

Article

Unraveling Diversity in Physical and Mineral Traits of Faba Bean (*Vicia faba* L.) Landraces Harvested at Immature Stages

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Abstract

Faba bean (*Vicia faba* L.) is a legume valued for its nutritional properties and adaptability, yet the effects of genotypic diversity among landraces and harvest stages on its physical and mineral traits remain insufficiently explored. This study evaluated 14 faba bean genotypes, (13 landraces and one commercial cultivar), harvested at two immature stages (baby and tender), to assess variation in seed volume, biomass-related parameters, and mineral composition, and to elucidate how developmental stage and genotype jointly shape nutritional profiles. Across genotypes, seed volume increased while the fresh-to-dry weight ratio (FW/DW) decreased from baby to tender stage, reflecting progressive tissue desiccation. Mineral concentrations, except for Mo and Na, generally declined during seed development, although several landraces (H9, H12, H20, H21, and H22) maintained stable values, indicating genotypic resilience to the dilution effect. Correlation analysis revealed high positive associations among mineral concentrations (e.g., Mg–Mn, K–S, P–S) and with FW/DW, suggesting that higher tissue hydration is associated with higher mineral accumulation. Principal Component Analysis (PCA) further distinguished three clusters per harvest stage, separating mineral-rich landraces (H21, H11, H9) from those with lower concentrations (H4, H7), thus highlighting developmental reorganization and genotype-dependent mineral retention. Overall, both genotype and harvest stage strongly determined the mineral profile of immature faba beans. Landraces emerged as valuable reservoirs of mineral-rich germplasm, while simple physical metrics such as FW/DW may serve as practical and economical proxies for mineral quality in breeding programs.

Keywords: *Vicia faba*; mineral content; genotype diversity; fresh-to-dry weight ratio; seed development; seed volume; immature faba beans



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1. Introduction

Faba bean (*Vicia faba* L.) is a globally important cool-season legume cultivated largely for human consumption and animal feed due to its high protein content, adaptability to diverse agroclimatic conditions, and role in sustainable agriculture through nitrogen fixation [1,2]. It contributes substantially to soil fertility by fixing atmospheric nitrogen, thus reducing the need for synthetic fertilizers, an attribute especially valuable in low-input cropping systems [3]. Beyond its agronomic significance, faba bean is valued for its nutritional qualities, being rich in proteins, with higher content than most pulses [4,5], dietary fiber, vitamins, and essential mineral micronutrients such as iron (Fe), zinc (Zn),

magnesium (Mg), potassium (K), and calcium (Ca), which are critical to human health and play vital physiological roles in the plant itself [6,7].

Faba beans can be harvested at different stages: as immature seeds (i.e., when pods and seeds are green) or as mature seeds (i.e., when pods dry out and only the seeds are kept) [8]. Immature seeds can be picked either at the baby stage, when they are smaller and younger, or at the tender stage, when they are larger but still not fully developed. Although the dry stage is the most common harvest form worldwide [9], the consumption of fresh pods and immature seeds remains popular in many regions [10,11]. In Mediterranean countries such as Spain and Italy, harvesting faba beans for fresh consumption is a long-standing practice. Recent studies highlight the importance of evaluating genotypic differences in the quality traits of faba beans destined for the fresh market, particularly their nutritional value. This interest stems from the fact that immature seeds are rich in essential mineral elements, including key macrolelements like potassium, magnesium, and calcium, as well as microelements such as iron [12–14]. Mineral deficiencies represent a crucial global health challenge, particularly iron and zinc deficiencies, which are among the most prevalent micronutrient malnutrition issues worldwide, and particularly in developing countries [15,16]. Legumes like faba bean are excellent targets for biofortification strategies aimed at improving mineral nutrient density to combat hidden hunger [17,18]. Identifying and exploiting natural variation in seed mineral composition has thus been highlighted as a crucial step toward breeding nutrient-dense legumes, integrating classical selection with genomics and phenomics tools to accelerate nutritional improvement [19].

Although immature faba beans are traditionally consumed at two distinct stages, baby and tender, which differ in both sensory and compositional traits, limited information is available on how mineral and nutritional profiles evolve between these developmental phases. Understanding these changes across genotypes provides valuable insights into the stability and plasticity of nutritional traits, which is essential for breeding programs targeting different market uses and consumption types. Previous studies have shown that mineral accumulation and nutrient partitioning during seed filling are strongly stage-dependent, and that developmental timing profoundly influences the final nutritional quality of legume seeds [20]. Evaluating such developmental variation helps identify genotypes with consistent mineral profiles, in line with the integrative breeding framework proposed by Jha et al. [19] for improving the nutritional value of food legumes.

In this context, faba bean landraces from the Mediterranean basin constitute an important reservoir of genetic diversity [12]. Several studies have shown that local populations exhibit high levels of polymorphism and distinct population structures [21–23], pointing to substantial variability in traits potentially associated with mineral uptake, seed development, and nutritional quality. This diversity, which is often preserved in traditional landraces, is underrepresented in modern breeding programs [24–26]. The use of these landraces is common when seeking to provide useful alleles for enhancing mineral accumulation and improving adaptation to specific environments [27–29]. Despite this relevance, studies explicitly addressing mineral composition in Mediterranean landraces remain scarce. Although traditional germplasm is recognized in the Valencian region, where *Vicia faba* var. *major* has long been cultivated for fresh consumption using local farmer selections [30], only limited published studies have reported on the mineral composition of these landraces, particularly at early developmental stages, nor compared them with commercial cultivars. Filling this gap, our objective is valorize local genetic resources and to explore their potential contribution to mineral and their relationship with early harvest stages improvement and fresh-market valorization.

2. Materials and Methods

2.1. Plant Material and Field Experiment

In a previous study, a pool of faba bean of 13 accessions was selected based on their agronomic potential (concretely higher productivity) and phenotypic traits (unpublished data). Six of the accessions used in this study were provided by the Institute for the Conservation and Improvement of Valencian Agrobiodiversity (COMAV, Polytechnic University of Valencia), while the remaining seven were supplied by the Valencian Institute for Agrarian Research (IVIA), from different origins (Table 1). These accessions correspond to traditional landraces and locally conserved germplasm representative of the Valencian region. A commercial variety (Mutxamel), widely cultivated for fresh consumption in eastern Spain, was used as a control.

Table 1. Geographical origin, identity, accession codes, and institution of the accessions characterized in this study.

ID	Local Name	Code	Origin	Institution
H4	Haba valenciana	IVIA-O19G	Catarroja (Valencia)	IVIA
H5	Haba valenciana	IVIA-263	Foios (Valencia)	IVIA
H6	Haba valenciana	IVIA-475	Millares (Valencia)	IVIA
H7	Haba de Elche	BGV-9859	Novelda (Alicante)	COMAV-UPV
H8	Haba Valenciana tardía	BGV-15037	El Perelló (Valencia)	COMAV-UPV
H9	Haba valenciana	BGV-15620	Valencia	COMAV-UPV
H11	Haba valenciana	BGV-16035	Moncada (Valencia)	COMAV-UPV
H12	Haba valenciana	BGV-16094	Moncada (Valencia)	COMAV-UPV
H17	Haba de Mutxamel	17-152	Mutxamel (Alicante)	IVIA
H20	Haba valenciana	H68-LG	Turis (Valencia)	IVIA
H21	Haba de Bétera	LG10-69	Bétera (Valencia)	IVIA
H22	Haba del terreno	Var-303	Valencia	IVIA
H23	Haba valenciana	BGV-9886	Valencia	COMAV-UPV
Mutxamel	Mutxamel comercial		Mutxamel (Alicante)	BATLLE

Plants were cultivated in a field located at the Valencian Institute for Agrarian Research (IVIA) in Moncada, Valencia (39°35'23.2" N, 0°23'40.2" W). On 11 December 2020, seeds were sown in seedbeds. A month later (15 January 2021), seedlings were transplanted following a randomized block design, with four blocks per genotype. Each block contained eight plants per genotype. Plants of the same genotype were spaced 1 m apart, with 1.2 m between accessions.

