Legume Science

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ORIGINAL ARTICLE OPEN ACCESS

Genetic and the Environmental Influences on the Concentrations Iron and Zinc in Small Seeded Common Bean (*Phaseolus vulgaris* L.) Varieties and Advanced Lines From Ethiopia

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Received: 4 April 2023 | **Revised:** 27 January 2024 | **Accepted:** 9 February 2024

Funding: This study is supported by Tropical Legume III (TL-III) and the Ethiopian Institute of Agricultural Research (EIAR).

Keywords: AMMI | biofortification | environment | genotype by environment interaction | iron | iron bioavailability | *Phaseolus vulgaris* L | zinc

ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is a grain legume rich in proteins and micronutrients, particularly iron and zinc. In this study, 30 small-seeded genotypes were planted in five locations in Ethiopia, following an alpha lattice design with three replications, to determine environmental and genotypic influence on the Fe and Zn concentration. Based on their Fe and Zn contents, bean cultivars were evaluated for adaptability and stability using AMMI analysis. The Fe concentrations of raw bean seed varied from 44.4 to 84.4 μg/g within the panel of small-seeded genotypes, with an average range of variance of 18 μg/g across environments, and its seed Zn concentrations varied from 19.7 to 32.3 μg/g, with an average range of variance of 12.6 μg/g across environments. The averages bean Fe concentration among the small-seeded genotypes across sites in Ethiopia was 62.2 and 26.1 μg/g for Zn concentrations. Results from the analysis of variance using the AMMI model indicated that genotypes accounted for 20.53% and 9.49% of the total variance in seed Fe and Zn concentrations, respectively. The environment had a greater impact, affecting 60.92% and 81.52% of total sum of squares for Fe and Zn concentrations, respectively. According to the broad-sense heritability, there appears to be some genetic control over Fe and Zn concentrations. However, the substantial effects of the environment and genotype-by-environment interaction on Fe and Zn concentrations in small-seeded genotypes indicates breeding for higher amounts of trace minerals in new bean varieties could be a challenging task. This means the notion that beans can be biofortified to have higher concentrations of Fe and Zn might not be achievable in Ethiopia. A shift in breeding strategies that focuses on traits to enhance the bioavailability of Fe and Zn from bean is warranted and could be a solution to enhance the delivery of iron from small-seeded beans produced in Ethiopia.

1 | Introduction

The most widely cultivated food legume crop globally is the common bean (*Phaseolus vulgaris* L.). It is primarily produced as a fresh vegetable (snap beans and green beans) or as a grain crop (i.e., dried beans). Global production of dry seeds and fresh pods in 2020 reached 27,545,942 tons and 23,276,716 tons, respectively. A total of 34,801,567 and 1,579,489 ha were designated for the production of these crops (FAOSTAT 2021).

The common bean is the most widely grown and consumed grain legume in Ethiopia, coming in second place only to Faba beans in terms of production. This is attributed to the yield of the Faba bean being higher than common bean. Approximately 311,583.58 ha, or 2.4% of Ethiopia's total grain crop area, are planted to produce common beans. This contributes 1.61% (552,564.074 tons) of Ethiopia's total grain production (CSA 2020).

In low- and mid-altitude regions of Ethiopia, nearly 3.0 million subsistence farmers cultivate the common bean (CSA 2020). There are wide ranges of common bean types grown in Ethiopia including mottled, red, white, speckled and black varieties (Ali et al. 2006). Most commercial varieties are pure red or pure white in colour beans and becoming the most commonly grown types in Ethiopia with increasing market demand (Ferris and Kaganzi 2008). On the other hand, coloured large-seeded (mottled and speckled) varieties are preferred for local markets and consumption (Legesse and Ayenew 2006).

Beans provide essential nutrition and income for nearly 3 million smallholder farmers who largely live hand to mouth in the country. Common beans, a nutrient-dense food that can be canned and exported, bring in three times as much revenue for farmers as other common commodities like maize (*Zea mays* L.). The common bean breeding programmes were conducted by the Ethiopian Institute of Agricultural Research (EIAR) in collaboration with other organizations aimed to improve the yield and quality of the crop. As a result, 58 improved common bean cultivars were made available up until 2017 (MoA 2017). With the help of these improved varieties and farmer training on best management practices, the seed yields increased considerably from an average of 0.5 tons/ha in 2004 to 1.78 tons/ha in 2020(CSA 2020).

The common bean is a significant source of protein, complex carbohydrates, B vitamins (especially folate), dietary fibre, and minerals such as calcium (Ca), magnesium (Mg), potassium (K), copper (Cu), iron (Fe) and Zinc (Zn) (Blair 2013; Broughton et al. 2003). Through symbiotic nitrogen fixation, common bean also aids in enhancing the health of the soil and the environment. Numerous bioactive compounds, such as flavonoids found in the seed coats of beans have been linked to the prevention of chronic illnesses that include obesity, diabetes, cancer and coronary heart disease (Messina 2014).

More than one-third of the world's population continues to struggle with micronutrient deficiency, a significant global health issue (Gonmei and Toteja 2018). The main causes of mineral malnutrition are deficits in dietary intakes of Fe and Zn. Iron is integral to oxygen transport proteins via haemoglobin and myoglobin (Fava, Piepoli, and Villani 2019). Iron deficiency, which affects 27% of the world's population, is the main source of anaemia (Ning and Zeller 2019). Iron deficiency and anaemia increases the risk of lowered baby birth weight, fatigue, immune system suppression, impaired brain development and higher mortality and morbidity rates (Cappellini, Musallam, and Taher 2020; Jamnok et al. 2020).

Estimates place the global population at risk for low zinc intake between 17.6% and 29.6%, with the largest incidence seen in South Asia, South-East Asia, Sub-Saharan Africa and Central America (Gupta, Brazier, and Lowe 2020). The immune system, cell division, cell growth, wound healing, carbohydrate metabolism, reproduction, and the sensations of taste and scent all depend on zinc. Children have been found to have a high mortality rate from infections linked to insufficient Zn intake. As a catalyst, structural, and regulatory ion, zinc plays important functions in biological systems (Grüngreiff, Gottstein, and Reinhold 2020). Zinc plays an important role in many metabolic pathways, which makes it essential for healthy bodily growth and development. Zn deficiency results in immune and epidermal disorders, hypogonadism, delayed and decreased development, and central nervous system dysfunction (Chasapis et al. 2020). Since Zn cannot be retained by the human body, it is advised to consume enough Zn to prevent deficiency (Chasapis et al. 2020; Grüngreiff, Gottstein, and Reinhold 2020). Dietary Reference Intakes (DRIs) developed by the Food and Nutrition Board (FNB) at the Institute of Medicine of the National Academies of the National Academies (United States) include intake recommendations for Zn, with the Recommended Dietary Allowance (RDA) for men and women in the >19 age groups being 8 mg/day and 11 g/day, respectively (Dixon, Winkleby, and Radimer 2001).

Common bean is a major staple and an important vehicle to deliver Fe and Zn into the diets of both the rural and urban populations. Beans are a staple food crop that has been targeted for Fe biofortification (Huertas et al. 2022). Therefore, in addition to having the required agronomic traits, like seed yield and resistance to biotic and abiotic stress, it is crucial to find and create bean varieties that provide enhanced Fe and Zn nutrition (Bailey-Serres et al. 2019). The seed Fe and seed Zn concentrations of common bean genetic resources have been shown to be influenced by G × E in studies conducted in different countries such as Central Africa (Blair et al. 2010), Mexico,(García-Díaz et al. 2018) and Turkish (Celmeli et al. 2018; Nadeem et al. 2020). However, in Ethiopia evaluation of released varieties and advanced lines genotypes have not been fully evaluated for micronutrient content to understand their variable or stable performance across different environments.

Recent research shows that common beans have not been sufficiently evaluated for genotype by environment and phenotype stability of Fe content across various agroecology (Glahn and Noh 2021). In Ethiopia, there are no studies that specifically address different agroecology environmental conditions, which can influence the concentration of Fe and Zn in small seed common bean genotypes. Also, in regard to bean Fe biofortification, the average baseline Fe and Zn concentrations has not been established for Ethiopia. Harvest Plus uses a global average of 50 μg/g for bean iron concentrations, however, a recent study (Glahn, Wiesinger, and Lung'aho 2020) revealed that the countries of East Africa did not represented by the average Fe concentration of 50 μg/g from the CIAT core collection, where the average bean Fe levels of non-biofortified varieties was approximately 71 μg/g, essentially equal to that of biofortified varieties.

