Ambit Journal of Agriculture.Vol 1(1) pp. 01-16 June, 2015.
http://www.ambitjournals.org/ajar

Full Length Research Paper

Characterization and Identification Of Potential Sources Of High Seed Iron and Zinc Content Among Uganda Common Bean Germplasm

Mukamuhirwa, F.[1], Tusiime,G[1] Richard E[1]., Mukankusi M.C[2], Gibson P[1]. and Edema,R.[1]

[1]Department of Agricultural production, Makerere University, P.O.Box 7062, Kampala, Uganda.
[2]CIAT Africa, National Agricultural Research Laboratories- Kawanda, P.O.Box 7065, Kampala, Uganda.

Accepted 10th May, 2015.

Though Uganda produces adequate amounts of food for its population the nutritional status is generally poor and undernourishment is considered as one of the major health problems. Despite the high prevalence of malnutrition, there is no literature that clearly documents the Uganda national food composition. The objective of this study was to identify potential sources of high iron (Fe) and zinc (Zn) content among bean germplasm of Uganda. An experiment of 187 genotypes was set at the National Agricultural Research Laboratories, Institute, Kawanda in the second season of 2011 in plots of 2 rows of 2 meters long in lattice design with 2 replications. Seed iron and zinc were analysed using XRF at Rwanda Agriculture Board (RAB). This study identified a high level of genetic diversity in Ugandan bean varieties ($p \leq 0.001$) that may be appropriate for using in plant breeding programs directed at increasing the nutritional quality of common beans with respect to Fe and zinc. Iron and zinc were strongly positively correlated ($r=0.51$) suggesting that genetic factors that increase iron concentration are co- segregating with genetic factors that increase zinc concentration. The present study suggests that genes responsible for these minerals are linked to other genes and are multigenically controlled.

Keywords: Micronutrient, Malnutrition, Bean.

INTRODUCTION

The constitution of the Republic of Uganda places a lot of emphasis on attaining food security and adequate nutrition for its citizens and the government is committed to fulfilling this goal so that all Ugandans can attain good health, social and economic well-being (Smith, 2006). Unfortunately, according to the Uganda Bureau of Statistics, even though Uganda

Corresponding Author: fmukamuhirwa@yahoo.com

produces adequate amounts of food for its population, the nutritional status of its people is generally poor and undernourishment is considered as one of the major health problems (Tidemann-Andersen *et al*., 2011). Widespread micronutrient malnutrition results in an enormous negative socioeconomic impact at the individual, community and national level (Stein, 2006). Beans are of major importance both as a source of food and income, Uganda being a major exporter in the regional market (Eastern and Central Africa Bean Research Network (ECABREN, 2003). Even though there is a need to develop other new varieties which combine acceptable agronomic and grain characteristics, resistance to biotic and abiotic stresses and high micronutrient content, (Buruchara *et al*., 2011) the availability of bean varieties with high seed iron and zinc contents within the farming systems in Uganda will go a long way to combat nutritional disorders associated with these nutrients. Unfortunately, the Fe and Zn content in bean seeds of the varieties currently grown in Uganda is not known. This hampers efforts to promote consumption of such bean types or their utilization in breeding for improved mineral content. In addition, high seed Fe and Zn bean varieties cannot be exploited unless they withstand other production constraints in the region. Some of these are diseases such as angular leaf spot (ALS), anthracnose, ascochyta, bean common mosaic virus (BCMV), bean common mosaic necrotic virus (BCMNV) common bacterial blight (CBB) and rust (Hagedorn and Inglis, 1986).

Based on the high consumption of beans in Uganda (Tidemann-Andersen *et al*., 2011), this study was conducted to investigate the micronutrient content (iron and zinc) in bean seed grown and consumed in Uganda and also to document the reaction of the genotypes to the common diseases.

MATERIALS AND METHODS

Research site

The study was conducted at the International Center for Tropical Agriculture (CIAT) based at the National Agricultural Research Laboratories Institute (NARL), Kawanda Uganda.

Soil sampling and analysis

Soil was sampled from the middle and each corner of each replicate using a hand auger at approximately 20 cm of depth (U.S.D.A, 2002; U.S.E.P.A., 2011). For each replicate, soil samples were carefully and thoroughly mixed in a plastic bucket, until a homogeneous sample was obtained. Each homogeneous sample was packed in a new paper bag, labelled twice and analyzed in Kawanda Agricultural Research Laboratories (KARL). Soil was analyzed for iron (Fe), zinc (Zn), acidity (pH), organic matter (OM), nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), and Magnesium (Mg) following the Soils and Soil Fertility Management Programme protocol of National Agricultural Research Laboratory, Kawanda.

Soils were characterized depending on the amount of each mineral found (Soil Fertility Interpretation, 2009; Rosen and Eliason, 2005; Gerwing and Gelderman, 2005; and Verheye, 2013).

Plant material

A total of 187 bean genotypes coded UGK1-187 that compromised of 153 bean landraces collected from farmers and traders in different parts of Uganda and sourced from the National Agricultural Crops Research Resources Institute (NaCRRI), 17 officially released varieties, 15 pre-released varieties and two universal checks (CAL 96; low Fe check and MIB 465; high Fe check) were evaluated for Fe and Zn content in this study (Appendix 1).

Trial Design

The 187 bean genotypes were planted in a Lattice design with two replications in plots of two rows each measuring two meters long at plant spacing of 20 x 50 cm for climbers and 10cm x 50 cm for bush beans. The trial was weeded twice at 17 and 53 days after planting. An insecticide, Dimethoate and a fungicide (either Benlate or Ridomil) were applied for insect pests and fungal disease control, respectively, up to the flowering stage. N.P.K. 17:17:17 fertilizer was applied at the rate of 100 kgha-1(a recommendation for the experimental site used).

