, DM= Days to Maturity, BCMV= Bean Common Mosaic virus, BCMNV= Bean Common Mosaic Necrotic Virus, CBB= Common Bacterial Bright, ALS= Angular leaf spot, ANTH= Anthracnose, ASCO= Ascochyta,

Appendix 2 ANOVA table for AMMI model: Mean Squares for different traits and G X E interaction in 4 environments

Source	DF	BCMV	BCMNV	CBB	100SW	Emergency	Vigor
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Genotypes	15	29.86 ***	2679.30 ***	2.40***	954.10 ***	658.10 ***	7.65***
Environments	3	15.02 ***	1283.70 ***	8.22 ***	239.10 NS	6855.80 ***	46.72 ***
Interactions	42	4.01 ***	382.80 ***	0.94**	96.20 NS	302.30 ***	2.51 **
IPCA	17	9.69	718.10	1.71	145.10	675.40	4.52
IPCA	15	0.63	231.70	0.69	83.20	125.60	1.60
Residuals	10	0.48	118.70	0.22	32.50	18.30	0.94
Error	54	0.75	100.10	0.44	69.10	100.30	1.18
CV		24.08	97.04	25.20	20.14	16.47	25.44
Mean		3.59	10.31	2.62	41.28	60.80	4.27
SEM		0.31	3.54	0.23	2.94	3.54	0.38
LSD		0.87	10.01	0.66	8.33	10.02	1.09

Appendix 3ANOVA table for AMMI model: Mean Squares for different traits and G X E interaction in 3 environments

The table above is for Yield components and fungal diseases

Source of variation	Genotypes	Environments	Interactions	IPCA	IPCA	Error	CV	Mean	SEM	LSD
DF	15	2	30	16	14	44				
Days to maturity	301.00 ***	12101.00***	26.00 ***	42.00	8.00	2.00	1.39	102.00	0.50	1.42
Harvested pants	686.00 ***	7812.00 ***	285.00 ***	367.00	192.00	128.00	25.62	44.16	4.62	13.16
Pods/plant	36.10 ***	2.38 ns	20.93 ***	35.35	4.45	2.98	24.03	7.19	0.61	1.74
Grains/pod	3.74 ***	9.89 ***	0.65 *	0.86	0.41	0.35	13.16	4.51	0.21	0.69
Yield/plant (grs)	198.90 **	1885.10 ***	145.80 *	262.40	12.60	72.50	54.41	15.65	3.01	8.58
Yield (kg/ha)	3934446.00 ***	16141511.00 ***	1249664.00 ***	2034716.00	352462.00	405585.00	37.98	1677	259.99	741.03
ALS AUDPC	1.38 ***	5.63 ***	0.65 **	0.91	0.36	0.30	25.25	2.18	0.23	0.64
ALS top score	3.83 ***	10.95 NS	2.45 ***	3.09	1.71	0.56	20.24	3.70	0.31	0.87
RUST AUDPC	1.47 ***	1.71 **	0.45 ***	0.58	0.30	0.11	24.06	1.37	0.13	0.38
RUST top score	4.51 ***	3.76 **	1.07 ***	1.41	0.70	0.28	31.69	1.67	0.22	0.61
ANTH AUDPC	1.13 ***	0.05 NS	0.60 ***	1.02	0.12	0.09	23.93	1.23	0.12	0.34
ANTH top score	1.36 ***	1.09 **	0.69 ***	1.02	0.32	0.21	34.38	1.32	0.19	0.53
ASCO AUDPC	1.98 ***	17.70 ***	1.19 ***	2.60	0.09	0.30	27.08	2.02	0.22	0.63
ASCO top	2.33 ***	32.09 ***	1.69 **	2.98	0.23	0.68	32.89	2.50	0.34	0.96

Appendix 4 G X E Interactions and Mean squares of different traits in 4 environments using a combined analysis of variance methodology

Source of variation	DF	Days to flowering	BCMV top score	BR %	CBB	Vigor	Emergency	100 seed weight
Environment	3	3717.15 ***	15.02 **	1283.70 **	8.22 *	46.72 *	6855.80 ***	242.23 ns
Location	1	11026.13 *	8.00 ns	3358.50 ns	2.94 ns	24.5 0ns	6976.80 ns	9.71 ns