Consequently, the main goal of this research was to ascertain whether high Fe and high Zn beans could be consistently grown in Ethiopia and to identify the average Fe and Zn concentration in Ethiopia from small seeded varieties popular in the Ethiopian diets and marketplace. The extent of genotype and genotype by environment interaction (GEI) for Fe and Zn concentrations in Ethiopian common bean genotypes was also evaluated in this study.

2 | Materials and Methods

This study evaluated 30 small-seeded common bean types, 25 of which were released over a time span of 46 years, and five advanced breeding lines. These bean varieties are representative of the majority of beans found in Ethiopia's food supply and market. The Melkasa Agricultural Research Center's (MARC) database for the common bean breeding programme at the Ethiopia Institute of Agricultural Research contained information on the five advanced breeding lines for seed yield results and yield-related traits (EIAR). The details of experimental materials are presented in Table 1.

Seeds of the candidate and released varieties were obtained from the federal and regional institutions of agricultural research and universities in Ethiopia. The field experiments were performed in five locations representing Ethiopia's main common bean-producing areas differing in altitude; 1550–1980 masl (meter above sea level). Table 2 lists the description of locations where the evaluations were performed and the corresponding sowing dates.

Before planting, three sub-samples of composite soil were taken from each trial plot along the field's diagonal, at a 0–20 cm depth. The samples were sent to Kampala, Uganda, where Aggrotech Analytical Laboratory Service LTD performed the

TABLE 1 | Description of the small-seeded common bean varieties and advanced lines from Ethiopia.

S. N	Breeders code	Variety name	Year of release	Maintainer	Seed colour	Grow	Gene pool
G1	G11239	Mexican-142	1973	MARC/EIAR	White	TypeIIa	Meso-Andean
G2	Red Wolaita	Red Wolaita	1974	MARC/EIAR	Red	Bush	Meso-Andean
G3	G445	Awash 1	1990	MARC/EIAR	White	Bush	Meso-Andean
G4	A176	Roba-1	1990	MARC/EIAR	Cream	Bush	Meso-Andean
G5	Melk 97	Beshbesh	1998	MARC/EIAR	Cream	Bush	Meso-Andean
G6	PAN182	Awash Melka	1999	MARC/EIAR	White	Bush	Meso-Andean
G7	A788	Tabor	1999	ARARC/SRARI	Cream	Bush	Meso-Andean
G8	DICTA 105	Nasir	2003	MARC/EIAR	Red	Bush	Meso-Andean
G9	DOR-554	Dimtu	2003	MARC/EIAR	Red	Bush	Meso-Andean
G10	812-BRC-28	Tibe	2004	BARC/OARI	Red	Climber	Meso-Andean
G11	AR04GY	Argene	2005	MARC/EIAR	White	TypeIIa	Meso-Andean
G12	TA04JI	Nazareth-2	2005	MARC/EIAR	White	TypeIIa	Meso-Andean
G13	EMP-376	Anger	2005	BARC/OARI	Red	Type-II	Meso-Andean
G14	XAN310	Dinknesh	2006	MARC/EIAR	Red	Bush	Meso-Andean
G15	STTT-165-92	Chore	2006	MARC/EIAR	White	Bush	Meso-Andean
G16	STTT-165-96	Chercher	2006	HU	White	Bush	Meso-Andean
G17	VAX-2	Gabisa	2007	BARC/OARI	Cream	Bush	Meso-Andean
G18	DOR811	Dursitu	2008	CIAT line/HU	Red	Bush	Meso-Andean
G19	SNNPR-120	HawassaDume	2008	HwRC/SARI	Red	Bush	Meso-Andean
G20	Awash-2	Awash-2	2013	CIAT line	White	Bush	Meso-Andean
G21	SER-119	SER-119	2014	MARC/EIAR	Red	Bush	Meso-Andean
G22	SER-125	SER-125	2014	MARC/EIAR	Red	Bush	Meso-Andean
G23	RWR-719	Omo-95	2003	ARARC/SRARI	Red	Bush	Meso-Andean
G24	CAW-02-04-11-4-1	SARI-1	2011	HwRC/SARI	Red	Bush	Meso-Andean
G25	Bifort small seeded – 15	Awash Mitin	2017	MARC/EIAR	White	Bush	Meso-Andean
G26	RAZ-11		Advanced lines	MARC/EIAR	White	Bush	Meso-Andean
G27	RAZ-42	Nekeze ayfere	Advanced lines	MARC/EIAR	White	Bush	Meso-Andean
G28	SCN-5	Awash Tikure	Advanced lines	MARC/EIAR	Black	Bush	Meso-Andean
G29	SCN-11		Advanced lines	MARC/EIAR	Black	Bush	Meso-Andean
G30	SCR-11		Advanced lines	MARC/EIAR	Red	Bush	Meso-Andean

Abbreviations: BARC = Bako Agricultural Research Center; EIAR = Ethiopian Institute of Agricultural Research; HU = Haramaya University; MARC = Melkasa Agricultural Research Center; OARI = Oromia Agricultural Research Institute; SRARI = South Regional Agricultural Research Institute.

TABLE 2 | Description of the research sites where evaluations were performed (2017).

Location	Soil type	Altitude (m.a.s.l)	Latitude	Longitude	Annual average		Rainfall (mm)	Sowing date
					Min (°C)	Max (°C)		
Alem Tena	Andosols	1610	8°18′N	38°57′E	12.9	29.8	728	19 July 2017
Aris Negele	Nitisols	1890	7°35′N	38°65′E	11.1	25.2	876	14 July 2017
Haramaya	Fluvisol	1980	9°26′N	42°03′E	12.3	24.3	790	24 July 2017
Melkasa	Andosols	1550	8°30′N	39°21′E	16	28.8	763	13 July 2017
Sirinka	Eutricvertisol	1880	11°08′N	39°28′E	15.3	28.3	806	1 August 2017

Abbreviations: E = east; m.a.s.l = meters above sea level; Max = maximum; Min = minimum; N = enorth.
Source: Melkasa Agricultural Research Centers and National Meteorology Agency.

soil analysis (ATALS). After being pounded and sieved through 2 mm to remove any debris, the air-dried soil samples were subjected to physical and chemical analysis using the established procedures outlined by Okalebo, Gathua, and Woomer (2002). Total N was found by Kjeldhal digestion, and soil pH was measured using a soil water solution ratio of 1:2.5. Organic matter was determined using the potassium dichromate wet acid oxidation method. Exchangeable bases from an ammonium acetate extract were determined by flame photometry (K^+ and Na^+), atomic absorption spectrophotometer (AAS) (Ca^{2+} and Mg^{2+}) and the Bouyoucos (hydrometer) technique. Extractable P was determined by the Bray PI method. Zn and Fe were determined by AAS using an ADTA sample.

Growing experiment were conducted in an alpha-lattice design, with three replicates, in each replication 5 columns with 6 rows, in four 4-m rows plots, 0.4 m apart between rows, 40 seeds sown per row and 0.1 m between plants within a row. To ensure optimal conditions for growth and yield, we applied Di-ammonium phosphate (DAP) fertilizer at the rate of 100 kg/ha (containing 18 kg N and 46 kg P2O5/ha). During the growing period, hand weeding was utilized to manage weeds. No pesticides or fungicides were used for the control of insects or diseases. The trials took place in a rain-fed environment. The central two rows were used for data collection. Yield, yield-related traits and agronomic traits such as days to 75% maturity were determined by counting the number of days from sowing to when 85% of plants in the plot had at least 90% of their pods have were dried or had changed colour from green to yellow or to the colour typical for the genotype.

For mineral analysis, 15 fully mature pods per plot were harvested, and the beans were threshed. Two hundred grams of raw seeds were wiped with wet wipes and cleaned thoroughly in distilled water and alcohol to remove dust, debris and non-edible material. Seed samples were sent to the USDA-ARS Robert W. Holley Center for Agriculture and Health located in Ithaca, New York for mineral analysis. Measurements for Fe and Zn concentration in raw bean seed were conducted using inductively coupled plasma emission spectroscopy (ICP-ES), via established protocols (Glahn, Wiesinger, and Lung'aho 2020).

2.1 | Data Analysis

In order to identify significant differences between varieties for the collected variable data, the R packages agricolae Version: 1.3–5 (De Mendiburu 2021) and lme4 Version: 1.1–20., Built: R 3.6.3 (Bates et al. 2014) were used to subject the Fe and Zn concentrations from 30 small seeded common bean genotypes from individual sites to analysis of variance (ANOVA). Tukey's tests were used to separate the seed Fe and Zn mean genotypes at a 5% threshold of probability. The agricolae package is used for statistical analysis of experimental designs. The lme4 package is used for fitting linear mixed models. Replications, blocks and environments were random factors while genotypes were fixed.