Data collection

Agronomic, phenological and morphological data collection

Data collected on agronomic traits include only plant vigour that was evaluated visually at flowering using 1 to 9 score where 1= excellent and 9 very poor (CIAT, 2012). For phenological traits, days to flowering were evaluated visually by counting the number of days from planting to the day when 50% of plants had at least one flower. Morphological traits, including growth habit, seed colour and seed size were also recorded.

Growth habit was evaluated at flowering and pod formation using 5 categories named from 1-5 where 1 = determinate bush, 2 = indeterminate bush habit, erect stems and branches, 3 = indeterminate bush habit with weak main stem and prostrate stem and branches, 4 = indeterminate climber habit with weak, long and twisted stem and branches, 5 = determinate climber. Primary seed colour was evaluated visually using 1 to 9 scale for the predominant seed colour where 1 = white; 2 = cream-beige; 3 = yellow; 4 = brown-maroon; 5 = pink; 6 = red; 7 = purple; 8 = black; 9 = others. Seed size was evaluated by weighing 100 seeds on an analytical scale in grams where small size: < 25g/100 seed, medium size = 25-40g/100 seed; large size >40g/100 seed (CIAT, 2012).

Evaluation of biotic stress under field conditions

Biotic traits, including resistance to angular leaf spot (ALS), bean common mosaic virus (BCMV), bean common mosaic necrotic virus (BCMNV), common bacterial blight (CBB), rust, anthracnose and ascochyta blight were recorded under field conditions using the new trait dictionary developed by CIAT (CIAT, 2012) by quantifying symptoms using 1-9 scale where 1 = no visible symptoms and 9 = severe to very severe symptoms causing considerable yield loss or plant death.

Seed iron and zinc analysis

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 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 and sent to Rubona Agriculture Research Station, Rwanda for XRF analysis.

XRF analysis: The 200gm seed samples were subdivided into smaller samples of 15-20gm each, and transferred to blue plastic cap tubes (HarvestPlus, 2008). Three small sub-samples were used per genotypes. The 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. According to Paltridge, *et al*. (2011) this process reduces Aluminum (Al) contamination from approximately 15 ppm to 2 ppm and by about 5 ppm for Fe contamination while Zn remains unchanged. 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 the sample cup to analyze Fe and Zn content and records intensities of emitted X-rays. Energy and intensity of fluorescence provide information about elemental make-up sample after sample. The screen tray rotates to place the samples being measured at the top and the results of the analysis are displayed automatically when all samples on the tray have been measured as described by Oxford Instruments, (2009).

Standard samples were run after every 100 samples to standardize the machine so as to produce reliable results. Standardization of the machine was done by the air method using Setting Up Samples (SUSs); SUSi10B and SUSi05B called GL50B and IO5B respectively (Oxford Instruments, 2009) with intensities of 106 to 4837 cps for iron and 8 to 109 cps for zinc. The analysis of two duplicates for twenty genotypes in two replications was done to assess error variation (Paltridge *et al*., 2011).

Data analysis

Genotype effects of zinc and iron were subjected to analysis of variance (ANOVA) statistical procedure of Gen Stat 14th Edition (GenStat 64-bit Release 14.1 (PC/Windows 7) 10 June 2012 12:59:13Copyright 2011, VSN International Ltd. Makerere University). Differences between genotypes were analyzed with the Least Significant Difference (LSD) test. Correlation and regression analysis were carried out to din bean seed.

RESULTS

Soil status of experimental site

Apart from low soil phosphorus with 7.7 ppm (but not critical), the experimental soil had high to very high levels of nutrients. However, the concentrations of minerals such as calcium (1598.77 ppm), magnesium (389.64 ppm), potassium (339.64 ppm) and zinc (7.4 ppm) were not sufficient. Organic matter (8.7%), nitrogen (0.38%) and iron (151.0ppm) concentrations were high to very high and sufficient.

Variability of seed iron and zinc content

The analysis of duplicate bean samples Zn and Fe for every entry revealed that the procedure was accurate and results thus reliable. The genotype effect was highly significant (p≤0.001) for both seed iron and zinc contents (Table 1).

Table 1 Mean squares for iron and zinc in Ugandan bean germplasms from which potential sources of iron were to be selected

Source of variation	DF	Mean squares	
		Iron content (ppm)	Zinc content (ppm)
Rep	1	0.1 ns	43.8 **
Genotype	186	116.2 ***	22.3***
Residual	183	27.2	4.9
Total	370		
Means		61.4	33.0
CV (%)		8.5	6.7
LSD		10.29	4.37

Ns= not significant, **: significant at 0.01 probability level ***: significant at 0.001 probability level

Table 2. ANOVA for duplicates to assess variations due to field plots and analysis in the laboratory

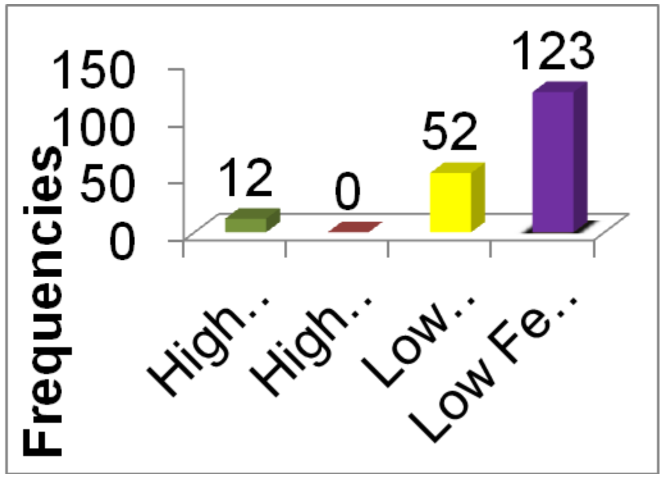
Source of variation	DF	Fe Mean Square	Zn Mean Square
Replication	1	2.0 ns	0.07 ns
Replication/duplicate	2	11.5 *	1.8 ns
Genotypes	19	278.3***	18.5**
Replication * Genotype (field error)	19	3.5	0.5
Replication*duplicate*genotype (lab error)	38	3.4	1.02
Total	79	5513.05	404.521

ns, *, **, ***: not significant, significant at 0.05, 0.01 and 0.001 probability level respectively.