Faba beans were harvested as fresh beans in two stages of immaturity: on 1 April 2021, for baby beans, and on 20 April 2021, for tender beans. Pods were peeled and the grains were used for analysis.

Throughout the trial period, the average minimum and maximum temperatures were as follows: 12–19 °C in November, 8–16 °C in December, 6–14 °C in January, 10–17 °C in February, 8–16 °C in March, and 10–18 °C in April (source <http://riegos.ivia.es/datos-meteorologicos> (accessed on 15 June 2021)).

2.2. External Quality Assessment

Seed volume was measured for each variety and harvest stage using the water displacement method. Four independent replicates ($n = 4$) of 10 seeds per genotype and harvest stage were submerged in a graduated cylinder containing a known volume of distilled water, and the displaced volume (in mL) was recorded.

Fresh weight (FW) was measured on the same basis (four replicates of 10 seeds per genotype and harvest stage). After weighing, seeds were dried in an oven at 65 °C for

five days. Subsequently, dry weight (DW) was recorded. Seed hydration was indirectly evaluated through the FW/DW ratio.

2.3. Internal Quality Assessment

2.3.1. Sample Preparation

Four replicate samples ($n = 4$), one per block, were used to evaluate the mineral composition of each genotype. Within each accession, each replicate consisted of 50 g of representative seeds. After conventional drying, the seeds were processed and transformed into bean powder using a mixer mill (MM400, Retsch, Hann, Germany) to use for analytical purposes.

2.3.2. Mineral Composition

Mineral concentrations were measured by an inductively coupled plasma-optical emission spectrometry (ICP-OES, iCAP 6000, Thermo Scientific, Cambridge, UK). This technique was used for all macro- and micronutrient determinations, with argon (purity higher than 99.995%) was employed as the plasmogen and carrier gas. The operating conditions of the ICP-OES equipment were previously established, and the instrument automatically selected the analytical emission lines.

Each replicate ($n = 4$) consisted of 0.5 g of faba bean powder, which was digested in a mixture of 70% HNO_3 - HClO_3 (2:1) in tubes. The tubes were kept at 200 °C for 15 to 20 min. Once digestion had finished, the extracts were diluted in 25 mL tubes and micronutrient concentration were analyzed in the ICP. The concentration was calculated through Equation (1).

$$\text{Micronutrient concentration} = \frac{(a - b) \times V}{P} \quad (1)$$

where a was the concentration of micronutrients in the solution of the digestion of the sample ($\text{mg} \cdot \text{L}^{-1}$); b was the concentration of these nutrients in the blank ($\text{mg} \cdot \text{L}^{-1}$); V was the final volume of digestion (25 mL); and P was the dry weight of the sample digested.

An aliquot of 0.5 mL was taken from the extraction solution to determine the macronutrients and made up to 10 mL with Milli-Q water (Milli-Q® IQ 7000 Ultrapure Water Purification System, Merk, Darmstadt, Germany). The concentration was calculated using Equation (2).

$$\text{Macronutrient concentration} = \frac{(a - b) \times V \times d}{P \times 1000} \quad (2)$$

where a was the concentration of the macronutrient in the aliquot from the digestion of the sample (mg L^{-1}); b was the concentration of these nutrients in the blank (mg L^{-1}); V was the final volume of digestion (25 mL); P was the dry weight of the sample digested; and d was the dilution factor.

A total of 32 elements were measured: Al, As, Be, Bi, B, Ca, Cd, Co, Cr, Cu, Fe, K, La, Li, Mg, Mn, Mo, Na, Ni, Pb, P, Rb, Sb, Se, Si, S, Sr, Ti, Tl, V, and Zn.

From this comprehensive dataset, a selection of the most relevant elements in faba bean was made for detailed analysis, based on their nutritional importance for human health and their recognized physiological roles in plant metabolism. These included six macroelements (Ca, K, Mg, Na, P, and S) and six microelements (Cu, Fe, Mn, Mo, Ni, and Zn).

To estimate the overall mineral content, all 32 measured elements were summed for each sample, and this value was referred to as the total mineral content or mineral sum. This provided a general indication of the total mineral concentration in each accession and harvest stage.

The content of macronutrients was expressed as g 100 g⁻¹ DW, while micronutrients were expressed as mg kg⁻¹ DW.

2.4. Statistical Analysis

Two-way ANOVA was performed using the results obtained from the evaluated parameters, considering plant genotype and harvest stage as factors. Analyses were conducted with Statgraphics Centurion XVIII 18.1.16 (Statistical Graphics Corporation, 2014, Englewood Cliffs, NJ, USA). When a significant interaction between factors was detected, a one-way ANOVA was carried out instead, using either plant genotype or harvest stage as a single factor. Results were expressed as means $\pm$ standard error (SE), and statistical comparisons were made using Scott–Knott test at a significance level of $p < 0.05$, performed in R software (version 2025.09.2+418, R Core Team, Viena, Austria).

To further explore the relationships between variables, simple linear regressions and Pearson correlation analyses were conducted. Regression outcomes were reported by the r and p -values. A correlation matrix was computed including all measured variables and visualized using the *corrplot* package (v0.92) in R [31]. Only significant correlations ($p < 0.05$) were considered.

Principal component analysis (PCA) was performed separately for the baby and tender seeds data. The PCA was developed to unravel the complex interplay among the variables analysed. All studied variables for each harvest stage (baby and tender) were incorporated into a unified matrix, upon which PCA was executed using the *prcomp* function in R (v4.3.2) [31], using scaled values. The information on component values, plotting and trait values contributing to the components were extracted and analysed with *FactoMineR* (v2.9) [32] and *Factoextra* (v1.0.7) [33] R libraries. The K-means and Silhouette algorithms from the R packages *cluster* (v2.1.4) [34] were used to determine the effective number of sample clusters in the PCA.

To complement multivariate analyses, a hierarchical heatmap was constructed to visualize overall similarity patterns among genotypes and traits across both developmental stages. Data were standardized (z-scores) prior to clustering to ensure comparability among variables. Clustering was based on Euclidean distance matrices using complete linkage as the agglomeration method. The heatmap and associated dendrograms were generated in R (v4.3.2) using the *ComplexHeatmap* package (v2.18.0) [35], allowing simultaneous grouping of both genotypes $\times$ stages (columns) and measured traits (rows). Color gradients represent standardized values, where positive and negative deviations indicate relative enrichment or depletion of each trait.

3. Results

3.1. Faba Beans Volume

The initial two-way ANOVA confirmed a significant interaction ($p < 0.01$) between accession and harvest stage for seed volume. Therefore, to dissect this interaction, data were analyzed separately for each stage. Significant differences were observed among accessions within both the baby and tender harvest stages (Figure 1).

In general, faba beans harvested at the tender stage exhibited greater volume than those harvested at the baby stage, with significant differences detected across all accessions ($p < 0.05$, see asterisks in Figure 1), according to one-way ANOVAs. The magnitude of this increase varied considerably among genotypes. Accession H12 showed the most pronounced increase (+280%), followed by H9 (+147.6%), Mutxamel (+120%), and H5 (+106.3%). In contrast, accessions H20 (+22.2%), H17 (+45.0%), and H4 (+52.4%) showed the lowest increases in volume.

