International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 25 No. 1 Dec. 2018, pp. 131-139

http://www.ijias.issr-journals.org/

Identification of biofortified beans (Phaseolus vulgaris L) : Case study on genetic diversity, relationship and rates of iron and zinc concentrations in farmer's accession, in eastern DR Congo

N. Mbikayi[1], A.D. Mumba[2], P.M. Kiman[3], R. Kizungu[4], and C. Mirindi[1]

[1]Institut National pour l'Etude et la Recherche Agronomiques (INERA), B.P. 2037 Kinshasa 1, RD Congo

[2]Université Pédagogique Nationale (UPN), Département de Phytotechnie, B.P. 8815, Kinshasa-Ngaliema, RD Congo

[3]University of Nairobi (OUN), Kenya

[4]Université de Kinshasa-11, Faculté d'Agronomie, RD Congo

ABSTRACT: The aim of the study was to determine variation in iron and zinc concentrations in farmer's accessions, relationship, and rates between the two essential minerals. Six sites were utilized for collecting accessions in two bean growing zones: South and North Kivu. The South Kivu comprised: Kabare, Walungu and Uvira and the North Kivu: sites of Goma, Rutshuru and Butembo. The CIAT Atlas "African Bean Environment (AFBE) was utilized to characterize the sites. Thousand collected accessions were characterized and went seed increase. The mineral analysis was carried out at Adailine Laboratory, Australia using the inductively coupled plasma (ICP)-method. The 383 genotypes selected went statistical analyses. The ANOVA and correlation analysis was performed using Pearson's product-moment method to determine the relationship between iron and zinc concentrations. The rates and trends of iron and zinc concentrations were calculated using respectively R software Analysis and the Scatter Diagram Technique. All data were analyzed using Statistix10 and R Development Core Team Software. High variation in iron and zinc concentrations with mean rages of 71.63 ppm for iron and 30.69 ppm for zinc was found. Highly significant correlation (r=0.94***) between iron and zinc concentrations. The effects of variety, environment and growth habit on the mineral concentrations of genotypes were highly significant (***). The rates calculated based on targets were: 3.4% at low concentration (40-50 ppm); 21.7% at baseline level (50-60ppm); 31.0% at increment target (60-70ppm); 21.4% at 1st wave target (70-80ppm); at 2nd wave target (80-90 ppm); and 3rd wave target ≤94 ppm).

KEYWORDS: Bean farmer's accessions, Biofortified beans, Mineral analysis.

1 BACKGROUND

Common bean is one of the staple foods in the Democratic Republic of Congo (RDC). It is regarded as the near-perfect food or "meat for the poor as farmer said. Bean provides cholesterol-free dietary proteins, energy, folic acid, fiber and micronutrient such as iron and zinc in a variety of dishes. More than 50% of the beans consumed in DR. Congo is produced in the eastern part of the country that encompasses the provinces of South and North Kivu. The sustainability of the bean production in the region has been based on the selection and utilization of cultivars from accessions maintained by farmers for many generations. Farmers maintain bean accessions as pure stands or mixtures of seeds depending up on objectives and environments. The pure stands accessions are oriented on market demand. However mixture accessions designated for home consumption and are more resilient to stresses.

The biofortified beans are rich in iron and zinc micronutrients and remain ways of reducing iron deficiency which the public health problem in the D.R. Congo and in the region of African. In the regions of the North and south Kivu, beans are staple food and the consumption has been estimated at 300 grams per capita per day **[1].** The surveys conducted on five main legume crops the most eaten, the beans ranked first with 83.8 %, followed by groundnut (8.1%), soybeans (6.3%), peas (0.9 %) and others (0.9%). The mean production of six years had showed high production of these four legume crops: 38.925,2 Tones for beans (75%); 11,707.8 Tones for the groundnut (22%); 862.3 Tones for Soybeans (1.7%); and 673.8 Tones or (1.3%) for the peas **[2].** However, in spite of theses crop productions, the prevalence of iron deficiency in the country was found higher: 36% of the pre-school children in North Kivu, and 47% in South Kivu had iron deficiency anemia **[3].** At the national level, the surveys conducted in 2005 have showed 71% iron deficiency among pre-school children, and 53% among pregnant women **[4].** In general people suffer from iron deficiency and iron deficiency anemia which may be provided by the poor diet food eaten by majority of people. The question is why bean has not contributed to food diet in spite of its micronutrient contents? This answer would be oriented on the presence of micronutrients in the farmer's beans accessions and availability in the consumed food. The quality of the famer's bean accessions should be known and the diet food should be improved by incorporating the biofortified bean varieties in the farmer's seeds.