Iron content varied from 45pp to 87 ppm with the mean of 61 ppm while zinc content varied from 22pp to 43 ppm with the mean of 33 ppm. The greatest proportion of the genotypes had Fe content ranging between 61-65pmm. Only 5% of genotypes under investigation have had Fe content greater than 75ppm. These mineral differences among varieties exist because they are genetically different from each other. These results revealed that the genetic diversity within the Ugandan genotypes for iron and zinc content was high, thus the importance of this region as an important source of nutritional bean germplasm. A significant (P≤0.001) strong (r=0.51) positive correlation between the concentrations of Fe and Zn in 187 Ugandan bean genotypes was found. This probably implies that selecting for high bean seed Fe concentrations also tended to increase Zn concentrations in bean seeds simultaneously

The variety UGK 116 and UGK4 had the highest iron content (87ppm and 85ppm respectively) with high zinc content of 37pp and 38ppm for UGK116 and UGK 4 respectively. The varieties UGK 43 (MIB456; high iron and zinc check), UGK45, UGK 47, UGK 92 and UGK 111 had the highest zinc content of 43ppm for UGK 43 and 40ppm for the rest (UGK45, UGK 47, UGK 92 and UGK 111).

Apart from UGK43 and UGK 111 which had 75 and 77 ppm, respectively, other varieties high in zinc (UGK45, UGK 47, UGK 92) were low in iron content with the respective values of 63, 62 and 61ppm



High Fe:>75ppm, high Zn:>35ppm,
Low Fe :<75ppm, low Zn;<35ppm

Figure 1: Fe and Zn variability.

The iron content for low (CAL 96) and high (MIB 465) checks were 56 and 75 ppm respectively while the zinc content for low (Cal 96) and high (MIB 465) checks were 30 and 43 ppm respectively.

UGK86, UGK10, UGK70, UGK13, UGK174 had both low iron and zinc (≤56 and ≤30 ppm, respectively) content when compared to the checks. UGK180 and UGK146 had only low iron content (≤56 ppm) than the low check while UGK17 and UGK14 had only low zinc content (≤30 ppm) than the low check. This resulted in the selection of eleven (11) genotypes high in both iron and zinc content in comparison to the checks.

3.2.1 Variability of other traits evaluated along with iron and zinc content

In addition to variability in seed iron and zinc concentrations, Ugandan germplasm also varied in agronomic and phenologic traits; and reaction to diseases. The summary of the performance of the germplasm for the above traits is presented in the table below:

Table 3 Summary of results of evaluated traits

Trait	Range	Mean	Mean square
Fe Content (ppm)	45-87	61	116.2 ***
Zn content (ppm)	22-43	33	22.3***
Days to Flowering	31-48	38	51.22***
100Seed Weight (gm)	15-54	29	181.96 ***
Vigor (1-9 scale)	1-5	3	1.41 ***
BCMNV (incidenc)	0-92	8	696.57 ***
BCMV(1-9 scale)	0-7	3	3.86 ***
ALS (1-9 scale)	1-5	2	0.87 ns
RUST (1-9 scale)	1-7	2	1.05 ***
Common bacterial blight (1-9 scale)	1-6	3	1.509 ns
Anthracnose (1-9 scale)	1-3	1	0.295ns
Ascochyta (1-9 scale)	1-3	1	0.1806 ns

***, ns=not significant and significant at 0.001 probability level respectively

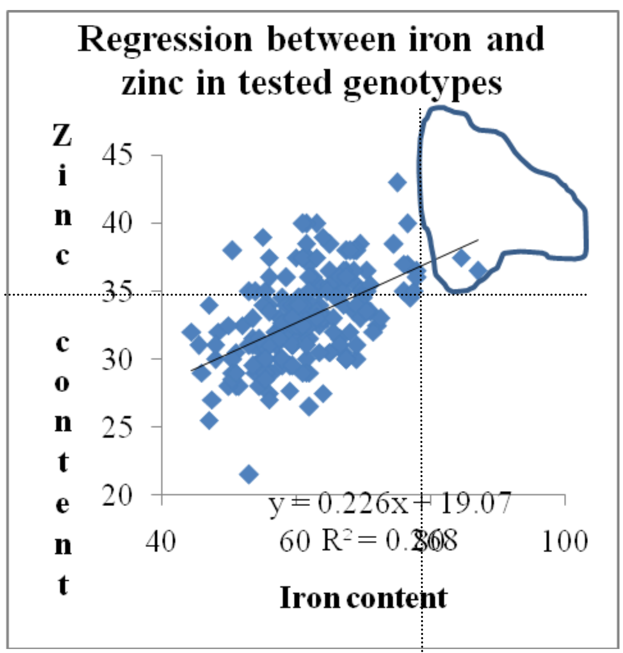
Genotypes significantly ($p<0.001$) influenced seed iron and zinc content; days to flowering, 100 seed weight, vigour, BCMNV incidence, BCMV severity and rust severity.

Differences in resistance to angular leaf spot, common bacterial blight, anthracnose and ascochyta diseases were not significant ($p>0.05$) among genotypes.