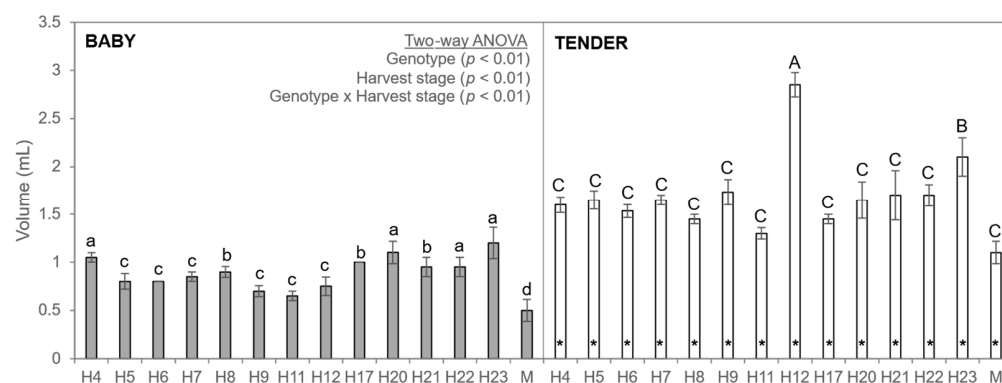


Figure 1. Seed volume (mL) in baby (dark bars) and tender (white bars) faba beans across 14 accessions. Values represent means $\pm$ SE ($n = 4$; each replicate consisted of 10 seeds per accession and harvest stage). Results from a two-way ANOVA, with genotype and harvest stage as factors, are shown within the graph. Separate one-way ANOVAs were conducted for each harvest stage, followed by Scott–Knott test ($p < 0.05$). Different letters indicate significant differences among accessions within each stage (lowercase letters for baby beans and uppercase letters for tender beans). Additionally, one-way ANOVAs performed for each accession, using harvest stage as a factor, are represented by asterisks. An asterisk indicates a significant difference between baby and tender stages, with the stage marked by the asterisk having a higher volume.

The accessions with the highest or lowest volumes varied depending on the harvest stage. At the baby stage, accessions H23, H20, H4, and H22 exhibited the largest volumes, whereas at the tender stage, accession H12 stood out as the largest, followed by H23. The control variety Mutxamel and accession H11 consistently showed the lowest seed volumes across both harvest stages.

3.2. Fresh-to-Dry Weight Ratio of Faba Beans

A significant interaction between genotype and harvest stage was found for fresh and dry weight (two-way ANOVA; $p < 0.01$). Consequently, data were analyzed per harvest stage and per genotype. Within each genotype, one-way ANOVA confirmed that tender beans had significantly higher FW and DW than baby beans in most accessions (see asterisks in Figures 2 and 3), reflecting the general trend of biomass accumulation during maturation. At both harvest stages, H23 exhibited the highest FW and DW values, whereas Mutxamel showed the lowest.

Additionally, the fresh-to-dry weight ratio (FW/DW) was significantly influenced by both genotype and harvest stage. A two-way ANOVA revealed a non-significant interaction but clear main effects, indicating that baby beans generally had a higher FW/DW ratio than tender beans. This reflects a higher relative water content at the earlier stage and its subsequent decrease during maturation. For consistency with other figures, we also performed one-way ANOVAs for each genotype and harvest stage (Figure 4). The difference between baby and tender beans was significant for most accessions (indicated by asterisks). The most substantial decreases in FW/DW were observed in H5 (−14.7%), H7 (−12.7%), and H6 (−11.5%), whereas H21 (−3.4%) and H20 (−3.5%) exhibited the smallest reductions. Generally, among the accessions, H9, Mutxamel, H11, H20 and H21 showed the highest FW/DW ratios, while H4 and H7 showed the lowest (indicated by letters, Figure 4).

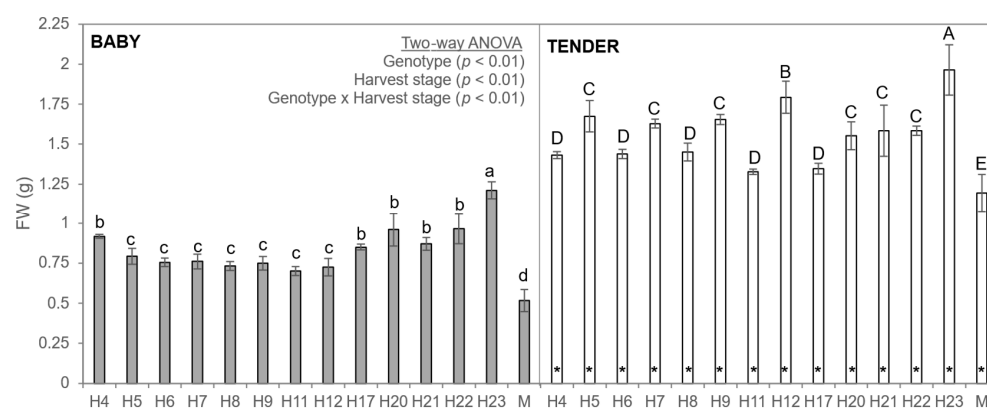


Figure 2. Fresh weight (FW) in baby (dark bars) and tender (white bars) faba beans across 14 accessions. Values are means $\pm$ SE ($n = 4$; each replicate consisting of 10 seeds per accession and harvest stage). Results from a two-way ANOVA, with genotype and harvest stage as factors, are shown within the graph. Separate one-way ANOVAs were conducted for each harvest stage, followed by Scott–Knott test ($p < 0.05$). Different letters indicate significant differences among accessions within each stage (lowercase letters for baby beans and uppercase letters for tender beans). Additionally, one-way ANOVAs performed for each accession, using harvest stage as a factor, are represented by asterisks. An asterisk indicates a significant difference between baby and tender stages, with the stage marked by the asterisk having a higher FW.

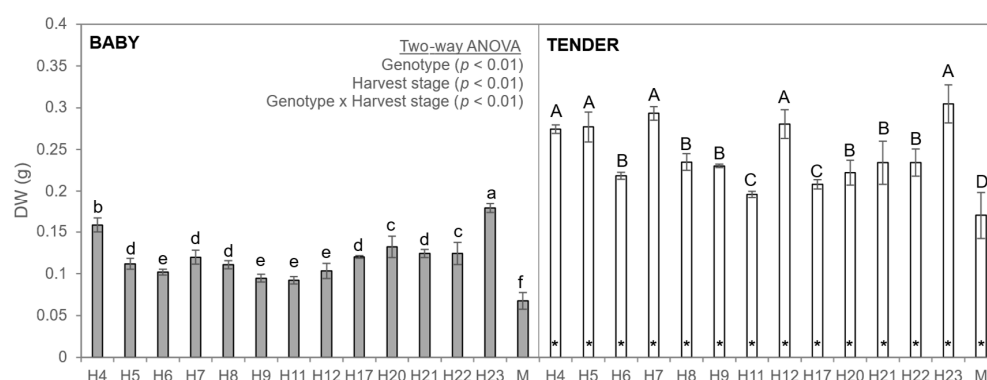


Figure 3. Dry weight (DW) in baby (dark bars) and tender (white bars) faba beans across 14 accessions. Values are means $\pm$ SE ($n = 4$; each replicate consisting of 10 seeds per accession and harvest stage). Results from a two-way ANOVA, with genotype and harvest stage as factors, are shown within the graph. Separate one-way ANOVAs were conducted for each harvest stage, followed by Scott–Knott test ($p < 0.05$). Different letters indicate significant differences among accessions within each stage (lowercase letters for baby beans and uppercase letters for tender beans). Additionally, one-way ANOVAs performed for each accession, using harvest stage as a factor, are represented by asterisks. An asterisk indicates a significant difference between baby and tender stages, with the stage marked by the asterisk having a higher DW.

3.3. Mineral Content

3.3.1. Total Mineral Content

A significant genotype $\times$ harvest stage interaction was identified for total mineral content (two-way ANOVA; $p < 0.01$). Consequently, data were subsequently analyzed using separate one-way ANOVAs for each harvest stage and for each genotype. In general, baby beans exhibited a higher total mineral percentage than tender beans, with statistically significant differences, shown by asterisks, found in 64% of the accessions (Figure 5). The largest decreases in mineral concentration from baby to tender stages were observed in H6 (−12.5%), H8 (−12.6%), and Mutxamel (−12.3%). Although a dilution effect was

observed in all genotypes, accessions H9, H12, H20, H21, and H22 did not show significant differences between stages.