Biofortification aims at generating nutritionally improved crop varieties through conventional plant breeding and modern biotechnology to achieve a measurable and positive impact on human health **[5].** It is a novel genetic approach that combines high concentration as one of the selection traits with other desirable agronomic traits.

Breeding biofortified bean varieties is a low cost breeding approach designed to combat public health problems such as iron deficiency in diets of the poor people. Moreover, incorporation of participatory breeding approaches can enhance rapid adoption and wider reach with these biofortified varieties **[6].**

The two sets of fast-truck bean lines composed of bush and climbing local germoplasme accessions were evaluated in the central Africa from 2004 - 2007 **[7].** The first generation (or Fast track) biofortified bean was targeted in 2008 with 70 ppm iron and 30 ppm zinc. In 2009, the development of second generation biofortified lines started with higher iron and zinc targets: iron 90 ppm and zinc 40 ppm. The current work is to screen germoplasme to identify lines or cultivars higher in iron and zinc than fast track bean lines having 94 ppm or more to fulfill daily intake requirement. Information has been focused on the baseline, target genotype and estimated biofortified target increment [8]. Previous germoplasme collections had showed high incidence of Fe and Zinc rich genotypes originated from this region **[9].**

The purpose of this study was to identify the target biofortified bean genotypes in the farmer's bean accessions in eastern DR. Congo and to estimate their rates in the collected bean accessions.

2 MATERIALS AND METHODS

The main activities were focused on characterization, evaluation and analysis of iron and zinc mineral concentrations in identified bean genotypes.

2.1 COLLECTION OF BEAN ACCESSIONS

Farmer's bean accessions were collected in the bean growing areas of eastern D.R. Congo. The accessions included landraces and improved varieties introduced by the National Bean Program to sustain production, consumption and trade of beans.

2.2 ZONES OF ACCESSION COLLECTIONS

Accessions were collected from 2006 to 2012. Samples were collected from Kabare, Walungu and Uvira in South Kivu, and Goma, Rutshuru and Butembo in North Kivu. These bean growing zones were described according to the CIAT Bean Atlas called "African bean environment (AFBE)" [10] as followed:- The South Kivu and North Kivu zones were covered by AFBE2, AFBE7 and AFBE8.

AFBE2: Sub humid highlands on acid soils at low latitudes, 1.0 S and 5.0 S. >1500 masl; >400 mm with available moisture; with soil pH <5.5; Bimodal/Photoperiod (with neutral effects on bean plants); on dystric Nitosol.

AFBE7: Sub humid areas at mid-altitudes and low latitudes are described as followed: At North-East and West Kivu on latitude 3.0 N and 0.0 of the orthic Ferralsol type; with 1000-1500 masl; >400 mm available moisture; with soil pH >5.5; bimodal/Photoperiod (with neutral effects on bean plants); on orthic Ferralsol and eutric Nitosol in the North-east and humic Nitosol and dystric Nitosol in west Kivu.

AFBE8: Sub humid areas at mid-altitudes and mid-latitudes, at latitude 5.0 S and 13.0 S. 1000-1500 masl; >400 mm available moisture; on rhodic Ferralsol, with soil pH >5.5: unimodal/Photoperiod (with probable effects on bean plants)

More than 1000 bean accessions were collected, characterized, evaluated step by step according to available spaces in the greenhouse. To avail seeds for mineral analysis, the selected genotypes seeds went seeds increase in the field. In the greenhouse, each genotype was planted by row on the sol benches. Each bench was filled with fertile soil collected from the field. From the thousand collected bean accessions, 880 genotypes were identified after mineral analysis **[11]**. The fields activities were carried out based on the following protocol: the area of 2700 m^2 covers bush and climbing bean types. A one row line planting, for the bean bush type, was utilized by genotype that gave a plot size of 3 m long and 0.4 m large that gave the plot size of 1.2m^2. For the climbing bean types, a one row line was also utilized as followed: a line of 3 m long with 0.5 m large that gave a plot of 1.5 m^2. The spacing within row was 0.10m. Evaluation parameters were based on the morphological and physiological stresses. After characterization and evaluation of genotypes, seeds samples were sent to laboratories of the Centre International d' Agriculture Tropical (CIAT), Cali, Colombia and at the Waite Analytical Services (WAS), Australia for mineral analyzes.