For the fungal diseases only one time assessment was used since we were forced to harvest prematurely. The means for Fe and Zn content, diseases scores, days to flowering (DF), 100 seed weight (100SW) and vigour are presented in the Appendix 1

3.2.2 Correlation between iron/zinc content and different traits

There was a significant relationship between iron and zinc content in bean seed. Iron and zinc content ($p<0.001$, r=0.51), iron content and days to flowering ($p<0.001$, r=0.44), zinc content and days to flowering ($p<0.001$, r=0.25), were moderately to strongly positively correlated. In contrast, iron content and rust ($p<0.001$, r=-0.26), iron content and 100 seed weight ($p<0.001$, r= -0.28), zinc content and 100 seed weight ($p<0.001$, r= -0.26) were moderately negatively correlated. The observed positive relationship between Fe/Zn content and days to flowering, negative relationship between iron and rust resistance, iron/zinc and anthracnose resistance observed suggest that genes responsible for these minerals are linked to other genes and are multigenically controlled.



r^2 is the coefficient of determination

Figure 1 Regression between iron and zinc content in tested materials (the solid line is the regression, the dashed lines indicate 75 ppm iron and 35 ppm zinc)

DISCUSSION

This study identified a high level of genetic diversity in Ugandan bean germplasm. This might be revealing a possibility of a diversity of bean landraces in the greater Eastern Africa region; and therefore the existence of valuable alleles for bean improvement including nutritional breeding (Asfaw *et al*., 2009). Of all the tested genotypes, only 11 genotypes combined high iron and zinc content in seed. Of these, UGK111, UGK4 and UGK116 had the highest concentrations of both iron and zinc. Among genotypes under investigation 6% and 34% were classified high, 50% and 53% medium, and 43% and 13% low in iron and zinc content respectively, providing yet more evidence of genetic variability.

In this study iron and zinc were strongly positively correlated (r=0.51) supporting the study of Nchimbi-Msolla and Tryphone (2010) and (Tryphone and Nchimbi-Msolla, 2010) who observed the strong positive correlation of Fe and Zn. The positive and highly significant correlation between the iron and zinc concentrations in seeds of bean suggests that genetic factors that increase iron concentration co-segregate with genetic factors that increase zinc concentration, therefore selecting for bean seeds with high concentration of either iron or zinc may contain high amounts of both elements (Nchimbi-Msolla and Tryphone, 2010). The negative relationship between iron/zinc and seed size was observed. Apart from this, most of the genotypes used in the study were from the Mesoamerican gene pool (87%) while the Andean gene pool represented only 13%. Ninety percent (90%) of the genotypes that exhibited high iron and zinc seed contents were from the MesoAmerican gene pool confirming the negative relationship between Iron/ zinc and seed size observed in Ugandan bean germplasm and suggesting that these micronutrients should be increased in Mesoamerican genpool. These results contradicted reports by (Blair *et al* 2010a) which showed that Andean beans had a tendency for higher average seed iron concentration, but significant lower seed zinc concentration than Mesoamerican beans. CIAT (2005) reported that the two major gene pools of common bean are represented in Africa, where Andean Gene pool represent 61% of cultivars and the rest are small and medium seeded types typical of MesoAmerican gene pool. López *et al.,* (2006) reported that large-seeded Andean cultivars predominate medium-seeded Middle American cultivars in the secondary centre of diversity of the common bean.

This contradiction may be the result of large genetic diversity observed in Ugandan germplasm. It may also be due to the environment under which the experiment was grown. A significant positive and strong relationship was also observed between Fe/Zn and days to flowering, significant moderate negative relationship between iron and rust resistance and a weak significant negative relationship between iron/zinc and anthracnose resistance. All these relationships observed suggest that genes responsible for these minerals are linked to other genes and are multigenically controlled (Blair *et al.,* 2009, Blair et al., 2010 b). Zinc appeared to be more consistent than iron supporting results in Anuradha *et al.* (2012) and since the correlation of Zn and Fe was pretty strong and Zn had less variability than iron and less experimental error, the first step in selection should be to go for high Zn. The identified genotypes may be appropriate for using in plant breeding programs directed at increasing the nutritional quality of common beans with respect to Fe and zinc content.

REFERENCES:

Anuradh *et al*. (2012). Evaluating rice germplasm for iron and zinc concentration in brown rice and seed. *Journal of Phytology* 2012, 4(1) , 19-25.

Asfaw A., Matthew W. Blair, Conny Almekinders. (2009). Genetic diversity and population structure of common bean (*Phaseolus Vulgaris* L.) landraces from the East African Highlands. *Theor Appl Genet*. 2009 Dec;120(1):1-12.Epub2009 Sep 16, 1-12.

Blair, Astudillo, Grusak, Graham and Beebe (2009). Inheritance of seed iron and zinc content in common bean (Phaseolus vulgaris L.). *Molecular Breeding* (2009)23, 197-207.

Blair, M., L. F. Gonzales, P. K. Kimani and L. Butare (2010). Genetic diversity, inter-gene pool introgression and nutritional quality of common beans (Phaseolus vulgaris L.) from Central Africa. *Theor Appl Genet*, 121: 237.

Blair, M. W.; J. I. Medina, C. Astudillo, R. Judith, B. E. Steve, G. Machado, and R.Gram, (2010). QTL for seed iron and zinc concentration and content in a Mesoamerican common bean (Phaseolus vulgaris L.) population. *Theor Appl Gener,* 121, 1059-1070.

Buruchara R., R. Chirwa, L. Sperling, C. Mukankusi, J.C. Rubyogo, (2011). Development and delivery of bean varieties in Africa: The Pan-Africa Bean Research Alliance (PABRA) Model. *African Crop Science Journal*, Vol. 19, No. 4, 2011, 227-245.

CIAT. (2005). Utilisation of Bean genetic diversity in Africa, Highlight CIAT in Africa. www.ciat.cgiar.org/work/Africa/Documents/Highlight21.pdf, accessed on 11 october 2012.

CIAT. (2012). Crop Ontology. https://integratedbreeding.net.

Eastern and Central Africa Bean Research Network (ECABREN). (2003). Beans, the final report of the Eastern and Central Africa Bean Research Network on Priority-Setting Workshop, held at the Hotel Intercontinental Nairobi, Kenya during 13-17 July 2003.