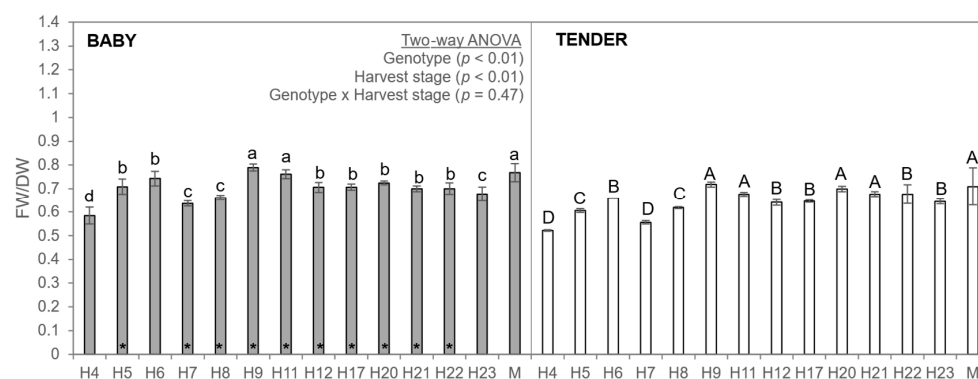


Figure 4. Fresh-to-dry weight ratio (FW/DW, g g^{-1}) in baby (dark bars) and tender (white bars) faba beans across 14 accessions. Values represent means $\pm$ SE ($n = 4$; each replicate consisting of 10 seeds per accession and harvest stage). Results from a two-way ANOVA, with genotype and harvest stage as factors, are shown within the graph. Separate one-way ANOVAs were conducted for each harvest stage, followed by Scott–Knott test ($p < 0.05$). Different letters indicate significant differences among accessions within each stage (lowercase letters for baby beans and uppercase letters for tender beans). Additionally, one-way ANOVAs performed for each accession, using harvest stage as a factor, are represented by asterisks. An asterisk indicates a significant difference between baby and tender stages, with the stage marked by the asterisk having a higher FW/DW.

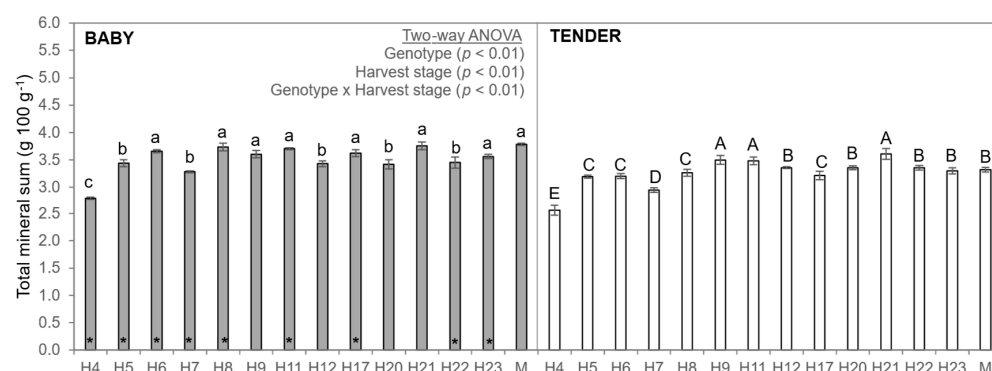


Figure 5. Total mineral content, expressed as the sum of all analyzed minerals ($\text{g } 100 \text{ g}^{-1}$), in baby (dark bars) and tender (white bars) faba beans across 14 accessions. Values are means $\pm$ SE ($n = 4$). Results of the two-way ANOVA, with genotype and harvest stage as factors, are shown within the graph. Separate one-way ANOVAs were conducted for each harvest stage, followed by Scott–Knott test ($p < 0.05$). Different letters indicate significant differences among accessions within each stage (lowercase letters for baby beans and uppercase letters for tender beans). Additionally, one-way ANOVAs performed for each accession, using harvest stage as a factor, are represented by asterisks. An asterisk indicates a significant difference between baby and tender stages, with the stage marked by the asterisk having a higher total mineral content.

Accession H4 consistently exhibited the lowest total mineral content across both stages. Among baby beans, accessions Mutxamel, H21, H8, H11, H6, H17, H9 and H23 recorded the highest mineral percentages. At the tender stage, H21, H9, and H11 stood out for their elevated mineral content.

3.3.2. Macro-Elements

The mean concentrations ($\pm$ SE) of macro-elements across all accessions and harvest stages are summarized in Tables 2 and 3. A two-way ANOVA performed for each element revealed a significant genotype $\times$ harvest stage interaction in all cases. Therefore, data

were subsequently analyzed using separate one-way ANOVAs for each harvest stage and for each genotype.

Table 2. Concentration of macro-elements (Ca, Mg, K) in the seeds of the different faba bean accessions (ID). M stands for the commercial genotype Mutxamel. Values ($\text{g } 100 \text{ g}^{-1}$) are presented as means $\pm$ standard error (SE) ($n = 4$). The bottom section of the table displays the p -values from the two-way ANOVA, with genotype (G) and harvest stage (HS) as factors. Due to the significant interaction, separate one-way ANOVAs were conducted for each harvest stage, using genotype as a factor, followed by Scott–Knott test ($p < 0.05$). Different lowercase letters indicate significant differences among accessions within each harvest stage. Additionally, one-way ANOVAs performed for each accession, using harvest stage as a factor, are represented by asterisks. An asterisk indicates a significant difference between baby and tender stages for that accession, with the stage marked by the asterisk having a higher mineral content.

