

Agronomic, nutritional and sensory assessment of biofortified bush bean genotypes (*Phaseolus vulgaris* L.) intercropped with coffee (*Coffea arabica* L.)

Santiago Jaramillo-Cardona

santiago.jaramillo@cafedecolombia.com

National Coffee Research Center-Cenicafé

Jairo Arcos

University of Florida (UF/IFAS)

Sonia Gallego

Alliance Bioversity and CIAT. CGIAR Consortium. Colombia Headquarters

Rubén Darío Medina

National Coffee Research Center-Cenicafé

Héctor Fabio Buendía

Alliance Bioversity and CIAT. CGIAR Consortium. Colombia Headquarters

Natali Rendón

Alliance Bioversity and CIAT. CGIAR Consortium. Colombia Headquarters

Research Article

Keywords: Intercropping, Biofortification, Coffee, Bush Bean, Nutritional quality, Plant Breeding

Posted Date: July 23rd, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7077021/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

This study evaluates agronomic, nutritional, and sensory attributes of biofortified bush bean (*Phaseolus vulgaris* L.) genotypes intercropped with coffee (*Coffea arabica* L.) in Colombia. Four advanced biofortified lines (BNA4, BNA8, BNA21, DAN15) and two commercial varieties (ICA Quimbaya and Calima) were assessed across contrasting environments. Key phenotypic traits such as growth habit and canopy architecture were prioritized for intercropping suitability. Nutritional quality was measured in terms of iron and zinc content, while sensory evaluation considered consumer acceptance. Phenological analyses revealed early maturity in BNA4 and BNA21, which are advantageous for escaping adverse conditions. BNA21 showed superior yield stability (up to 2,208 kg/ha), while BNA4 recorded the highest iron (73.9–82.1 mg/kg) and zinc (41.7–50.0 mg/kg) concentrations, surpassing commercial checks. Sensory tests highlighted unique grain attributes of BNA4 and BNA21, contributing to consumer acceptance and market differentiation. Genotype x Environment (GxE) interactions significantly influenced performance, underscoring the role of tailored agronomic practices. BNA21 and BNA8 showed the highest stability across environments. Biofortified lines demonstrated enhanced contributions to dietary iron and zinc, meeting up to 68% and 38% of daily requirements for children, respectively. The findings support the potential of biofortified bush beans as a nutritional crop for coffee-growing systems, enhancing food security and income generation for smallholder farmers. This work reinforces the importance of integrated breeding and agronomic strategies in optimizing intercropped systems.

1. INTRODUCTION

Colombia is the third largest coffee producer in the world and the first global producer of mild wet-processed Arabica coffees (ICO, 2022). Social relevance of Colombian coffee is supported by the activity of more than half a million families of small farmers (less than 1.3 hectares per producer) who cultivate the grain in 840,000 hectares (FNC, 2023). For these peasant coffee production systems, the planting of short cycle food crops such as bush bean (*Phaseolus vulgaris* L.) intercropped between coffee rows during the unproductive stage (18 to 24 months until the start of the first coffee harvest), represents an opportunity to improve family food security and to generate complementary incomes that can cover up to 50% of the costs of establishing the coffee plantation (Jaramillo & Salazar, 2021).

Common bean (*Phaseolus vulgaris* L.) is the most important food legume for direct human consumption, representing 50% of the legume grain consumed worldwide (Broughton et al., 2003; Saltzman et al., 2013; Beebe, 2012). In the Andean zone, beans are considered a basic food of the peasant economy represented by small and medium producers (Pachón, 2010), and have been cataloged by national and international organizations as one of the mandatory crops for food security programs due to its high protein content and high micronutrients concentration (Arcos and Rojas, 2019). The main types of bush beans planted in Colombia are Radical and Nima-Calima, these short cycle beans (70–90 days from sowing to harvest) are well adapted to a wide range of agroecological conditions at altitudes between 800–1800 m and have good commercial acceptance (Arcos and Rojas, 2019). In Latin America, more than 70% of beans are produced under the intercropping system with crops such as corn, potatoes, coffee and other species (Ann et al., 1985; Brooke et al., 2015). Currently, there is a new trend in the conventional breeding of bean varieties that seeks to combine agronomic performance and yield potential with better nutritional quality through a process called biofortification (Blair, 2013; Ray et al., 2014). This promising strategy of modern agriculture allows more people to access more nutritious foods with the use of fewer resources (Bouis and Welch, 2010; Blair et al., 2013; Vaz-Tostes et al., 2016). Following this approach, CIAT biofortification program released in 2019, an iron-biofortified bush bean variety named BIO-101 that contain 60% more iron (82 ppm) and 50% more zinc (43 ppm), than traditional variety ICA -Quimbaya that contain an average of 50 ppm of iron and 28 ppm of zinc (Beebe, 2020; Brigide et al., 2014). Following the release of BIO-101, five observation fields were established in March 2020 under an intercropped system with coffee at the La Catalina Experimental Station of Cenicafé. After a 90-day evaluation cycle, it was concluded that the growth habit of the variety (indeterminate, with medium-long tendrils) did not adapt well to the intercropping system because the bean climbed over the coffee trees.

For the particular case of bush beans, the search for materials with better adaptation to the intercropping system with coffee begins with the prioritization of the particular characteristics that determine agronomic and productive performance in typical conditions of coffee growing areas. The type of growth habit and planting density are the two factors that most affect the architecture of the bush bean canopy, since they condition the development of the plants and modify light interception, influencing plant health and competition for space, water and nutrients (Ricaurte et al., 2016; Hampton et al., 1997; Debouck and Hidalgo, 1986; Nienhuis and Singh, 1985). The bush bean genotypes with type I determinate growth habit develop a canopy architecture that is well adjusted to the conditions of an intercropped system with coffee, since they do not climb coffee branches and do not require staking (Jaramillo et al., 2023).

Biofortified bush bean genotypes are obtained through conventional non-transgenic genetic improvement techniques. The process begins at seed banks of the Alliance Bioversity-CIAT with the identification and characterization of seeds that naturally present higher contents of minerals such as iron and zinc; subsequently, these seeds are crossed with genotypes with high yield potential and good adaptability to biotic and abiotic factors, in order to obtain highly productive progenies with better nutritional quality (Saltzman et al., 2013). Zinc is an essential micronutrient for human nutrition, its deficiency is common in rural communities with limited economic resources that cannot access natural sources of this element such as meat, fish and seafood. The role of zinc in nutrition is mainly related to strengthening people's immune system and is an essential micronutrient for pregnant women and children (Bhatt et al., 2020). In Colombia, 36% of children between the ages of 1 and 4 years have zinc deficiencies. Iron is another essential mineral for nutrition given its importance as the main constituent element of hemoglobin (National Nutrition Situation Survey - ENSIN, 2015). Its deficiency is very accentuated in poor rural populations, causing problems such as anemia, cognitive problems, sedentary lifestyle, low birth weight, premature birth and maternal mortality (Connorton & Balk, 2019). Colombia is a country highly vulnerable to iron deficiency; the latest studies report that 24.7% of children under 5 years of age and 50% of pregnant women suffer from iron deficiencies (National Nutrition Situation Survey - ENSIN, 2015). Both zinc and iron deficiencies can be counteracted with the regular consumption of beans biofortified with these micronutrients since it is a cheap food and a traditional constituent of the rural diet of Colombian families.

For Colombian coffee growers, intercropping represents an opportunity to produce staple food crops and to generate additional income that can partially cover the costs of establishing the coffee plantation. Profitability is a determining factor for coffee growers when making decisions about which crop to establish since their main interest is to have a positive flow of additional income without affecting coffee productivity. Other factors influencing the adoption of intercropping practice are related to the availability of capital, achievable yields, production costs, ease of marketing, sales price and risks associated with the production of new crops (Duque-Orrego et al, 2021).

The objective of this study was to evaluate agronomic, nutritional quality (represented by iron and zinc grain contents) and sensory performance of biofortified bush beans genotypes under an intercropping system with coffee in Colombia. Particularly, we explore differences in phenotypic variables, yield potential, nutritional quality and sensory particularities that allow the advanced genotypes to become commercial varieties recommended for coffee intercropping systems.

2. MATERIALS AND METHODS

The research was established in four localities in Colombia corresponding to Cenicafé experimental stations: (a) Naranjal Station- Caldas, (b) La Catalina Station- Risaralda, (c) El Rosario Station- Antioquia and (d) El Tambo Station- Cauca; with contrasting geographical and climatic conditions during two stages: two seed multiplication cycles and two evaluation cycles between 2020 and 2021 (Fig. 1 and Table 1). The genotypes evaluated were BNA4, BNA8, BNA21, DAN15; corresponding to four biofortified bush bean advanced lines (F8/F10 progenies) developed by the Alliance Bioversity International & CIAT and two commercial varieties used as checks, ICA Quimbaya and Calima. (Table 2).

Table 1
Geographic and climatological conditions of the evaluation sites

Condition / Experimental Station (ES)	Naranjal ES	La Catalina ES	El Rosario ES	El Tambo ES
Country	Colombia	Colombia	Colombia	Colombia
Region	Caldas	Risaralda	Antioquia	Cauca
Municipality	Chinchiná	Pereira	Venecia	El Tambo
Latitude	04°58'N	04°45'N	05°58'N	02°24'N
Longitude	75°39'W	75°44'W	75°42'W	76°44'W
Altitude (m.a.s.l)	1381	1321	1635	1735
Average Temperature (°C)	21.2	21.7	20.4	18.9
Average Relative Humidity (%)	80	77	75	80
Average solar brightness (hours)	1636	1621	1935	1662
Average annual precipitation (mm)	2939	2282	2644	2078
Source: (Federación Nacional de Cafeteros, 2023). Information obtained from climatological normal (1990–2019)				

Evaluation phase corresponded to two production cycles of the advanced lines in La Catalina, El Rosario and El Tambo stations, where growth habit types and other phenotypic variables were evaluated according to the methodology described by Schoonhoven and Corrales in 1991. For the response variable associated with the yield of each genotype, the total bean production of each 25 m²/plot was obtained and converted to kilograms per hectare at 14% moisture. In addition, the nutritional quality was evaluated by estimating the content in parts milligrams per kilogram (mg/kg) of iron and zinc of the grain samples by genotype per plot, and was determined by the X-ray fluorescence spectrometry technique (XRF) at CIAT nutritional quality laboratory.

