CROP BREEDING AND APPLIED BIOTECHNOLOGY

ARTICLE

Genotype x environment interaction in the iron and zinc biofortification of common bean grains

Crop Breeding and Applied Biotechnology
23(2): e43782321, 2023
Brazilian Society of Plant Breeding.
Printed in Brazil
http://dx.doi.org/10.1590/1984-70332023v23n2a13

Gustavo Ligarreto[1*]

Abstract: *Bean breeding programs have focused on the generation of early bush cultivars with higher yields, commercial acceptance and resistance to crop-limiting diseases. This study prioritized the nutritional quality in terms of iron (Fe) and zinc (Zn) content in bean grains grown in three high tropical environments. Two bush bean cultivars and five climbing bean cultivars were evaluated. The mineral content of the grains was determined with atomic absorption spectroscopy. The results indicated that the bush genotypes Bacatá and Bianca are biofortified with Fe and Zn and the climbing cultivar Sutagao is biofortified with Zn. Statistical differences were found between genotypes (G), environments (E) and GxE interactions. According to the AMMI stability value (ASV), the cultivars Sutagao, Bianca and Iraca were stable, whereas Bacatá, which was the one with the highest nutritional quality, was moderately stable. The Simijaca environment showed an adequate edaphic contribution to bean production with nutritional quality.*

Keywords: *Grain quality traits, multi-environment, SREG, AMMI stability value, stability index*

INTRODUCTION

The global deficiency of micronutrients iron (Fe) and zinc (Zn) is a moderate to severe public health problem since it causes anemia and immunodeficiency disorders that limit a person's ability to work and affects more than two billion people worldwide, that is, about 33% of the human population (Rehman et al. 2021). Therefore, this deficiency is a prevalent health concern in developing countries (Blair et al. 2009, Powers and Thavarajah 2019). The main sources of these minerals are foods with animal origin; however, because of their high cost, low-income populations cannot afford them, but the consumption of common beans, which is a cheap source for preventing mineral deficiency, is an option (Andrade et al. 2012).

To mitigate this problem, strategies such as supplementation with pharmaceutical products, food fortification and biofortification, which maintain and improve nutritional levels, have been used (Brigide et al. 2014). The last-mentioned strategy uses genetic improvement to increase micronutrients in grains, roots and tubers, which is sustainable. In addition, biofortification is the most affordable way to combat malnutrition in low- and middle-income countries and to offer an adequate nutrition option for people following vegetarian diets in developed countries, which, in turn, can prevent socioeconomic losses and

***Corresponding author:**
E-mail: galigarretom@unal.edu.co
ORCID: 0000-0001-9372-6094

Received: 12 October 2022
Accepted: 04 March 2023
Published: 24 May 2023

[1] Universidad Nacional de Colombia, Facultad de Ciencias Agrarias - Sede Bogotá, Carrera 30 # 45-03, Bogotá 111321, Colombia

reduced work performance and productivity, which ultimately affects national economies (Astudillo and Blair 2008, Katuuramu et al. 2021).

Common bean cultivars vary in grain mineral accumulation, with iron concentrations ranging from 30 to 120 ppm and zinc concentrations ranging from 20 to 60 ppm (Islam et al. 2002). The range of mineral accumulation in the two gene pools of the Andean and Mesoamerican common bean is similar, although many Andean beans or hybrids between gene pools have a higher iron concentration than Mesoamerican beans (Islam et al. 2002).

Iron is available in abundance in almost all types of soils and can be absorbed by plants through the reduction of Fe^{3+} to Fe^{2+} or via chelation of iron ions; the latter strategy is also used for Zn. The concentrations of micronutrients Fe and Zn in the grain are influenced by environmental conditions and by genotype. Therefore, extensive knowledge will aid the development of improved cultivars that maintain higher concentrations of micronutrients in grains in adverse environments (Waters and Sankaran 2011, Stanton et al. 2021, Portilla et al. 2022).

In previous studies for agricultural practices, Astudillo and Blair (2008) determined that the concentration of iron in bean grains increased with applications of phosphorus, but, for zinc, there were no significant responses when compared to the controls. In other studies, Andrade et al. (2013) reported genetic control for the concentrations of Fe and Zn in bean grains, where expression was mainly of the variance of dominance for Fe, and the additive allelic interaction explained the variation between genotypes for Zn. Glahn and Noh (2021), in turn, suggest that higher Fe concentration in beans does not always equate to higher uptake, that there is a strong effect of environment and genotype-environment interaction on Fe concentration and that there are few studies addressing composition of nutrients in soils, environmental factors and the stability of the genotype in the environments.

The novelty of this study is given by: 1. Prioritizing climbing beans, whose cultivation, according to studies by Katungi et al. (2018), has contributed in tropical countries to improving the well-being of poor farmer households, 2. It is one of the few works carried out in the world on nutritional quality related to microminerals (Fe and Zn) in climbing beans and 3. It is one of the few investigations that address the genotype x environment interaction in the nutritional quality of beans.