ID	Ca ($\text{g } 100 \text{ g}^{-1}$)		Mg ($\text{g } 100 \text{ g}^{-1}$)		K ($\text{g } 100 \text{ g}^{-1}$)	
	Baby	Tender	Baby	Tender	Baby	Tender
H4	0.167 $\pm$ 0.003 c *	0.147 $\pm$ 0.001 b	0.143 $\pm$ 0.001 f *	0.128 $\pm$ 0.003 h	1.734 $\pm$ 0.015 e	1.603 $\pm$ 0.060 e
H5	0.123 $\pm$ 0.002 f	0.122 $\pm$ 0.005 d	0.188 $\pm$ 0.004 e	0.176 $\pm$ 0.001 e	2.167 $\pm$ 0.043 c *	1.985 $\pm$ 0.017 c
H6	0.126 $\pm$ 0.001 f *	0.113 $\pm$ 0.001 e	0.218 $\pm$ 0.001 b *	0.177 $\pm$ 0.002 e	2.355 $\pm$ 0.021 b *	2.044 $\pm$ 0.031 c
H7	0.155 $\pm$ 0.004 d	0.150 $\pm$ 0.002 b	0.187 $\pm$ 0.001 e *	0.152 $\pm$ 0.002 g	2.036 $\pm$ 0.008 d *	1.834 $\pm$ 0.027 d
H8	0.127 $\pm$ 0.003 f *	0.110 $\pm$ 0.002 e	0.224 $\pm$ 0.005 b *	0.189 $\pm$ 0.004 d	2.458 $\pm$ 0.047 a *	2.140 $\pm$ 0.039 b
H9	0.136 $\pm$ 0.003 e	0.149 $\pm$ 0.004 b *	0.193 $\pm$ 0.004 e	0.189 $\pm$ 0.005 d	2.410 $\pm$ 0.046 b	2.333 $\pm$ 0.051 a
H11	0.082 $\pm$ 0.002 h	0.096 $\pm$ 0.002 f *	0.239 $\pm$ 0.001 a *	0.224 $\pm$ 0.005 a	2.470 $\pm$ 0.012 a *	2.306 $\pm$ 0.047 a
H12	0.186 $\pm$ 0.004 b *	0.135 $\pm$ 0.000 c	0.213 $\pm$ 0.004 c *	0.203 $\pm$ 0.001 b	2.142 $\pm$ 0.033 c	2.140 $\pm$ 0.010 b
H17	0.137 $\pm$ 0.003 e	0.129 $\pm$ 0.003 c	0.200 $\pm$ 0.003 d *	0.169 $\pm$ 0.004 f	2.293 $\pm$ 0.040 b *	2.040 $\pm$ 0.050 c
H20	0.123 $\pm$ 0.002 f	0.131 $\pm$ 0.000 c	0.182 $\pm$ 0.004 e	0.166 $\pm$ 0.001 f	2.197 $\pm$ 0.056 c	2.175 $\pm$ 0.022 b
H21	0.110 $\pm$ 0.001 g	0.125 $\pm$ 0.003 d *	0.209 $\pm$ 0.004 c	0.208 $\pm$ 0.006 b	2.494 $\pm$ 0.048 a	2.362 $\pm$ 0.065 a
H22	0.203 $\pm$ 0.007 a	0.189 $\pm$ 0.005 a	0.199 $\pm$ 0.006 d	0.197 $\pm$ 0.003 c	2.192 $\pm$ 0.063 c	2.094 $\pm$ 0.028 c
H23	0.109 $\pm$ 0.001 g	0.114 $\pm$ 0.002 e	0.211 $\pm$ 0.002 c *	0.185 $\pm$ 0.003 d	2.378 $\pm$ 0.025 b *	2.201 $\pm$ 0.043 b
M	0.150 $\pm$ 0.003 d *	0.124 $\pm$ 0.003 d	0.218 $\pm$ 0.002 b *	0.160 $\pm$ 0.003 g	2.408 $\pm$ 0.017 b *	2.168 $\pm$ 0.025 b
Average	0.138 $\pm$ 0.009	0.131 $\pm$ 0.006	0.202 $\pm$ 0.006	0.180 $\pm$ 0.007	2.267 $\pm$ 0.056	2.102 $\pm$ 0.054
	0.134 $\pm$ 0.005		0.191 $\pm$ 0.005		2.184 $\pm$ 0.041	
G	$p < 0.01$		$p < 0.01$		$p < 0.01$	
HS	$p < 0.01$		$p < 0.01$		$p < 0.01$	
G $\times$ HS	$p < 0.01$		$p < 0.01$		$p < 0.01$	

Calcium (Ca)

In most accessions, baby beans showed higher Ca concentrations than tender beans, particularly in H4, H6, H8, H12, and Mutxamel. The greatest decreases in Ca concentration from baby to tender stage were observed in H12 (−27.5%) and Mutxamel (−16.9%). However, the opposite trend was found in H9, H11, and H21, where Ca concentrations increased by 9.8%, 17.5%, and 13.9%, respectively. In the remaining accessions, no significant differences in Ca concentration were detected between harvest stages.

Accession H22 showed the highest Ca content at both stages, while H11 consistently exhibited the lowest values.

Magnesium (Mg)

Baby beans exhibited higher Mg concentrations in most accessions, including H4, H6, H7, H8, H11, H12, H17, H23, and Mutxamel. The most pronounced dilution effects were observed in Mutxamel (−26.7%), H6 (−18.9%), and H7 (−19.0%). No significant differences between harvest stages were found in H5, H9, H20, H21, and H22.

Accession H11 consistently exhibited the highest Mg content, while H4 showed the lowest values.

Potassium (K)

Most baby beans had higher K concentrations than their tender counterparts, particularly in H5, H6, H7, H8, H11, H17, H23, and Mutxamel. The strongest dilution effects from baby to tender stage were observed in H6 (−13.2%), H8 (−12.9%), and H17 (−11.1%). No significant differences between harvest stages were detected in the remaining accessions.

Genotypes H21, H11 and H8 exhibited the highest K content at the baby stage, while H21, H9, and H11 remained among the highest at the tender stage.

Accession H4 consistently showed the lowest K concentrations across both stages.

Table 3. Concentration of macro-elements (P, S, Na) in the seeds of the different faba bean accessions (ID). M stands for the commercial genotype Mutxamel. Values ($\text{g } 100 \text{ g}^{-1}$) are presented as means $\pm$ standard error (SE) ($n = 4$). The bottom section of the table displays the p -values from the two-way ANOVA, with genotype (G) and harvest stage (HS) as factors. Due to the significant interaction, separate one-way ANOVAs were conducted for each harvest stage, using genotype as a factor, followed by Scott–Knott test ($p < 0.05$). Different lowercase letters indicate significant differences among accessions within each harvest stage. Additionally, one-way ANOVAs performed for each accession, using harvest stage as a factor, are represented by asterisks. An asterisk indicates a significant difference between baby and tender stages for that accession, with the stage marked by the asterisk having a higher mineral content.

ID	P ($\text{g } 100 \text{ g}^{-1}$)		S ($\text{g } 100 \text{ g}^{-1}$)		Na ($\text{g } 100 \text{ g}^{-1}$)	
	Baby	Tender	Baby	Tender	Baby	Tender
H4	0.528 $\pm$ 0.004 e	0.502 $\pm$ 0.023 d	0.155 $\pm$ 0.001 d *	0.144 $\pm$ 0.004 f	0.032 $\pm$ 0.000 c *	0.020 $\pm$ 0.000 f
H5	0.728 $\pm$ 0.013 b *	0.678 $\pm$ 0.005 a	0.182 $\pm$ 0.002 c	0.174 $\pm$ 0.002 c	0.026 $\pm$ 0.000 e	0.034 $\pm$ 0.000 c *
H6	0.676 $\pm$ 0.005 c *	0.620 $\pm$ 0.009 c	0.205 $\pm$ 0.002 a *	0.172 $\pm$ 0.003 c	0.049 $\pm$ 0.000 b	0.053 $\pm$ 0.001 b *
H7	0.684 $\pm$ 0.004 c *	0.606 $\pm$ 0.008 c	0.179 $\pm$ 0.001 c	0.168 $\pm$ 0.002 d	0.019 $\pm$ 0.001 f	0.016 $\pm$ 0.000 h
H8	0.653 $\pm$ 0.013 d *	0.589 $\pm$ 0.013 c	0.189 $\pm$ 0.004 c *	0.156 $\pm$ 0.003 e	0.057 $\pm$ 0.001 a	0.058 $\pm$ 0.001 a
H9	0.636 $\pm$ 0.013 d	0.607 $\pm$ 0.015 c	0.192 $\pm$ 0.004 b	0.178 $\pm$ 0.005 c	0.016 $\pm$ 0.000 g	0.019 $\pm$ 0.000 f *
H11	0.680 $\pm$ 0.003 c *	0.639 $\pm$ 0.011 b	0.180 $\pm$ 0.001 c *	0.169 $\pm$ 0.003 d	0.028 $\pm$ 0.000 d *	0.024 $\pm$ 0.001 e
H12	0.662 $\pm$ 0.008 d	0.638 $\pm$ 0.005 b	0.184 $\pm$ 0.002 c	0.185 $\pm$ 0.002 b	0.018 $\pm$ 0.000 f	0.028 $\pm$ 0.000 d *
H17	0.761 $\pm$ 0.014 a *	0.675 $\pm$ 0.015 a	0.193 $\pm$ 0.004 b *	0.164 $\pm$ 0.004 d	0.010 $\pm$ 0.000 i	0.010 $\pm$ 0.000 i
H20	0.704 $\pm$ 0.018 b	0.676 $\pm$ 0.009 a	0.182 $\pm$ 0.004 c	0.178 $\pm$ 0.002 c	0.007 $\pm$ 0.000 j	0.009 $\pm$ 0.000 j *
H21	0.718 $\pm$ 0.014 b	0.681 $\pm$ 0.016 a	0.192 $\pm$ 0.003 b	0.193 $\pm$ 0.005 a	0.011 $\pm$ 0.000 h	0.018 $\pm$ 0.001 g *
H22	0.643 $\pm$ 0.020 d	0.668 $\pm$ 0.006 a	0.186 $\pm$ 0.006 c	0.179 $\pm$ 0.003 c	0.006 $\pm$ 0.000 k	0.006 $\pm$ 0.000 k
H23	0.647 $\pm$ 0.008 d *	0.595 $\pm$ 0.010 c	0.188 $\pm$ 0.002 c *	0.173 $\pm$ 0.002 c	0.008 $\pm$ 0.000 j	0.010 $\pm$ 0.000 i *
M	0.764 $\pm$ 0.003 a *	0.648 $\pm$ 0.010 b	0.199 $\pm$ 0.001 a *	0.175 $\pm$ 0.003 c	0.018 $\pm$ 0.000 f *	0.017 $\pm$ 0.000 g
Average	0.678 $\pm$ 0.016	0.630 $\pm$ 0.013	0.189 $\pm$ 0.003	0.172 $\pm$ 0.003	0.022 $\pm$ 0.004	0.023 $\pm$ 0.004
	0.654 $\pm$ 0.011		0.179 $\pm$ 0.003		0.022 $\pm$ 0.003	
G	$p < 0.01$		$p < 0.01$		$p < 0.01$	
HS	$p < 0.01$		$p < 0.01$		$p < 0.01$	
G $\times$ HS	$p < 0.01$		$p < 0.01$		$p < 0.01$	