GEA-R was used to analyse the yield data and create the additive main effects and multiplicative interaction (AMMI) statistical model, which resulted in a biplot displaying both main and interaction effects for both genotypes and environments (Alvarado et al. 2020). This allowed us to evaluate the adaptability and stability of common bean genotypes across different environments.

2.1.1 | AMMI Model

The AMMI model was used to investigate GEI. The model AMMI equation is

$$Y_{ge} = \mu + \alpha_g + ß_e + \Sigma n\, \lambda_n\, \gamma_{gn}\, \delta_{en} + \rho_{ge}$$

where ***Yge*** is the concentration of iron or zinc for genotype g in environment e, μ is the grand mean, μg the mean for genotype g (over environments), and μe the mean for environment e (over genotypes), $\alpha g = \mu_g - \mu$ be the genotype deviation and deviation and $\beta e = \mu_e - \mu$ is the environment deviation, λ_n the singular value for n component, γ_{gn} be the eigenvector value for genotype g and let and let δ_{en} be the eigenvector value for environment e, ρ_{ge} is the residual term.

2.1.2 | Identification of Stable and High Yielding Genotypes

The identification of stable and high yielding (Fe and Zn concentrations) common bean genotypes is done based on AMMI stability value (ASV) and genotype selection index (GSI).

2.1.3 | AMII Stability Value (ASV)

After testing the significance of the GEI mean square for yield and AMMI stability analysis ASV for each genotype was calculated using the following formula:

$$\text{ASV} = \sqrt{\left[\frac{SS\ IPCA1}{SS\ IPCA2} \times IPCA1\ Score\right]^2 + (IPCA2\ score)^2}$$

(Purchase, Hatting, and Van Deventer 2000).

where ASV = AMMI stability value; SS = sum of the square; IPCA1 and IPCA2 = the first and the second interaction principal component axes, respectively. Genotypes with lower values of ASV were considered to be stable.

2.1.4 | Genotype Selection Index (GSI)

The most stable genotypes might not always have the greatest yield performance, according to Mohammadi and Amri (2008), so methods that combine mean yield and stability into a single index are required. GSI, a novel strategy for the joint selection of yield and stability, was suggested by Farshadfar (2008). GSI was calculated by the following formula:

$$\text{GSI}_\text{i} = \text{RASV}_\text{i} + \text{RM}_\text{i}$$

Genotype Stability Index (GSI_i) of each common bean genotype in terms of iron and zinc was calculated based on; the rank of the ith genotype across environments based on AMMI Stability Value (RASV_j) and rank of the ith genotype based on mean iron and zinc concentration across environments (RM_i).

For the combined analyses, the broad sense heritability is calculated as

$$\text{H}^2 = \frac{\sigma^2}{\sigma^2_\text{g} + \sigma^2_{\text{ge}} / \text{nEnvs} + \sigma^2_\text{e} / (\text{nEnvs x nreps})}$$

(Henderson 1975)

where nreps is the number of repetitions, and $\sigma^2{}_\text{g}$ and $\sigma^2{}_\text{e}$ are the genotype and error variance components, respectively. Where nEnvs is the number of environments included in the analysis, and the novel term '$\sigma^2{}_\text{ge}$' refers to the GEI variance component. For well-known traits and environments, the predicted broad-sense heritability (repeatability) offers useful insight into the effectiveness of a breeding programme.

3 | Results

3.1 | Soil Properties and Weather of Experimental Locations

The total amount of rainfall, mean maximum and minimum temperature, and other annual average weather parameters varied between the five locations during the growing season

TABLE 3 | Physical and chemical characteristics of the soil at the test locations.

	Location							
Soil properties	**AN**	**AT**	**MK**	**SR**	**Mean**	**LSD**	**cv%**	**F pr**
Clay	14.55	18.48	14.29	33.30	20.05	1.87	18.5	<.001
Silt	40.16	42.42	38.00	39.77	40.09	3.16	15.6	0.054
Sand	45.29	39.10	47.65	27.47	39.98	3.62	17.9	<.001
Ca	5.29	6.65	5.16	11.78	7.18	0.65	17.9	<.001
Fe	124.91	132.79	107.00	107.30	118.18	14.28	23.8	<.001
K	0.34	0.35	1.07	1.97	0.92	0.05	10.3	<.001
Mg	1.61	2.04	1.63	3.51	2.18	0.19	17.2	<.001
N	0.23	0.16	0.14	0.24	0.19	0.02	19	<.001
OM	3.93	2.55	2.13	3.43	3.01	0.37	24.4	<.001
P	12.55	10.45	77.48	140.57	59.61	5.36	17.8	<.001
PH	6.15	6.54	6.35	6.04	6.27	0.11	3.6	<.001
Zn	11.19	7.57	6.64	8.55	8.49	8.49	40.9	<.001

Note: A soil sample from the Haramaya location was sent to the laboratory; however, its results did not become available.
Abbreviations: AT = Alem Tena; AN = Aris Negele; CV = coefficient of variance; LSD = list significance difference; MK = Melkasa; SR = Sirinka.

(Table 2). Aris Negele followed by Sirinka and Haramaya, had the highest rainfall (876 mm), while Alem Tena had the lowest rainfall (728 mm). The lowest maximum and minimum temperatures (11.1°C and 25.2°C) were recorded at Aris Negele, whereas Alem Tena had the highest maximum (12.9°C and 29.8°C).

Table 3 provides information about the soil's physical and chemical characteristics at the experiment sites. When comparing the soils of the study locations based on soil particle size distribution, sand and clay showed a significant difference ($p < 0.01$), but not in silt distribution. Clay loam was used to describe the soils at Alem Tena, Melkasa, and Sirinka, while loam was used to describe the soils at Aris Negele. The majority of the measured soil chemical characteristics were found to vary significantly ($p < 0.01$) from one place to another. Among the tested location the highest Fe at Alem Tena (132.79) followed by Aris Negele (124.91), in Zn the highest at Aris Negele (11.19) followed by Sirinka (8.55). The lowest soil Fe and Zn was measured at the Melkasa location 107 and 6.64 respectively.

3.2 | Analysis of Variance for Bean Fe and Zn Concentrations

Evaluation of variance for seed iron concentrations at Aris Negele, Haramaya, and Melkasa locations confirmed significant difference ($p < 0.001$) among harvested common bean genotypes and at Alem Tena location significant difference ($p < 0.01$) while non-significant at Sirinka location (Table 4). Analysis of variance for Zn contents at Aris Negele, and Melkasa locations showed a highly significant difference ($p < 0.001$) among harvested common bean genotypes and at Alem Tena location significant difference ($p < 0.05$) while non-significant at Haramaya and Sirinka for Zn contents in all testing environments (Table 4). The range of iron concentrations at Alem Tena, Aris Negele, and Haramaya was 44.4–69.5 μg/g; 45.1–67.6 μg/g; 60.2–84.4 μg/g respectively while it ranged from 56.3 to 74.4 μg/g at Melkasa. Alternatively, bean iron concentrations ranged from 52.6 to 67.1 μg/g at Sirinka. Overall, Nazrrit-2 (white), SCN 5 (black), Red Wolaita (red), SARI 1 (red), and Awash 2 (white) Fe concentrations were greater in Haramaya than in the other locations in this study.

In common bean seeds, the Zn content varied from 21.3 to 29.2 μg/g at Alem Tena and from 28.3 to 36.8 μg/g at Aris Negele, from 16.4 to 23.3 μg/g at Haramaya, and from 25.5 to 33.9 μg/g at Melkasa and from 20.7 to 25.2 μg/g at Sirinka. Among the tested locations, genotypes produced in the Aris Negele location showed higher Zn contents than the remaining locations. Genotypes that recorded higher Zn contents at Aris Negele are SER 125 (red), SCN 5 (black), Argen (white), Awash 2 (white), and RAZ 42 (white). Tables 5 and 6 provide the Fe and Zn information on the 30 common bean at each location.

3.3 | Variance Study Using AMMI for Seed Fe and Zn

AMMI analysis in this study showed that, the main effects of genotypes and environment accounted for 20.5% and 60.9%, respectively, of the total sum of squares of the seed Fe, while the impact of the genotype × environment interaction effect accounted for 18.5% of the total sum of squares of the seed Fe. The effects of genotypes and environment on the Fe and Zn concentrations of common bean seeds were extremely significant ($p < 0.001$) across locations. These results suggest that the genotypes vary in how they react to the various environments. The GEI for seed Fe contents was accounted for by the two-interaction principal component axis (IPCA 1 and IPCA 2), which were both very highly significant ($p < 0.001$) for seed Fe (Table 7).