MATERIAL AND METHODS

Environment assessments

This study was carried out in 2016 and 2017 in three environments in the Andean zone, in the Ubaté and Guavio regions of the Department of Cundinamarca, Colombia. In the Ubaté region, sowing was carried out in the municipality of Simijaca (lat 5° 30’ 36.3” N, long 73° 51’ 28.0” W, alt 2,554 m asl), with 382 mm of precipitation during the 180-day cultivation cycle, with an average temperature of 14.2 ºC during the months of February and August 2016. The rainy season begins in February, and the dry season starts in August, when farmers sow bean crops. Sowing was also done in the Guavio region, in the municipalities of Gama (lat 4° 44’ 22.60” N, long 73° 35’ 36.19” W, alt 2,350 m asl), with 454 mm of precipitation during the crop cycle and a temperature of 18.6 **ºC**, and Gachalá (lat 4° 39’ 45.37” N, long 73° 30’ 58.28” W, alt 1,800 m asl), with 781 mm of rainfall and an average temperature of 18.2 ºC during the months of August 2016 to February 2017, which was the dry season.

Description of the soils

Table 1 shows the results of the soil analyses for the farms where the experimental bean plots were established. In general, the soils had a high content of organic matter, with clayey and loamy clay textures. However, they were acidic, with a pH lower than or equal to 5.3, creating low availability and deficiencies of elements such as P, Ca and Mg for plants (Restrepo et al. 2017). The Simijaca soil in the Ubaté region had high levels of N, P, K, Ca, Mg, Zn, and Fe, while in the Gama and Gachalá environments, the soils had deficiencies of P, Mg, Zn, Cu, Mn, and B and high Al contents. There was a high Ca/Mg ratio in Gachalá.

Bean cultivars

Seven improved bean cultivars delivered to farmers in Colombia in 2016 were evaluated, which have been widely accepted by producers from small cultivation areas; including two bush cultivars (habit I), called Bacatá (Red kidney, Radical Cerinza type) and Bianca (white grain), and five climbing or indeterminate habit cultivars: (habit IV) Chíe, Hunza, Iraca and Sutagao, with red grains (Red round shape, Bola Rojo type), and Serrania, with pinto grains (Cargamanto Rojo type). They are commercial-type grains for the American market, large grain with a weight of 100 grains greater than 66 g (Ligarreto-Moreno and Pimentel-Ladino 2022).

Planting systems

The seeds were sown in plots with three rows with 7 linear meters; the experimental design used was randomized complete blocks with three replications. Densities of 62,500 plants ha^{-1} were used for cultivars with growth habit I and densities of 30,300 plants ha^{-1} were used for cultivars with growth habit IV, according to the distances used by producers. The harvest in Simijaca with type I beans was carried out 135 days after sowing (DAS) and from 155 to 180 DAS in types IV. In Gama and Gachalá, it was done at 120 DAS and 155 DAS, respectively. Thirty days before sowing, a pH correction was made to 5.3 in the Gama and Gachalá environments with a dolomite lime dose of 5.7 kg $plot^{-1}$ (2 t ha^{-1}). At 30 days after emergence (DAE), when weeding and hilling was carried out, the plants were fertilized with a physical mixture of edaphic fertilizers: i) Di-ammonium phosphate (DAP) at a dose of 1.1 g $plant^{-1}$ (70 kg ha^{-1}), ii) Urea at a dose of 1.2 g $plant^{-1}$ (80 kg ha^{-1}) and iii) potassium chloride at a dose of 0.8 g $plant^{-1}$ (50 kg ha^{-1}). No irrigation was used.

Grain nutritional quality analysis

When the cultivars reached maturity in each central row of the plots, the plants were harvested and shelled manually to minimize any contamination by metals and soil; seeds were hand sorted to remove any impurities such as immature, wrinkled, discolored, or damaged seed and stored in a humidity-controlled cold room (4 °C; 75 % relative humidity) (Glahn et al. 2020). For each bean cultivar, Fe and Zn were quantified with a 5 g seed sample that was washed, dried in an oven at 50 °C, and ground in a Retsch mill (Haan, Germany), modified with Teflon chambers and zirconium pellets without the use of metal pieces to avoid contamination. The sample was then transferred to 30 mL plastic containers for storage in a chamber at 20 °C. The concentration of the minerals was determined with atomic absorption spectroscopy (AAS), measured in mg kg^{-1}, in a Unicam Solaar 969 (Burladingen, Germany) in the Laboratory of Analytical Services at CIAT, Palmira. To determine the homogeneity of the samples, two subsamples of the ground powder were evaluated. The readings were compared to standard curves prepared from iron diluted to a concentration of 100 mg L^{-1} and zinc diluted to 50 mg L^{-1}.

Statistical analysis

The Pearson degree of association (r) was estimated between the averages of the Fe and Zn contents of the bean grains with the soil nutrients of the three environments and with the average yield. An analysis of variance by environment and combined for environments was used to show the contribution of genotypes, localities and interaction. In the analyses by environment, the effects of blocks and genotypes (G) were fixed effects, and, in the combined analyses, the genotypes were fixed, and the effects of the environments (E) were random. The comparison of the means was carried out using the Tukey test ($P<0.05$).

