Advances in Crop Science and Technology
ISSN: 2329-8863

Mukamuhirwa and Rurangwa E et al., Adv Crop Sci Tech 2018, 6:2
DOI: 10.4172/2329-8863.1000344

Research Article | Open Access

Evaluation for High Iron and Zinc Content among Selected Climbing Bean Genotypes in Rwanda

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Received date: February 23, 2018; **Accepted date:** March 03, 2018; **Published date:** March 09, 2018

Abstract

Daily consumption of beans in Rwanda may offer the opportunity for alleviating micronutrient malnutrition since other sources of these elements are expensive and/ or unavailable. In light of improving the nutritional status of many Rwandans, identification of new bean varieties able to accumulate high Fe/Zn content in addition to high yield performance would be good news for the country. The objective of this study was to identify and select climbing bean genotypes that have high mean yield, iron and or zinc content that should be the candidates for release for the traits of interest. Trials of seven climbing bean genotypes were conducted in ten research centers of Rwanda Agriculture Board (RAB) in 2017A and 2017B cropping seasons in randomized complete block design with 3 replications. Grain yield differed significantly ($P \leq 0.001$) among the seven climbing bean genotypes evaluated and environments. Strong interactions between genotype and environment were observed ($P \leq 0.001$) for yield, iron and zinc content. Grain yield averaged over the two seasons varied from 554 to 4,906 kg ha-1. The least grain yield was achieved with RWV 2365-2 in Ngoma while greater yield was achieved with RWV 2350-2B in Muhoza. The across environments means revealed that Nyiramagorori had the least grain yield (1966 kg ha^{-1}) while MBC 71 had greater yield (2,502 kg ha^{-1}). Mean iron and zinc content in seeds differed significantly ($P \leq 0.001$) among the seven climbing bean genotypes and ten environments. The mean iron content varied between 54.0 and 91.7 ppm. The genotype 665SI-4/1 averaged a low iron content of 63.0 ppm while 665SI-4/1 averaged a greater iron content of 76.4 ppm. In general three climbing bean genotypes among the seven evaluated showed superior performance for the three traits evaluated (yield, iron and zinc content) across environments. These genotypes include Rwibarura, RWV 2350-2B and MBC71.

Keywords: Common bean; Micronutrient; Accumulation; Malnutrition; Genotype

Introduction

Common beans are staple food in Rwanda and they are grown in almost all parts of the country [1]. There are many criteria that guide farmer's preferences including high yield, early maturity, wide adaptation, resistance to disease and pest, tolerance to drought and other abiotic stresses, good colour and size with high nutritional content (Fe and Zn). Daily consumption of beans in Rwanda may offer the opportunity for alleviating micronutrient malnutrition since other sources of these elements are expensive and/ or unavailable [2]. Beans are used in mineral bio fortification program since they have high iron and zinc content without any prior mechanical processing. Bio fortified beans are sustainable inexpensive and cost effective health food for low and high income people. Bio fortified beans have been tested in diverse agro ecological zones of Rwanda after release of 10 varieties with iron content ranging between 75-94.8 ppm. 14 Bio fortified beans have advantages of being high yielding and high in nutritional quality thus playing an important role in economic development and contributing to food and nutrition security, and may contribute to national nutrition security initiatives.

Though both soil and genotype affect iron accumulation in bean seed [3], their interaction (G × E) is very important in bean iron/zinc content breeding since it demonstrates the changes in the relative performance of the genotypes across different environments. Interaction between genotype and environment either in beans or in other crops may complicates identification of superior genotypes for a range of environments and calls for evaluation of different genotypes in many environments to determine the true genetic potential [4,5].

Though genotype by environment interaction was observed for bean nutritional traits, biofortification is required to meet the demand for food with health benefits [6]. There are many reports on the correlation between iron and zinc content in bean [4,7]. Promoting beans with potential of accumulating high Fe/Zn content may be one of the sustainable strategies to reduce iron and zinc deficiencies in bean eating populations since they provide sufficient genetic variability in iron and zinc concentration which is the basic requirement for biofortification [8].

Eating biofortified beans has a significant impact on iron level in the blood. The study by Hass [2] found an estimate body iron increase of 0.50 mg per kilogram higher in women that consumed biofortifed beans compared to the control group which has eaten normal beans. The same source revealed a significant reduction in iron deficiency and also an increase in haemoglobin levels of all women who ate iron beans. This study showed the most impressive increase in iron uptake than all other staple crops such as rice and pearl millet. According to Nsengiyumva et al. [9] high iron beans have potential of high production and income generation than local bean varieties. It is therefore recommended to increase the diversity of high iron beans and replace the common bean varieties with more nutritious bio fortified varieties.