Gerwing and Gelderman. (2005). Fertilizer Recommendations Guide. Cooperative Extension Service, South Dakota State University, U.S. Department of Agriculture , 1-28.

HarvestPlus. (2008). Crop sampling protocal for micronutreint analysis: precautionary notes on avoiding contamination. www.harvestplus.org , 1-2.

López R, Asension C, Gilbertson RL (2006). Phenotypic and Genotic Diversity in Strains of common Blight Bacteria (Xanthomonas campestris pv. Phaseoli and X. campestris pv.phaseoli var.fuscans) in a secondary Center of Diversity of the common bean Host Suggests multiple Introduction Events. P*hytopathology* 2006 Nov;96(11):1204-1212, 1204-1212.

Nchimbi-Msolla S. and George Muhamba Tryphone. (2010). The Effects of the Environment on Iron and Zinc Concentrations and Performance of Common Bean (Phaseolus vulgaris L.) Genotypes. *Asian Journal of Plant Sciences*, 9, 455-462.

OXFORD INSTRUMENTS. (March 2009). X-Supreme800 User's Guide. The Busness of Science.

Paltridge N, L. Palmer and J. Stangoulis, (2011). Micronutrient analysis in the HarvestPlus program. Rwanda: Harvest plus training on how to use XRF machine.

Rosen and Eliason. (2005). Nutrient Management for Commercial Fruit & Vegetable Crops in

Minnesota. University of Minnesota Extension Service, 1-40.

Smith. E. (November 2006). Nutrition Information Systems in Uganda: National Nutrition Surveillance Systems in Uganda, A Review and Recommendation Report Supported by UNICEF ESAR.

Soil Fertility Interpretation. (2009). Agronomy Technical Note 76. (http://www.nm.nrcs.usda.gov/technical/handbooks/iwm/nmiwm.html) rudy.garcia.2009, 1-9.

Stangouilis J and Cristina Sison. (2008). Crop Sampling protocals for Mirconutrient analysis. Harvest Plus Technical Monograph series 7. ISBN 978-0-9818176-0-6, 1-20.

Stangoulis J., (2010). Technical aspects of Zinc and Iron analysis in biofortification of the staple food crops, wheat and rice. 19th World congress of soil science, soil solutions for a changing world 1-6 August 2010, Brisbana, Australia Published on DVD.

Stein A.J., (2006). Micronutrient malnutrition and the impact of modern plant breeding on public health in India: How cost-effective is biofortification? Göttingen: Cuvillier Verlag. ISBN: 3865379478.

Tidemann-Andersen, Hedwig Acham, Amund Maage and Marian K malde (2011). Iron and zinc content of selected foods in the diet of schoolchildren in Kumi district, East of Uganda: a cross-sectional study. *Nutrition Journal* 2011 10:81.

Tryphone and Nchimbi-Msolla. (2010). Diversity of common bean (Phaseolus vulgaris L.) genotypes in iron and zinc contents under screen house conditions. *African Journal of Agricultural Research* Vol. 5(8), 738-747.

U S. Department. of Agriculture, Natural Resources Conservation Service, (2002). Soil sampling . Fact Sheet MN-NUTR3, 1-2.

U.S. Environmental Protection Agency. (2011). Soil sampling. Science and Ecosystem Support Division , Operating procedure , 1-25.

Verheye, W. (2013). management of Agricultural land: Chemical and fertility aspects, Land use, land cover and soil science vol IV. http://www.eolss.net/Eolss-sampleAllChapter.aspx, 1-10.

Appendix 1: Summary table of means for Fe and Zn, different diseases, agronomic, phenological and morphological data of the varieties under investigation.

New code	Name	Category	Fe (ppm)	Zn (ppm)	BCMNV (%)	BCMV	ALS	Rust	CBB	Anthr	Asco	DF	100SW	Vigor
UGK1	Coffee	Landrace	64	35	0	2	1	1	3	1	1	48	19	3
UGK2	Cream	Landrace	68	37	40	1	1	1	3	1	1	44	19	4
UGK3	Cream and black speckles	Landrace	61	32	0	4	1	1	3	1	1	35	31	5
UGK4	Black	Landrace	85	38	0	4	1	1	3	1	1	41	25	3
UGK5	Kakhi	Landrace	67	36	0	3	1	1	3	1	1	39	28	3
UGK6	Red	Landrace	69	36	0	5	3	2	2	1	2	41	24	4
UGK7	Yellow	Landrace	54	31	13	5	2	2	4	1	1	32	34	4
UGK8	Yellow	Landrace	57	31	40	0	1	1	4	1	1	43	17	3
UGK9	Red	Landrace	71	37	0	2	5	1	3	1	1	45	22	4
UGK10	Kanyebwa	Landrace	46	29	0	3	3	2	4	1	1	35	32	3
UGK11	Coffee	Landrace	70	34	0	4	1	1	3	1	1	40	25	4
UGK12	Cream with pink stripes	Landrace	70	35	0	2	2	1	3	1	2	37	31	3
UGK13	Kanyebwa	Landrace	48	27	0	3	3	2	2	2	1	31	30	3
UGK14	Yellow	Landrace	53	22	0	5	2	1	3	1	3	31	31	4
UGK15	Kanyebwa large kidney	Landrace	54	30	0	4	2	1	2	1	1	32	34	3
UGK16	Kanyebwa	Landrace	67	32	0	3	1	2	3	1	1	32	32	2
UGK17	Namunye (cream coffee striped	Landrace	62	27	2	3	2	2	4	1	1	43	33	3
UGK18	Red	Landrace	69	37	0	6	3	1	3	1	2	44	23	3
UGK19	Calima short (nambale)	Landrace	70	39	-1	3	2	3	3	1	1	34	35	5
UGK20	Kanyebwa large kidney	Landrace	51	38	0	5	1	7	2	1	1	39	44	4
UGK21	Yellow	Landrace	52	33	0	2	2	2	3	3	1	31	35	4
UGK22	White	Landrace	69	34	22	0	4	1	4	1	1	39	20	2
UGK23	Pink with black speckles	Landrace	61	35	0	3	3	1	4	1	1	33	29	2
UGK24	Coffee	Landrace	58	33	28	0	3	2	2	1	1	37	17	2
UGK25	Taso(nkijakwanama) roba 1	Landrace	67	34	32	1	2	2	2	1	1	45	19	2
UGK26	Taso(nkijakwanama)* roba 1	Landrace	62	32	55	0	2	1	3	1	2	44	19	2