The genotype and environment interaction (GxE) and the analyses of phenotypic stability and identification of the representative environments were carried out with the regression model of SREG environments, which, according to Ligarreto-Moreno and Pimentel-Ladino (2022), provides greater discrimination of genotypes by environment than the AMMI model. SREG generates a biplot that contains the effects of G plus the effects of the GxE interaction, which is why it is called a “GGE biplot”. The AMMI stability value (ASV) was calculated, as suggested by Purchase et al. (2000), and the stability index for Fe and Zn (MSI) was adapted from the yield stability index (YSI), as proposed by Bose et al. (2014). Statistical software R (RStudio 2014) was used through an application developed by the International Maize and Wheat Improvement Center (CIMMYT), called GEA-R (genotype x environment analysis with R for Windows) Version 4.1 (Pacheco et al. 2015).

RESULTS AND DISCUSSION

The degrees of association between the nutritional elements of the soil and the contents of Fe and Zn in the grains were mostly positive, except OC, N, Al and the Ca/Mg ratio, which were negative. With grain yield, the association was negative with Fe and positive with Zn, while the correlation between the Fe and Zn mineral contents in the grains was positive (Table 1).

The Fe levels in the soils of the three environments were above the optimal level of 80 ppm (Tofiño-Rivera et al. 2016). For Zn, only Simijaca exceeded the optimal level (8 ppm). This characteristic of the local environment directly affected the nutritional contents of these minerals in the bean grain, since the properties of the chemical composition of the soil and the climatic patterns influence the absorption of nutrients in the plants, by affecting the solubility and availability of nutrients in the root zone (Katuuramu et al. 2021). There was a significant association of Fe and Zn in the grain with the Fe and Zn present in the soil, a result that coincides with what was found by Katuuramu et al. (2021) in a study of nine farms in Uganda, of which bean seeds with high average Fe and Zn also had the highest average Fe and Zn in soil over two field seasons. This behavior is also consistent with what was reported by Moraghan et al. (2002), who report that the characteristics of the soil and the genotype affect the accumulation of minerals in the seed. The content of P in the soil, which was higher in Simijaca with 45.30 ppm versus 1.63 and 17.30 ppm in the Gama and Gachalá environments, respectively, may be another factor in the increase in concentrations of Fe and Zn in the grains, which showed a positive correlation (r), as also reported by Astudillo and Blair (2008).

The positive and significant associations (*r*) of several of the soil chemical parameters in this study with the nutritional contents of Fe and Zn in the grains may indicate that the latter are markedly influenced by the conditions of the cultivation, such as soil fertility, as reported for beans by other authors in Brazil (Silva et al. 2012, Brigide et al. 2014). Moraghan et al. (2002) showed that the characteristics of the soil and genotype affect the accumulation of iron in grains. Thus, beans produced in soils with pH 6.0 contain 25% more Fe in the grains than those produced in calcareous soils with pH 8.2. This result is consistent with Tofiño-Rivera et al. (2016), who indicates that the agronomic and nutritional behavior of biofortified products is associated with the characteristics of soils with a slightly acidic pH, moderate availability of Fe and Zn, and a medium to high content of organic matter, and with Astudillo and Blair (2008), who showed that the availability of K influences the stimulation of root growth and the populations of microorganisms that contribute to the mobility and capture of minerals by plants.

Table 1. Physicochemical parameters of soils from three environments in the high tropics with correlations with yield and mineral content in bean grains

Variable	**Unit**	**Location**			**Correlation** (r)	
		Simijaca	**Gama**	**Gachalá**	**Fe in grain**	**Zn in grain**
Texture		Clay	Loam	Clay loam		
pH		5.30	4.50	4.70	+*	+
OC[1]	%	6.37	12.10	6.75	-	-
N	%	0.55	1.04	0.58	-	-
P	mg kg^{-1}	45.30	1.63	17.30	+	+
K	meq 100 g	2.26	0.54	0.47	+	+**
Ca	meq 100 g	8.52	3.08	7.34	+	+
Mg	meq 100 g	4.93	1.06	1.11	+	+*
Fe	mg kg^{-1}	393	191	220	+*	+
Zn	mg kg^{-1}	9.18	0.59	0.99	+	+*
Na	meq 100 g	0.54	0.10	0.07	+	+*
Al	meq 100 g	0.35	5.29	2.11	-	-
ECEC	meq 100 g	16.60	10.10	11.10	+*	+
Cu	mg kg^{-1}	0.92	0.16	0.75	+	+
Mn	mg kg^{-1}	13.80	3.67	2.42	+	+*
B	mg kg^{-1}	0.31	<0.12	<0.12	+	+*
Ca/Mg		1.73	2.91	6.61	-	-
Grain yield	t ha^{-1}	3.34	3.12	2.67	-0.60**	0.21
Fe in grain	mg kg^{-1}					0.41

[1] OC = oxidizable organic carbon; N: high = > 0.50, medium = 0.25-0.50; P: high = > 40, medium = 20-40; K: high = 0.35, medium = 0.15-0.35; Ca: high = > 6, medium = 3-6; Mg: = high = > 2.5, medium = 1.5-2.5; Fe: high = > 20, medium = 10.1-20; Zn: high = > 3, medium = 1.6-3; Cu: high = > 3, medium = 1.1-3; Mn: high = >10, medium = 5.1-10; B: high = > 0.6, medium = 0.26-0.6; ECEC = effective cation exchange capacity; + - = positive and negative correlation; *, ** = significant at P < 0.05 and P < 0.01, respectively.