In light of improving the nutritional status of many Rwandans, identification of new bean varieties able to accumulate high Fe/Zn content in addition to high yield performance would be good news for Rwanda which is the highest per capita bean consumption in the world but also for the region. In Rwanda, nutritional security is of public health concern. In that framework, national initiatives that complement dietary diversification and contributing to better life have been promoted and include eating fruits, vegetables in home gardens, one cup per child, one cow per poor family program, Umurenge vision 2020 program, biofortification of beans as staple food crop [10]. The nutritional status of under five year old children has improved with lower percentages of wasted, stunted and underweight children. Stunting which is the indicator of chronic malnutrition and a key nutritional issue in Rwanda has decreased from 44% in 2010 to 38% in 2015. The prevalence of wasting decreased from 5% to 1.7% and underweight decreased from 11% to 8% (Rwanda Demographic and Health Survey [11,12].

Biofortification of beans and other staple foods is a globally accepted strategy to address micronutrient malnutrition in nutritionally vulnerable groups. Despite this global initiative, NISR [13] has reported a low adoption of improved seed by small scale farmers who represent the majority of bean growers which would lead to food insecurity due to the fact that the total productivity for improved bean seeds ranges from 2-5 t ha^{-1} compared to 0.8-1 t ha^{-1} for landraces [13,14].

Bean breeding desires stable genotypes with good agronomic performance across all environmental conditions. Understanding the variety performance and yield adaptation in diverse zones is of high importance for variety selection and improvement. Existence of negative correlation between plant yield and iron/zinc accumulation ability [4] suggests that selection may go by yield and then iron content for subsequent seasons/evaluations.

The effects of global unpredicted climate change may contribute to changes in crop nutrient content variation as well as crop yield. Therefore, the role of plant breeding to generate and select new plant varieties with high yield, iron and zinc content that can contribute to improving food and nutrition security is highly recognized.

The present study will contribute to food and nutrition security in Rwanda by revealing the most promising new bean varieties adapted to different conditions of Rwanda. Therefore the objectives of this study were (1) to identify climbing bean genotypes that have high mean yield, Fe and or zinc content (2) to select climbing bean genotypes with stability on yield, Fe and Zn content in diverse environments of Rwanda and based on observed results decide the candidates for release for the traits of interest.

Materials and Methods

Trials evaluating the adaptation, yield performance, iron and zinc content of seven climbing bean genotypes were conducted in ten research centres of Rwanda Agriculture Board (RAB) including Rubona, Muhanga, Akanyirandoli, Kitabi, Rwerere, Kinigi, Muhoza, Ngoma, Karama and Nyagatare. The trials were established in 2017A and 2017B cropping seasons. The experiments were laid down in randomized complete block design with 3 replications at each site in plots of 5 m having 5 lines spaced with 50 cm between rows and 20 cm within rows. Sowing in both seasons followed the recommended sowing times (October for Season A and March for Season B). Mineral fertilizer was applied at recommended rates of 50 kg ha^{-1} of DAP combined with 25 MT ha^{-1} of organic manure at planting, and 50 kg ha^{-1} of Urea at weeding as top dressing. Weeds were controlled using a hand hoe, reflecting farmer's common practice in Rwanda.

At crop maturity, iron and zinc content in seeds was analysed using XRF machine located at RAB-Rubona centre. Before the main harvest, 10 well-filled pods from the middle parts of plants of each genotype and free from soil were randomly harvested and put in clean new paper bags (to avoid contamination with dust and dirty while uprooting plants and threshing in bulk). The harvested pods were hand threshed under conditions that keep the seeds as clean as possible [15]. From each genotype, a seed sample weighing about 200 grams was taken [16], cleaned with distilled water, packed in new paper bags. The sampled seeds were further surface cleaned by rubbing them between clean cloths 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. This process reduces Aluminium (Al) contamination from approximately 15 ppm to 2 ppm and by about 5 ppm for Fe contamination while Zn remains unchanged [17]. Thereafter, each sample was oven-dried at 60°C 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 (20-25 setting) and then grinding again on finer setting (0-5 setting). 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 [18]. The ground samples to be analysed were then carefully transferred into small sample cups on the tray, positioned in the machine's tray and identified by labelling them on the screen tray with the sample number. The amount of iron and zinc in the seed was determined by XRF spectrometry by scanning each sample for 100 seconds with spinning of sample cup to analyse Fe and Zn content and records intensities of emitted X-rays. 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). In addition to Fe and Zn content data, yield data was calculated in kg ha^{-1} after harvesting, and all recorded following CIAT new trait dictionary [19].