UGK27	Cream taso* roba1	Landrace	66	35	30	2	2	1	3	1	1	43	19	2
UGK28	Tindalwesire*roba1	Landrace	58	30	67	0	2	1	3	1	1	42	18	3
UGK29	Yellow	Landrace	55	30	0	3	2	1	4	1	1	31	37	4
UGK30	Yellow	Landrace	54	33	0	3	2	2	3	1	1	32	33	3
UGK31	Cream(*roba1)	Landrace	62	29	92	0	2	1	5	1	1	42	18	3
UGK32	Kabonge(pink with black str.)	Landrace	63	33	0	2	2	1	3	1	1	37	18	3
UGK33	Kakoodo dark purple	Landrace	61	33	70	0	2	1	4	1	1	37	16	3
UGK34	Yellow	Landrace	62	38	0	5	3	1	2	2	1	33	32	3
UGK35	Kanyebwa pink with mar. Sp	Landrace	51	31	0	3	1	3	4	1	1	31	38	3
UGK36	Marrow	Landrace	55	31	37	0	2	1	3	1	2	43	17	3
UGK37	Kanyebwa	Landrace	47	34	0	3	3	2	4	1	1	34	29	2
UGK38	Marrow(nabe10c)	Landrace	72	33	0	5	1	1	4	1	1	45	23	4
UGK39	Coffee	Landrace	76	35	0	5	2	1	3	1	1	43	28	3
UGK40	White	Landrace	67	37	32	0	3	1	4	1	1	44	20	2
UGK41	Pink with marrow speckles	Landrace	51	29	0	3	3	1	4	3	1	31	32	2
UGK42	Cream with coffee speckles	Landrace	58	35	0	6	3	4	3	1	1	43	22	4
UGK43	MIB465(High Fe/Zn Check)	Landrace	75	43	48	0	1	1	2	1	1	45	21	4
UGK44	Yellow(bajogore)	Landrace	55	39	2	5	3	1	5	1	1	31	32	5
UGK45	Red	Landrace	63	40	0	4	2	1	3	1	1	48	22	4
UGK46	Orange with coffee str.	Landrace	54	30	0	3	2	1	4	1	1	32	30	4
UGK47	Pink with white speckles	Landrace	62	40	0	4	2	2	3	1	1	35	32	5
UGK48	Cream(roba1)	Landrace	51	28	0	3	2	1	2	1	1	31	33	2
UGK49	Cream	Landrace	62	33	0	4	3	2	3	1	1	39	22	2
UGK50	Coffee	Landrace	71	35	30	1	1	1	3	1	3	34	18	4
UGK51	Pink with marrow speckles(kanyebwa)	Landrace	52	28	0	3	1	1	5	1	1	32	31	3
UGK52	Black	Landrace	64	34	3	2	1	1	3	1	1	41	19	3
UGK53	Cream with black stripes	Landrace	59	36	50	4	1	2	3	1	1	33	49	3
UGK54	Purple large	Landrace	53	35	0	4	2	1	4	1	1	31	46	5
UGK55	Cream with coffee stripes	Landrace	56	34	0	5	1	3	3	1	2	36	27	3
UGK56	Mazongotto/nabe12c	Landrace	59	30	0	2	2	3	3	1	1	46	46	1
UGK57	Calima	Landrace	62	33	0	3	2	1	4	1	1	36	35	3
UGK58	Cream with coffee speckles	Landrace	54	29	1	6	3	1	3	1	1	33	39	4
UGK59	Nabufumbo(pink)	Landrace	62	37	0	4	2	2	2	1	1	43	24	3
UGK60	Calima(tanzania)	Landrace	59	28	0	4	2	2	3	1	1	32	44	4
UGK61	Black	Landrace	69	38	90	0	1	1	1	1	1	45	16	3
UGK62	Purple	Landrace	58	30	0	3	2	2	3	1	1	33	51	4