2.3 STATISTICAL ANALYSIS

Two types of bean samples were utilized for the statistical analyses: 1) 383 genotypes having at least 20 ppm iron and zinc concentrations were selected to study variation in iron and zinc concentrations, relationships and rates of the iron and zinc concentrations in the bean sample **[12],** and their trends have also been studied [**3]** 2) The second set of twelve realized bean varieties identified among the collected bean genotypes samples were utilized to study the effects of variety, growth habit and environment on the iron and zinc concentrations. Thus, ANOVA, correlations analysis based on Pearson's product-moment method and Scatter Diagram Technique were performed utilizing the Statistix 10 and the R Software Analysis.

3 RESULTS

Two sets of samples that were composed of 383 genotype samples and second set of 12 released bean varieties collected among thousand accessions. The first set of accessions was used to study the diversity of genotypes, relationship, rates and trends of iron and zinc concentrations. Two Tables: 1 and 2 have shown data on different concentration targets for iron and zinc. The second set of released bean varieties, identified in the farmer's bean accession was utilized to study effects of varieties, growth habits and environments on the mineral concentrations in the two main bean growing zones that were South Kivu and North Kivu.

3.1 VARIATION IN IRON CONCENTRATION

The study has observed variation in morphological and physiological characteristics of genotypes in the farmer's accession sample. The diversity of genotypes was confirmed by the mineral analysis conducted on iron and zinc concentrations of the bean sample. The Table 1 are divided in five colons: showing : concentration targets, Iron scope, number of genotypes and the % iron increment or arte. The concentration target started with: low iron concentrations, baseline, iron increment, 1st wave, 2nd wave and 3rd wave target.

At low iron concentration target level, the iron scope varied from 40 to 50 ppm in 13 genotypes and the iron increment rate was 3.4% in the bean sample. At baseline level, the iron scope was 50-60 ppm in 83 genotypes and their increment rate was 21.7%. At iron increment target, the iron scope was 60-70 ppm in 119 genotypes and their increment rate was 31.0%. At 1st wave target, the iron scope was 70-80 ppm in 82 genotypes increment rate of 21.4%. At 2nd wave, the iron scope was 80-90 ppm in 45 genotypes and their increment was 11.8%. In the 3rd wave, the scope was equal and more than 94ppm in 41 genotypes and their increment rate was 10.7%. All genotypes having iron concentration of 90 ppm or above in comparison with the baseline were considered as the targeted ones.

Previous study has shown that genotype that has iron concentration of 44 ppm above the baseline was targeted as one that can meet a large proportion of the recommended daily intake of iron **[13], [14].** The Table 1 below has shown 41 target genotypes at 3rd wave target which have 90 ppm or more concentration at the concentration rate of 10.7 %. These bean genotypes were: 1)29-4A 5(Fe=95 ppm); 2)39-2A (Fe=105 ppm); 3)60-1A: (Fe=95 ppm); 4)61-3A (Fe=105ppm); 5)66-2A (Fe=100 ppm); 6) CAB2(Fe=103ppm); 7)COD65-5(CODMLB166/05)Fe=95ppm); 8)COD66-1(CODMLB167/05 (Fe=100ppm); 9)COD74-1(CODMLB175/05) Fe=105ppm; 10)COD85-1(CODMLB186/05) Fe= 95ppm; 11)COD88-7(CODMLB189/05) Fe=100ppm; 12)CODMLB166/05(COD65-5) Fe=95ppm); 13)CODMLB167/05(COD66-1) Fe=100ppm; 14)CODMLB175/05(COD74-1) Fe=105ppm); 15)CODMLB186/05 (COD85-1) Fe=95ppm); 16)CODMLB377/08 Fe=98 ppm; 17)CODMLB389/08 Fe=119ppm; 18)CUARENTINO0817 Fe=101ppm); 19)DIANIANIA Fe=100ppm); 20)KARARINA-ROUGE Fe=95ppm); 21)KIBULANGITI