The association between Fe and Zn in the grain that was positive with r = 0.41 is similar to that reported in previous studies (Moraghan et al. 2002, Blair et al. 2009, Katuuramu et al. 2021), which can be useful in the development of common bean varieties that have high concentrations of Fe and Zn in the seeds. According to Katuuramu et al. (2021), this association between Fe and Zn may be because there is a similar genetic control of transport of minerals in plants and their final accumulation in seeds.

The negative or low correlation between the contents of Fe and Zn in the grains with yield, as well as the low association between the contents of Fe and Zn in the grains, have also been reported by other authors, such as Phuke et al. (2017). Good grain filling leads to high yield, likely with a diluting effect on grain Fe and Zn, causing opposite associations (Portilla et al. 2022). However, greater knowledge is needed on factors that affect the accumulation of microminerals in grains, and cultivars should be tested in various environments to obtain good estimators of the degrees of association and to determine local adaptation for new biofortified varieties (Andrade et al. 2013, Portilla et al. 2022).

There were highly significant statistical differences ($P<0.01$) between the evaluation environments, between the cultivars, and for the GxE interaction in the Fe and Zn contents in the grains (Table 2). The overall average of the minerals in the grains was 61.02 ppm for Fe and 34.58 ppm for Zn.

Table 2 shows the comparison of averages, where the Simijaca environment was the most favorable for beans with a greater amount of Fe and Zn, with averages of 66.51 and 39.29 ppm, respectively, with statistically significant differences in the Gama and Gachalá environments, which in turn did not show statistical differences.

The significant differences in Fe and Zn minerals between the grains of the genotypes indicated variability in the bean cultivars for biofortification, as reported by Andrade et al. (2012), who found significant differences, especially for Mn, Zn and Fe, in the bean germplasm from Brazil, attributing such differences to the effect of genotypes and the environment. Beans with a bush habit stood out for their high contents of Fe and Zn, while those with a climbing habit stood out for their high Zn content. The high concentrations of Fe and Zn in the grains of the Bacatá and Bianca cultivars with an Andean origin were attributed to the fact that they have the ICA Cerinza cultivar as their common

Table 2. SREG, AMMI variance components and averages of the concentration of Fe and Zn in grains by bean cultivar and evaluated environments

Sources of variation	**df**	**SREG, Mean squares**		**AMMI, Mean squares**	
		Fe	**Zn**	**Fe**	**Zn**
Environment (E)	2	493.96**	349.60**	493.96**	349.60**
Genotype (G)	6	758.78**	20.09**	758.78**	20.09**
G x E	12	68.92**	10.45**	68.92**	10.45**
IPCA1	7	752.57**	22.00**	109.24**	13.54**
IPCA2	5	12.50	12.65**	12.47	6.10
IPCA3	3	16.43	9.52*	0.00	0.00
Residuals	42	7.25	3.33	7.25	3.13

	Fe ($mg\ kg^{-1}$)			**Zn** ($mg\ kg^{-1}$)		
Genotype	**Simijaca**	**Gama**	**Gachalá**	**Simijaca**	**Gama**	**Gachalá**
Bacatá	94.00 a[1]	67.63 a	71.07 a	39.90 ab	30.70 de	30.93 bcd
Bianca	73.27 b	63.27 b	63.57 b	40.83 a	32.20 bcd	30.33 cd
Chíe	59.40 d	53.83 d	58.63 bc	36.00 b	33.80 abc	33.7 abc
Hunza	47.77 e	47.43 e	48.37 d	39.13 ab	28.50 e	29.53 d
Iraca	60.97 cd	55.80 cd	56.87 c	39.97 ab	31.73 cd	33.90 ab
Serrania	64.50 cd	58.13 c	55.93 c	38.00 ab	34.70 ab	30.93 bcd
Sutagao	65.67 c	55.07 cd	60.30 bc	41.20 a	35.30 a	35.50 a
Mean	66.51 a[2]	57.31 b	59.25 b	39.29 a	32.41 b	32.04 b
Overall mean ($mg\ kg^{-1}$)		61.02			34.58	

*, ** = significant at $P < 0.05$ and $P < 0.01$, respectively. [1] Means of the same column with the same letter do not show significant differences according to Tukey's test at $P < 0.05$. [2] Means of the same row with the same letter do not show significant differences according to Tukey's test at $P < 0.05$.