UGK63	Cream	Landrace	65	37	0	3	3	1	3	1	1	44	21	3
UGK64	Cream	Landrace	62	32	0	4	3	1	1	1	1	41	33	3
UGK65	Yellow	Landrace	59	32	0	4	1	2	3	1	1	31	35	2
UGK66	Coffee	Landrace	55	30	42	1	2	2	2	2	1	38	16	2
UGK67	Cream with black speckles	Landrace	61	33	0	1	2	1	3	1	1	42	23	3
UGK68	Cream	Landrace	77	35	0	2	3	1	5	2	1	33	31	1
UGK69	Kanyebwa pink with brown speckles	Landrace	55	30	0	3	2	3	5	1	2	31	34	4
UGK70	Kanyebwa	Landrace	50	28	-1	3	2	1	3	1	2	31	35	4
UGK71	Cream with pink stripes	Landrace	64	36	0	3	2	2	4	1	1	32	29	3
UGK72	Cream	Landrace	77	37	32	2	2	2	3	1	1	44	20	3
UGK73	Nabufumbo(pink)	Landrace	62	39	0	3	2	1	3	1	1	39	22	3
UGK74	White	Landrace	67	36	32	1	1	1	4	1	1	44	19	2
UGK75	U00054	Landrace	67	31	40	1	2	1	3	1	1	38	19	3
UGK76	Coffee/matokwor(duukadde asiwuse)	Landrace	54	35	77	2	2	1	4	2	1	35	15	2
UGK77	Cream	Landrace	65	36	0	2	1	1	4	1	2	44	16	3
UGK78	Coffee	Landrace	61	35	42	1	4	1	4	1	1	36	16	4
UGK79	Large red kidney	Landrace	51	30	0	3	2	1	3	2	1	32	50	4
UGK80	Cream(mary meda)	Landrace	69	30	75	0	2	1	4	1	1	42	18	2
UGK81	Cream(agwede agwede)*roba1	Landrace	70	35	15	2	2	1	3	1	1	44	17	2
UGK82	White	Landrace	56	35	0	4	3	1	3	1	1	39	16	4
UGK83	Red	Landrace	68	30	3	1	2	2	3	1	2	45	20	2
UGK84	Marrow with cream speckles	Landrace	57	32	0	3	2	1	3	1	1	36	19	5
UGK85	Calima short	Landrace	75	39	5	4	4	2	4	2	1	35	54	4
UGK86	Calima	Landrace	48	30	0	2	2	4	3	1	1	38	32	3
UGK87	Pink with marrow stripes(kanyebwa)	Landrace	49	32	0	3	2	3	3	1	1	38	29	2
UGK88	Cream with purple stripes	Landrace	61	33	0	4	2	1	5	1	1	32	27	5
UGK89	Pink with marrow stripes(kanyebwa)	Landrace	57	29	0	2	2	2	4	2	1	32	35	3
UGK90	Cream	Landrace	67	31	0	4	2	1	4	2	1	41	23	3
UGK91	Pink with marrow speckles	Landrace	51	29	0	3	3	2	4	1	1	32	25	4
UGK92	Cream with black speckles	Landrace	61	40	0	4	2	2	3	1	1	32	25	4
UGK93	Masindi yellow round	Landrace	54	30	5	2	2	2	4	1	1	31	36	3
UGK94	Calima(sayitoti)	Landrace	71	36	18	3	2	1	3	1	1	34	38	4
UGK95	Coffee	Landrace	78	35	0	3	2	1	3	1	1	44	24	3
UGK96	Yellow	Landrace	56	30	0	3	3	3	4	2	1	32	32	3
UGK97	Calima(seed engufu)	Landrace	63	34	0	4	2	2	3	1	1	32	31	3
UGK98	Calima	Landrace	55	31	0	2	1	2	2	1	1	33	29	2

UGK99	Nabufumbo	Landrace	65	35	0	3	3	1	4	1	1	40	25	2
UGK100	Purple with cream speckles	Landrace	60	33	0	4	2	3	3	1	1	34	31	2
UGK101	Cream with black speckles	Landrace	62	30	0	3	3	1	3	1	1	38	30	4
UGK102	Cream with pink stripes	Landrace	70	34	0	3	3	1	3	1	1	44	27	4
UGK103	Black	Landrace	78	37	0	5	2	1	5	1	1	47	22	2
UGK104	Orange	Landrace	62	29	2	2	2	1	4	1	1	46	20	2
UGK105	Red large kidney	Landrace	60	33	0	5	1	2	3	1	1	31	36	5
UGK106	Coffee	Landrace	63	36	0	3	2	2	3	1	1	31	50	3
UGK107	Cream	Landrace	67	35	0	2	2	1	2	1	1	46	16	3
UGK108	Cream	Landrace	64	33	0	3	2	1	3	1	2	43	34	3
UGK109	Kanyebwa	Landrace	51	29	0	3	1	2	2	2	1	31	31	3
UGK110	Mcm1015	Landrace	64	33	5	3	2	1	3	1	2	34	16	3
UGK111	Grey(akola amero)	Landrace	77	40	18	2	2	1	3	1	1	45	18	2
UGK112	Black	Landrace	68	32	55	1	2	2	2	1	1	44	17	3
UGK113	Manyigamulimi	Landrace	56	36	8	3	4	1	4	2	3	39	42	4
UGK114	Mixture	Landrace	69	34	0	1	2	1	4	1	1	42	17	4
UGK115	White	Landrace	60	38	0	2	3	3	4	1	1	39	18	2
UGK116	Coffee*	Landrace	87	37	2	2	2	1	3	1	1	44	26	2
UGK117	Cream(like roba1)	Landrace	76	37	17	2	2	2	4	1	1	43	18	3
UGK118	White	Landrace	60	31	0	1	1	1	4	1	1	44	19	2
UGK119	Grey	Landrace	68	30	75	2	2	1	2	1	1	40	15	3
UGK120	Kanyebwa	Landrace	51	29	0	5	2	1	3	1	3	32	30	4
UGK121	Pink	Landrace	61	38	0	4	2	1	5	1	1	43	21	4
UGK122	Coffee	Landrace	65	31	0	2	3	1	3	1	1	43	16	4
UGK123	Orange	Landrace	65	39	0	4	2	2	3	1	1	33	23	4
UGK124	Red small	Landrace	68	36	0	5	2	2	4	1	1	39	23	4
UGK125	Pink	Landrace	54	32	0	3	1	2	3	1	1	41	20	3
UGK126	Coffee small	Landrace	59	34	53	1	3	1	2	3	1	36	16	3
UGK127	Purple with white speckles	Landrace	63	34	28	2	2	2	6	1	1	34	38	5
UGK128	Kanyebwa	Landrace	56	29	8	3	2	2	4	2	1	32	33	4
UGK129	Pink mixture	Landrace	62	36	0	3	2	1	4	1	1	39	20	4
UGK130	Orange	Landrace	57	32	0	4	1	2	4	1	1	40	22	3
UGK131	Orange with marrow stripes	Landrace	65	33	0	3	1	3	1	1	1	42	39	3
UGK132	Orange	Landrace	58	33	0	4	1	2	4	1	1	42	22	2
UGK133	Red	Landrace	69	31	2	2	3	1	3	1	1	42	21	2
UGK134	Pink with white speckles	Landrace	69	38	0	4	3	3	2	1	1	39	24	2