Table 2
Information of bush bean genotypes evaluated under intercropping system with coffee

Genotype	Designation	Pedigree	Origin
BNA 4	NA 27019-1CF2-4C-(CM)C-1C-(CM)Q-(M)C	(NUA 666 x NUA 681) x NUA 35	CIAT
BNA 8	NA 27019-7CF2-4C-(CM)C-2C-(CM)Q-(M)C	(NUA 666 x NUA 681) x NUA 35	CIAT
BNA 21	SANA27336-(M)CF2-4C-(CM)Q-(CM)C	DAN 26 x DAN 32	CIAT
DAN 15	NA 25491-10WF2-(CM)Z-(CM)Z-2Z	(SAB 725 x KATB1) x NUA 424	CIAT
ICA Quimbaya	Commercial Check	Canadian Wonder x (Perú 69 x Vermelho)	ICA
Calima	Commercial Check	(Perú 5 x Ant10) x (Ant 19 x ANT10)	ICA

Source: (Jaramillo & Jaramillo-Jiménez, 2025). Bean Breeding Program of Alliance Bioversity-CIAT.

2.1 Coffee-Bean intercropping management

The experimental plots were established under a minimum tillage system in coffee seed production fields. To obtain a bush bean planting density that did not affect coffee production, a population in an intercropped system of 160,000 plants per hectare was adjusted with a spatial arrangement of double furrows spaced 40 cm and located in the center of the coffee rows. Fertilizer application was based on soil analysis results presented in Table 3 and split in two moments:

(i) 5 days after bean emergence applying a fertilizer mix per hectare of 20 kg N. ha⁻¹, 15 kg P₂O₅. ha⁻¹ and 20 kg K₂O. ha⁻¹ and (ii) 30 days after bean emergence with 20 kg N. ha⁻¹. Before bean flowering, an evaluation of weed growth was carried out to determine the need for weed manual control. Once bean plants showed a percentage of defoliation above 90% and grain moisture was around 20%, manual harvesting was carried out by uprooting complete plants followed by mechanical shelling of the dry pods. The grains were cleaned by air stream supplied by a fan, subsequently the grains were dried in solar parabolic dryers until the grains reached a moisture of 14%.

The coffee fields where intercropping was established corresponded to a free sun exposure system with a spatial arrangement of 1.4 m x 1.0 m (1.4 meters between rows and 1.0 meter between plants) to obtain a coffee planting density of 7,142 plants per hectare. Due to a variable terrain slope of the different locations, integrated weed management was conducted to reduce soil losses due to rainwater runoff, this type of management includes practices such as mechanical-manual control between coffee rows and selective chemical management of highly interfering species to promote the establishment of noble weed coverage. Before the establishment of the experiment plots, random soil samples were taken for physical and chemical analysis. Based on the results of the soil analysis, the doses of dolomitic lime were adjusted to correct soil acidity and fertilizer doses per plant were defined for the coffee plants following the recommendations of Technical Bulletin No. 32 published by Cenicafé (Sadeghian, 2008).

Table 3
Physical and chemical soil properties of the evaluation sites.

Site - Experimental Station	pH	OM	N	P	Al	K	Ca	Mg	S	Clay	Silt	Sand	Texture
		%		mg.kg	cmol (+).kg				mg.kg	%			
Naranjal	4.8	14.3	0.51	20	1.6	0.11	0.9	0.4	17.4	25.0	18.0	57.0	SICL
La Catalina	5.1	11.2	0.44	24	1.1	0.31	2.1	0.9	11.3	27.0	24.0	49.0	SCL
El Rosario	4.6	11.4	0.45	10	3.2	0.47	1.3	0.8	14.3	50.0	16.0	34.0	C
El Tambo	4.1	19.9	0.63	6	1.5	0.24	2.3	0.7	13.1	37.0	12.0	51.0	L

Analysis Methods: pH: Potentiometric-soil: Water 1:1; N: Calculated; OM: Walkley-Black-Colorimetry; K, Ca, Mg: 1N ammonium acetate pH 7.0-EAA; Al:KCl IM-titration; P:Bray II-Bray Kurtz colorimetry; Texture: Bouyoucos with sodium pyrophosphate - USDA triangular diagram classification - L (loamy), C (clayey), SI (silty), S (sandy). Soil taxonomy of the locations Naranjal, La Catalina and El Tambo are Typic Dystrandep; El Rosario corresponds to a Typic Dystrandep dystropepts

2.2 Phenotypic and agronomic characterization

During the experiment, different variables related to the life cycle, phenotypic characteristics, seed traits, as well as the yield performance of the four advanced lines and two commercial checks were evaluated. The phenotyping evaluation was conducted using the Standard Evaluation System (SES) for bean germplasm developed by CIAT in 1987. The phenological variables evaluated in the genotypes and checks were days from sowing to 50% emergence, days from emergence to flowering, days from emergence to physiological maturity and days from emergence to harvest. Phenotypic variables included growth habit, plant height, branching type, leaf type, flower color, pod length, pod width, pod shape, number of pods per plant, number of seeds per pod, seed type, seed size, seed color, seed shape. Yield variables included grain yield per plot.

2.3 Nutritional and sensory assessment

Nutritional quality of advanced lines and controls was carried out by assessing the grain contents of iron and zinc using x-ray spectrophotometry technique and analyzing the grain concentration of phytic acid under colorimetric determination by UV-Vis spectrophotometry, since this compound acts as an inhibitor of the absorption of these minerals after human ingestion; these analyses were carried out at CIAT's nutritional quality and food processing laboratory. Besides, the potential contribution of bush bean genotypes to daily iron and zinc intake and to Estimated Average Requirement (EAR) was calculated based on average bean consumption in the Central region of Colombia (ENSIN, 2015), where most coffee-growing families are located, the EARs for children (4–6 years) and women of childbearing age and the iron and zinc bioavailability, as recommended by European Food Safety Authority (EFSA, 2014, 2015).

For the sensory analysis of this study we used an affective/hedonic test selecting two advanced lines (BNA 4 and BNA 21) and a commercial check (ICA Quimbaya) widely consumed in the Colombian coffee regions; the lines were prioritized for having exhibited good agronomic performance, higher micronutrients contents and for presenting novel grain formats that were traditionally only found in climbing bean varieties and not in bush beans. The study consisted in a sensory test of the three genotypes whose objective was to identify and select those lines with the greatest acceptability by regular bean consumers who were native to the Colombian coffee zone. Since the test was made with humans, the study was previously approved by Institutional Review Board (IRB) of the Alliance Bioversity International and CIAT to ensure that it does not pose a risk of physical or moral harm to the participants or that there would be any type of abuse of the information shared during the survey. The test was performed at Cenicafé headquarters in Chinchiná (Caldas) and included 122 participants who voluntarily signed an informed consent form that described the purpose and methodology of the study and filled a survey designed to collect information on age, sex, marital status, bean consumption frequency, aspects considered at the time of purchasing beans and a questionnaire with a seven-point hedonic scale to determine the acceptability of the materials based on a level of like or dislike for each of the attributes perceived (color, size, smell, flavor and texture) in randomized samples prepared with an standardized recipe for the three bean genotypes. The hedonic scale was composed by (i) three points of dislike: Dislike it a lot [1], Dislike it [2], Dislike it a little [3]; (ii) a neutral or indifference point: Neither like it nor Dislike it [4]; and (iii) three points of acceptance or liking: Like it a little [5], Like it [6] and Like it a lot [7].

2.4 Experimental design

The six bush bean genotypes (four advanced lines and two commercial checks) were defined as treatments and were arranged in a randomized block design with nine replicates (blocks) at four locations, where the blocking factor was adjusted based on the slope of the terrain, the total of the experimental units for each experiment were fifty-four in each location. The experimental units had an area of 30 m² (6 m x 5 m) and the total area of the experiment per intercropping cycle at each location was 2,500 m², where treatments were established with shared borders. The spatial arrangement corresponded to 6 m long plots with 6 double rows of bush beans spaced at 0.4 m and intercropped in the center of the coffee furrows.

2.5 Data Collection

For data collection, destructive sampling was carried out for yield and lab determinations related to nutritional, cooking and sensory quality, while phenological and phenotypic measurements were carried out through non-destructive sampling.

Phenological variables were (i) Days from sowing to 50% emergence: in each plot, the number of days from sowing until 50% of the plants emerged was recorded; (ii) Days from emergence to flowering: flowering date was recorded in the R6 stage when 50% of the plants in the plot have one or more open flowers; (iii) Days from emergence to physiological maturity: the ripening date was recorded in R9 stage, when 50% of the plants have reached their physiological maturity, that is, when pods lose their pigmentation and begin to dry (iv) Days from emergence to harvest: the harvesting date was recorded when the seed had a moisture between 16–18% and the plants had 90% defoliation.

Phenotypic variables included: (v) Growth habit: it was determined at the beginning of the R6 stage for determinate genotypes and at R9 stage for indeterminate genotypes. All the tested materials presented a Type IA growth habit without tendrils, except for BNA8, which exhibited a Type IB growth habit which is prostrate; (vi) Plant height: it refers to the length of the main stem (from the insertion of the roots to the apex of the last floral cluster) in centimeters; this variable was taken at the end of flowering stage; (vii) Branching type: was defined according to the density of the lateral branches of the genotypes and was classified as compact or open. All the advanced lines presented a compact branching type, except BNA8 which presents an open branching; (viii) Leaf type: depending on the stage of development, the bean can present simple and alternate primary leaves (cotyledon leaves) in the seedling state. After the first trefoil appeared, it was determined that the leaves are compound. Leaf size was determined in the central leaflet, determining its length from the insertion point on the petiole to the apex of the leaf; (ix) Flower color: flowers appear in lateral or terminal inflorescences in which the peduncle and rachis can be distinguished in addition to the flower buds. The bean flower is a typical papilionaceous flower composed of the pedicel, the calyx and the corolla; however from a descriptive point of view the most important parts for defining the color are the wings and the banner, which can be colored white, lilac and purple; (x) Pod length: it was measured in centimeters from the pedicel insertion point to the end of the apex; (xi) Pod width: it was measured in centimeters in the widest part of the sheath, between the dorsal and ventral sutures; (xii) Pod shape: when the pod dries, its profile acquires a shape that can be defined as straight, medium curved, curved and recurved; (xiii) Number of pods per plant: the number of pods that have at least one viable seed on each sampled plant were counted; (xiv) Number of seeds per pod: to determine this, the number of viable seeds contained in each pod was counted; the same pods were used to determine pod length and width; (xv) Seed type: depending on the format that the seed exhibits on its testa, it was classified as mottled, smooth, marbled, striated, spotted or dotted. (xvi) Seed size: it was expressed according to the weight in grams of 100 seeds chosen randomly. This measure was determined following the international standards of the ISTA (International Seed Testing Association) and classified as small (less than 25g per 100 seeds), medium (between 25-40g per 100 seeds) and large (more than 40g per 100 seeds); (xvii) Seed color: the seed closest to the apex of the selected pod was taken to make the previous determinations. When a seed has more than one color, it is necessary to determine the primary color (background color) and the secondary color with the colorimetry method. These colors were determined in the freshly harvested dry seed and classified with a color chart as Cream - Beige [2], Coffee-Brown [4], Pink [5] and Red [6]; (xviii) Seed shape: the seeds were observed longitudinally and any similarity in their shapes was determined and classified as round, ovoid, elliptical, almost square, elongated-ovoid, elongated almost square, kidney-shaped (straight next to the hilum) and kidney-shaped (curved to the opposite side of the hilum).