An analysis of the total sum squares for zinc concentrations revealed that the environment contributed 81.5%, genotypes contributed 9.4%, and interaction contributed 8.9%. Environmental diversity is indicated by a high sum of squares, with the wide range of environmental factors accounting for the majority of the variation in Zn concentrations. The GEI for seed Zn concentrations was explained by the two interplay main aspect axes (IPCA 1 and IPCA 2), which were both extremely significant ($p < 0.001$) and accounted for 37.4 and 28.3%, respectively.

3.4 | AMMI Stability for Seed Fe and Zn

Mean seed Fe concentrations varied between 53 and 70 μg/g at the five locations, with a mean value of 62.6 μg/g. The average seed Zn content across all sites was 26.14 μg/g, ranging from 23.7 to 28.3 μg/g of dry weight. Among the tested genotypes 12 genotypes scores were greater in Fe concentrations than the grand mean. Similarly, among the tested genotypes 16 genotypes' scores for Zn concentrations were greater than the

TABLE 4 | Analysis of variance for bean Fe and Zn concentrations (μg/g) of 30 small seeded common bean genotypes at five locations.

	Location	Alem Tena	Aris Negele	Haramaya	Melkasa	Sirinka
Fe	Mean Sq	53.39	71.501	103.36	50.208	35.521
	Residual/SE	0.915	0.888	0.905	0.898	0.885
	Mean	56.4	57	72.4	66.8	60.4
	F value	2.5181	4.3425	8.1194	3.6988	1.5979
	Pr (>F)	0.002581 **	3.854e-06 ***	1.646e-09 ***	6.139e-05 ***	0.06396
Zn	Mean Sq	8.7206	12.455	4.8331	11.486	3.2021
	Residual/SE	0.972	0.966	0.97	0.968	0.966
	Mean	25.8	32.3	19.7	30.2	22.7
	F value	2.1051	5.3621	1.6313	3.7256	0.6297
	Pr (>F)	0.01676 *	2.222e-07 ***	0.07865	4.976e-05 ***	0.9072

*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

TABLE 5 | Mean Fe concentrations (μg/g) of 30 common bean varieties grown in five locations.

Alem Tena seed Fe concentrations		Aris Negele seed Fe concentrations		Haramaya seed Fe concentrations		Melkasa seed Fe concentrations		Sirinka seed Fe concentrations	
Genotype	**Mean**	**Genotype**	**Mean**	**Genotype**	**Mean**	**Genotype**	**Mean**	**Genotype**	
SARI-1	69.5^{a}	SARI-1	67.6^{a}	Nazareth-2	84.4^{a}	Red Wolaita	74.4^{a}	Mexican-142	67.1^{a}
Nazareth-2	65.4^{ab}	Nazareth-2	67.3^{ab}	SCN-5	82.9^{ab}	Dimtu	74.3^{a}	SER-119	65^{a}
Argene	63.5^{ab}	Tabor	63.8^{abc}	Red Wolaita	80.5^{abc}	Gabisa	73.3^{ab}	Red Wolaita	65^{a}
Awash-2	62.5^{ab}	SCN-5	63.6^{abc}	SARI-1	79.6^{abc}	Argene	72.7^{ab}	SCN-5	64.4^{a}
Bifort small seeded-15	60^{ab}	Gabisa	63.1^{abc}	Awash-2	78.6^{abc}	Awash Melka	72.3^{ab}	Tabor	64.3^{a}
Roba-1	60^{ab}	Awash-2	62.6^{abc}	SER-125	78.2^{abcd}	Beshbesh	71.1^{abc}	Roba-1	64.1^{a}
Red Wolaita	59.6^{ab}	Chore	60.1^{abcd}	Dimtu	78^{abcde}	Nazareth-2	70.5^{abc}	SCR-11	63.2^{a}
RAZ-11	58.9^{ab}	Argene	59.7^{abcd}	Bifort small seed-15	77.7^{abc}	Tabor	69.9^{abc}	Nazareth-2	63^{a}
Tabor	58.2^{ab}	Roba-1	59.6^{abcd}	Gabisa	75.9^{abcde}	Roba-1	69.6^{abc}	Awash 1	62^{a}
Dimtu	57.9^{ab}	SCR-11	58.9^{abcde}	Beshbesh	$75.5^{abcdefg}$	Awash-2	69.4^{abc}	Beshbesh	62^{a}
Beshbesh	57.6^{ab}	Beshbesh	58.7^{abcde}	Nasir	$75.1^{abcdefghij}$	Chore	68.6^{abcd}	RAZ-11	62^{a}
SER-125	56.7^{ab}	SER-125	58.6^{abcde}	Tabor	$75.1^{abcdefghi}$	SCN-5	67.4^{abcd}	RAZ-42	61.8^{a}
Omo-95	56.4^{ab}	Tibe	57.9^{abcde}	Anger	$74.8^{\text{abcdef h}}$	SARI-1	67.4^{abcd}	Gabisa	61.5^{a}
SCN-5	56.2^{ab}	Omo-95	57.7^{abcde}	SER-119	$74.7^{abcdefghi}$	Dinknesh	66.8^{abcd}	SARI-1	61.3^{a}
Gabisa	56.2^{ab}	RAZ-11	57.4^{abcde}	Awash Melka	$74.4^{abcdefghij}$	Anger	66.3^{abcd}	Dursitu	61^{a}
Chercher	55.5^{ab}	Bifort small seeded-15	56.9^{abcde}	Argene	$74.3^{abcdefghij}$	SER-125	66.2^{abcd}	Nasir	60.8^{a}
RAZ-42	55.5^{ab}	Red Wolaita	56.6^{abcde}	Dursitu	$73.9^{abcdefghij}$	RAZ-42	66.2^{abcd}	SER-125	60.4^{a}
Nasir	54.8^{ab}	Mexican-142	56^{abcde}	Tibe	$72.5^{abcdefghijk}$	Dursitu	65.7^{abcd}	Bifort small seeded-15	59.6^{a}
Anger	54.3^{ab}	SER-119	54.6^{abcde}	Dinknesh	$72.2^{abcdefghijk}$	SCR-11	65.3^{abcd}	Dimtu	59.3^{a}
Chore	54.2^{ab}	Awash Melka	54.4^{abcde}	Roba-1	$72.1^{abcdefghijk}$	SCN-11	64.9^{abcd}	Anger	59.1^{a}
Mexican-142	54.1^{ab}	Awash 1	54.1^{abcde}	Omo-95	$71.8^{bcdefghijk}$	Mexican-142	64.6^{abcd}	Omo-95	58.7^{a}
Tibe	54.1^{ab}	Dursitu	53.4^{bcde}	Chore	$70^{bcdefghijk}$	Awash 1	64.6^{abcd}	Awash Melka	58.7^{a}
SER-119	53.7^{ab}	RAZ-42	53.2^{abcde}	Chercher	$68.4^{cdefghijk}$	Nasir	64.4^{abcd}	Awash-2	58.4^{a}
Dursitu	53.4^{ab}	Anger	53.1^{abcde}	RAZ-42	$63.4^{defghijk}$	Tibe	64^{abcd}	Argene	58.3^{a}
Awash 1	53.1^{b}	Nasir	52.9^{cde}	SCN-11	62.8^{fghijk}	Bifort small seeded-15	63.4^{abcd}	Hawassa Dume	57.9^{a}
SCN-11	52.6^{b}	Dimtu	52.8^{bcde}	RAZ-11	$62.4^{\text{g ijk}}$	RAZ-11	63^{abcd}	SCN-11	56.2^{a}
Dinknesh	52.6^{ab}	Chercher	52.7^{bcde}	Awash 1	$62.2^{efghijk}$	Chercher	61.8^{abcd}	Chore	56^{a}

(Continues)

TABLE 5 | (Continued)

Alem Tena seed Fe concentrations		Aris Negele seed Fe concentrations		Haramaya seed Fe concentrations		Melkasa seed Fe concentrations		Sirinka seed Fe concentrations	
Genotype	**Mean**	**Genotype**	**Mean**	**Genotype**	**Mean**	**Genotype**	**Mean**	**Genotype**	
SCR-11	51.6^{b}	SCN-11	51.3^{cde}	Mexican-142	61.6^{jk}	Omo-95	61.3^{bcd}	Dinknesh	55^{a}
Awash Melka	51^{b}	Dinknesh	47.8^{de}	Hawassa Dume	61.1^{hijk}	SER-119	58.2^{cd}	Tibe	54.6^{a}
Hawassa Dume	44.4^{b}	Hawassa Dume	45.1^{e}	SCR-11	60.2^{k}	Hawassa Dume	56.3^{d}	Chercher	52.6^{a}

Note: Means followed by the same letter(s) are not significantly different according to DNMRT ($p \leq .05$).

grand mean, and from genotype Awash Miten (Bifort small seeded-15; this genotype released due to good Fe and Zn concentrations) 21 genotypes were scored greater in Zn concentration (Tables 8 and 9).