Fe=100ppm); 22)KIJAMBERE1 Fe=95ppm); 23)KINIZO Fe=100ppm); 24)KINYARUKA Fe=105ppm); 25)MAC442 Fe=97ppm); 26)MAGHAVIRI Fe=100ppm); 27)MLB-49-89A Fe=124ppm); 28)MUKARA Fe=95ppm); 29)NABUZIRO Fe=100ppm); 30)RWV3316 Fe=97ppm); 31)SMC18 Fe=107ppm); 32)SMC21 Fe=99ppm); 33)U10-7 Fe=100ppm); 34)U2-11 Fe=95ppm); 35)U3-8 Fe=95ppm); 36)U4-4 Fe=95ppm); 37)U8-16 Fe=105ppm); 38)U8-17 Fe=95ppm); 39)UI-4 Fe=95ppm); 40)UI-7 Fe=95ppm); 41)UI10-6 Fe=95ppm).

Table 1. Concentration targets (waves) of iron in the farmer's bean accessions

Targets	Iron. scope	Number of genotypes	% ppm iron increment
Low iron concentration	40-50 ppm	13	3.4
Baseline level	**50-60 ppm**	**83**	**21.7**
Iron increment	60-70 ppm	119	31.0
1st wave	70-80 ppm	82	21.4
2nd wave	80-90 ppm	45	11.8
3rd wave	**≤ 94 ppm**	**41**	**10.7**
TOTAL	**-**	**383**	**100.0**

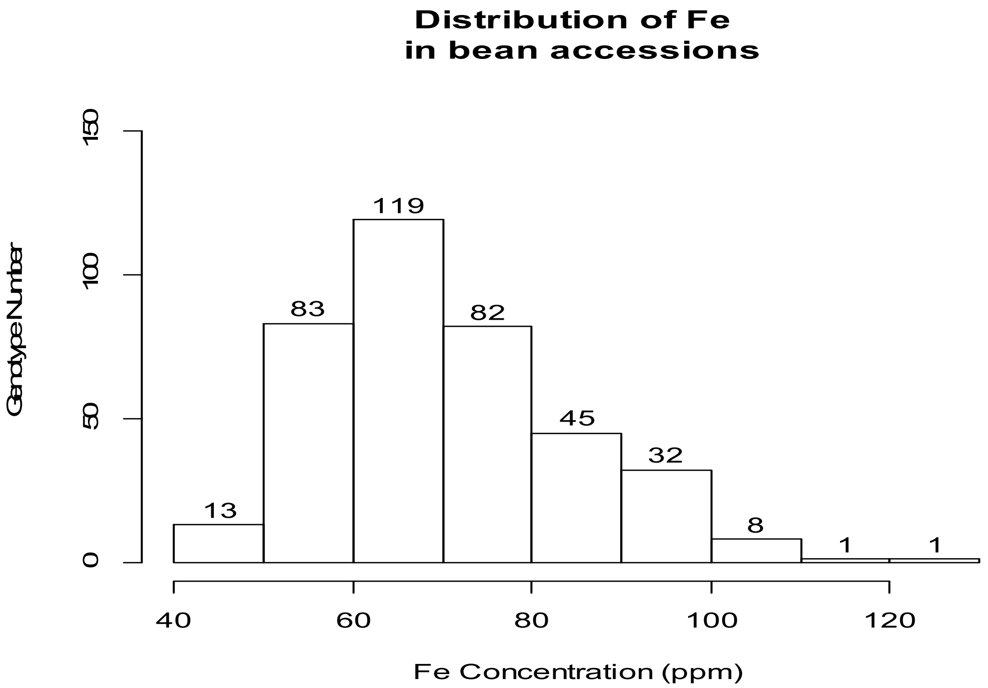

Fig. 1. Distribution of iron concentration in bean genotype sample

The distribution of data has shown clearly detailed characteristics and concentration targets. However the figure has shown an outlier which has more than 120 ppm as shown in the Figure 1.

3.2 VARIATION IN ZINC CONCENTRATION

The variation in zinc concentration was also observed with much less concentration comparing to the iron concentration (Table 2). The trend in zinc concentration was similar as with iron concentration as explained by the correlation cited above.