Phosphorus (P)

Significant differences in P content were found between harvest stages, with baby beans generally showing higher concentrations, particularly in H5, H6, H7, H8, H11, H17, H23, and Mutxamel. The dilution effect during seed development was most pronounced in Mutxamel (−15.1%). No significant differences were observed in H4, H9, H12, H20, H21, and H22.

At the baby stage, accessions H17 and Mutxamel recorded the highest P levels. At the tender stage, H21, H5, H20, H22, and H17 stood out. Accession H4 consistently exhibited the lowest P content across both stages.

Sulfur (S)

Higher S concentrations were observed in baby beans of accessions H4, H6, H8, H11, H17, H23, and Mutxamel. The most pronounced decrease in S concentration from baby to

tender stage was found in H8 (−17.5%). No significant differences between harvest stages were detected in the remaining genotypes.

Accessions Mutxamel and H6 exhibited the highest S content at the baby stage, while H21 stood out at the tender stage. Accession H4 showed the lowest S content across both stages.

Sodium (Na)

Significant differences between harvest stages were observed in 10 of the 14 accessions (71.4%). In general, baby beans had higher Na concentrations in H4, H11, and Mutxamel, whereas tender beans showed higher values in H5, H6, H9, H12, H20, H21, and H23. No significant differences between harvest stages were detected in the remaining accessions.

This indicates that few genotypes experienced a dilution effect during seed development, most notably H4 (−38.1%), while most of them showed mineral reconcentration, with H12 (+50.6%) and H21 (+55.3%) standing out.

Accession H8 exhibited the highest Na content overall, while H22 consistently showed the lowest values at both stages.

3.3.3. Micro-Elements

The concentration of micro-elements across accessions and harvest stages is presented in Tables 4 and 5. For all micronutrients analyzed, the initial two-way ANOVA indicated a significant genotype × harvest stage interaction ($p < 0.01$). This significant interaction warranted further dissection of the data through separate one-way ANOVAs for each individual genotype.

Copper (Cu)

Significant differences in Cu content between harvest stages were observed in 50% of the accessions. Baby beans exhibited higher Cu concentrations in H6, H7, H8, H11, H12, and Mutxamel, while tender beans showed higher values in H22. These results indicate that a dilution effect occurred during seed development in most genotypes, particularly in Mutxamel (−15.9%) and H6 (−14.9%), whereas a reconcentration effect was observed in H22 (+12.9%). No significant differences were detected between stages in the remaining genotypes.

Accession H11 exhibited the highest Cu content overall, while H21 and H23 consistently recorded the lowest values at both stages. Additionally, H17 ranked among the lowest at the baby stage.

Iron (Fe)

Fe content varied significantly in 64.3% of the accessions. Higher Fe concentrations in baby beans were observed in H5, H6, H7, H8, H23, and Mutxamel, while H9, H12, and H17 showed higher values at the tender stage. These patterns suggest a dilution effect during seed growth in most genotypes, particularly in H6 (−26.9%), and a reconcentration effect in others, with H12 (+69.5%) standing out. No significant differences were detected between stages in the remaining genotypes.

Among accessions, H6 exhibited the highest Fe content at the baby stage, while H12 showed the highest values at the tender stage. Accession H4 consistently recorded the lowest Fe levels across both stages.

Manganese (Mn)

Significant differences between harvest stages were found in 42.8% of the accessions. Baby beans had higher Mn levels in H4, H6, H7, H23, and Mutxamel, while H9 showed higher Mn content at the tender stage. These results indicate a dilution effect during seed

development in most genotypes, most notably in Mutxamel (−18.9%), and a reconcentration effect in H9 (+9.2%). No significant differences were observed between stages in the remaining genotypes.

At the baby stage, accessions H6, H8, H12, H21, H22, and Mutxamel exhibited the highest Mn concentrations. At the tender stage, the highest Mn content was recorded in H9, H11, H12, H21, and H22. Accession H4 consistently showed the lowest Mn levels at both stages.

Table 4. Concentration of micro-elements (Cu, Fe, Mn) in the seeds of the different faba bean accessions (ID). M stands for the commercial genotype Mutxamel. Values ($\text{g } 100 \text{ g}^{-1}$) are presented as means $\pm$ standard error (SE) ($n = 4$). The bottom section of the table displays the p -values from the two-way ANOVA, with genotype (G) and harvest stage (HS) as factors. Due to the significant interaction, separate one-way ANOVAs were conducted for each harvest stage, using genotype as a factor, followed by Scott–Knott test ($p < 0.05$). Different lowercase letters indicate significant differences among accessions within each harvest stage. Additionally, one-way ANOVAs performed for each accession, using harvest stage as a factor, are represented by asterisks. An asterisk indicates a significant difference between baby and tender stages for that accession, with the stage marked by the asterisk having a higher mineral content.