The 30 common bean genotypes that had been harvested from different locations were ranked from least to most stable based on the amount of Fe and Zn in the seeds using the Genotype Stability Index (GSI), stability value (ASV), additive main effects, and multiplicative interaction (AMMI). The genotype stability index (GSI), which ranks genotypes according to their mean seed Fe concentrations and AMMI stability values, placed Gabisa (cream) as the most stable high seed Fe-containing genotype, followed by Beshebes (cream), SER-119 (red), Tabour (cream), and Argene white (Table 8). The genotype with the highest seed Zn content according to the Genotype Stability Index was SCN-5 (black), which was closely followed by RAZ_42 white, Red Wolalaita (red), Tibe (red), and Nasir (red) (G8) (Table 9). In this study among tested genotypes, the top five high Fe contents are G12, G24, G17, G20 and G5 and in Zn content G22, G9, G27, G28 and G26. However, due to lack of stability only two of them G17 and G5 in case of Fe, and G27 and G28 in case of Zn were selected based on GSI.

3.5 | Genotype Stability and Environment Evaluation

The first two AMMI multiplicative components' genotypic and environmental scores were used in the current research to create the AMMI2 biplot, which was used to cross-validate the interaction pattern of the 30 small-seed common bean genotypes across five environments (Figure 1). According to the genotypic and environmental IPC scores, a biplot with seven parts was seen using AMMI2, but the test environments were divided into four sectors for iron contents and three sectors for Zn (Figures 1 and 2). The straight line drawn from the biplot origin to the placement of a genotypes or an environment is called a ‘vector’. The best genotype for an environment is the vertex genotype for the sector in which the environment resides An ideal genotype is one that shows superior performance in mean yield and that has high performance in constancy is called winner genotype.

In environments where the markers fall into the appropriate sector, the vertex genotypes in that sector are regarded as the finest. Environments in the same sector are considered with similar genotypes for winners. In this instance, G21, G25, G14, G7 and G22 for Zn expressed either positively or negatively high interactive behaviour and were thought to have contributed more to the exhibited G × E interaction than G6, G9, G12, G24, G26, G26, G30 and G1 for Fe (Figure 1). To find the best genotypes with regard to environments, genotype-environment affinity is represented as orthogonal projections of the genotypes on the environmental vectors. The genotypes G26, G30, G3, G27 and G1 performed best in the Sirinka environment. Alem Tena and Aris Negele were better suited to the genotypes G24, G12, G20 and G25. G6, G9, G2 and G14 were also more suited to Haramaya (Figure 1). The best genotypes for the Sirinka environment in terms of Zn contents were G12 and G20. Melkasa and Aris Negele environment were more suitable to the genotypes G17, G1, G9, G5, and G13. G25 and G19 were also preferable to

TABLE 6 | Mean seed Zn concentrations (μg/g) of 30 common beans varieties grown in five locations.

Alem Tena seed Zn concentrations		Aris Negele seed Zn concentrations		Haramaya seed Zn concentrations		Melkasa seed Zn concentrations		Sirinka seed Zn concentrations	
Genotype	**Mean**	**Genotype**	**Mean**	**Genotype**	**Mean**	**Genotype**	**Mean**	**Genotype**	**Mean**
Chercher	29.2^{a}	SER-125	36.8^{a}	SCN-5	23.3^{a}	Tabor	33.9^{a}	SER-125	25.2^{a}
SER-125	28.7^{a}	SCN-5	36.2^{ab}	Red Wolaita	21.9^{a}	Dimtu	33.5^{a}	Chore	25^{a}
Red Wolaita	28.4^{a}	Argene	35.7^{abc}	Dursitu	21.7^{a}	Beshbesh	32.9^{ab}	RAZ-42	24.4^{a}
Argene	28.2^{a}	Awash-2	34.9^{abcd}	Bifort small seeded15	21.5^{a}	Dursitu	32.8^{abc}	RAZ-11	24.1^{a}
RAZ-11	28.1^{a}	RAZ-42	34.8^{abcd}	SCR-11	21.3^{a}	Red Wolaita	32.7^{ab}	Tibe	24^{a}
SCN-5	27.8^{a}	Red Wolaita	34.8^{abcd}	Chercher	21.2^{a}	SCN-5	32.4^{abcd}	Beshbesh	23.9^{a}
Dursitu	27.5^{a}	RAZ-11	34.5^{abcde}	SCN-11	21.1^{a}	SCR-11	32^{abc}	SARI-1	23.6^{a}
Awash-2	27.4^{a}	Chore	34.3^{abcde}	Dimtu	20.9^{a}	Chercher	31.5^{abcd}	Nasir	23.4^{a}
RAZ-42	27.4^{a}	Awash 1	34.2^{abcde}	RAZ-11	20.9^{a}	Argene	31.4^{abcd}	Dimtu	23.3^{a}
Dimtu	27.3^{a}	Dimtu	33.1^{abcdef}	SARI-1	20.8^{a}	RAZ-11	31.4^{abcd}	Dursitu	23.2^{a}
SARI-1	27.3^{a}	SCN-11	33^{abcdef}	Argene	20.6^{a}	SER-125	31^{abcd}	SCR-11	23^{a}
Beshbesh	26.2^{a}	SER-119	32.7^{abcdef}	Awash-2	20.5^{a}	Mexican-142	30.8^{abcd}	Red Wolaita	22.9^{a}
SCR-11	26.2^{a}	Tabor	32.4^{abcdef}	Beshbesh	20.3^{a}	Gabisa	30.6^{abcd}	Argene	22.9^{a}
Nasir	26.2^{a}	SCR-11	32.3^{abcdef}	Awash Melka	19.9^{a}	Awash Melka	30.4^{abcd}	SCN-11	22.9^{a}
Bifort small seeded15	26.1^{a}	Dursitu	32.3^{abcdef}	Chore	19.8^{a}	SCN-11	30.2^{abcd}	Bifort small seeded-15	22.7^{a}
Dinknesh	25.8^{a}	SARI-1	31.9^{abcdef}	Nasir	19.6^{a}	Chore	30.2^{abcd}	Nazareth-2	22.6^{a}
Tabor	25.8^{a}	Chercher	31.4^{abcdef}	SER-119	19.5^{a}	RAZ-42	30.2^{abcd}	SER-119	22.5^{a}
Chore	25.5^{a}	Beshbesh	31.4^{abcdef}	Dinknesh	19.3^{a}	Nasir	30^{abcd}	Awash Melka	22.5^{a}
Omo-95	25.4^{a}	Nazareth-2	31.3^{bcdef}	Omo-95	19.3^{a}	Omo-95	29.7^{abcd}	Chercher	22.4^{a}
Tibe	25.3^{a}	Omo-95	31.1^{bcdef}	Hawassa Dume	19.1^{a}	Tibe	29.4^{abcd}	Roba-1	22.3^{a}
SCN-11	25.1^{a}	Nasir	31.1^{bcdef}	Roba-1	18.7^{a}	Anger	29.2^{abcd}	Awash-2	22.2^{a}
Mexican-142	24.1^{a}	Mexican-142	30.9^{bcdef}	RAZ-42	18.6^{a}	Dinknesh	29^{abcd}	Gabisa	21.9^{a}
Roba-1	23.8^{a}	Gabisa	30.9^{bcdef}	Tabor	18.5^{a}	Awash 1	29^{abcd}	Awash 1	21.9^{a}
Gabisa	23.8^{a}	Tibe	30.6^{cdef}	Nazareth-2	18.3^{a}	SARI-1	28.7^{abcd}	Tabor	21.9^{a}
Anger	23.7^{a}	Roba-1	30.2^{def}	Mexican-142	18^{a}	Roba-1	28.2^{abcd}	Dinknesh	21.5^{a}
SER-119	23^{a}	Awash Melka	30.1^{cdef}	Tibe	17.9^{a}	Bifort small-seeded-15	27.8^{abcd}	SCN-5	21.5^{a}
Awash 1	22.9^{a}	Bifort small seedes-15	29.9^{def}	Awash 1	17.7^{a}	Awash-2	27.8^{abcd}	Omo-95	21.1^{a}
Hawassa Dume	22.9^{a}	Hawassa Dume	28.5^{f}	Gabisa	17.5^{a}	Hawassa Dume	26.6^{bcd}	Anger	21^{a}

(Continues)

TABLE 6 | (Continued)

Alem Tena seed Zn concentrations		Aris Negele seed Zn concentrations		Haramaya seed Zn concentrations		Melkasa seed Zn concentrations		Sirinka seed Zn concentrations	
Genotype	**Mean**	**Genotype**	**Mean**	**Genotype**	**Mean**	**Genotype**	**Mean**	**Genotype**	**Mean**
Awash Melka	22.4^{a}	Anger	28.5^{ef}	Anger	17.2^{a}	Nazareth-2	26.4^{cd}	Hawassa Dume	20.9^{a}
Nazareth-2	21.3^{a}	Dinknesh	28.3^{f}	SER-125	16.4^{a}	SER-119	25.5^{d}	Mexican-142	20.7^{a}

Note: Means followed by the same letter(s) are not significantly different according to DNMRT ($p \leq .05$).