At low iron concentration target level, the zinc scope varied from 20 to 25 ppm in 83 genotypes and the iron increment rate was 21.7% in the bean sample. At baseline level, the zinc scope was 25-30 ppm in 150 genotypes and their increment rate was 39.2%. At iron increment target, the zinc scope was 30-35 ppm in 96 genotypes and their increment rate was 25.0%. At 1st wave target, the scope was 35-40 ppm in 38 genotypes increment rate of 9.9%. At 2nd wave, the zinc scope was 40-45 ppm in

11 genotypes and their increment was 2.9%. In the 3rd wave, the scope was equal and more than 47ppm in 5 genotypes and their increment rate was 1.3% ppm. All genotypes having iron concentration of 47 ppm or above in comparison with the baseline were considered as the targeted ones. Those target genotypes were:1)CODMLB085 Zinc=65ppm; 2)MLB-49-89A Zinc=55ppm; 3)Zebra Zinc=49ppm; 4)NUA134 Zinc=49ppm; 5)NUA131 Zinc=50ppm. The zinc mineral was considered, in the study as a second variable in comparison to the Fe mineral. These target zinc genotypes that represented 1.3% had fulfilled the requirement of daily intake for zinc micronutrient.

Table 2. Concentration targets of zinc in the farmer's bean accessions

Targets	Zinc scope	Number of genotypes	% ppm zinc increment
Low zinc concentration	20-25 ppm	83	21.7
Baseline level	**25-30 ppm**	**150**	**39.2**
Zinc increment	30-35 ppm	96	25.0
1st wave	35-40 ppm	38	9.9
2nd wave	40-45 ppm	11	2.9
3rd wave	**≤ 47 ppm**	**5**	**1.3**
TOTAL	**-**	**383**	**100.0**

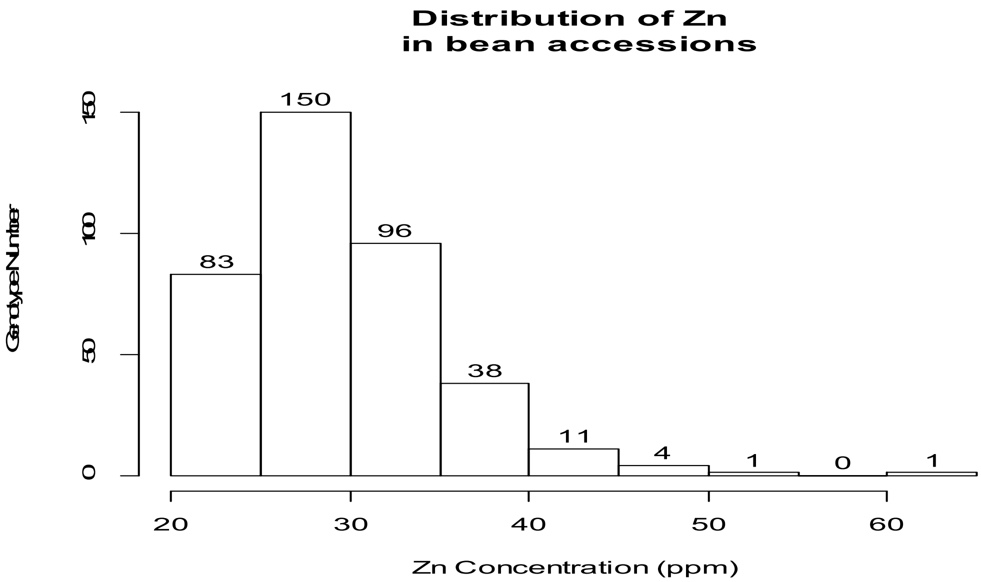

Fig. 2. Distribution of zinc in bean accessions

3.3 CORRELATION BETWEEN IRON AND ZINC CONCENTRATION

In this study the Fe element was the important variable comparing to the zinc element because the iron deficiency has been the most prevalent micro nutrition problem in the word affecting more than 2 billion people using beans as staple food [15]. It has been an essential element to improvement of nutritional status of human being. The relationship between these elements has been evaluated to help decide on the breeding strategy to be undertaken in development of biofortified bean varieties. The iron element was considered as independent variable, most important to zinc element in this study.