UGK135	K20	Landrace	59	33	0	2	1	1	2	1	1	40	36	3
UGK136	Black	Landrace	61	34	3	2	2	1	3	1	1	44	18	3
UGK137	Large white coffee stripes	Landrace	64	33	0	4	1	3	2	1	1	41	54	3
UGK138	Yellow large kidney	Landrace	60	31	0	2	2	2	4	1	1	35	33	4
UGK139	Cream with black speckles	Landrace	72	32	5	6	3	1	3	1	1	33	31	4
UGK140	Marrow	Landrace	64	39	0	7	2	1	2	1	1	42	22	4
UGK141	Cream with pink spickles	Landrace	55	28	0	2	3	2	3	1	1	44	35	2
UGK142	Cream small	Landrace	66	35	0	2	1	1	2	1	1	45	17	3
UGK143	Pink with white speckles(kahura)	Landrace	55	34	0	3	3	2	3	3	1	39	18	4
UGK144	Orange	Landrace	68	38	0	4	2	1	3	1	1	35	23	4
UGK145	Cream with black stripes	Landrace	68	38	0	4	2	1	2	1	1	42	19	3
UGK146	Calima purple with white speckles	Landrace	45	32	0	4	2	3	2	1	1	40	31	4
UGK147	Red	Landrace	61	38	0	6	2	2	3	1	1	41	23	5
UGK148	Cream coffee striped	Landrace	56	38	2	5	1	1	1	1	1	38	30	3
UGK149	Black	Landrace	78	36	0	5	1	2	2	1	1	42	21	3
UGK150	Kanyebwa	Landrace	48	31	0	3	1	2	2	1	1	38	32	3
UGK151	Orange with coffee stripes	Landrace	64	28	2	4	2	1	3	1	1	47	37	2
UGK152	Red	Landrace	56	31	0	5	2	1	1	1	1	46	25	4
UGK153	Calima(sofia sofia)	Landrace	58	33	5	2	2	2	4	1	1	32	32	3
UGK154	Black	Landrace	70	33	3	4	2	1	2	1	1	41	20	5
UGK155	K131	Released	58	32	2	2	1	1	3	1	1	43	19	3
UGK156	K132/Cal96 (Low Fe/Zn check)	Released	56	30	0	4	2	1	3	3	1	33	46	4
UGK157	NABE 1	Released	62	31	8	2	2	1	2	1	1	34	39	3
UGK158	NABE 2	Released	62	33	8	3	2	1	2	1	1	39	18	2
UGK159	NABE 3	Released	71	34	0	2	2	1	3	1	1	46	19	3
UGK160	NABE 4	Released	61	29	0	3	1	2	2	1	1	35	44	4
UGK161	NABE 5	Released	60	34	0	3	1	2	2	1	1	35	50	2
UGK162	NABE 6	Released	63	33	0	2	1	1	2	1	1	44	20	2
UGK163	NABE 7C	Released	58	30	0	2	2	1	3	1	1	42	25	2
UGK164	NABE 8C	Released	65	31	0	3	2	2	2	1	1	47	39	3
UGK165	NABE 9C	Released	56	29	0	3	1	1	2	1	1	43	41	2
UGK166	NABE 10C	Released	61	35	2	3	1	1	2	1	1	44	22	2
UGK167	NABE 12C	Released	58	30	2	3	1	2	3	3	1	46	46	1
UGK168	NABE 13	Released	50	33	20	2	1	1	2	1	1	37	31	1
UGK169	NABE 14	Released	62	35	0	3	1	2	2	2	1	38	41	3
UGK170	NABE 15	Released	53	31	0	4	2	2	3	1	1	32	34	3

UGK171	NABE 16	Released	56	28	0	2	2	2	4	2	1	34	40	3
UGK172	NABE 11	Released	64	31	0	2	2	2	3	2	1	34	43	3
UGK173	NARBRL 252	Pre-released	71	34	0	4	2	1	4	1	1	33	38	4
UGK174	NARBRL 50-1	Pre-released	47	26	3	3	1	1	2	1	1	37	41	4
UGK175	NARBRL 220	Pre-released	56	27	0	4	2	1	3	1	1	32	44	5
UGK176	NARBRL 110-2	Pre-released	66	33	0	4	2	1	3	1	1	33	37	4
UGK177	NARBRL 224-1	Pre-released	56	34	25	2	2	1	2	1	1	36	34	4
UGK178	NARBRL 122-1	Pre-released	60	34	0	3	2	2	3	1	1	32	44	4
UGK179	NARBRL 53-3	Pre-released	63	38	2	3	3	2	4	1	1	33	35	4
UGK180	NARBRL 60	Pre-released	46	31	0	3	2	2	3	1	1	32	29	3
UGK181	UGNAM 40-5/6	Pre-released	60	33	0	4	2	2	3	1	1	32	31	4
UGK182	UGNAM 30-3/5	Pre-released	55	32	0	4	1	1	1	1	1	34	45	3
UGK183	UGNAM 40-1/5	Pre-released	60	30	0	3	2	2	3	3	2	34	36	2
UGK184	UGNAM 10-3/5	Pre-released	61	33	0	3	1	1	2	1	1	34	42	3
UGK185	UGNAM 10-4/5	Pre-released	73	33	3	3	2	2	3	1	1	32	46	1
UGK186	UGNAM 40-2/22	Pre-released	61	34	0	3	2	3	3	1	1	32	32	2
UGK187	UGNAM 10-2/1	Pre-released	66	32	0	3	2	1	2	2	1	35	33	3