Yield variables included: (xix) Grain yield per plot: the weight in kilograms of the beans harvested in each plot was registered and grain moisture was determined with a Kett PM450® moisture meter. Final weight was transformed to yield in kg per hectare at 14% grain moisture; (xx) Lodging: it was registered from flowering to physiological maturity and when winds caused plants to overturn, this was reported with the date of the event and the number of plants affected per plot. Nutritional variables included: (xxi) Iron grain content in milligrams per kilogram; (xxii) Zinc grain content in milligrams per kilogram; (xxiii) Phytic acid concentration in milligrams per gram; (xxiv) Contribution to EAR for iron in percentage; (xxv) Contribution to EAR for zinc in percentage. Sensory variables included acceptance and hedonic perception of participants in terms of: (xxvi) Color; (xxvii) Size; (xxviii) Smell and (xxix) Flavor.

2.6 Statistical analysis

Statistical analysis of yield and nutritional quality data was conducted using R statistical software (version 4.1.2; R Core Team, 2021). To evaluate differences in yield, phenotypic traits, and iron and zinc contents among advanced lines and control groups across four evaluation locations over two production cycles an analysis of variance (ANOVA) was conducted using a 5% randomized complete block design model to assess the yield, phenotypic traits and nutritional quality. Models examined the interaction between the factors to identify Genotype (advanced breeding lines) × Environment (locations) interaction, analysis for each factor was reported for each level of interaction. For each location,

average values and standard deviations for the response and complementary variables were calculated and reported in violin graphs. Statistical differences among lines, locations, and production cycles were identified using the Least Significant Difference (LSD) test with significance levels set at p-values of 0.05, 0.001, and 0.0001. Interactions between genotype factor (advanced breeding lines) and environment (locations) were analyzed using the GGE biplot method combined with AMMI (Additive Main effects and Multiplicative Interaction) analysis. This was accomplished using the R packages “metan” and “agricolae,” with graphical representations generated using the “ggplot2” package. Principal Component 1 – Genotype (PC1) and Principal Component 2 - Location (PC2) were reported for each case study. For the data analysis of the sensory assessment, individual evaluations were performed for each attribute (color, size, smell, taste and texture) assigning ordinal values that were compared using the nonparametric Friedman Test, the objective of which was to determine the medians of the scores to express the central tendency of the data set analyzed. For weather data an analysis was carried by calculating average values and standard deviations for Average temperature (°C), Average Relative Humidity (%), Daily Solar Brightness (hours/day), Daily precipitation (mm/day) available in <https://agroclima.cenicafe.org/>

3. RESULTS AND DISCUSSION

Phenological variables were analyzed to estimate the differences in the growth of biofortified advanced breeding lines compared to those recorded in the commercial checks; although the duration of these stages is highly influenced by the environmental conditions recorded in the different locations, there are genetic differences such as precocity that can influence the differences in the duration of the different stages recorded for the system intercropped with coffee. The duration of the days to germination did not show significant differences between the lines with respect to the control, since at the time of sowing it was ensured that the soil moisture was at field capacity to guarantee a uniformity in the imbibition of the seeds as well as in the emergence of the bean seedlings. For the variable days from sowing to 50% emergence, the average values recorded fluctuated between 6.3 and 7.3 days. For the phenological variable days from seedling emergence to 50% flowering recorded at stage R6, a higher precocity was observed associated with lines BNA 4 and BNA 21 (34.7 and 35.7) compared to genotypes DAN 15, BNA 8 and the ICA Quimbaya control (39 and 40 days); this behavior was consequently reflected in the length of days elapsed to physiological maturity and days to harvest (Figure 2). Precocity is a phenological trait that has been associated with competitive advantages such as the escape of plants from extreme climatic factors and obtaining the harvest in a shorter time.

The evaluation of the differences in the phenotypic traits of the advanced lines compared to the controls, allowed to observe that in terms of the growth habit all the genotypes except BNA 8 exhibited a type IA habit without tendril, a trait that allows them to adapt ideally to the system intercropped with coffee, since they did not climb the stem or the branches of the plants without causing any physical damage to floral glomeruli where the coffee cherries develop. In terms of plant height, the advanced lines BNA 4, BNA 21 and DAN 15 and ICA Quimbaya control fluctuated between 56 to 58 cm above the ground surface; unlike the BNA 8 line, had an average height of 98 cm and a growth type IB, condition that accentuated the lodging of the plants after the start of the grain filling stage. Regarding flower color, the BNA 4 and BNA 21 lines presented lilac-colored flowers, while the other genotypes presented white flowers; this behavior was reflected in the novel grain color formats such as the mottled pink format of the BNA 4 line and the intense red of the BNA 21 line; characteristics that give them a higher price differentiation in the Colombian market. Regarding the type of seed, all the genotypes presented a kidney-shaped bean shape, unlike the BNA 21 line which presented a round shape similar to that of the commercial climbing bean variety Bola Roja (Table 4). This trait was highly novel for breeders, since previously this seed type was only present in climbing varieties that grow at altitudes above 1800 meters above sea level.

Table 4. Phenotypic traits of biofortified bush bean advanced lines and commercial check intercropped with coffee in three locations

Phenotypic Trait														
Genotype	Growth habit	Plant height (cm)	Branching type	Leaf type	Flower color	Pod length (mm)	Pod width (mm)	Pod shape	Number of pods per plant	Number of seeds per pod	Seed type	Seed Length (mm)	Width seed (mm)	Seed Color
BNA4	Type IA	58.4	Compact	Trifoliolate	Lilac	104.69	9.7	Type 3	20.4	3.5	Kidney	15.92	8.01	Pink
BNA21	Type IA	54.4	Compact	Trifoliolate	Lilac	98.98	10.56	Type 2	17.81	3.87	Rounded	13.06	8.77	Red
DAN15	Type IA	56.1	Compact	Trifoliolate	White	124.23	8.8	Type 3	13.19	4.04	Kidney	15.82	7.92	Brown-Red
BNA8	Type IB	98.1	Open	Trifoliolate	White	105.57	8.16	Type 3	20.18	3.6	Kidney	16.02	7.33	Speckled red
ICA Quimbaya	Type IA	58.8	Compact	Trifoliolate	White	114.75	7.7	Type 3	14.83	3.91	Kidney	16.04	7.44	Red

The ANOVA results reveal several critical insights into the main effects of advance genetic lines, environmental factors, and their interactions on crop yield and micronutrient content are presented in table 5. Notably, factor advanced breeding lines significantly influence both yield (kg/ha) and the micronutrient levels of iron (Fe) and zinc (Zn) in ppm. For yield, significant differences among lines include highly significant results for Catalina ($p < 0.0001$) and noticeable effects for Rosario ($p = 0.01369$) and Cycle 1 ($p = 0.00275$), while Tambo ($p = 0.1895$) and Cycle 2 ($p = 0.06875$) did not show significant differences. The influence of crop cycles on yield was profoundly significant ($p < 0.0001$), suggesting that the timing of the crop cycle is a pivotal factor in yield outcomes.

Environmental factors such as location also demonstrated a strong influence on both yield and micronutrient content, with significant location-specific variations ($p < 0.0001$ for yield, $p = 0.0175$ for Fe and Zn). The interaction between genetics and environment (GxE) further underscores the complexity, showing significant interactions that affect both yield ($p = 0.0044387$) and micronutrient concentrations ($p < 0.0001$ for both Fe and Zn), indicating that the optimal performance of genetic lines varies across different environmental conditions. Interestingly, the block effect was significant for yield ($p < 0.0001$) but not for micronutrient levels ($p = 0.442$ for both Fe and Zn), indicating consistent experimental conditions across blocks for Fe and Zn measurements. However, micronutrient levels did not show significant variability across blocks, highlighting a uniformity in conditions that did not affect these nutrients significantly. These findings emphasize the intricate interplay of genetics, environment, and management practices in agricultural production and suggest targeted breeding and management strategies could be optimized based on specific environmental conditions and genetic characteristics.

Table 5. ANOVA Results: influence of genotype, environmental factors and their interactions on yield and micronutrient content

Source of variation	kg/ha		Fe (ppm)		Zn (ppm)	
	DF	p-value	DF	p-value	DF	p-value
Advanced Breeding Line (G)	5	0.000546	4	<0.0001	4	<0.0001
G - Tambo	5	0.1895	4	<0.0001	4	0.000143
G - Catalina	5	<0.0001	4	0.002626	4	0.000263
G - Rosario	5	0.01369	4	<0.0001	4	<0.0001
G - Cycle 1	5	0.00275	-		-	
G - Cycle 2	5	0.06875	-		-	
Crop cycle (C)	1	<0.0001	-		-	
Locations (E)	2	<0.0001	2	0.0175	2	0.0175
E - Cycle 1	2	<0.0001	-		-	
E - Cycle 2	2	<0.0001	-		-	
GxE	10	0.004439	8	<0.0001	8	<0.0001
GxE - Cycle 1	10	0.05806	-		-	
GxE - Cycle 2	10	<0.0001	-		-	
GxC	5	0.16829	-		-	
GxC - Tambo	5	0.1449	-		-	
GxC - Catalina	5	0.007658	-		-	
GxC - El Rosario	5	0.10126	-		-	
ExC	2	<0.0001	-		-	
GxExC	10	0.019521	-		-	
Block (B)	8	<0.0001	4	0.442	4	0.442
B - Tambo	8	<0.0001	4	0.6467	4	0.6467
B - Catalina	8	0.0746	4	0.8341	4	0.8341
B - Rosario	8	<0.0001	4	0.4972	4	0.4972
B - Cycle 1	8	<0.0001	-		-	
B - Cycle 2	8	<0.0001	-		-	
Residuals	830		59		59	

3.1 Yield Performance

The yield of the genotypes (kg/ha) showed a wide variability between cycles and between locations, mainly attributed to both the differences in the environmental offer recorded during the development of the crop and particular differences in soil and altitudes of the contrasting sites.

At El Tambo station, the yields of all genotypes were higher during the first production cycle (November to February 2020) compared to the values recorded during the second production cycle (March to June 2021). During the first production

cycle, significant differences ($p < 0.001$) were observed in terms of yield, where the lines BNA 21 (1,852 kg/ha) and BNA 8 (1,774 kg/ha) had the highest average yields, followed by Calima (1,632 kg/ha) and BNA 4 (1,631 kg/ha), surpassing the ICA Quimbaya control (1,537 kg/ha); while for the second cycle there were no significant differences in the yield of the lines compared to the controls (2). This behavior is mainly explained because during the first production cycle the highest levels of daily solar brightness were recorded between flowering and grain filling stages, a condition that positively affected plant photosynthesis and decreased the incidence of foliar diseases (Figure 4).