Data analysis

Statistical analysis with a general ANOVA was performed with site × genotype as fixed factors and replication as random factor. Analysis of variance (ANOVA) was used to compare treatments in a RCBD design using Genstat 16th Edition. Treatment means were compared using the Least Significant Difference (LSD) at $P \leq 0.05$ significance level.

Results

Grain yield assessment

Grain yield differed significantly ($P \leq 0.001$) between the seven climbing bean genotypes evaluated and environments (Table 1). Grain yield averaged over the two seasons varied from 554 to 4,906 kg ha^{-1}. The least grain yield was achieved with RWV 2365-2 in Ngoma while greater yield was achieved with RWV 2350-2B in Muhoza. Averaged across the ten environments, Nyiramagorori genotype had the least grain yield (1966 kg ha^{-1}) while greater yield was achieved with MBC 71 (2,502 kg ha^{-1}). For all the genotypes, greater yields were achieved in sites located in the high lands (Muhoza, Rwerere, Kinigi and Akanyirandoli) and least in low and mid altitude (Ngoma, Karama, Nyagatare, Muhanga, Rubona and Kitabi). The low yield observed in low and mid altitude maybe attributed to the differences in rainfall during the cropping seasons.

Environment\Genotype	665SI-4/1	MBC 71	NYIRAMAGORORI	RWIBARURA	RWV 1129	RWV 2350-2B	RWV 2365-2
Akanyirandoli	3,000	3,000	1,800	1,300	2,400	1,600	2,500
Karama	1,600	1,600	800	2,000	1,200	2,000	1,400
Kinigi	3,850	4,367	3,359	2,842	3,750	3,650	3,984
Kitabi	714	1,571	1,571	1,714	2,786	1,357	1,214
Muhanga	2,775	1,890	1,788	1,285	2,040	2,300	1,360
Muhoza	4,634	4,492	3,234	3,567	4,550	4,917	4,292
Ngoma	747	738	606	1,169	748	653	554
Nyagatare	1,170	1,454	667	956	251	1,480	1,130
Rubona	1,683	1,488	1,461	1,317	1,395	1,754	1,602
Rwerere	4,031	4,418	4,375	4,656	4,906	3,500	3,723
Max	4,634	4,492	4,375	4,656	4,906	4,917	4,292
% Over check	-6	-8	-11	-5	0	0	-13
Min	714	738	606	956	251	653	554
% Over check	184	194	141	281	0	160	121
Mean	2420	2502	1966	2080	2403	2321	2176
% Over check	1	4	-18	-13	0	-3	-9
LSD=220	Grand mean 2,225		%CV=16		G^{***}	E^{***}	GxE^{***}

Table 1: Mean yield of 7 bean genotypes evaluated in 10 locations in national performance trial (NPT) of climber during 2017 A and B seasons. G: Genotype; E: Environment; GxE; ***: Significant at 0.001.

Iron and zinc accumulation

Mean iron and zinc content in seeds differed significantly (P ≤ 0.001) between the seven climbing bean genotypes and 10 environments (Tables 2 and 3). There was large variability within and across environments for both iron and zinc content. For instance mean iron content varied between 54.0 and 91.7 ppm, with the least iron content achieved with RWV 2350-2B in Kanyirandoli and greater iron content with Nyiramagoroli in Karama site. Averaged across environments, a low iron content of 63.0 ppm was observed with 665SI-4/1 genotype against a greater iron content of 76.4 ppm with Rwibarura genotype. In general four climbing bean genotypes among the seven evaluated registered a mean iron content above the reported threshold of 70 ppm. These genotypes include Rwibarura (76.4 ppm), RWV 2350-2B (74.3 ppm), MBC 71 (71.5 ppm) and RWV 1129 (70.7 ppm) which is the RAB check. Although Nyiramagoroli genotype showed the greatest iron content in Karama, its capacity to accumulate iron was critical in other environments. The same applies for 665 SI-4/1 and RWV 2365-2 genotypes.

In terms of zinc content, there was also a large variability within and across environments. For instance, zinc content varied between 25 and 39 ppm, with the least zinc content observed with 665SI-4/1 and RWV 23501-2B both in Rwerere site. Four climbing bean genotypes accumulated greater zinc content across environments over the remaining. These genotypes include Nyiramagoroli (33.4 ppm), Rwibarura (33.3), RWV 2350-2B (32.7) and MBC 71 (32.0). Generally all the genotypes accumulated greater zinc than the RAB check.