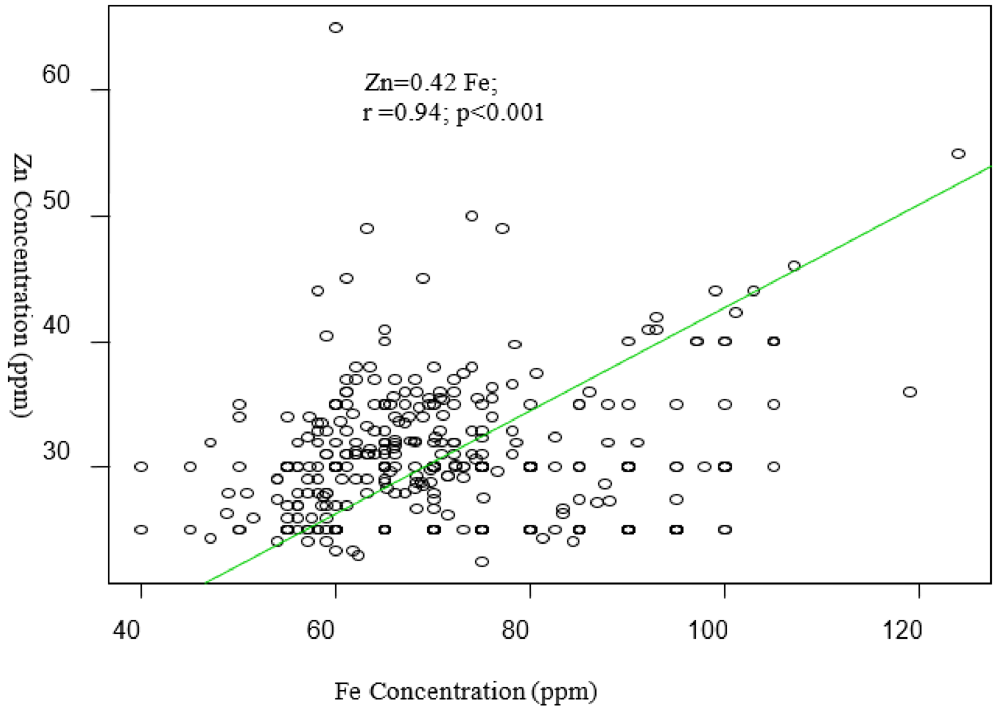

Fig. 3. Relation between iron and zinc and wave iron concentrations of genotypes

N.B: Pearson's product – moment correlation

The relationship between the iron and zinc elements as the nutritional components in the bean grain was one of the key messages for the bean breeding activity. The results of this study have shown a positive and significant correlation (r^2 = 0.94; P<0.001) according to Pearson's product – moment correlation in Figure 3. The implication of this correlation is the fact that some genetic factors are co-segregating, and that a selection for one element such as iron will in fact result in an increase of other element such as zinc. Previous studies had revealed positive and significant correlation that exists between iron and zinc concentrations in beans [15], [16], [17], [18].

3.4 EFFECTS OF GROWTH HABIT, VARIETY, ENVIRONMENT AND THEIR INTERACTIONS

Statistical analysis was conducted with 12 released bean varieties evaluated in the two main bean growing regions of North and South Kivu. The objective was to know effects of growth habit, variety, environment and their interaction on the Fe and Zn concentrations.

The results had shown significant effects of these variables on the Fe concentration (Table 3) which implied these variables should be evaluated in a precise experiment to get more information that may orient the breeding program.

Table 3. ANOVA of Fe concentration with 12 released bean varieties

Sources	DF	Sum of Squares	Mean Square	F values	P
Growth habit	1	344	344	145.42***	0.000
Variety	11	7099	645.3	272.84***	0.000
Environment	1	1716	1716.4	725.68***	0.000
Growth habit x Environment	1	265	265.2	112.13***	0.000
Vatiety x Environment	10	1788	178.8	75.58***	0.000
Residuals	47	111	2.4		

****Level of significance at 0.001.*

The results had showed that they were highly significant differences (***) between the growth habits (bush and climbing bean varieties), among varieties, between environments (North and South Kivu) and their interactions between growth habit, variety and environment were highly significant. All these sources of variation had showed highly significant effects on the Fe concentrations (Table 3).

As concern the results on the variable zinc, the results were similar to those of zinc, which has confirmed that thes mineral elements were together and acted the same ways. (Table4).