Table 3. Classification of seven bean genotypes according to the average concentration of Fe and Zn in the grains, AMMI stability value (ASV), and mineral stability index (MSI)

Genotype	Mean	MC	IPCA1	IPCA2	ASV	RASV	MSI	MSI rank
Fe (mg kg^{-1})								
Bacatá	77.57 a[1]	1	-1.000	-0.060	12.27	7	8	4
Bianca	66.70 b	2	-0.100	0.160	1.24	3	5	2
Chíe	57.29 d	6	0.300	-0.300	3.69	5	11	6
Hunza	47.86 e	7	0.500	0.060	6.13	6	13	7
Iraca	57.88 cd	5	0.220	0.080	2.70	4	9	5
Serrania	59.52 cd	4	0.060	0.400	0.84	2	6	3
Sutagao	60.34 c	3	0.020	-0.340	0.42	1	4	1
Zn (mg kg^{-1})								
Bacatá	33.84 bc	6	-0.460	0.000	1.43	3	9	5
Bianca	34.45 b	4	-0.460	0.560	1.53	4	8	3
Chíe	34.32 b	5	1.000	-0.260	3.12	7	12	6
Hunza	32.39 c	7	-0.740	-0.140	2.30	6	13	7
Iraca	35.20 b	2	-0.140	-0.620	0.76	1	3	2
Serrania	34.54 b	3	0.560	0.720	1.88	5	8	4
Sutagao	37.33 a	1	0.260	-0.240	0.84	2	3	1

MC = mineral classification (Fe and Zn) in all environments, IPCA1 score = interaction principal component axis one score, IPCA2 score = interaction principal component axis two score, ASV = AMMI stability value, RASV = ranking of the ASV, and MSI = mineral stability index (Fe and Zn). [1] By nutrient, means of the same column with the same letter do not show significant differences according to Tukey's test at $P < 0.05$.

parent, documented as biofortified with the two minerals (Astudillo and Blair 2008). Something additional is the color of the seed of the Bianca cultivar since, according to Katuuramu et al. (2021), yellow and white seeds have a higher bioavailable Fe value for human health. The lower Fe bioavailability in red common bean grains is associated with the presence of tannins in the grain coat.

The Bacatá and Bianca cultivars, with a bush growth habit, had higher Fe contents, which, in turn, differed statistically from each other and from the other cultivars. Bacatá reached an average of 77.57 ppm, which is why it is considered a biofortified grain for Fe according to Brigide et al. (2014) (Tables 2 and 3). Meanwhile, the cultivars with climbing growth habits showed averages between 47.86 and 60.34 ppm, with the Hunza cultivar having the lowest value, and Sutagao having the highest value. For the Simijaca environment, the cultivars showed high Fe contents in the grains, except Hunza.

The concentration of Zn in the grains had averages that were similar among the cultivars, varying between 37.33 ppm in Sutagao and 32.39 in Hunza. The bush cultivars Bacatá and Bianca had an intermediate behavior, similar to other cultivars, with 33.84 and 34.45 ppm of Zn, respectively (Table 3). For the differential behavior of the cultivars in the three environments, the concentration of Zn in the bean grains was higher in the Simijaca environment, as seen for Fe, with values between 36.00 ppm in Chíe and 41.20 ppm in Sutagao, as compared to Gama and Gachalá, with average values of 32.41 and 32.04 ppm, respectively (Table 2). The average grain yields of the three environments were 1.90, 1.77 and 3.03 t ha^{-1} for Bacatá, Bianca and Sutagao, respectively, data reported by Ligarreto-Moreno and Pimentel-Ladino (2022), and higher yields were seen in the Simijaca environment with 3.34 t ha^{-1}, with 3.12 t ha^{-1} in Gama and 2.67 t ha^{-1} in Gachalá (Table 1).

The climbing bean cultivars Chíe, Iraca, Serrania and Sutagao, which are hybrids of Bola Rojo and Cargamanto Rojo with an Andean origin and G2333 with a Mesoamerican origin, showed better contents of Fe and Zn than the Andean cultivar Hunza, without a response to the accumulation of Fe in the grains. This is an important result for producers and consumers because, in the group of Andean x Mesoamerican hybrid gene pool cultivars, the characteristics of resistance to anthracnose and high yield provided by the Mesoamerican parent G2333 are combined with the good amount of Fe and Zn and the commercial quality of large grains from the parents Bola Rojo and Cargamanto Rojo from the northern Andean gene pool, as reported by Islam et al. (2002) for iron concentration and resistance to anthracnose in beans, and by Tamayo-Vélez et al. (2020) for biofortified NUA, CAL, MAC and AFR genotypes developed by CIAT. Islam et al. (2002) mention that the red or red speckled bean genotypes, especially from the Andean gene pool, have been previously reported to accumulate more Fe in the seeds than other types of beans in the markets, with the disadvantage of having less bioavailability of Fe, but it can deliver more absorbable Fe if served with certain foods in the diet, since Fe release and absorption is often a function of other nutrients present in the food matrix (Glahn et al. 2020).