Three climbing bean genotypes among the seven evaluated in general showed superior performance for the three traits evaluated (yield, iron and zinc content) across environments. These genotypes include Rwibarura, RWV 2350-2B and MBC71. Nyiramagoroli genotype seemed to be unstable and achieved poor yield and iron content.

Strong interactions between genotype and environment were observed (P ≤ 0.001) for yield, iron and zinc content. This suggests the strong influence of the environment on climbing bean iron and zinc accumulation which is in line with the large variability observed among the genotypes evaluated across environments.

Environment/Genotype	665SI-4/1	MBC 71	NYIRAMAGORORI	RWIBARURA	RWV 1129	RWV 2350-2B	RWV 2365-2

Akanyirandoli	51.9	61.0	56.0	67.5	71.7	54.0	59.9
Karama	86.3	72.3	91.7	70.5	58.6	91.2	69.3
Kinigi	62.7	56.6	66.3	70.4	61.4	77.4	63.4
Kitabi	62.0	72.5	66.2	77.7	72.8	74.7	67.4
Muhanga	60.5	74.4	65.5	79.9	79.8	84.2	64.3
Muhoza	62.6	84.1	63.8	84.8	78.3	81.0	68.4
Ngoma	63.5	71.2	65.5	79.6	67.9	74.7	76.4
Nyagatare	64.5	78.8	64.7	74.6	69.7	70.9	63.6
Rubona	65.3	80.1	70.0	82.5	88.7	72.5	73.3
Rwerere	51.1	64.1	63.6	76.3	58.4	62.0	59.0
Max	86.3	84.1	91.7	84.8	88.7	91.2	76.4
% Over check	-2.7	-5.2	3.4	-4.4	0.0	2.8	-13.8
Min	51.1	56.6	56.0	67.5	58.4	54.0	59.0
% Over check	-12.5	-3.1	-4.1	15.6	0.0	-7.5	1.0
Mean	63.0	71.5	67.3	76.4	70.7	74.3	66.5
% Over check	-10.9	1.1	-4.8	8.0	0.0	5.0	-6.0
LSD=2.6	Grand mean=70.47	%CV=6.2			G^{***}	E^{***}	GXE^{***}

Table 2: Mean iron content of 7 bean genotype evaluated in 10 locations in national performance trial (NPT) of climber during 2017 A and B seasons.

Environments/Genotype	665SI-4/1	MBC 71	NYIRAMAGORORI	RWIBARURA	RWV 1129	RWV 2350-2B	RWV 2365-2
Akanyirandoli	28.1	27.2	30.4	30.7	29.5	29.9	29.9
Karama	39.4	35.4	39.0	37.4	33.6	36.7	33.9
Kinigi	31.3	32.8	37.7	35.1	35.2	38.2	29.6
Kitabi	30.7	32.3	33.1	33.3	30.6	32.8	31.3
Muhanga	32.0	35.2	33.3	33.8	30.1	35.8	32.8
Muhoza	28.4	34.1	29.7	32.7	31.0	28.6	31.5
Ngoma	29.9	31.8	32.1	33.1	30.4	34.5	32.4
Nyagatare	29.2	29.7	29.7	31.0	28.3	30.7	28.0
Rubona	35.2	35.0	38.6	38.6	33.4	34.7	35.6
Rwerere	25.2	27.0	30.8	27.2	25.5	25.3	26.0
Max	39.4	35.4	39.0	38.6	35.2	38.2	35.6
% Over check	11.9	0.6	10.8	9.6	0.0	8.5	1.0
Min	25.2	27.0	29.7	27.2	25.5	25.3	26.0
%Over check	-1.2	5.8	16.3	6.7	0.0	-0.8	2.1
Average	30.9	32.0	33.4	33.3	30.8	32.7	31.1
%Over check	0.6	4.2	8.7	8.2	0.0	6.4	1.1

LSD=1.066	Grand mean=32.00			%CV=5.5	G***	E***	GXE***

Table 3: Mean zinc content of 7 bean genotypes evaluated in 10 locations in national performance trial (NPT) of climber during 2017 A and B seasons.

Discussion

Yield differed significantly (P ≤ 0.001) among the seven climbing bean genotypes evaluated and environments. The interaction (G × E) effects were highly significant at P ≤ 0.001 on yield, iron and zinc content. The significant differences on yield observed among tested genotypes within and across environments have been reported elsewhere [20-22]. Rainfall variability in the study sites may have affected the performance of the tested genotypes. This may be explained by the specific adaptation of some genotypes to the high lands of the Northern Province which have relatively higher rainfall compared to the low and mid altitude.