TABLE 7 | Sum of squares (SS) and mean of squares (MS) partitioning from AMMI analysis of 30 small seeded genotypes with Fe and Zn contents assessed across five environments.

Source of variation (Fe)	DF	SS	MS	TSS explained %	Source of variation (Zn)	DF	SS	MS	TSS explained %
ENV	4	14,978.96	3744.74***	60.92	ENV	4	9076.78	2269.19***	81.52
GEN	29	5048.55	174.11***	20.53	GEN	29	1056.50	36.43***	9.49
ENV×GEN	116	4560.67	39.32***	18.55	ENV×GEN	116	1001.37	8.63 ns	8.99
PC1	32	2383.70	74.49***	45.76	PC1	32	295.35	9.23***	37.49
PC2	30	1308.35	43.61***	25.11	PC2	30	223.42	7.44***	28.36
PC3	28	976.98	34.89***	18.75	PC3	28	146.42	5.23	18.59
PC4	26	540.60	20.79***	10.38	PC4	26	122.57	4.71	15.56
Residuals	268	5993.63	22.36		Residuals	268	2586.49	9.65	

Abbreviations: DF = degree of freedom; ENV = environment; GEI = genotype by environment interaction; GEN = genotype; MS = mean square; PC = principal component; SS = sum square; TSS = total sum square.
*Significant at $p < .05$, **significant at $p < 0.01$, and ***Significant at $p < 0.001$.

TABLE 8 | Mean iron, ASV, GSI values and ranks of small-seeded size common bean genotypes in five environments 2017.

Genotypes code	Genotypes name	Colour	Mean Fe	Fei	IPCA1	IPCA2	ASV	RASVi	GSi	RGSIi
G1	Mexican-142	White	61.59	17	−1.00	−0.01	1.82	30	47	26
G2	Red Wolaita	Red	66.54	6	0.24	0.55	0.71	16	22	10
G3	Awash 1	White	58.50	26	−0.70	0.06	1.27	27	53	30
G4	Roba-1	Cream	63.03	12	−0.24	−0.03	0.44	11	23	11
G5	Beshbesh	Cream	66.73	5	0.07	0.15	0.19	3	8	2
G6	Awash Melka	White	61.80	15	0.19	0.70	0.79	18	33	16
G7	Tabor	Cream	66.23	7	−0.10	−0.18	0.25	5	12	4
G8	Nasir	Red	56.02	27	0.20	0.22	0.42	9	36	19
G9	Dimtu	Red	61.36	20	0.41	0.71	1.02	23	43	24
G10	Tibe	Red	54.85	29	0.30	−0.23	0.60	13	42	21
G11	Argene	White	66.17	8	0.18	−0.07	0.33	7	15	5
G12	Nazareth-2	White	70.93	1	0.52	−0.46	1.05	24	25	13
G13	Anger	Red	64.64	10	0.26	0.26	0.54	12	22	9
G14	Dinknesh	Red	60.00	23	0.33	0.54	0.80	20	43	23
G15	Chore	White	61.75	16	0.02	−0.10	0.10	1	17	6
G16	Chercher	White	58.88	25	0.20	−0.24	0.43	10	35	18
G17	Gabisa	Cream	68.41	3	0.07	0.15	0.20	4	7	1
G18	Dursitu	Red	62.51	13	0.10	0.25	0.31	6	19	7
G19	Hawassa Dume	Red	53.31	30	−0.44	0.33	0.86	22	52	29
G20	Awash-2	White	66.92	4	0.43	−0.33	0.86	21	25	12
G21	SER-119	Red	65.93	9	−0.01	−0.18	0.18	2	11	3
G22	SER-125	Red	61.37	19	0.35	−0.02	0.64	15	34	17
G23	Omo-95	Red	62.31	14	0.06	−0.39	0.40	8	22	8
G24	SARI-1	Red	69.55	2	0.33	−0.99	1.16	26	28	14
G25	Bifort small seeded-15	White	64.64	11	0.40	−0.31	0.79	19	30	15
G26	RAZ-11	White	60.77	22	−0.69	−0.45	1.33	28	50	28
G27	RAZ-42	White	59.83	24	−0.60	0.14	1.11	25	49	27
G28	SCN-5	Black	61.30	21	0.42	−0.07	0.77	17	38	20
G29	SCN-11	Black	55.99	28	−0.34	0.10	0.64	14	42	22
G30	SCR-11	Red	61.45	18	−0.94	−0.10	1.72	29	47	25

Abbreviations: AMMI = additive main effects and multiplicative interaction; ASV = AMMI stability value; GSI = genotype stability index; IPCA1 = first interaction principal component axis; IPCA2 = second interaction principal component axis; RGSIi = rank of genotype stability index; RASVi = rank of ASV.

TABLE 9 | Mean Zn, ASV, GSI values and ranks of small-seeded size common bean genotypes in five environments 2017.

Genotypes code	Genotypes name	Colour	Mean Zn	Rini	IPCA1	IPCA2	ASV	RASVi	GSi	RGSIi
G1	Mexican-142	White	24.5	26	0.26	−0.40	0.53	13	39	20
G2	Red Wolaita	Red	28.7	6	0.06	−0.24	0.25	6	12	3
G3	Awash 1	White	27.2	10	−0.64	−0.25	0.89	26	36	19
G4	Roba-1	Cream	26.4	14	0.02	0.29	0.29	8	22	9
G5	Beshbesh	Cream	26.1	19	0.50	−0.18	0.69	21	40	22
G6	Awash Melka	White	24.7	24	0.35	0.19	0.50	12	36	18
G7	Tabor	Cream	26.1	18	0.39	−0.88	1.02	28	46	26
G8	Nasir	Red	26.9	11	0.16	0.13	0.25	5	16	5
G9	Dimtu	Red	29.9	2	0.38	−0.37	0.63	17	19	8
G10	Tibe	Red	26.6	13	−0.01	0.03	0.03	1	14	4
G11	Argene	White	28.1	7	−0.34	−0.31	0.54	15	22	10
G12	Nazareth-2	White	23.8	28	−0.52	0.51	0.85	23	51	28
G13	Anger	Red	21.0	30	0.39	−0.13	0.54	14	44	25
G14	Dinknesh	Red	24.4	27	0.61	0.28	0.85	24	51	29
G15	Chore	White	26.4	15	−0.46	0.06	0.61	16	31	15
G16	Chercher	White	24.7	23	0.50	−0.02	0.67	19	42	24
G17	Gabisa	Cream	25.7	20	0.15	−0.37	0.42	9	29	14
G18	Dursitu	Red	27.2	9	0.48	−0.08	0.64	18	27	12
G19	Hawassa Dume	Red	25.6	21	0.18	0.63	0.67	20	41	23
G20	Awash-2	White	26.7	12	−0.66	0.26	0.92	27	39	21
G21	SER-119	Red	22.0	29	−0.76	0.73	1.24	29	58	30
G22	SER-125	Red	31.2	1	−1.00	−0.87	1.58	30	31	16
G23	Omo-95	Red	24.6	25	0.20	−0.04	0.27	7	32	17
G24	SARI-1	Red	26.2	17	−0.02	0.48	0.48	11	28	13
G25	Bifort small seeded-15	White	25.2	22	0.26	0.81	0.88	25	47	27
G26	RAZ-11	White	28.8	5	−0.15	−0.09	0.22	4	9	2
G27	RAZ-42	White	29.4	3	−0.57	−0.27	0.80	22	25	11
G28	SCN-5	Black	29.0	4	−0.06	−0.12	0.15	2	6	1
G29	SCN-11	Black	26.2	16	−0.06	0.21	0.22	3	19	7
G30	SCR-11	Red	27.7	8	0.34	0.01	0.45	10	18	6

Abbreviations: AMMI = additive main effects and multiplicative interaction; ASV = AMMI stability value; GSI = genotype stability index; IPCA1 = first interaction principal component axis; IPCA2 = second interaction principal component axis; RGSIi = rank of genotype stability index; RASVi = rank of ASV.