In the GGE biplot of Figure 1A, the vertex genotypes of the polygon are: 1-Bacatá, 7-Sutagao, 3-Chíe, 4-Hunza and 6-Serrania, and the dotted and perpendicular lines on each side of the polygon make up the sectors for each genotype vertex. The three Simijaca, Gama and Gachalá environments are found in a single sector with vertex 1 genotype (Bacatá). Therefore, the Bacatá genotype was the best for Fe content in the three environments, showing the highest absolute value in the PC1 coordinate. In contrast, the Hunza genotype of the opposite vertex had the lowest concentration of Fe, followed by the Chíe and Iraca genotypes. The Bacatá and Iraca genotypes were stable in all environments because they had a small absolute value in the PC2 coordinate, and the Bianca, Sutagao and Serrania genotypes formed a second group with less stability, but with a good concentration of Fe in the grains. For environments, Simijaca was the most discriminating because of its higher absolute value of PC1 and lower contribution in PC2.

Figure 1B shows that the vertex genotypes of the polygon are: 4-Hunza, 3-Chíe, 7-Sutagao and 2-Bianca, and there is a sector with the Sutagao vertex genotype for the three environments. The contrasting genotypes for Zn were the extremes, with Sutagao with a higher concentration, which was unstable in the environments, and Hunza with the lowest Zn in the grains, which was not adapted to the environments. The Bacatá, Bianca and Iraca cultivars were more stable in all environments, of which Iraca had a high average Zn concentration (37.33). The Chíe and Serrania genotypes showed a high Zn average, but were unstable because of their higher absolute value in the PC2 coordinate. The Gama and Simijaca environments showed a higher absolute value in PC1, but differed from each other, where Simijaca had a greater contribution to the PC2 coordinate.

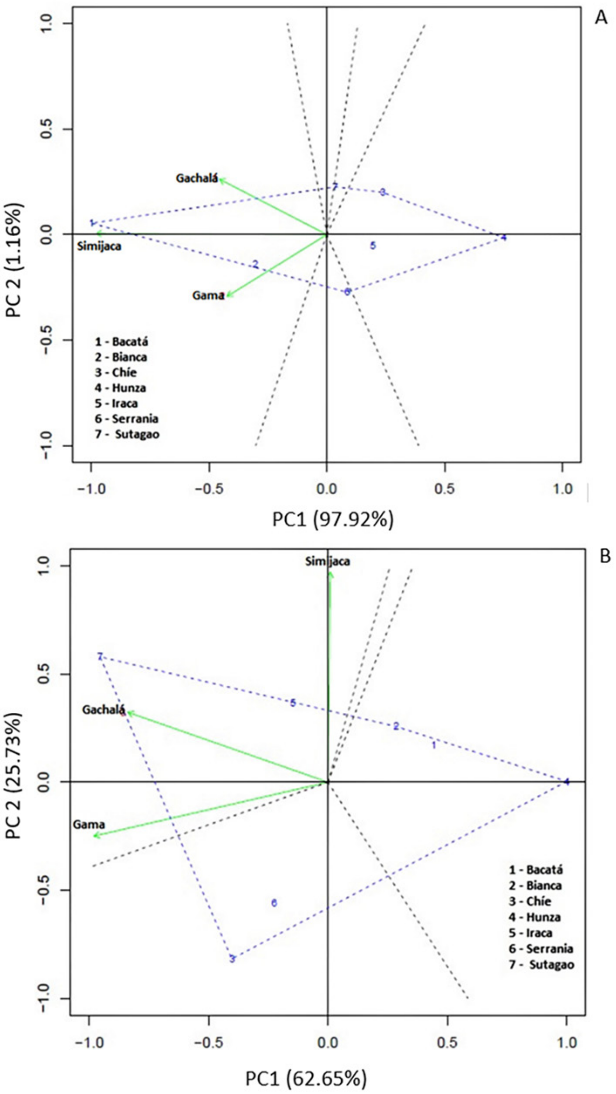

Figure 1. GGE Biplot for biofortified grains from bean cultivars in different environments, A. Fe concentration (mg kg-1), B. Zn concentration (mg kg-1).

The AMMI biplot for Fe content (Figure 2A), unlike the GGE biplot (Figure 1A), shows that the three environments Simijaca, Gama and Gachalá are found in different sectors, where the Bacatá genotype has a high correlation with the Simijaca environment and Chíe has a good correlation with the Gachalá environment. The Bacatá genotype was the best in Fe content in the three environments, showing the highest absolute value in the PC1 coordinate, and together with the Iraca cultivar, they were the most stable in all environments, showing the lowest absolute value in the PC2 coordinate.

In the AMMI case, for the Zn content, it can be seen in Figure 2B that the three environments appear in different sectors with the Hunza genotype as the vertex of the polygon in the Simijaca environment, the Serrania cultivar as the vertex in the Gama environment, and Chíe in Gachalá. A contrast was found with the GGE Biplot (Figure 1B) in that the cultivar Sutagao in AMMI was stable in the three environments and Hunza with low Zn in the grain was stable in the Simijaca environment. There was a coincidence with the GGE biplot in that Simijaca was the most discriminating environment due to its longer vector in PC1 and lesser contribution in PC2.