Season	1	116.28 ns	30.03 ns	463.90 ns	0.09 ns	21.12 ns	11007.60 ns	151.89 ns
Loc*Seas	1	9.03 *	7.03 **	28.70 ns	21.61 ***	94.53 ***	2583.00 ***	565.09 **
Rep/L*S	4	1.23 ns	0.77 ns	52.90 ns	1.12 *	7.08 ***	93.90 ns	224.03 *
Genotype	15	231.04 ***	29.83 ***	2679.30 ***	2.4 **	7.65 **	658.10 *	974.11 ***
G*E	45	32.86 ***	4.01 ***	382.80***	0.94 **	2.51 **	302.31 ***	98.66 ns
G*L	15	25.24 ns	5.53 ns	809.30 **	0.79 ns	1.38 ns	142.90 ns	126.47 ns
G*S	15	40.69 ns	3.46 ns	152.40 ns	1.13 *	1.51 ns	612.30 **	84.51 ns
G*L*S	15	32.65 ***	3.03 ***	186.70 *	0.44 ns	4.65 ***	151.70 ns	106.23 ns (12 df)
Error	60	1.42	0.75	100.10	0.44 (59df)	1.18	100.30	69.05(54 df)
CV		2.23	24.08	97.04	25.19	25.44	16.47	20.12
Mean		53.28	3.59	10.31	2.62	4.27	60.80	41.30
SEM		2.03	0.71	6.92	0.34	0.56	6.15	3.51
LSD		5.77	2.02	19.70	0.98	1.60	17.51	10.00

The G X E is assessed using the analysis of variance

Appendix 5 G X E Interactions and Mean squares of different traits in 3 environments using conventional analysis of variance procedure

The table is applied for fungal diseases an yield parameters due the slashing event that destroyed one environmental data

Source of variation	Environment	Rep/Environment	Genotype	G*E	Error	CV	Mean	LSD
DF	2	3	15	30	45			
Yield (kg/ha)	15404735.50 *	690977.00 ns	3934882.00 **	1245284.90 **	405585.00	37.98	1677.00	1315.79
Yield/plant (grs)	1853.12 **	41.83 ns	198.48 ns	145.51 *	72.50	54.41	15.65	14.22
Grains/pod	9.45 ns	1.01 *	3.76 ***	0.64 *	0.35	13.17	4.51	0.94
Pods/plant	1.62 ns	9.18 *	35.02 ns	20.89 ***	2.98	24.01	7.19	5.39
Plants emerged	731.57 *	35.44 ns	435.34 ns	384.12 ***	96.42	14.40	68.20	23.11
Harvested pants/plot	8207.60 ns	872.10 ***	693.00 *	285.73 *	133.50	26.32	43.90	19.93
Survivor %	19399.70 *	1533.60 ***	1374.80 **	400.79 **	156.00	18.92	66.00	23.61
Days to maturity	12101.45 ***	2.85 ns	301.17 ***	26.15 ***	2.14	1.43	102.04	6.03
ASCO top score	32.09 **	0.52 ns	2.33 ns	1.69 **	0.68	32.90	2.50	1.53
ASCO AUDPC	17.69 **	0.57 ns	1.98 ns	1.19 ***	0.30	27.07	2.02	1.29
ANTH top score	1.18 ns	0.18 ns	1.39 *	0.66 ***	0.21	34.37	1.32	0.96
ANTH AUDPC	0.088 ns	0.09 ns	1.14 ns	0.57 ***	0.09	23.93	1.23	0.89
RUST top score	3.76 ns	0.48 ns	4.51***	1.072 ***	0.28	31.70	1.67	1.22
RUST AUDPC	1.71 ns	0.25 ns	1.47 **	0.45 ***	0.11	24.06	1.37	0.79
ALS top score	10.95 ns	10.09 ***	3.83 ns	2.45 ***	0.56	20.24	3.70	1.84
ALS AUDPC	5.63 ns	0.99 *	1.38 *	0.65 **	0.30	25.25	2.18	0.95