ID	Cu (mg kg^{-1})		Fe (mg kg^{-1})		Mn (mg kg^{-1})	
	Baby	Tender	Baby	Tender	Baby	Tender
H4	17.68 $\pm$ 0.18 d	17.97 $\pm$ 0.23 b	43.53 $\pm$ 0.90 g	48.37 $\pm$ 2.52 e	23.94 $\pm$ 0.14 d *	21.66 $\pm$ 0.09 f
H5	17.78 $\pm$ 0.29 d	16.99 $\pm$ 0.16 c	67.38 $\pm$ 0.95 d *	61.46 $\pm$ 0.39 d	26.87 $\pm$ 0.44 c	25.48 $\pm$ 0.19 d
H6	19.47 $\pm$ 0.22 b *	16.57 $\pm$ 0.33 c	92.00 $\pm$ 5.17 a *	67.16 $\pm$ 0.82 c	30.08 $\pm$ 0.27 a *	25.52 $\pm$ 0.37 d
H7	17.58 $\pm$ 0.10 d *	15.59 $\pm$ 0.27 d	64.85 $\pm$ 0.82 e *	60.60 $\pm$ 0.67 d	26.28 $\pm$ 0.15 c *	23.28 $\pm$ 0.32 e
H8	17.38 $\pm$ 0.27 d *	15.63 $\pm$ 0.33 d	63.02 $\pm$ 1.31 e *	58.14 $\pm$ 1.06 d	30.32 $\pm$ 0.49 a	28.51 $\pm$ 0.47 b
H9	15.78 $\pm$ 0.35 e	15.59 $\pm$ 0.40 d	58.74 $\pm$ 1.20 f	69.17 $\pm$ 3.41 c *	27.11 $\pm$ 0.57 c	29.62 $\pm$ 0.66 a *
H11	21.15 $\pm$ 0.06 a *	18.76 $\pm$ 0.30 a	70.03 $\pm$ 0.75 c	79.80 $\pm$ 5.69 b	28.55 $\pm$ 0.21 b	29.83 $\pm$ 0.50 a
H12	18.33 $\pm$ 0.27 c *	17.11 $\pm$ 0.08 c	64.28 $\pm$ 0.85 e	108.94 $\pm$ 1.30 a *	29.33 $\pm$ 0.45 a	29.63 $\pm$ 0.12 a
H17	14.88 $\pm$ 0.24 f	15.25 $\pm$ 0.44 d	72.22 $\pm$ 1.48 c	80.02 $\pm$ 2.59 b *	28.44 $\pm$ 0.41 b	25.76 $\pm$ 1.11 d
H20	15.67 $\pm$ 0.14 e	15.30 $\pm$ 0.32 d	64.80 $\pm$ 4.02 e	72.80 $\pm$ 2.41 c	27.17 $\pm$ 0.43 c	25.94 $\pm$ 0.15 d
H21	14.24 $\pm$ 0.24 f	14.26 $\pm$ 0.44 e	61.31 $\pm$ 1.40 f	65.01 $\pm$ 2.72 d	29.84 $\pm$ 0.95 a	30.00 $\pm$ 0.78 a
H22	15.48 $\pm$ 0.48 e	17.47 $\pm$ 0.13 c *	66.65 $\pm$ 1.84 d	68.30 $\pm$ 1.85 c	29.87 $\pm$ 0.99 a	30.31 $\pm$ 0.37 a
H23	14.79 $\pm$ 0.17 f	14.64 $\pm$ 0.22 e	60.41 $\pm$ 0.74 f *	58.30 $\pm$ 1.31 d	28.83 $\pm$ 0.27 b *	27.17 $\pm$ 0.43 c
M	18.76 $\pm$ 0.09 c *	15.77 $\pm$ 0.31 d	83.79 $\pm$ 2.24 b *	70.06 $\pm$ 1.25 c	30.23 $\pm$ 0.19 a *	24.49 $\pm$ 0.48 d
Average	17.07 $\pm$ 0.54	16.21 $\pm$ 0.35	66.64 $\pm$ 3.03	69.15 $\pm$ 3.82	28.35 $\pm$ 0.50	26.94 $\pm$ 0.74
	16.64 $\pm$ 0.32		67.90 $\pm$ 2.40		27.64 $\pm$ 0.46	
G	$p < 0.01$		$p < 0.01$		$p < 0.01$	
HS	$p < 0.01$		$p < 0.01$		$p < 0.01$	
G $\times$ HS	$p < 0.01$		$p < 0.01$		$p < 0.01$	

Molybdenum (Mo)

Mo content differed significantly between harvest stages in 12 of 14 of the accessions. Tender beans had higher Mo concentrations than baby beans in H5, H6, H7, H9, H11, H12, H17, H20, H22, H23, and Mutxamel. In these accessions, a reconcentration effect occurred during seed development, with H17 (+57.7%) and H11 (+46.0%) standing out.

The opposite trend was observed in H21, which showed a dilution effect (−27.7%), while no significant differences were found in H4 and H8.

Accessions H17 and H11 exhibited the highest and lowest Mo content, respectively, at both stages, with H4 also ranking among the lowest at the tender stage.

Nickel (Ni)

Significant differences in Ni content between stages were found in 42.9% of the accessions. Baby beans had higher Ni concentrations than tender beans in H5, H7, and Mutxamel, while tender beans showed higher values in H9, H12, and H17. These results

indicate that some genotypes experienced a decrease in seed Ni concentration during development, with the most pronounced dilution in Mutxamel (−23.7%), while others showed an increase, most notably H12 (+96.4%). No significant differences were observed in the remaining accessions.

Among genotypes, H7 exhibited the lowest Ni content at both stages, with H4, H9, H11, and H12 also ranking among the lowest at the baby stage. The highest Ni concentrations in baby beans were recorded in H22, H23, H5, H17 and Mutxamel, while H12 had the highest value at the tender stage.

Zinc (Zn)

Zn content varied significantly between harvest stages in 50% of the accessions. Baby beans exhibited higher Zn concentrations in H6, H7, H11, H20, H23, and Mutxamel, while H21 showed higher values at the tender stage. These results indicate a dilution effect during seed development in most genotypes, particularly in H7 (−15.7%), and a reconcentration effect in H21 (+11.8%). No significant differences were found in the remaining genotypes.

Accession H12 exhibited the highest Zn content at both developmental stages, followed by Mutxamel and H11 at the baby stage. In contrast, the lowest Zn concentrations were consistently recorded in H20, H22, and H23, with H21 also showing low values at the baby stage.

Table 5. Concentration of micro-elements (Mo, Ni, Zn) in the seeds of the different faba bean accessions (ID). M stands for the commercial genotype Mutxamel. Values ($\text{g } 100 \text{ g}^{-1}$) are presented as means $\pm$ standard error (SE) ($n = 4$). The bottom section of the table displays the p -values from the two-way ANOVA, with genotype (G) and harvest stage (HS) as factors. Due to the significant interaction, separate one-way ANOVAs were conducted for each harvest stage, using genotype as a factor, followed by Scott–Knott test ($p < 0.05$). Different lowercase letters indicate significant differences among accessions within each harvest stage. Additionally, one-way ANOVAs performed for each accession, using harvest stage as a factor, are represented by asterisks. An asterisk indicates a significant difference between baby and tender stages for that accession, with the stage marked by the asterisk having a higher mineral content.