Table 4. ANOVA of Zn concentration with 12 released bean varieties

Sources	DF	Sum of Squares	Mean Square	F values	P
Growth habit	1	17.8	17.8	7.726**	0.000
Variety	11	524.7	47.7	20.664***	0.000
Environment	1	551.5	551.5	238.896***	0.000
Growth habit x Environment	1	36.9	36.9	16.002***	0.000
Variety x Environment	10	453.6	45.4	19.649***	0.000
Residuals	47	108.5	2.3		

****Level of significance at 0.001*

As concern the variable Zn, the same trends were observed in that variables variety, environment and interactions in between growth habits, varieties were highly significant. Their interactions were also highly significant (***). They were a high difference between growth habits too (**), (Table 4).

3.5 RELATIONSHIP BETWEEN IRON AND ZINC AS TWO COMPONENT VARIABLES

To observe the relationship between the two components, iron and zinc in the farmer's bean accessions, the scatter diagram technique was utilized to compare the trends of the two variables. The scatter diagram is the simplest and most commonly used procedure for examining the relationship between two variables in Figure 4. The increment of the iron concentration up to 120 ppm was much higher than the zinc increment at 60 ppm as shown by the quantile plot. **[3]**.This technique has allowed identifying the outliers of the two variables which have shown the presence of the concentration target levels in the Figure 1 and 2.

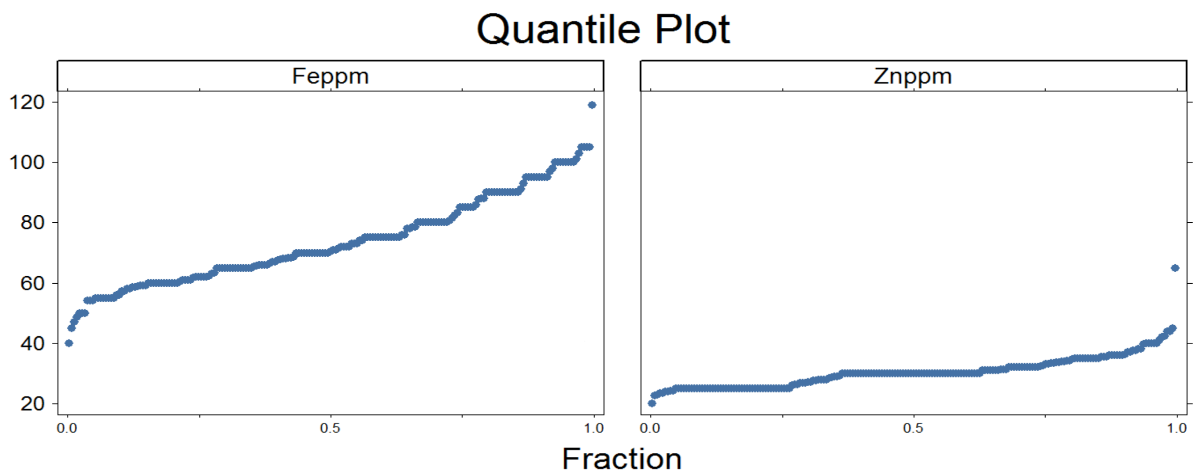

Fig. 4. Comparison of relation trends between Fe and Zn concentrations in the bean farmer's accession

The trend of iron concentration varied from 40 ppm to more than 100 ppm with outlier located at 120 ppm. However the zinc trend has showed a small increment varying from 20 ppm up to 40 ppm with outlier at more than 60 ppm. The same message on the relationship between iron and zinc in the quality of the farmer's bean accessions was confirmed by the above figure 4.

4 Discussion

The study has tackled same hypotheses which were based on the presence or absence of the genetic variability in the farmer's bean sample; the relationship between iron and zinc concentration as two important variables in the bean grain and the effects of variety (genotype), growth habit and environments on iron and zinc concentrations.

Based on these hypotheses, characterization and evaluation of the genotypes in the farmer's bean accession were shown variation in morphological and physiological characteristics. This observation was confirmed by the mineral analysis conducted on the identified genotypes in the farmer's bean sample. The variation in iron and zinc concentrations varied, for iron, from 40 ppm to 124ppm with mean of 71.63ppm. As concern the zinc concentration, the variation in concentration varied from10 ppm to 65ppm with mean at 30.69ppm. The relationship between the iron and zinc concentrations was significantly positive (r=0.94***), which implied that the improvement in quality of an element may affect another during gametic segregation. The trend of the two mineral concentrations was positive at different concentration based of the mineral element.