Iron and zinc content in seeds of the seven genotypes tested were variable. Our results support the findings by Silva et al. [23] who observed that accumulation of minerals in seeds of beans is influenced by many factors including genetic variability and environmental conditions. Three climbing bean genotypes among the seven evaluated in general showed superior performance for the three traits evaluated (yield, iron and zinc content) across environments. These genotypes include Rwibarura, RWV 2350-2B and MBC71. Nyiramagoroli genotype seemed to be unstable and achieved poor yield and iron content. Iron content of the studied genotypes ranged between 54.0 and 91.7 ppm. This range was reported by Steckling et al. [24] where they observed iron range of 63.00 to 140.68 ppm. Zinc content varied between 25 and 39 ppm, which is superior to the means of Fe and Zn content observed in Tanzania where Fe and Zn averaged to 57.4 and 23 ppm respectively.

Genotype x Environment effects observed on iron and zinc content in the present study were observed elsewhere in beans, sorghum and wheat [25-28]. In this study the tested genotypes showed differences for Fe, Zn and yield. Differences in iron and zinc concentration found in each genotype, within and across environments confirm the results by Susan et al. [29] and may be due to their differences in genetic makeup, the uptake capacity and partitioning of nutrients in the different parts of the plant. Significant interaction between genotypes and environments indicates that the accumulation of these minerals in the seed vary among genotypes and environments. The existence of genetic variability among common bean varieties in regards to mineral content allows the identification of varieties for use in food with high accumulation capacity of such micronutrients. Moraghan and Gratfon [30] when assessing seed of eight common bean cultivars of different gene pools in five locations in the united states, found significant cultivar location interaction for iron, calcium and potassium content.

Selected genotypes will contribute to high iron bean varieties leading to food and nutrition security in Rwanda and neighbouring countries through formal or informal trading. ASARECA in 2012 reported that bean in east African countries is recognized as an important source of human dietary protein including quality globulin and major source of fiber, complex carbohydrates and micronutrients especially iron and zinc, and vitamins. Over the last years, demand and trade in beans within East Africa has been growing faster than productivity, which is good for farmers who produce surplus for sale, but threatens to make beans less affordable by poorer households who primarily depend on it for their nutritional security as export trade will exert pressure on domestic prices. One variety cannot outperform other varieties on more than a few attributes with preferred attributes varying among the most popular improved varieties [31]. This could exacerbate the malnutrition challenges in East African countries that currently stand at more than 40% stunting among children below five years of age [32]. Similarly, Annicchiarico [33] reported that the inadaptability of several genotypes in specific sites is due to the fact that it is impossible for a genotype to contain all the genes responsible for best performance in any environment.

Variations in environments or plant genetic makeup can affect the accumulation of different multiple elements concurrently. Some parts of the plant and different soil types may results in micronutrient variability emphasizing the importance of environment factors on quantitative traits. Differences in mean performances indicated the existence of variability between genotypes. Availability of a wide range for both kinds of nutritional and productive traits indicated the opportunity of development and selection of Fe and Zn rich genotypes in high yielding background of beans as crop improvement depends on the magnitude of genetic variability. Studies also showed that Fe and Zn content accumulation in wheat grain depend largely on environmental conditions, particularly soil variability [26,27]. The selection of common bean genotypes with high iron content may contribute significantly to the quality of nutritional recommendations, especially in the prevention of micronutrient deficient related diseases.

Conclusion

The current study showed significant differences among the tested genotypes across environment for the three traits (yield, iron and zinc content) studied. The yields achieved for all the genotypes were largely influenced by environment. Our results also demonstrate the importance of both genotype and environment on iron and zinc accumulation in bean seeds. Three climbing bean genotypes among the seven evaluated in general showed superior performance for the three traits evaluated across environments. Our findings suggest that breeding and selection for high mineral content should be location specific.

The tested genotypes should be used as source of genes in future breeding work. Our results show a good potential for the tested genotypes for the people in Rwanda, particularly the most malnourished group.

Acknowledgements

The authors are grateful to the HarvestPlus project for funding this research through a grant to Rwanda Agriculture Board (RAB). We also thank researchers of the bean sub-program: Edouard Murwanashyaka, Alfred Rumongi, Martin Nizeyimana, Evariste Gasigwa and Viateur Ndayizeye for their cooperation in data collection.

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