Appendix 6 Variance components and BSH of different traits in 4 environments

		Variance components	variance components %	BSH	Basis
DF	variety	24.72	58.98	0.59	(individual plot basis)
	G*S	-1.85	-4.42	0.60	(entry mean within environment basis)
	G*L	2.01	4.80	0.86	(entry mean across environment basis)
	G*L*S	15.62	37.26		
	Error	1.42	3.38		
BCMV	variety	2.98	53.20	0.53	(individual plot basis)
	G*S	0.63	11.16	0.57	(entry mean within environment basis)
	G*L	0.11	1.93	0.84	(entry mean across environment basis)
	G*L*S	1.14	20.35		
	Error	0.75	13.36		
BR	variety	238.04	45.04	0.45	(individual plot basis)
	G*S	155.65	29.45	0.50	(entry mean within environment basis)
	G*L	-8.58	-1.62	0.80	(entry mean across environment basis)
	G*L*S	43.30	8.19		
	Error	100.10	18.94		
CBB	variety	0.11	14.05	0.14	(individual plot basis)
	G*S	0.09	10.82	0.19	(entry mean within environment basis)
	G*L	0.17	21.45	0.49	(entry mean across environment basis)
	G*L*S	0.00	0.00		
	Error	0.44	53.68		
VIGOR	variety	1.18	47.26	0.47	(individual plot basis)
	G*S	-0.82	-32.82	0.62	(entry mean within environment basis)
	G*L	-0.79	-31.56	0.87	(entry mean across environment basis)
	G*L*S	1.74	69.75		
	Error	1.18	47.36		
Emergence	variety	6.83	2.78	0.03	(individual plot basis)
	G*S	-2.20	-0.90	0.03	(entry mean within environment basis)
	G*L	115.15	46.85	0.13	(entry mean across environment basis)
	G*L*S	25.70	10.46		
	Error	100.30	40.81		
100 seed weight	variety	108.67	55.46	0.55	(individual plot basis)
	G*S	5.06	2.58	0.67	(entry mean within environment basis)
	G*L	-5.43	-2.77	0.89	(entry mean across environment basis)
	G*L*S	18.59	9.49		
	Error	69.05	35.24		

Appendix 7 Variance components and BSH of yield parameters and days to maturity in 3 environments

Trait	Source of variation	Variance components	Variance components %	BSH	Basis
Yield (kg/ha)	variety	448266.17	35.19	0.35	(individual plot basis)
	G X E	419850.00	32.96	0.42	(entry mean within environment)

					basis)
	Error	405585.00	31.84	0.68	(entry mean across environment basis)
	Total	1273701.17	100.00		
Yield/plant (grs)	variety	8.83	7.49	0.07	(individual plot basis)
	G X E	36.50	30.98	0.11	(entry mean within environment basis)
	Error	72.50	61.53	0.27	(entry mean across environment basis)
	Total	117.83	100.00		
Grains/pod	variety	0.52	51.26	0.51	(individual plot basis)
	G X E	0.14	14.06	0.62	(entry mean within environment basis)
	Error	0.35	34.69	0.83	(entry mean across environment basis)
	Total	1.02	100.00		
Pods plant	variety	2.36	16.48	0.16	(individual plot basis)
	G X E	8.96	62.67	0.18	(entry mean within environment basis)
	Error	2.98	20.85	0.40	(entry mean across environment basis)
	Total	14.29	100.00		
Survivor %	variety	162.33	36.83	0.37	(individual plot basis)
	G X E	122.40	27.77	0.45	(entry mean within environment basis)
	Error	156.00	35.40	0.71	(entry mean across environment basis)
	Total	440.73	100.00		
Plants emerged	variety	8.54	3.43	0.03	(individual plot basis)
	G X E	143.85	57.82	0.04	(entry mean within environment basis)
	Error	96.42	38.75	0.12	(entry mean across environment basis)
	Total	248.81	100.00		
Harvested plants/plot	variety	67.88	24.46	0.24	(individual plot basis)
	G X E	76.10	27.43	0.32	(entry mean within environment basis)
	Error	133.50	48.11	0.59	(entry mean across environment basis)
	Total	277.48	100.00		
Days to maturity	variety	45.84	76.42	0.76	(individual plot basis)
	G X E	12.00	20.01	0.78	(entry mean within environment basis)
	Error	2.14	3.57	0.91	(entry mean across environment basis)
	Total	59.98	100.00		