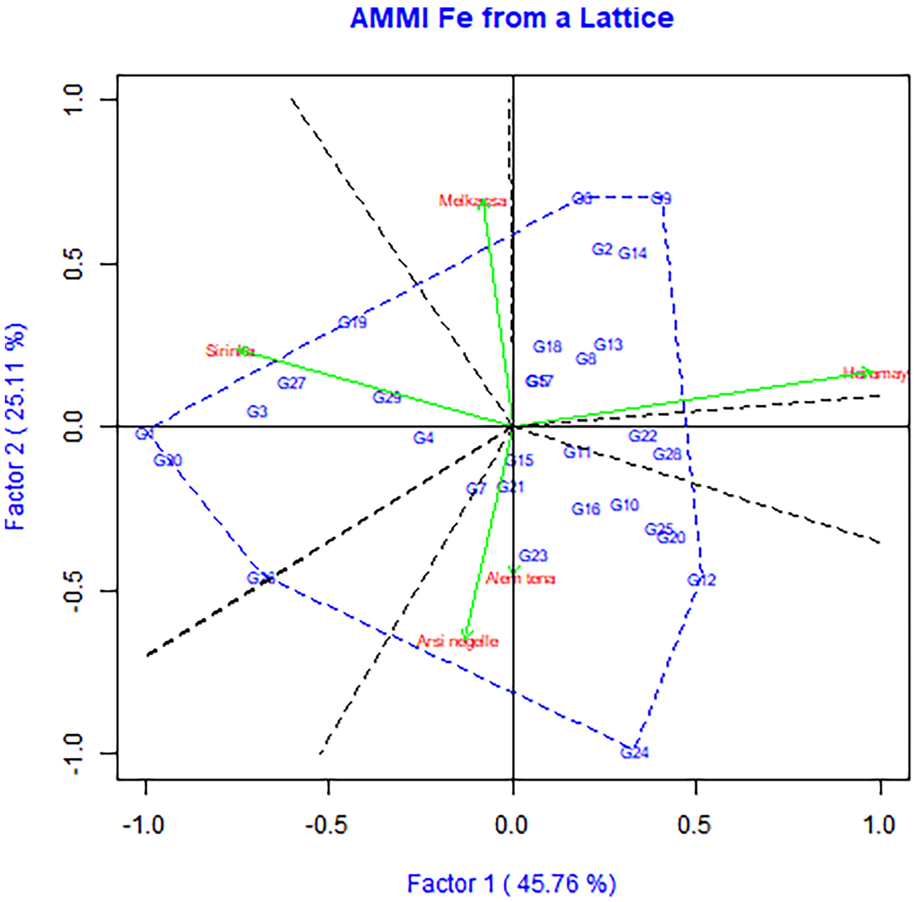

FIGURE 1 | A biplot of the first interaction principal component axis (IPCA1) versus the second interaction principal component axis (IPCA2) is displayed for types of small-seeded common beans with Fe contents. Abbreviations for genotypes are shown in Table 8.

Haramaya (Figure 2). The genotypes G4, G22, G15, G11, G5, G17, G7 and G21 for Fe contents and the genotypes G28, G10, G29, G2, G8 and G26 for Zn showed low interactive action over environments as they were closer to the biplot origin and less responsive than the vertex genotypes.

Environment Aris Negele, denoted by higher negative IPC1 values for Fe contents, were totally ineffective at differentiating between genotypes. Environment Haramaya, on the other hand, showed greater cultivar discriminating ability and was discovered to be less representative of the typical environment as evidenced by the greatest distance between their markers and the biplot origin (Figure 1). Figure 2 shows that environments Haramaya and Aris Negele, which had longer vectors, were more interactive and distinguished genotypic differences more effectively than other environments, which had shorter vectors. According to Yan (2002), the shorter vector environments were also less interactive and offered little insight into the variations in seed yield performances among the lines.

4 | Discussion

The current research sought to determine the baseline levels of Fe and Zn that are found in beans common to the food system of Ethiopia. The study's main goal was to ascertain how the amounts of Fe and Zn in Ethiopian beans were influenced by genotype, environment, and their interactions. This information is highly relevant to the success of bean biofortification in Ethiopia as it defines the biofortification breeding targets for Fe and Zn in beans. However, it is important to consider the following: The traditional approach to bean Fe biofortification is based on achieving sustainable increases in Fe that are substantially higher than the baseline values of a particular region. At present, in regard to Zn, beans are not considered a target crop for biofortification; hence the following points apply only to bean Fe biofortification.

The 'high Fe' approach requires that several key assumptions are met (Glahn and Noh 2021). First, in regard to bean Fe biofortification, enhanced levels of Fe must be sufficient, at least 22 μg/g higher than the varieties common to the area to be considered 'biofortified' (Boy et al. 2017). Furthermore, this approach also assumes that the enhanced amount of Fe represents a stable trait and that the Fe bioavailability of the biofortified variety is not significantly less than common marketplace varieties to negate the benefit of the additional Fe. It is also important to note that the recent PAS (Publicly Available Specification) assumes a global average of 50 μg Fe/g in non-biofortified beans (HarvestPlus 2022). Thus, by the PAS global standard, a biofortified bean should have at least 72 μg/g, assuming that the global average of 50 μg/g is representative of a given location. The Fe concentration value of 72 μg/g represents a Class III variety of beans, the lowest level of biofortification that is assumed to have nutritional benefit, which to our knowledge has not been evaluated in any human studies, relative to a global average variety.

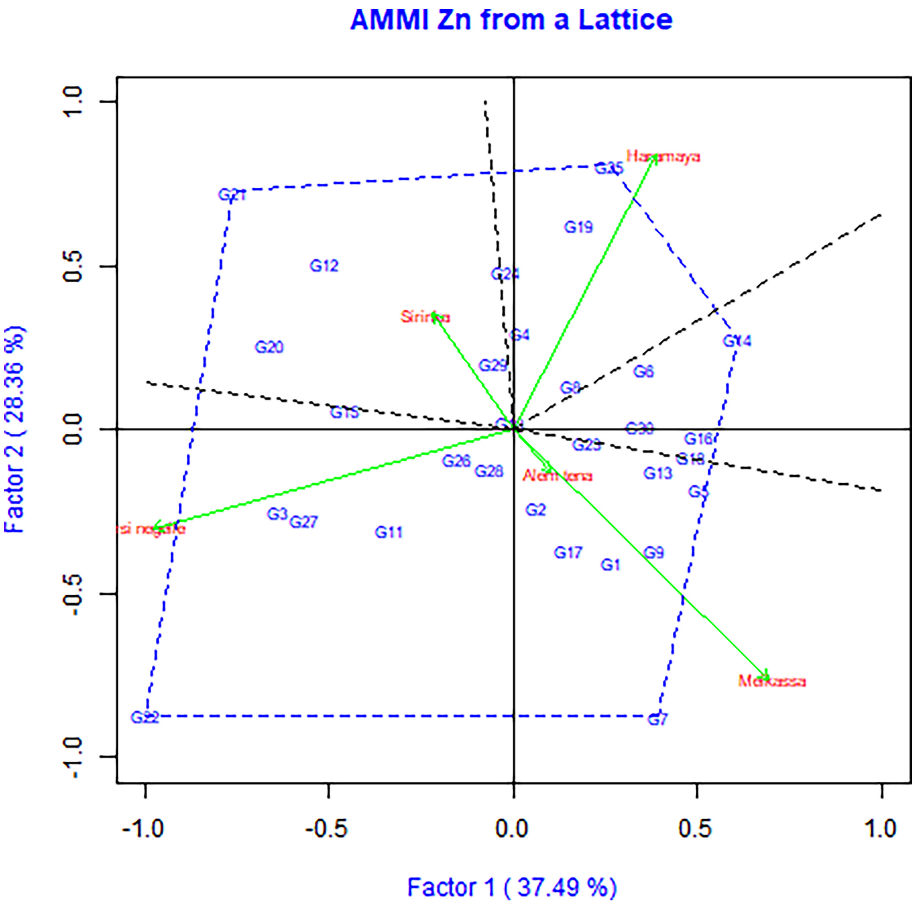

FIGURE 2 | Biplot of the Zn concentration of small-seeded common bean genotypes for the first interaction principal component axis (IPCA1) versus the second interaction principal component axis (IPCA2). Table 8 provides genotypes abbreviations.