At La Catalina station, the yields registered during the first production cycle were lower compared to the yields recorded during the second cycle; this behavior is largely explained by the high rainfall recorded (Figure 3), which favored the proliferation of foliar diseases that affected grain filling and caused the loss of plants due to lodging. During the first production cycle, the genotype with the highest yield was Calima (1,225 kg/ha) followed by BNA 4 (1,118 kg/ha), ICA Quimbaya (1,123 kg/ha), BNA 21 (1,106) and DAN 15 (1,078 kg/ha); while the lowest yield was observed in the BNA 8 line (1,013 kg/ha). During the second cycle, there was also an affectation of the yields in this location due to high rainfall (Figure 3); where the genotype with the highest yield was Calima (1,653 kg/ha) followed by ICA Quimbaya (1,350 kg/ha), BNA 21 (1,290 kg/ha), BNA 8 (1,222 kg/ha) and DAN 15 (1,214 kg/ha); while the lowest yield recorded for this cycle was presented in the BNA 4 line (996 kg/ha) (Figure 3).

At the El Rosario station, the yields obtained during the first production cycle were the highest yields recorded in all locations in all cycles, mainly due to a better offer of solar radiation evidenced by high values of solar brightness from flowering to grain filling. During the first production cycle, the genotypes with the highest yields were BNA 21 (2,208 kg/ha) and BNA 8 (2,197 kg/ha) followed by Calima (1,984 kg/ha) and ICA Quimbaya (1,945 kg/ha); while the lowest yields were found in the lines DAN 15 (1,744 kg/ha) and BNA 4 (1,722 kg/ha). During the second cycle, there was an impact on yields in this location due to high rainfall and low solar brightness (Figure 4) which influenced the fact that there were no significant differences between the lines and the controls (Figure 3). Weather data of the localities was analyzed by calculating average values and standard deviations for minimum, average and maximum temperature ($^{\circ}\text{C}$), relative humidity (%), accumulated solar brightness (hours/day), accumulated precipitation (mm/day) per production cycle which is available in <https://agroclima.cenicafe.org/>

3.2 Nutritional assessment

Nutritional quality of the biofortified bush bean lines mainly represented by the iron and zinc grain contents, showed significant differences between genotypes and between locations. Regarding the iron grain contents (mg/kg), significant differences were observed between the biofortified lines compared to the controls. In all the evaluation locations, BNA 4 line presented the highest iron contents (73.9 - 82.1 mg/kg), followed by BNA 8 line (72.4 - 76.5 mg/kg), BNA 21 line (66.5 - 72.4 mg/kg) and DAN 15 line (62.5 - 70.2 mg/kg); surpassing the control ICA Quimbaya (57.6 - 69.1) (Figure 5). In terms of zinc contents (mg/kg) present in the grains, significant differences were also observed between the biofortified lines compared to the controls. In all evaluation locations, the BNA 4 line presented the highest zinc contents (41.7 - 50.0 mg/kg), followed by the DAN 15 line (39.3 - 46.3 mg/kg), and by the BNA 8 line (37 - 46.3 mg/kg); the lowest zinc contents were recorded in the BNA 21 line (33.5 - 43.2) and in the ICA Quimbaya control (35.1 - 40 mg/kg) (Figure 5).

In order to estimate the impact of beans on the diet of populations at high risk of mineral deficiency such as growing children and women of childbearing age, the percentage contribution of the genotypes evaluated in the study was determined based on their content of iron, zinc, and phytic acid, in relation to the Estimated Average Requirement (EAR) for iron and zinc established by the European Food Safety Authority (EFSA). The EAR represents the average dietary intake level that meets the physiological needs of 50% of individuals. With this reference value, it is possible to determine whether a population is obtaining enough essential nutrients, such as iron and zinc, to prevent nutritional deficiencies that can lead to health problems.

The potential contribution of biofortified bush bean lines to the iron and zinc Estimated Average Requirement (EAR) is presented in Table 6. The biofortified lines BNA 4 and BNA 8 are estimated to provide between 65-68% of the iron EAR in children under 6 years and up to 63-66% in women, compared to 55% in children and 53% in women provided by the commercial variety ICA Quimbaya. Regarding zinc, it is estimated that the biofortified line BNA 4 can provide up to 38% and 27% of the Estimated Average Requirement in children aged 4–6 years and women, respectively, compared to the commercial variety, with a contribution of 33% and 23%, respectively. In summary, the greatest contribution to the EAR would be presented by the BNA 4 variety, since it is estimated that can provide up to 13% more to the iron Estimated Average Requirement than the commercial variety ICA Quimbaya, one of the most consumed by coffee-growing families in Colombia.

Table 6. Mineral and phytic acid (PA) content and contribution of bush bean advanced lines and commercial check to mineral daily intake and Percentage of Estimated Average Requirement (EAR) for children 4–6 years old and women of childbearing age.

Population	Genotype	Fe (mg/kg)	Zn (mg/kg)	PA (mg/g)	Mineral Daily Intake ^{ab}		Contribution to EAR ^{c,d}	
					Fe (mg)	Zn (mg)	Fe (%)	Zn (%)
Children	BNA4	78.50	44.53	15.30	3.41	1.74	68	38
	BNA8	74.50	39.67	15.14	3.24	1.55	65	34
	DAN15	67.27	41.67	16.99	2.93	1.63	59	35
	BNA21	69.17	36.83	15.51	3.01	1.44	60	31
	ICA Quimbaya	62.67	38.33	17.83	2.73	1.50	55	33
Women	BNA4	78.50	44.53	15.30	4.64	2.36	66	27
	BNA8	74.50	39.67	15.14	4.41	2.10	63	24
	DAN15	67.27	41.67	16.99	3.98	2.21	57	25
	BNA21	69.17	36.83	15.51	4.09	1.95	58	22
	ICA Quimbaya	62.67	38.33	17.83	3.71	2.03	53	23

^a Central region of Colombia: Children 5-12 y consumption = 50 g; Women consumption = 68 g (ENSIN 2015)

^b Iron cooking retention = 87%; Zinc cooking retention = 78% (Hummel et al., 2020)

^c EFSA Iron Estimated Average Requirements (EAR): Children 1-6 y = 5 mg/d; Women = 7 mg/d, assuming a 10% absorption efficiency

^d EFSA Zinc Estimated Average Requirements (EAR): Children 4-6 y = 4.6 mg/d; Women = 8.9 mg/d, assuming a 30% absorption efficiency

3.3 Genotype x Environment Analysis

To evaluate the stability of the genotypes across the different locations during the two evaluation cycles, a principal component analysis was performed for yield kg/ha (Figure 6) iron and zinc contents in mg/kg (Figure 7-8). The obtained results in terms of yield show that principal component PC1 explains 74.69%, which highlights the significant influence of environmental factors on genotype performance, of the total variation in the data, while PC2 explains 17.74% of the variation, and reflects genotype stability. Together PC1 and PC2 account for a significant portion (almost 92.43%) of the variation making this biplot a reliable representation of the genotype by environment interaction. The plot ranges from approximately -400 to 400 on the PC1 axis and -400 to 400 on the PC2 axis, showing a balanced spread of data points in all four quadrants.

The horizontal axis (PC1) represents the average yield of each genotype. Genotypes with points further to the right along the PC1 axis have higher mean yields, while those to the left have lower mean yields. Genotypes like BNA4 and DAN15 are positioned on the right side along the PC1 axis, suggesting that they perform significantly better in terms of yield compared to those near the origin or on the left in the environments tested such as ICA Quimbaya, BNA 21 and BNA 8. To evaluate stability, the vertical axis (PC2) is used. Genotypes closer to the horizontal line (PC2 = 0) are more stable because they exhibit less interaction with the environment, showing consistent performance across environments. For instance, La Catalina - Cycle 2, located lower on the PC2 axis might indicate less stability across environments tested compared to those closer to the horizontal zero line which signifies average stability such as La Catalina - Cycle 1 and Tambo Cycle 2.

Genotypes further from the horizontal axis show more variability meaning they are less stable, ICA Quimbaya is positioned far away from the horizontal line indicating low stability. BNA21 and BNA8 presents low yields but is highly stable across environments due to their proximity to the PC2 axis. Complementing this, the plot of eigenvalues versus accumulated variance further elucidates the underlying data structure. The first principal component (PC1) possesses the highest eigenvalue, underscoring its significant role in capturing the majority of variance within the dataset. Subsequent components exhibit a marked decrease in eigenvalues, with the accumulated variance reaching near completeness by PC4. This trend indicates that while PC1 provides the most substantial insight into data variance, incorporating up to PC4 ensures a comprehensive understanding, capturing nearly all the variability present (Figure 6).

Iron (Fe) content results show that principal component PC1 explains 81.25% of the total variation in the data. while PC2 explains 16.18% of the variation. Together, PC1 and PC2 account for a significant portion (almost 97.43%) of the variation, making this biplot a reliable representation of the genotype by environment interaction. The horizontal axis (PC1) represents the average of Fe content in grain for each genotype. Genotypes with points further to the right along the PC1 axis have higher iron content in grain, while those to the left have lower iron content. La Catalina and El Tambo are positioned further to the right, indicating high mean iron content in grain along with good stability. BNA4 and BNA8 are positioned near the horizontal line, suggesting high stability. BNA21 and DAN15 show relatively high iron grain content with reasonable stability. Finally, ICA Quimbaya (Check) shows both low iron grain content and poor stability across locations. The eigenvalue and accumulated variance graph provide a succinct overview of the principal components analysis conducted on Fe concentration data. The first principal component (PC1) exhibits a notably high eigenvalue, capturing a substantial portion of the dataset's variance, indicative of its significant role in summarizing the primary factors influencing Fe levels. In contrast, the second principal component (PC2), despite having a lower eigenvalue, still contributes meaningfully, as evidenced by the accumulated variance reaching nearly 100% with the inclusion of PC2 (Figure 7).