According to the stability value of the additive main effect and the AMMI multiplicative interaction (ASV), the lowest Fe value in the grains was that of Sutagao, indicating that it was the most stable for Fe but was the second most stable in the three environments for Zn. Chíe and Hunza were more unstable (Table 3). The sum of the classifications of the mineral content (MC) and the stability of AMMI (RASV) classified Sutagao, Bianca and Iraca as stable genotypes, and Bacatá, which had the highest nutritional quality, as moderately stable (Table 3). This finding contributes to the permanent understanding of the interaction between genotypes and the environment for more reliable recommendations for producers (Balestre et al. 2009, Bose et al. 2014, Ligarreto-Moreno and Pimentel-Ladino 2022). However, as suggested by Glahn et al. (2020) in the case of bean biofortification with high Fe content, the release of cultivars with high Fe content is not enough; it is also necessary to quantify bioavailability to ensure additional dietary Fe in consumption.

CONCLUSIONS

Genetic biofortification for iron and zinc contents is a complex trait that can be offered in new improved bean cultivars since it is dependent on genetic and environmental factors and the GxE interaction. Among the seven evaluated genotypes, the bush cultivars Bacatá and Bianca were biofortified with Fe and Zn, and the climbing cultivar Sutagao was biofortified with Zn. These cultivars should be promoted in markets since they enrich the basic diet of people and, because of their genetic potential for high yields and resistance to anthracnose, are an option for use by producers with limited land. The Simijaca environment was favorable, with an edaphic supply for the production of beans with good nutritional quality. The Gama and Gachalá environments need fertilization with micronutrients and major elements that increase biofortification levels.

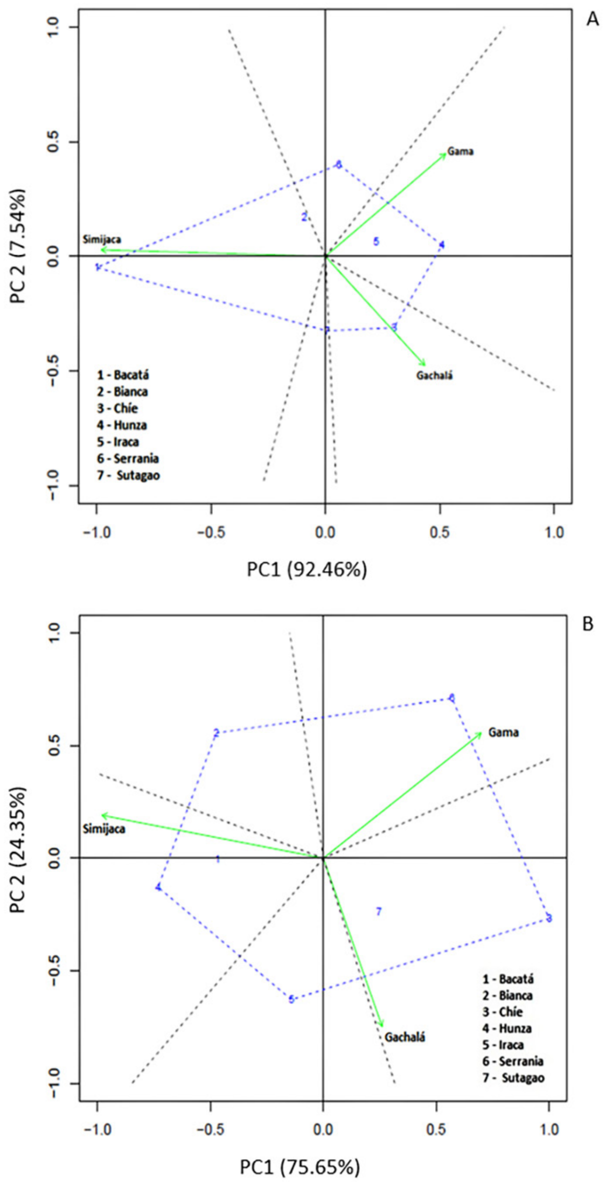

Figure 2. AMMI Biplot for biofortified grains from bean cultivars in different environments, A. Fe concentration (mg kg-1), B. Zn concentration (mg kg-1).

ACKNOWLEDGEMENTS

I would like to thank the team of the project "Mejoramiento de la Competitividad de los Cultivos de Fríjol y Maíz en las Regiones de Ubaté y Guavio en el Departamento de Cundinamarca - CTA Derivado 2" for their support in the field. I would like to thank the Bean Breeding team of the Bean Program of the International Center for Tropical Agriculture (CIAT) for all their contributions.

REFERENCES

Andrade C, Barbosa AF and Patto MA (2013) Genetic control of zinc and iron concentration in common bean seeds. **African Journal of Agricultural Research 8**: 1001-1008.

Andrade C, Barbosa AF, Patto MA and Silva LG (2012) Chemical composition as related to seed of common bean. **Crop Breeding and Applied Biotechnology 12**: 132-137.

Astudillo C and Blair MW (2008) Contenido de hierro y zinc en la semilla y su respuesta al nivel de fertilización con fósforo en 40 variedades de frijol colombianas. **Agronomía Colombiana 26**: 71-476.

Balestre M, Souza JC, Pinho RGV, Oliveira RL and Paes JMV (2009) Yield stability and adaptability of maize hybrids based on GGE biplot analysis characteristics. **Crop Breeding and Applied Biotechnology 9**: 219-228.