Appendix 8 Variance components and BSH of Fungal diseases in 3 environments

Trait	Source of variation	Variance components	Variance components %	BSH	Basis
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ASCO top score	variety	0.11	8.25	0.08	(individual plot basis)
	G X E	0.51	39.38	0.11	(entry mean within environment basis)
	Error	0.68	52.37	0.27	(entry mean across environment basis)
	Total	1.29	100.00		
ASCO AUDPC	variety	0.13	14.92	0.15	(individual plot basis)
	G X E	0.45	51.12	0.18	(entry mean within environment basis)
	Error	0.30	33.96	0.40	(entry mean across environment basis)
	Total	0.88	100.00		
ANTH top score	variety	0.12	22.03	0.22	(individual plot basis)
	G X E	0.23	40.67	0.27	(entry mean within environment basis)
	Error	0.21	37.30	0.53	(entry mean across environment basis)
	Total	0.55	100.00		
ANTH AUDPC	variety	0.10	22.62	0.23	(individual plot basis)
	G X E	0.24	56.96	0.25	(entry mean within environment basis)
	Error	0.09	20.43	0.50	(entry mean across environment basis)
	Total	0.42	100.00		
RUST top score	variety	0.57	45.91	0.46	(individual plot basis)
	G X E	0.40	31.73	0.52	(entry mean within environment basis)
	Error	0.28	22.36	0.76	(entry mean across environment basis)
	Total	1.25	100.00		
RUST AUDPC	variety	0.17	38.11	0.38	(individual plot basis)
	G X E	0.17	37.80	0.43	(entry mean within environment basis)
	Error	0.11	24.09	0.70	(entry mean across environment basis)
	Total	0.45	100.00		
ALS top score	variety	0.23	13.26	0.13	(individual plot basis)
	G X E	0.94	54.43	0.16	(entry mean within environment basis)
	Error	0.56	32.32	0.36	(entry mean across environment basis)
	Total	1.73	100.00		
ALS AUDPC	variety	0.12	20.29	0.20	(individual plot basis)
	G X E	0.18	29.23	0.27	(entry mean within environment basis)
	Error	0.30	50.47	0.53	(entry mean across environment basis)
	Total	0.60	100.00		

Appendix 9 Table of correlation between different traits

*, **,*** indicate significance at P< 0.05,0.01,0.001 respectively; ns: no significant differences

	Zinc	DM	Yield/plant(grs)	Yield (kg/ha)	DF	BCMV	BR	CBB	vigor	100seed weight	ALS	RUST	ANTH	ASCO
Iron	0.59 **													
Zinc		0.20 ns	0.27 ns	0.15 ns	0.22 ns	-0.16 ns	-0.26 ns	0.19 ns	-0.08 ns	0.23 ns	-0.13 ns	0.48 *	-0.10 ns	-0.16 ns
Days to maturity		-0.23 ns	-0.02 ns	-0.19 ns	0.24 ns	-0.22 ns	0.32 ns	-0.17 ns	-0.17 ns	-0.20 ns	0.24 ns	0.50 *	-0.18 ns	-0.26 ns
Yield per plant (grs)			0.44 ns	0.11 ns	0.83 ***	0.66 **	-0.16 ns	0.25 ns	0.58 *	0.12 ns	-0.63 **	0.27 ns	-0.40 ns	-0.68 **
Yield (kg/ha)				0.88 ***	0.36 ns	0.34 ns	-0.57 *	0.41 ns	-0.26 ns	0.26 ns	-0.56 *	0.18 ns	-0.37 ns	-0.57 *
DF					0.22 ns	0.25 ns	-0.53 ns	0.39 ns	-0.33 ns	0.26 ns	-0.45 *	0.18 ns	-0.20 ns	-0.40 ns
BCMV						0.38 ns	-0.26 ns	0.35 ns	0.34 ns	-0.25 ns	-0.46 *	0.10 ns	-0.59 **	-0.69 **
BR							-0.43 ns	0.52 *	0.78 ***	0.13 ns	-0.34 ns	0.26 ns	-0.12 ns	-0.31 ns
CBB								-0.33 ns	0.27 ns	-0.87 ***	0.39 ns	-0.03 ns	0.26 ns	0.06 ns
Vigor									0.15 ns	0.24 ns	-0.34 ns	0.21 ns	-0.41 ns	-0.35 ns
100seed weight										-0.26 ns	-0.22 ns	-0.22 ns	-0.24 ns	-0.02 ns
ALS top score											-0.10 ns	0.09 ns	0.16 ns	-0.16 ns
RUST top sc.												0.25 ns	0.57 *	0.63 **
ANTH													-0.22 ns	-0.12 ns
ASCO														0.63 **