Zinc (Zn) content results show that principal component PC1 explains 80.82% of the total variation in the data, while PC2 explains 18.92% of the variation. Together, PC1 and PC2 account for a significant portion (almost 99.74%) of the variation, making this biplot a reliable representation of the genotype by environment interaction. The horizontal axis (PC1) represents the average zinc content in grain of each genotype. Genotypes with points further to the right along the PC1 axis have higher Zn content in grain. while those to the left have lower zinc content. La Catalina is positioned further to the right. indicating high mean zinc content in grain along with good stability. BNA4 and DAN15 are positioned near the horizontal line and further to the right. indicating high zinc grain content and good stability. BNA8 and BNA21 exhibit good stability but lower zinc grain content. In contrast. ICA Quimbaya (Check) shows both low zinc grain content and poor stability. Additionally, the eigenvalues and accumulated variance for a dataset analyzing zinc (Zn) concentration, provides key insights through its visual representation of principal components analysis (PCA). The first principal component (PC1) shows a high eigenvalue, indicating its substantial role in capturing the predominant variance within the dataset, which suggests that PC1 predominantly encapsulates the major influencing factors on Zn levels. Interestingly, PC1 alone accounts for a significant portion of the total variance, evidenced by the teal line which nearly reaches 100% of the accumulated variance with the inclusion of PC1. This demonstrates the effectiveness of PC1 in summarizing the critical information in the data (Figure 8).

3.4 Sensory assessment

To evaluate the sensory attributes like color, size, smell, flavor, and texture among the bush bean genotypes, a nonparametric Friedman rank test was performed in SAS software (SAS Institute Inc., Cary, NC, USA). This test was performed because the response variable has a measurement on an ordinal scale, so the data do not meet the assumption of normality and homogeneity of variances is not assumed. In the analysis, the panelist was included as a block, which improves the precision of the analysis, controlling possible variations that are not directly related to the treatments (bush bean genotypes). To obtain a more precise estimate of the p-value, an exact calculation was performed

using the Monte Carlo method with 10,000 simulations. Subsequently, for each of the attributes evaluated (color, size, smell, taste and texture) a Dunn post hoc test was applied to identify which pairs of genotypes presented significant differences. This test showed statistically significant differences in their distributions in favor of the ICA Quimbaya witness compared to the BNA 4 genotype for the five sensory attributes evaluated [$q > q(0.05)$] (Table 7).

Table 7. Comparative analysis of the hedonic scores of the evaluated genotypes in sensory test according to Friedman's nonparametric rank test and Dunn's post hoc test

Genotype	No. Obs	Color			Size			Smell			Flavor			Texture		
		Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max
BNA 4	103	5	1	7	6	2	9	5	3	7	6	2	7	6	2	7
ICA Quimbaya	103	6	3	8	6	3	7	6	3	7	6	2	7	6	2	9
BNA 21	103	6	4	7	6	3	7	6	3	7	7	3	7	6	3	7

Comparison group	Diff	q	q(0.05)	Diff	q	q(0.05)	Diff	q	q(0.05)	Diff	q	q(0.05)	Diff	q	q(0.05)
ICA Quimbaya vs BNA 4	104.87*	8,8	2,394	36.33*	3,11	2,394	10.27*	2,52	2,394	54.04*	4,58	2,394	39.66*	3,36	2,394
ICA Quimbaya vs BNA 21	1,28	0,1	2,394	27	2,31	2,394	6,06	0,51	2,394	29.76*	2,52	2,394	18,14	1,54	2,394
BNA 4 vs BNA 21	103.6*	8,69	2,394	9,33	0,8	2,394	24,21	2,02	2,394	24,28	2,06	2,394	21,52	1,82	2,394

* For each variable indicates differences per pairs according to Dunn's test, $\alpha=0.05$
 $q(0.05)$: Critical value $\alpha=0.05$
q: Calculated test statistic

4. CONCLUSION

This research highlights the potential of biofortified bush bean genotypes intercropped with coffee to address food security, economic sustainability, and nutritional deficiencies among smallholder farmers in Colombia. The results demonstrate significant advancements in agronomic performance, nutritional value, and adaptability of biofortified genotypes compared to traditional varieties. Among the genotypes, BNA 4 and BNA 21 emerged as the most promising, these lines exhibited determinate growth habits, which minimized physical interference with coffee plants, and showed early maturity, enabling efficient resource utilization. The genotypes consistently achieved higher yields in most locations, particularly under favorable environmental conditions; these traits make them well-suited for intercropping systems in coffee-dominated regions, offering a practical solution for diversifying income streams during coffee's unproductive phase. Nutritionally, the biofortified genotypes demonstrated significantly higher levels of iron and zinc compared to the commercial varieties, with BNA 4 consistently achieving the highest concentrations, this type of improvement is critical for addressing micronutrient deficiencies, particularly in rural Colombian communities where diets often lack sufficient iron and zinc. Sensory assessment revealed that the biofortified genotypes possess unique attributes, such as novel grain colors and shapes, that can differentiate them in local markets, while consumer acceptance was comparable to commercial varieties, further efforts are needed to enhance their marketability and promote adoption among farmers and consumers alike.

The study underscores the value of intercropping biofortified beans with coffee as a sustainable agricultural practice, this approach not only diversifies income sources but also enhances soil health, reduces dependency on synthetic inputs, and promotes resilience in agroecosystems. The findings emphasize the importance of integrating agronomic traits, nutritional quality, and consumer preferences into breeding programs to maximize the impact of biofortification initiatives. Ultimately, the study highlights the potential of biofortified bush beans to transform smallholder coffee systems by improving family nutrition, increasing profitability, and contributing to sustainable rural livelihoods, where the scalability of this approach could have far-reaching implications for addressing global challenges in nutrition and sustainable agriculture.

Declarations

Acknowledgments

The authors gratefully acknowledge Dr. Gerardo Montenegro and Dr. Álvaro Gaitán of Cenicafe - Colombian Coffee Growers Federation (FNC); Dr. Carolina González and Dr. Steve Beebe of Alliance of Bioversity International and CIAT

- Funding: This work was supported by Fondo Nacional del Café - National Coffee Research Center (Cenicafe) (Crossref Funder ID 100019597).
- Conflicts of interest/Competing interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
- Ethics approval: this research is adhered to the principles outlined in the International Center for Tropical Agriculture (CIAT) Research Ethics Policy and was conducted in accordance with the Code of Conduct for Responsible Research of the CGIAR and the International Code of Conduct for the Sustainable Use of Fertilizers and Plant Genetic Resources.
- Consent to participate: This research was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and followed the guidelines for responsible research established by Institutional Review Board (IRB) of Alliance of Bioversity International and CIAT (Approval number of the ethics clearance: 2022-IRB35). All procedures involving human participants (e.g., interviews, surveys) were carried out with their informed consent, ensuring anonymity, voluntary participation.
- Clinical Trial: Not applicable.
- Consent for Publication: All authors have reviewed and approved the final version of the manuscript and provided their informed consent prior to inclusion in the study.
- Availability of data and material : The datasets generated and/or analyzed during the current research are available from the corresponding author upon reasonable request. All relevant agronomic, nutritional, and sensory data used in this study are stored in institutional repositories and can be made available in accordance with institutional and data-sharing policies.
- Code availability : Code will be made available on request to santiago.jaramilloc@cafedecolombia.com
- Author contributions: Santiago Jaramillo-Cardona: Corresponding Author; SJ; JA: conceptualization, methodology, analysis, research, data curation and writing original draft; SJ; JA; SG; RM.: analysis, methodology and writing—review and editing; SJ; JA; SG; RM; HFB; NR: revised the manuscript. All the authors read and approved the final manuscript.
- Declaration of Competing Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Ann, C. E., & Francis, C. A. (1985). Bean-Maize intercrops: A comparison of bush and climbing bean growth habits. *Field Crops Research*, 1, 319-335. [https://doi.org/10.1016/0378-4290\(85\)90023-1](https://doi.org/10.1016/0378-4290(85)90023-1)
2. Arcos, J., & Rojas, D. C. (2019). Recomendaciones para la producción de grano de frijol biofortificado en Colombia. HarvestPlus, CIAT & CGIAR Research Program on Agriculture Nutrition and Health. <https://hdl.handle.net/10568/100818>
3. Bhatt, R., Hossain, A., & Sharma, P. (2020). Zinc biofortification as an innovative technology to alleviate the zinc deficiency in human health: A review. *Open Agriculture*, 5(1), 176–187. <https://doi.org/10.1515/opag-2020-0018>
4. Beebe, S. (2012). Common bean breeding in the tropics. In *Plant Breeding Reviews* (Vol. 36, pp. 357-426). John Wiley & Sons. <https://doi.org/10.1002/9781118358566.ch5>
5. Beebe, S. (2020). Biofortification of common bean for higher iron concentration. *Frontiers in Sustainable Food Systems*, 4, Article 573449. <https://doi.org/10.3389/fsufs.2020.573449>
6. Blair, M. W. (2013). Mineral biofortification strategies for staples: The example of common bean. *Journal of Agricultural and Food Chemistry*, 61(35), 8287-8294. <https://doi.org/10.1021/jf400774y>