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.). **Molecular Breeding 23**: 197-207.

Bose LK, Jambhulkar NN, Pande K and Singh ON (2014) Use of AMMI and other stability statistics in the simultaneous selection of rice genotypes for yield and stability under direct seeded conditions. **Chilean Journal of Agricultural Research 74**: 1-9.

Brigide P, Canniatti-Brazaca SE and Silva MO (2014) Nutritional characteristics of biofortified common beans. **Food Science and Technology, Campinas 34**: 493-500.

Glahn RP and Noh H (2021) Redefining bean iron biofortification: A review of the evidence for moving to a high Fe bioavailability approach. **Frontiers in Sustainable Food Systems 5**: 682130.

Glahn RP, Wiesinger JA and Lung'aho MG (2020) Iron concentrations in biofortified beans and nonbiofortified marketplace varieties in east Africa are similar. **The Journal of Nutrition 150**: 3013-3023.

Islam FMA, Basford KE, Jara C, Redden RJ and Beebe SE (2002) Seed compositional and disease resistance differences among gene pools in cultivated common bean. **Genetic Resources Crop Evolution 49**: 285-293.

Katungi EM, Larochelle C, Mugabo JR and Buruchara R (2018) The effect of climbing bean adoption on the welfare of smallholder common bean growers in Rwanda. **Food Security 10**: 1-19.

Katuuramu DN, Wiesinger JA, Luyima GB, Nkalubo ST, Glahn RB and Cichy KA (2021) Investigation of genotype by environment interactions for seed zinc and iron concentration and iron bioavailability in common bean. **Frontier in Plant Science 12**: 670965.

Ligarreto-Moreno G and Pimentel-Ladino C (2022) Grain yield and genotype x environment interaction in bean cultivars with different growth habits. **Plant Production Science 25**: 232-241.

Moraghan JT, Padilla J, Etchevers JD, Grafton K and Acosta-Gallegos JA (2002) Iron accumulation in seed of common bean. **Plant and Soil 246**: 175-183.

Pacheco A, Vargas M, Alvarado G, Rodríguez F, Crossa J and Burgueño J (2015) GEA-R (Genotype x environment analysis with R for windows) version 4.1. Available at <https://data.cimmyt.org/dataset.xhtml?persistentId=hdl:11529/10203>. Accessed on July 21, 2021.

Phuke RM, Anuradha K, Radhika K, Jabeen F, Anuradha G, Ramesh T, Hariprasanna K and Mehtre SP (2017) Genetic Variability, genotype × environment interaction, correlation, and GGE Biplot Analysis for grain iron and zinc concentration and other agronomic traits in RIL population of sorghum (*Sorghum bicolor* L. Moench). **Frontiers in Plant Science 8**: 1-13.

Portilla AE, Mayor-Duran VM, Buendía HF, Blair MW, Cichy K and Raatz B (2022) Climbing bean breeding for disease resistance and grain quality traits. **Legume Science 4**: e122.

Powers SE and Thavarajah D (2019) Checking agriculture's pulse: Field pea (*Pisum Sativum* L.), sustainability, and phosphorus use efficiency. **Frontiers in Plant Science 10**: 1-8.

Purchase JL, Hatting H and Van Deventer CS (2000) Genotype x environment interaction of winter wheat (*Triticum aestivum* L.) in South Africa: II. Stability analysis of yield performance. **South African Journal of Plant and Soil 17**: 101-107.

Rehman A, Masood S, Khan NU, Abbasi ME, Hussain Z and Ali I (2021) Molecular basis of iron biofortification in crop plants; A step towards sustainability. **Plant Breeding 140**: 12-22.

Restrepo H, Ligarreto GA and Sánchez AD (2017) **Manejo de la nutrición en el cultivo del maíz**. Universidad Nacional de Colombia. Facultad de Ciencias Agrarias, Bogotá, 12p.

Silva CA, Abreu AF, Ramalho MAP and Correa AD (2012) Interaction genotype by season and its influence on the identification of beans with high content of zinc and iron. **Bragantia 71**: 336-341.

Stanton C, Sanders D, Kramewr U and Podar D (2021) Zinc in plants: Integrating homeostasis and biofortification. **Molecular Plant 15**: 65-85.

Tamayo-Vélez A, Santana-Fonseca GE, Blair MW and Ortiz-Muñoz C (2020) Agronomic evaluation of biofortified beans in Antioquia producers' farms. **Revista Facultad Nacional de Agronomía Medellín 73**: 9143-9149.

Tofiño-Rivera AP, Pastrana-Vargas IJ, Melo-Ríos AE, Beebe S and Tofiño-Rivera R (2016) Rendimiento, estabilidad fenotípica y contenido de micronutrientes de fríjol biofortificado en el Caribe seco colombiano. **Corpoica Ciencia y Tecnología Agropecuaria 17**: 309-329.

Waters BM and Sankaran RP (2011) Moving micronutrients from the soil to the seeds: Genes and physiological processes from a biofortification perspective. **Plant Science 180**: 562-574.