The concentration rate at different concentration target has shown the quality of the farmer's bean sample. The baseline level for iron concentration was improved from 50 ppm with 83 genotypes to 70 ppm with 119 genotypes. But the requirement iron intake was reached at 94ppm or more with 41genotypes at the rate of 10.7% which implied that much work should be done by breeding program in releasing of the more target biofortified bean to improve quality of the farmer's bean accessions.

The effects of variety (genotype), growth habit and environment on iron and zinc concentrations were highly significant which implied that different variable should be study differently to get more information as concern action of each factor or variable.

5 Conclusion

The genetic variability on common bean has been observed in the South and North Kivu based on the results of this study. Same bean landraces collected in the two zones were high mineral concentration, specially iron such as: 1)DIANIANIA (100ppm); 2)KINYARUKA (105ppm); 3)MAGHAVIRI (100 ppm); 4)NABUZIRO (100ppm)...etc. But the quality of the farmer's bean accessions should be improved to combat iron deficiency and improving diets of poor population. With 10.1% as rate of the target genotypes in the farmer's bean accessions, it is impossible to provide 30% of the mean daily iron requirement through normal consumption habits. Thus more breeding work should be undertaken to improve the rate of target bean genotypes in the farmer's accessions by releasing more biofortified bean varieties.

References

[1] HarvestPlus Product Profile 2004-2007
[2] Annuaire des Statistiques Agricoles 2006 – 2011, MEDIASPAUL – Kinshasa Mars 2013. www.mediaspaul.cd
[3] Beebe, S. 2009. Iron Bean Brochure. CIAT A.A. 6713 Cali, Columbia. (www.ciat.org)
[4] Programme National de Nutrition "PRONANUT ». (2005) Enquête sur la prévalence de l'anémie en République Démocratique du Congo. Ministère de la Santé.
[5] Pfeiffer, W.H. and McClafferty, B. (2007) HarvestPlus: Breeding Crops for better nutrition. Crop Science 47: (3) S88 – S105.
[6] HarvestPlus 2011 Annual Report (Breaking Ground).
[7] HarvestPlus Product Profile Report 2004-2007.
[8] CIAT Malawi
[9] Blair MW, Gonzalez L.F, Kimani P.M, Butare L (2010) Genetic diversity, inter-gene pool introgression and nutritional quality of common beans (*Phaseolus vulgaris* L.) from Central Africa. Theor Appl Genet (2010) 121:237-248.
[10] Wortmann CS, RA Kirkby, CA Eledu, DJ Allen (1998) Atlas of common bean (*Phaseolus vulgaris L.*) production in Africa. CIAT, Cali, Colombia, p 133 (CIAT publication; No 297) ISBN 958-9439-94-2.
[11] Mbikayi, N.T., Kijana R., Koleramungu, Mirindi, T., Bakulikira, R. E., and Soda, K. (2006) Caractérisation des Variétés de Haricot commun. "AKORPRESS" à Kinshasa-Limete.
[12] R Development Core Team (2012). R: A language and environment for statistical computing. R: Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.
[13] Graham R, Senadhira D, Beebe S, Iglesias C, Monasterio l (1999) Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. Field Crops Res 60: 57-80.
[14] Welch RM, House WA, Beebe S, Cheng Z (2000). Genetic selection for enhanced bioavailable levels of iron in bean (*Phaseolus vulgaris* L.) J Agr Food Chem 48: 3576-3580.

[15] Welch RM, and Graham RD 1999. A new paradigm for word agriculture: Meeting human needs, productive, sustainable, and nutritious. Field Crops Res. 60:1-10.
[16] Beebe, S., A.V. Gomez, and J. Renfigo 2000 Research on trace minerals in the common bean. Food Nutr Bull.21:387-391.
[17] House, W.A., R.M. Welch, S. Beebe. Z. Cheng. 2002. Potential for increasing the amounts of bioavailable zinc in dry beans through plant breeding. J. Sci. Food Agric. 82:1452-1457.
[18] Blair MW, Astudillo C, Grusak MA, Graham R, and Beebe SE (2009) Inheritance of seed iron and zinc concentrations in common bean (*Phaseolus vulgaris* L.). Mol Breeding(2009) 23:197-207.
[19] Muhamba GT and Nchimbi-Msolla S (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), pp. 738-747, 18 April, 2010.
[20] Statistix 10 User's Manual: Copyright 1985-2013; Analytical Software.