7. Bouis, H. E., & Welch, R. M. (2010). Biofortification—A sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. *Crop Science*, 50(S1). <https://doi.org/10.2135/cropsci2009.09.0531>
8. Brigide, P., Canniatt-Brazaca, S., & Silva, M. O. (2014). Nutritional characteristics of biofortified common beans. *Food Science and Technology*, 34(3), 493-500. <https://doi.org/10.1590/1678-457x.6245>
9. Brooker, R. W., & Others. (2015). Improving intercropping: A synthesis of research in agronomy, plant physiology and ecology. *New Phytologist*, 206, 107–117. <https://doi.org/10.1111/nph.13132>
10. Broughton, W. J., Hernández, G., Blair, M., Beebe, S., Gepts, P., & Vanderleyden, J. (2003). Beans (*Phaseolus* spp.)—Model food legumes. *Plant and Soil*, 252, 55-128. <https://doi.org/10.1023/A:1024146710611>
11. Connorton, J. M., & Balk, J. (2019). Iron biofortification of staple crops: Lessons and challenges in plant genetics. *Plant and Cell Physiology*, 60(7), 1447–1456. <https://doi.org/10.1093/pcp/pcz079>
12. Debouck, D., & Hidalgo, R. (1986). Morphology of the common bean plant *Phaseolus vulgaris*. CIAT, Cali, Colombia. <https://hdl.handle.net/10568/54185>
13. Duque-Orrego, H., Salazar, H. M., Rojas-Sepúlveda, L. A., & Gaitán, Á. (2021). Análisis económico de tecnologías para la producción de café en Colombia. Cenicafé. <https://doi.org/10.38141/cenbook-0016>
14. EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies). (2014). Scientific opinion on dietary reference values for zinc. *EFSA Journal*, 12(10):3844. <https://doi.org/10.2903/j.efsa.2014.3844>
15. EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies). (2015). Scientific opinion on dietary reference values for iron. *EFSA Journal*, 13(10):4254. <https://doi.org/10.2903/j.efsa.2015.4254>
16. ENSIN. (2015). Encuesta Nacional de la Situación Nutricional- 2015. Ministerio de Salud, Departamento para la Prosperidad Social y ICBF. <https://www.icbf.gov.co/bienestar/nutricion/encuesta-nacional-situacion-nutricional>
17. Federación Nacional de Cafeteros de Colombia- FNC. (2023). Informe de Gestión 2023. <https://federaciondecafeteros.org/wp/informe-de-gestion-2023/>
18. Federación Nacional de Cafeteros de Colombia, Centro Nacional de Investigaciones de Café. (2023). Anuario Meteorológico Cafetero 2022. Cenicafé. <https://doi.org/10.38141/10782/anu2022>
19. George, T. S., Hawes, C., Newton, A. C., McKenzie, B. M., Hallett, P. D., & Valentine, T. A. (2014). Field phenotyping and long-term platforms to characterize how crop genotypes interact with soil processes and the environment. *Agronomy*, 4, 242-278. <https://doi.org/10.3390/agronomy4020242>
20. Hampton, J., Mesquita, F., & Hill, M. (1997). The effect of genotype and plant density on grain yield and cooking quality in common bean (*Phaseolus vulgaris* L.). *Proceedings of the Agronomy Society of New Zealand*, 27, 109–114.
21. Hill, J. (1996). Breeding components for mixture performance. *Euphytica*, 92, 135–138. <https://doi.org/10.1007/BF00022838>
22. Hummel, M., Talsma, E. F., Taleon, V., Londoño, L., Brychkova, G., Gallego, S., Raatz, B., & Spillane, C. (2020). Iron, zinc, and phytic acid retention of biofortified, low phytic acid, and conventional bean varieties when preparing common household recipes. *Nutrients*, 12(3), 658. <https://doi.org/10.3390/nu12030658>
23. International Coffee Organization - ICO. (2022). World coffee statistics database. Retrieved from <https://ico.org/what-we-do/world-coffee-statistics-database/>
24. Jaramillo, S., & Salazar, H. M. (2021). Cultivos intercalados: Una alternativa para aumentar los ingresos y la sostenibilidad de cafetales. *Avances Técnicos Cenicafé*, 534, 1–8. <https://doi.org/10.38141/10779/0534>
25. Jaramillo, S. (2023). Recomendaciones para el manejo agronómico del cultivo de fríjol arbustivo en el sistema intercalado con café. *Avances Técnicos Cenicafé*, 551, 1-12. <https://doi.org/10.38141/10779/0551>
26. Jaramillo, S., & Jaramillo-Jiménez, A. (2025). Nuevas variedades de fríjol arbustivo biofortificado adaptadas al sistema intercalado con café. *Avances Técnicos Cenicafé*, 571, 1–8. <https://doi.org/10.38141/10779/0571>

27. Nienhuis, J., & Singh, S. P. (1985). Effects of location and plant density on yield and architectural traits in dry beans. *Crop Science*, 25, 579–584. <https://doi.org/10.2135/cropsci1985.0011183X002500040001x>
28. Pachón, H. (2010). El impacto nutricional de cultivos biofortificados o cultivos con mayor calidad nutricional. Cali, Colombia: CIAT. ISBN 978-958-694-100-6.
29. Postma, J., & Lynch, J. P. (2012). Complementarity in root architecture for nutrient uptake in ancient maize/bean and maize/bean/squash polycultures. *Annals of Botany*, 110, 521–534. <https://doi.org/10.1093/aob/mcs082>
30. Ray, H., Bett, K., Tar'an, B., Vandenberg, A., Thavarajah, D., & Warkentin, T. (2014). Mineral micronutrient content of cultivars of field pea, chickpea, common bean, and lentil grown in Saskatchewan, Canada. *Crop Science*, 54(4), 1698–1708. <https://doi.org/10.2135/cropsci2013.08.0568>
31. Ricaurte, J., Clavijo, J. A., Sinclair, T. R., Idupulapati, M. R., Rao, M., & Beebe, S. E. (2016). Sowing density effect on common bean leaf area development. *Crop Science*, 56. <https://doi.org/10.2135/cropsci2016.01.0056>
32. Sadeghian, K., S. (2008). Guía práctica de fertilidad del suelo y nutrición del café en Colombia. (Boletín Técnico No 32). Manizales: CENICAFÉ. <http://hdl.handle.net/10778/587>
33. Saltzman, A., Birol, E., Bouis, H. E., Boy, E., De Mouea, F. F., Islam, Y., & Pfeiffer, W. H. (2013). Biofortification: Progress toward a more nourishing future. *Global Food Security*, 2(1), 9–17. <http://doi.org/10.1016/j.gfs.2012.12.003>
34. Sarandón, J. S., & Chamorro, A. M. (2003). Policultivos en los sistemas de producción de granos. In E. Satorre, A. R. Benech, G. A. Slafer, E. de la Fuente, D. Miralles, M. E. Otegui, & R. Savin (Eds.), *Producción de cultivos de granos. Bases funcionales para su manejo* (pp. 783). Buenos Aires: Editorial Facultad de Agronomía.
35. Schoonhoven, A., & Corrales, P. (1991). Sistema estándar para la evaluación de germoplasma de frijol. *Guía de evaluación del Centro Internacional de Agricultura Tropical (CIAT)*, pp.12-13. <https://hdl.handle.net/10568/69699>
36. Vaz-Tostes, M. G., Verediano, T. A., de Mejia, E. G., & Costa, N. M. B. (2016). Evaluation of iron and zinc bioavailability of beans targeted for biofortification using in vitro and in vivo models and their effect on the nutritional status of preschool children. *Journal of the Science of Food and Agriculture*, 96(4), 1326–1332. <https://doi.org/10.1002/jsfa.7226>

Figures

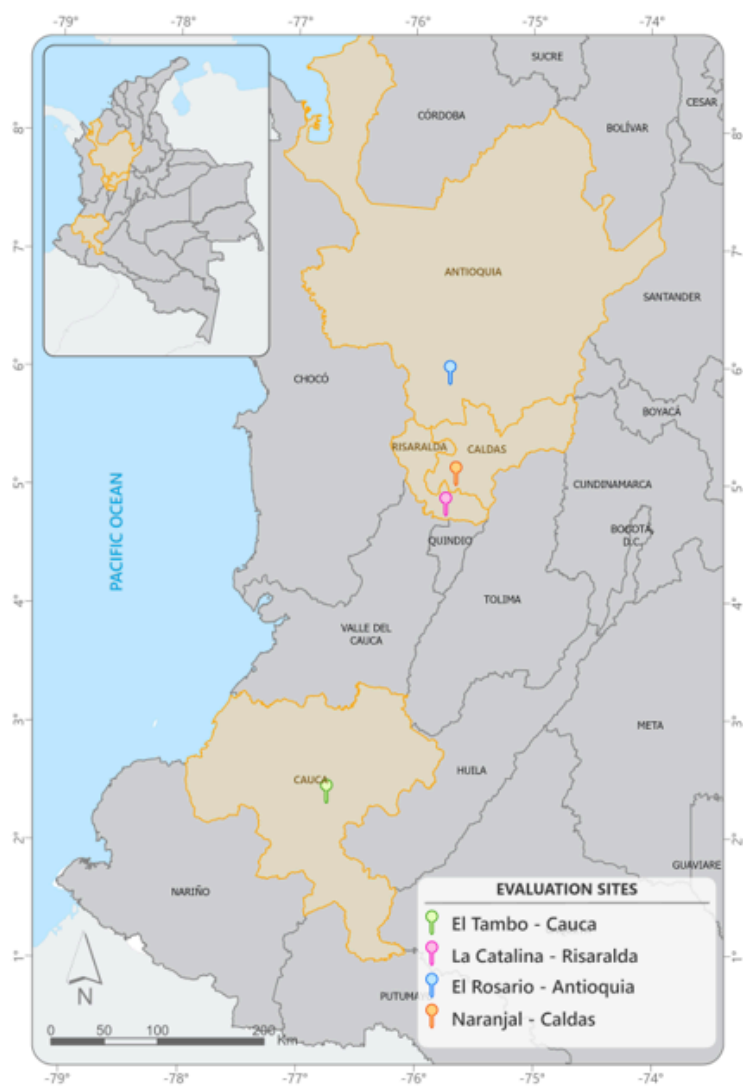


Figure 1

Study area map with the evaluation sites corresponding to Cenicafé experimental stations located in Colombia