The current research shows that Ethiopia's average Fe concentration for the 30 small seeded varieties is 62.6 μg/g, which is higher than the global average of 50 μg/g for the 30 small seeded varieties that are widely available. Furthermore, by extension the PAS mentioned above, the present study also suggests that for Ethiopia, a Class III biofortified variety should have at least 84 μg/g to be considered biofortified. It is also important to note that the 'biofortified' designation also assumes that the additional Fe is more bioavailable so as to provide nutritional benefit relative to an average variety. According to recent studies, evaluating the bioavailability of bean iron is crucial to figuring out whether a biofortified variety is more nutritious. (Glahn, Wiesinger, and Lung'aho 2020). The biofortified varieties that have been published have not undergone such evaluation using a recognized model, such as the Caco-2 cell bioassay (Harvest Plus, 2022). Thus, the recent research indicates that assessment of Fe content and bioavailability should now be monitored for a variety to be defined as biofortified (Glahn and Noh 2021). Such standardization of bean Fe bioavailability is already in place in programmes focusing on Fe nutrition from yellow beans (Wiesinger et al. 2019).

According to the present research, significant ($p < 0.01$) genotype and environment effects were responsible for significant variations in bean Fe and Zn concentrations. Similarly, the study of Philipo, Ndakidemi, and Mbega (2020) showed that highly significant effects of bean genotypes, environments, and GEI were observed for both seed Fe and Zn concentrations. The significant genotype × location interaction showed that the 30 genotypes examined in this study had distinct genetic characteristics. This is because of variations in the uptake and partitioning of nutrients due to variations in environmental factors (i.e., temperatures and soil characteristics) among the experimental locations (Tables 2 and 3). Similar results were demonstrated in other studies (Mukamuhirwa and Rurangwa 2018). (Manzeke et al. 2012) and (Blair et al. 2010) described that The soil characteristics of the sites may have contributed to differences in Fe and Zn content in the various environments,. Furthermore, weather patterns have been known to affect nutrient uptake in plants because they affect nutrient solubility and availability in the root zone. Therefore, the environment and the genotype × environment interaction (G × E), which affects the selection of winning genotypes adapted to broader regions, condition a genotype's superiority. According to research by Katuuramu et al. (2021), no single genotype displayed stability for all of the characteristics examined under various environments. Consequently, Fe concentration was not a constant characteristic across numerous sites in a relatively small region like Uganda.

For bean Fe and Zn, respectively, broad-sense heritability across environments was 78.7% and 80.1%. The range of variation in Fe and Zn concentration, which is reportedly caused by the strong effects of environment and genotype by environment, may make breeding for enhanced Fe and Zn concentration an impractical goal for beans, according to statistical evidence that suggests some genetic control over Fe and Zn concentrations. In other words, the biofortification assumption that greater Fe and Zn levels are sustainable and therefore provide more absorbable Fe and Zn may be refuted by the significant variation in Fe and Zn content caused by the E and GxE effects.

The GSI and AMMI II biplot were employed to show the stability of the genotype as well as the proportional magnitude of interaction effects of each genotype and environment. Genotypes Gabisa, Beshbesh (cream seed cote), SER-119 (red), Tabor (cream) and Aregeni (white) for grain Fe, while SCN-5 (black), RAZ 11 (white), Red wolayta, Tibe and Nasir (red) for grain Zn concentration. Had higher average performance for the trait and adaptable to favourable environments, according to the genotype selection index GSI. Through a polygon view of the AMMI II biplot analysis, crossover and non-crossover genotype-by-environment interaction as well as potential mega environments under multiple-location experiments were found. Utilizing IPCA 1 and IPCA 2 values, the AMMI II biplot was created (Figures 1 and 2). The distances from the biplot origin (0, 0) represent the degree of interplay genotypes and environments (Voltas et al. 2002). According to Figure 1, genotypes G4, G22, G15, G7, G11, G17 and G5 G21 for Fe contents and Figure 2, for Zn, genotypes G28, G10, G29, G2, G8 and G26 for Zn contents were shown to have minimal interactive action over environments. From a statistical perspective, this shows that these genotypes showed had less variation in response to variations in the growing environment or the best genotypes. However, from a nutritional perspective, the quantity of variation in a given variety is quite significant when considering the assumptions of the high Fe or Zn approach to biofortification.

Because they are adapted to that particular environment, Caligari (2001) recommended that the highest seed Fe and Zn-containing common bean genotypes at each experimental site can be used for specific environmental breeding purposes. In line with this, the best genotypes for environment Sirinka were G26, G30, G3, G27 and G1; G24, G12, G20 and G25 for Alem Tena and Aris Negele; and G6, G9, G2 and G14 for Haramaya are specifically adaptable to an environment (Figure 1). In the case of Zn contents, the best genotypes concerning environment Sirinka were G12 and G20. Genotypes G17, G1, G9, G5, G22 and G13 were better adapted to environments Melkasa and Aris Negele. Similarly, G25 and G19 were better adapted to Haramaya (Figure 2). Similarly, best performer genotypes and three mega environments were noticed through biplot analysis. Environment Melkasa was strongly correlated with higher positive IPC1 values, suggesting a better capacity to distinguish between genotypes with differing Fe and Zn concentrations. Also, Alem Tena, which are closest to the origin, showed less cultivar discrimination than the other environments and were found to be more representative of the typical environment.

The identification of some very interesting genotypes when considering relatively higher average performance for the seed Fe and Zn-containing and adaptability to favourable environments is one of the major outcomes of the current study, even though the results only reflect 1 year of data. Varieties Gebisa, Beshebes, Tabour and Roba-1 cream seed coat colour, SER-119 and SER-125 of red seed colour, and Chore and Argene (white colour) were characterized by the highest Fe content and adaptable to favourable environments. The highest Zn content and relatively stable were determined in genotypes RAZ_42 (white colour), Red wolaita, Tibe and Nasir (red colour), and SCN-11 and SCN-5 (black colour). In addition, the study found out that among the tested genotype Nazareth 2 and SER-125 were better adaptable to favourable environments Alem Tena and Aris Negele. For Zn Genotypes SER-125, and Dimtu were better adapted to environments Melkasa and Aris Negele. For breeding purposes in those particular environments, the genotypes that recorded the highest bean Fe and Zn concentrations at each experimental location may be used.

5 | Conclusion

Breeders need to be able to categorize plant genetic resources in order to take advantage of the diversity and execute effective breeding strategies. This study's wide variation across 30 small seeded bean genotypes for Fe and Zn concentrations in five locations in Ethiopia can be tapped into breeding programmes aimed at increasing bean Fe and Seed Zn concentrations. In Ethiopia, the average Fe concentration of 62.6 μg/g and Zn concentration of 26.2 μg/g were observed among the small seeded released and advanced lines. According to the research, environmental variation had the greatest impact on the Fe and Zn contents of small-seeded common beans, with GEI and genotypic effect having the least impact. These findings clearly show that Fe concentration is not a constant characteristic across various Ethiopian sites. The findings suggest that a different strategy should be used to biofortify beans with Fe, one that focuses on evaluating Fe delivery using recognized screening methods.

The introduction of new alleles controlling seed Fe and Zn concentration into the genetic background with high Fe bioavailability should generally be the emphasis of Ethiopian common bean breeding. Ethiopian bean varieties that have been biofortified, however, have not been the subjected to Fe bioavailability measurements. Additional investigation is required to examine the potential impacts of the environment, soil components, and physical characteristics on the Fe and Zn concentrations of common beans.

Author Contributions

Girum K. Ejigu: Conceptualization; Data curation; Formal analysis; Methodology; Writing – original draft; Writing – review and editing. **Raymond P. Glahn:** Conceptualization; Funding acquisition; Methodology; Project administration; Resources; Supervision; Validation; Writing – review and editing. **Clare M. Mukankusi:** Conceptualization; Data curation; Funding acquisition; Project administration; Resources; Supervision; Writing – review and editing. **Berhanu A. Fenta:** Conceptualization; Funding acquisition; Project administration; Resources; Supervision. **Jason A. Wiesinger:** Data curation; Formal analysis; Visualization.

Acknowledgments

The authors acknowledge financial support from Tropical Legume III (TL-III) and the Ethiopian Institute of Agricultural Research (EIAR) for this study. Workers in the field for the common bean breeding programme at the Melkasa Agricultural Research Centre and other partner organizations. We are very appreciative of the support in study design and the platform for micronutrient analysis provided by Cornell University in Ithaca and the Alliance of Bioversity International.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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