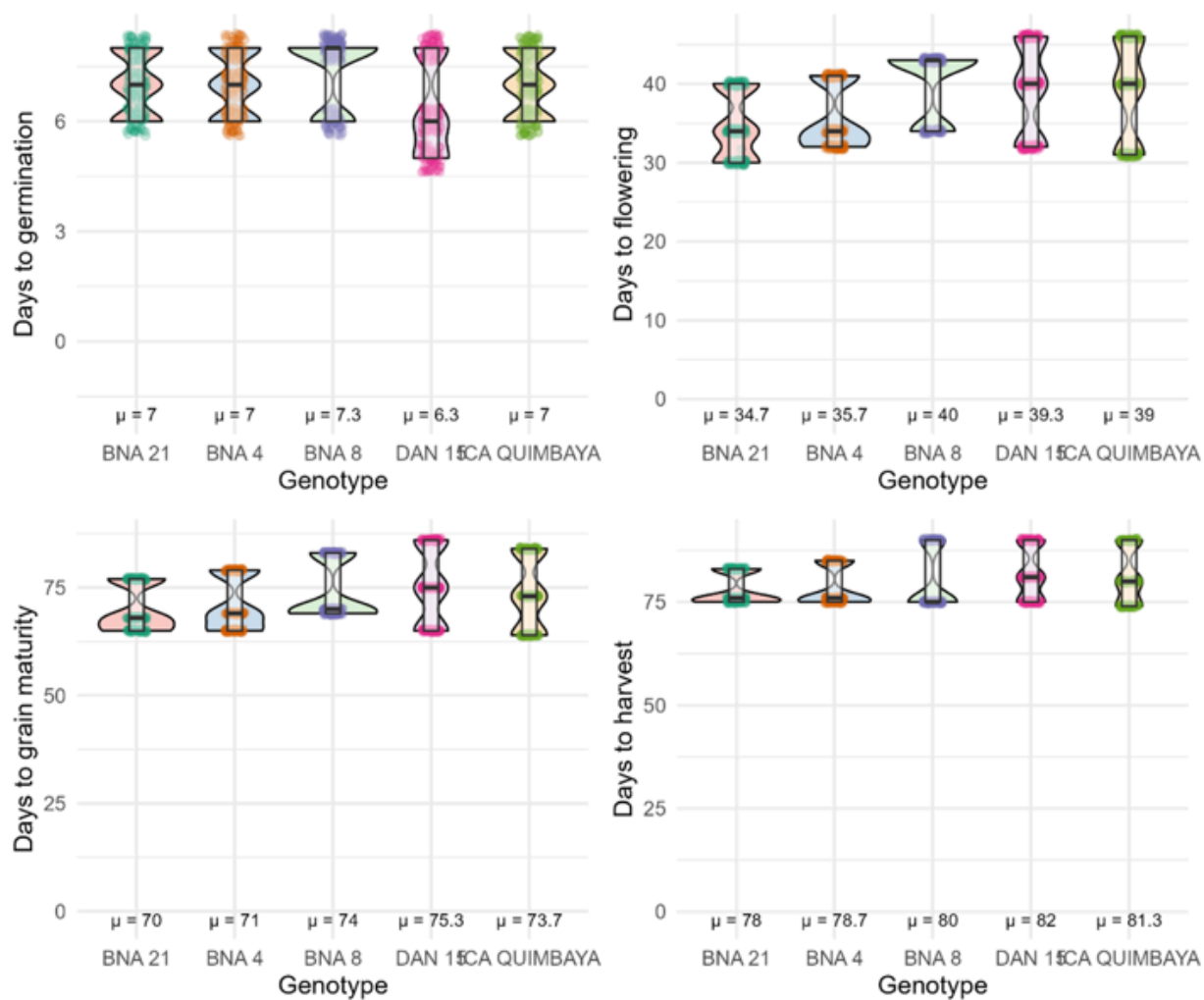
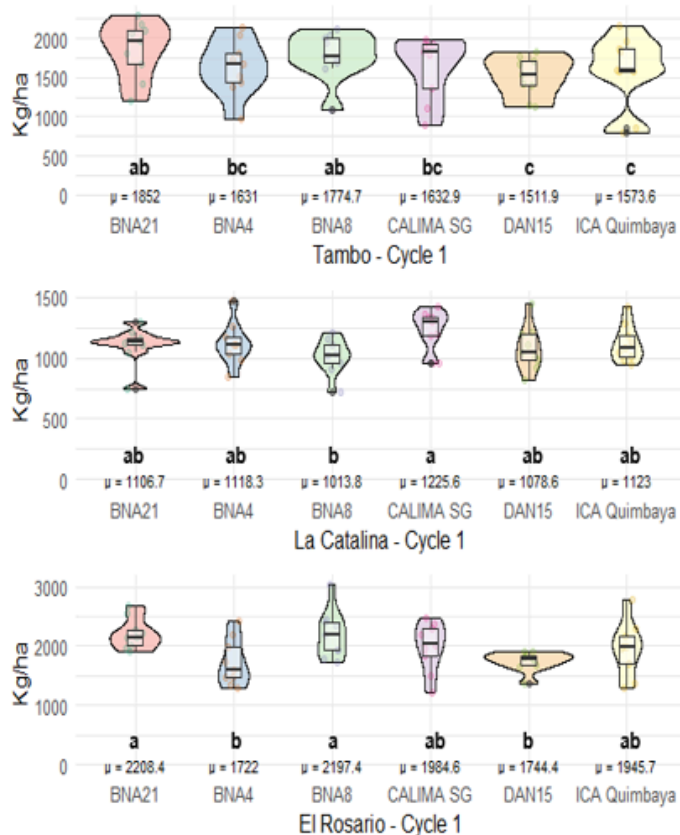


Figure 2

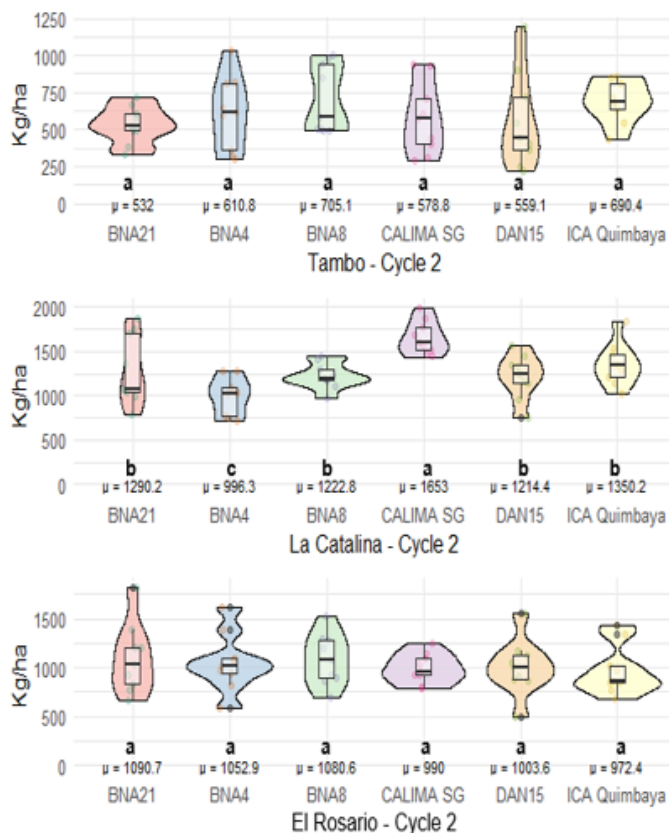
Phenological behavior of biofortified bush bean advanced lines and commercial check intercropped with coffee

Yield (kg/ha) in Tambo, Catalina, and Rosario in Cycle 1



* Letters represent LSD (Least Significant Difference) comparisons are a post-hoc analysis method used following an ANOVA (Analysis of Variance) test. The LSD test is designed to identify which specific means are significantly different from each other.

Yield (kg/ha) in Tambo, Catalina, and Rosario in Cycle 2



* Letters represent LSD (Least Significant Difference) comparisons are a post-hoc analysis method used following an ANOVA (Analysis of Variance) test. The LSD test is designed to identify which specific means are significantly different from each other.

Figure 3

Yield differences (kg/ha) between bush bean advanced lines and commercial checks intercropped with coffee in three locations and two production cycles

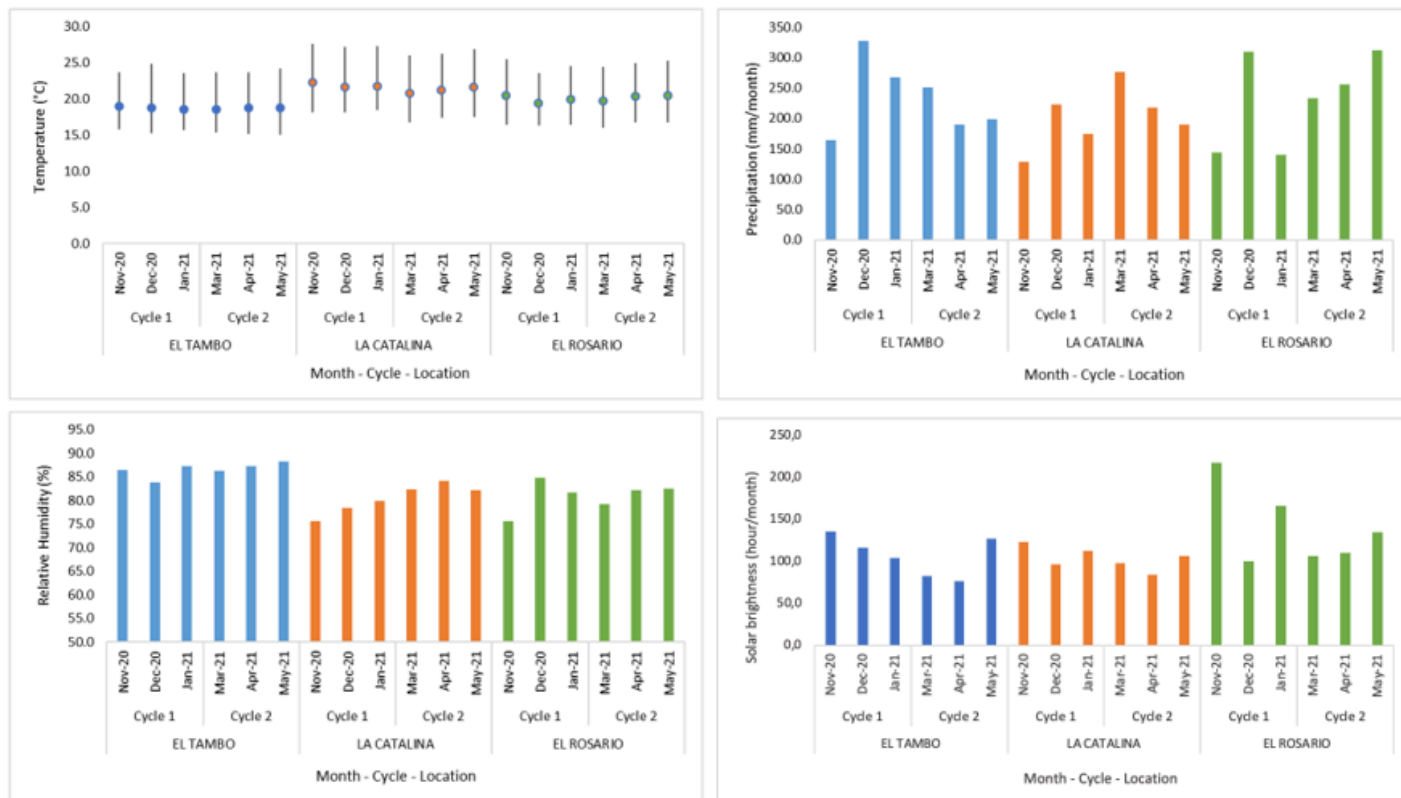


Figure 4

Weather variables registered in the three locations during two evaluation cycles: (a) Temperature - min, avg, max (°C); (b) accumulated precipitation (mm/month); (c) average relative humidity (%) and (d) accumulated solar brightness (hours/month)

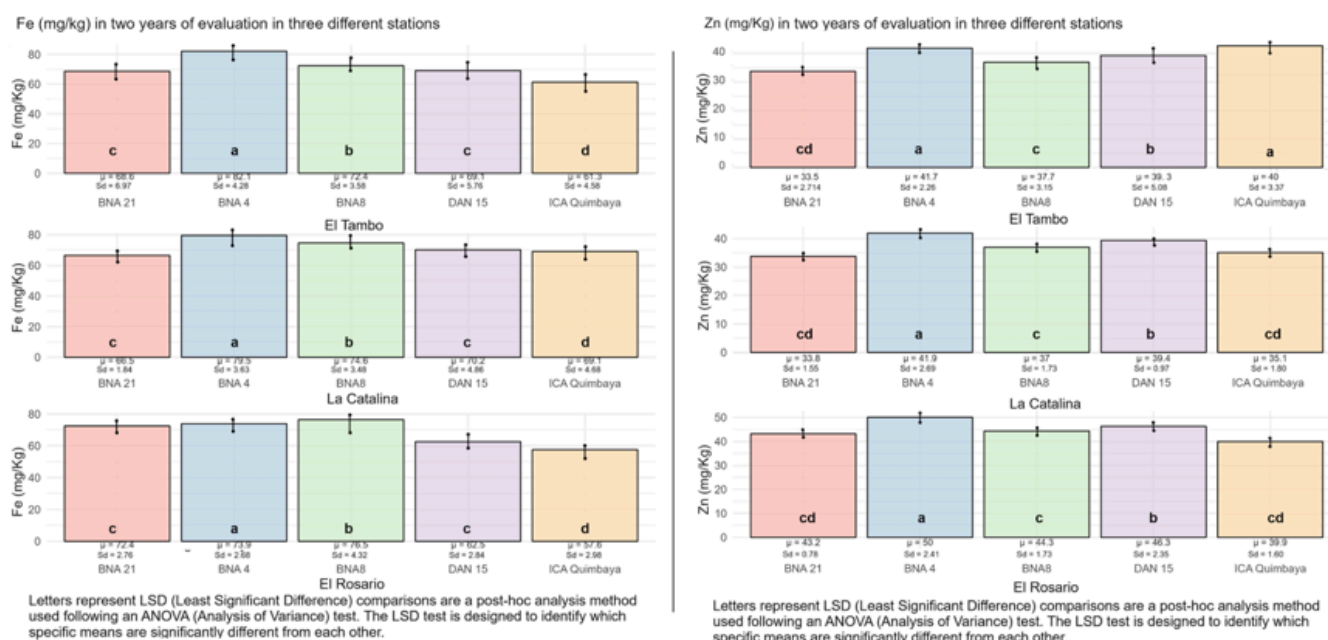


Figure 5

Iron and zinc contents (mg/kg) present in the grain of the advanced lines of bush beans and in the commercial control ICA Quimbaya in the three evaluation locations

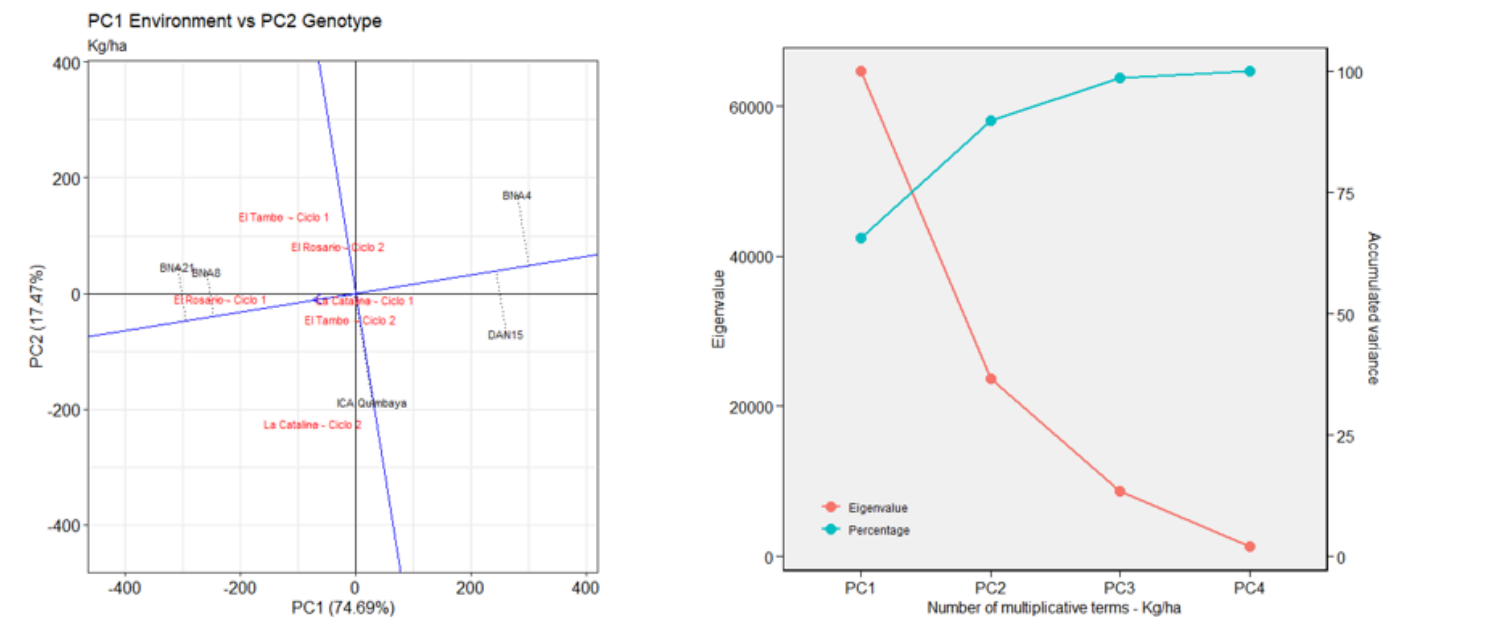


Figure 6

GGE biplot represents the genotype x environment interaction for yield (kg/ha), in addition to the comparisons between eigenvalues and accumulative variance of each PC.

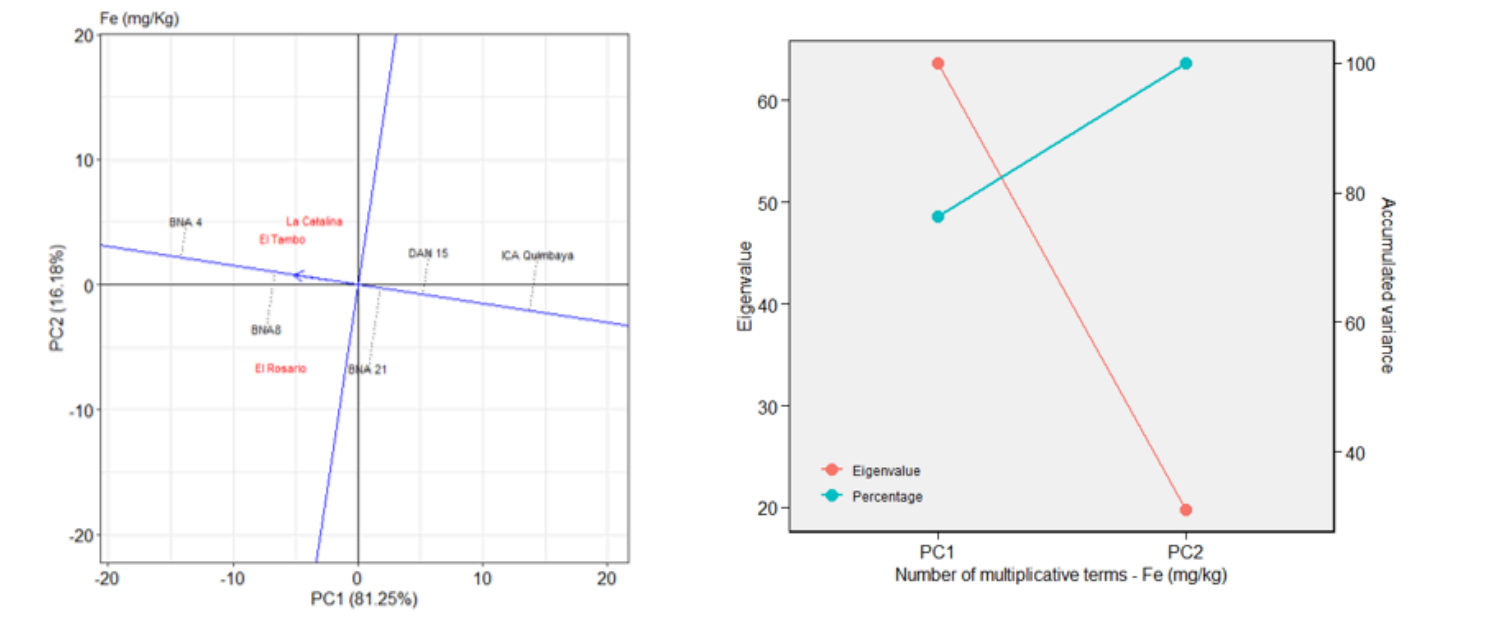


Figure 7

GGE biplot represents the genotype x environment interaction for Fe (mg/kg), in addition to the comparisons between eigenvalues and accumulative variance of each PC.

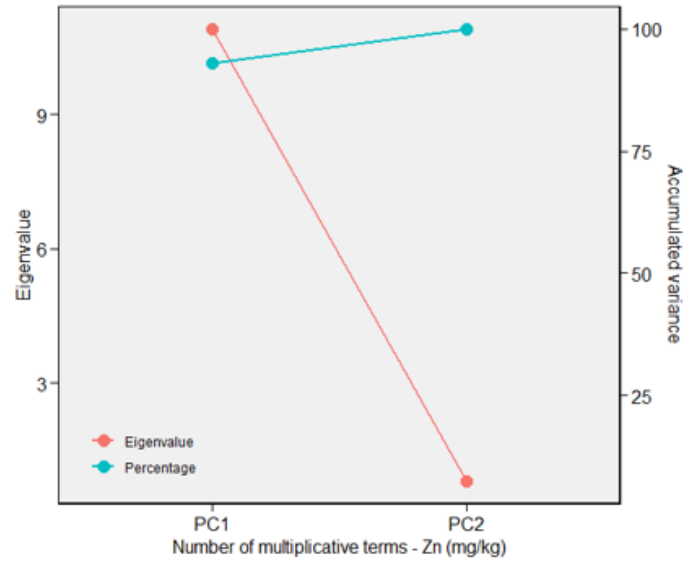
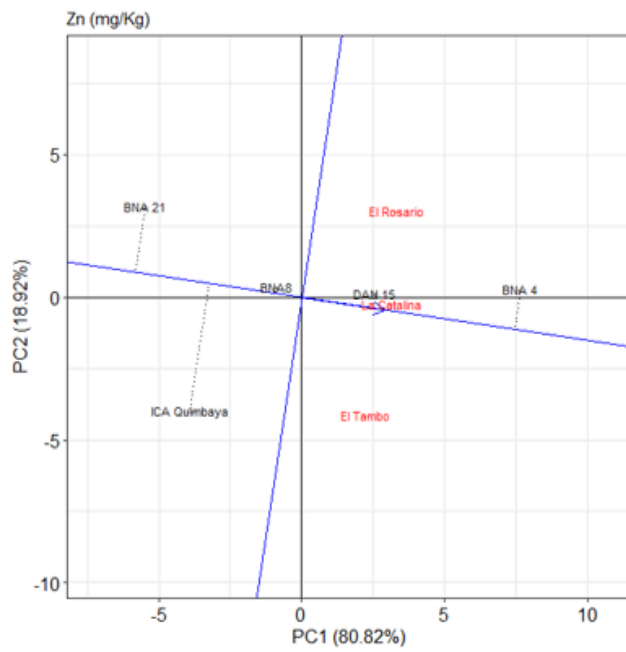


Figure 8

GGE biplot represents the genotype x environment interaction for Zn (mg/kg), in addition to the comparisons between eigenvalues and accumulative variance of each PC.