ID	Mo (mg kg^{-1})		Ni (mg kg^{-1})		Zn (mg kg^{-1})	
	Baby	Tender	Baby	Tender	Baby	Tender
H4	5.59 $\pm$ 0.07 h	6.15 $\pm$ 0.33 j	1.71 $\pm$ 0.04 c	1.88 $\pm$ 0.07 d	48.19 $\pm$ 0.51 f	47.82 $\pm$ 1.03 f
H5	6.52 $\pm$ 0.13 g	7.28 $\pm$ 0.03 i *	2.52 $\pm$ 0.15 a *	1.96 $\pm$ 0.06 d	58.54 $\pm$ 0.88 c	56.46 $\pm$ 1.53 c
H6	5.94 $\pm$ 0.05 h	7.67 $\pm$ 0.13 i *	2.35 $\pm$ 0.20 b	1.84 $\pm$ 0.04 d	63.33 $\pm$ 0.46 b *	55.03 $\pm$ 0.85 c
H7	10.2 $\pm$ 0.07 e	12.6 $\pm$ 0.18 e *	1.58 $\pm$ 0.06 c *	1.33 $\pm$ 0.03 e	59.29 $\pm$ 0.40 c *	49.98 $\pm$ 0.54 e
H8	9.80 $\pm$ 0.42 e	10.8 $\pm$ 0.24 f	2.14 $\pm$ 0.08 b	2.18 $\pm$ 0.07 c	60.61 $\pm$ 1.81 b	55.07 $\pm$ 1.19 c
H9	6.56 $\pm$ 0.13 g	8.99 $\pm$ 0.77 h *	1.80 $\pm$ 0.03 c	2.23 $\pm$ 0.05 c *	51.56 $\pm$ 1.21 e	53.00 $\pm$ 1.19 d
H11	4.40 $\pm$ 0.04 i	6.43 $\pm$ 0.39 j *	1.71 $\pm$ 0.05 c	1.86 $\pm$ 0.04 d	67.70 $\pm$ 1.58 a *	60.35 $\pm$ 0.90 b
H12	8.89 $\pm$ 0.10 f	9.98 $\pm$ 0.10 g *	1.63 $\pm$ 0.03 c	3.20 $\pm$ 0.02 a *	71.44 $\pm$ 1.98 a	66.85 $\pm$ 0.38 a
H17	24.5 $\pm$ 0.45 a	38.7 $\pm$ 0.47 a *	2.34 $\pm$ 0.04 a	2.66 $\pm$ 0.05 b *	55.19 $\pm$ 1.08 d	53.47 $\pm$ 0.86 d
H20	18.6 $\pm$ 0.41 b	21.0 $\pm$ 0.27 b *	2.04 $\pm$ 0.18 b	2.06 $\pm$ 0.13 d	41.67 $\pm$ 0.52 g *	39.37 $\pm$ 0.63 h
H21	14.1 $\pm$ 0.26 d *	10.2 $\pm$ 0.21 g	2.10 $\pm$ 0.07 b	2.29 $\pm$ 0.18 c	40.82 $\pm$ 0.54 g	45.64 $\pm$ 1.12 g *
H22	14.7 $\pm$ 0.49 d	16.2 $\pm$ 0.16 d *	2.69 $\pm$ 0.07 a	2.69 $\pm$ 0.12 b	44.08 $\pm$ 1.44 g	41.54 $\pm$ 0.53 h
H23	15.4 $\pm$ 0.15 c	18.2 $\pm$ 0.30 c *	2.52 $\pm$ 0.05 a	2.50 $\pm$ 0.12 b	42.30 $\pm$ 0.46 g *	40.04 $\pm$ 0.46 h
M	8.77 $\pm$ 0.16 f	11.3 $\pm$ 0.20 f *	2.51 $\pm$ 0.09 a *	1.91 $\pm$ 0.10 d	68.48 $\pm$ 1.09 a *	61.15 $\pm$ 1.06 b
Average	11.00 $\pm$ 1.54	13.26 $\pm$ 2.29	2.12 $\pm$ 0.10	2.18 $\pm$ 0.12	55.23 $\pm$ 2.83	51.84 $\pm$ 2.21
	12.13 $\pm$ 1.37		2.15 $\pm$ 0.08		53.53 $\pm$ 1.79	
G	$p < 0.01$		$p < 0.01$		$p < 0.01$	
HS	$p < 0.01$		$p < 0.08$		$p < 0.01$	
G $\times$ HS	$p < 0.01$		$p < 0.01$		$p < 0.01$	

3.4. Correlation Analysis

Correlation analysis was conducted to explore the relationships between physical traits (volume and FW/DW) and mineral content in faba bean seeds. An overall correlation analysis was developed (Figure 6), along with a separate analysis for baby (Supplementary Figure S1) and tender (Supplementary Figure S2) data. Only significant correlations ($p < 0.05$) were considered in the analysis.

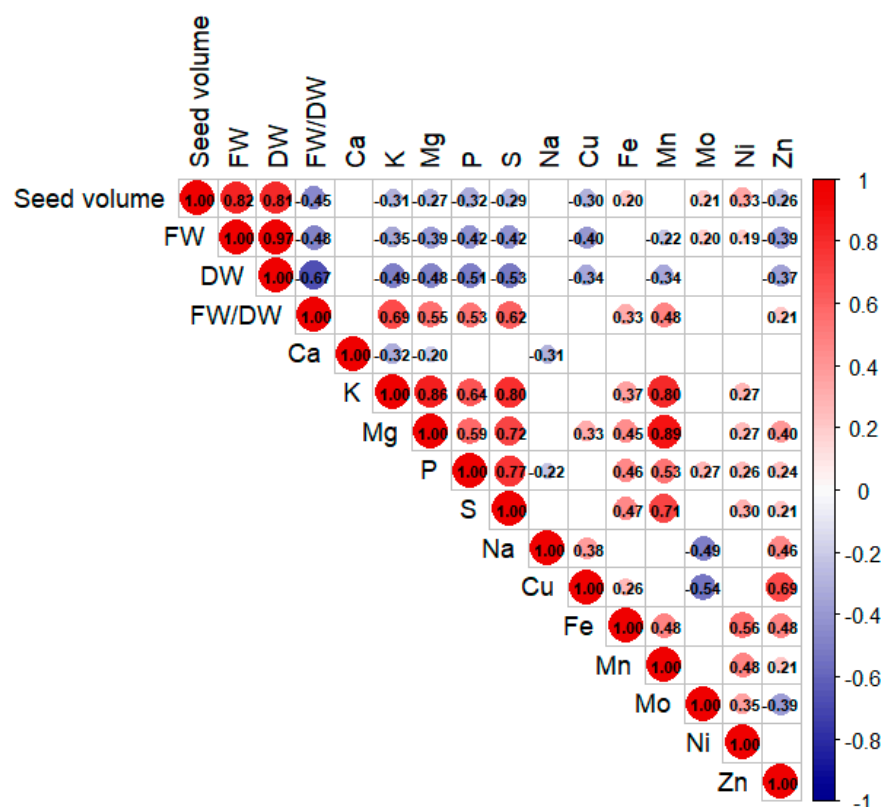


Figure 6. Pearson correlation matrix among all the variables measured in this study. Red dots indicate positive correlation while blue dots correspond to negative correlation. Only significant correlations (p -value < 0.05) are shown. Circle size increases with higher significant levels. The colored bar on the right shows the correspondence between the p -value and the different colors. Variables measured in this study and represented here are physical parameters (seed volume, fresh weight (FW), dry weight (DW) and the FW/DW ratio) and mineral concentration of Ca, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S and Zn.

3.4.1. Overall Correlations

Across all samples, a low but significant negative correlation [36] was observed between seed volume and total mineral content ($r = -0.38$), indicating a general trend in which smaller seeds tended to have a higher concentration of minerals. This pattern appeared to be primarily driven by harvest stage, as baby beans exhibited both lower volume and higher mineral concentrations.

Additionally, positive correlations were found between the fresh-to-dry weight ratio (FW/DW) and several mineral elements, including K ($r = 0.69$), S ($r = 0.62$), Mg ($r = 0.55$), P ($r = 0.53$), Mn ($r = 0.48$), Fe ($r = 0.33$), and Zn ($r = 0.21$), suggesting that seeds with higher relative water content also tended to accumulate more minerals. On the other hand, seed volume showed negative correlations with several macronutrients, including P ($r = -0.32$), S ($r = -0.29$), K ($r = -0.31$), and Mg ($r = -0.27$).

Notably, the highest correlations ($r > 0.7$) were all positive and occurred, generally, between individual minerals: Mn–S ($r = 0.71$), Mg–S ($r = 0.72$), P–S ($r = 0.77$), K–Mn ($r = 0.80$), K–S ($r = 0.80$), Mg–K ($r = 0.86$), and Mn–Mg ($r = 0.89$), indicating strong co-accumulation

in mineral accumulation. Additionally, high or very high correlations [36] were found between seed volume and both fresh weight (FW) ($r = 0.82$) and dry weight (DW) ($r = 0.81$), as well as between FW and DW ($r = 0.97$).

Other significant but mainly moderate correlations [36] among individual minerals were both positive (Zn–Cu, $r = 0.69$; K–P, $r = 0.64$; Mg–P, $r = 0.59$) and negative (Na–Mo, $r = -0.49$; Cu–Mo, $r = -0.54$; K–Ca, $r = -0.32$). To minimize this stage-related effect and focus exclusively on differences attributable to varietal diversity, two separate correlation analyses were performed—one for each harvest stage (Supplementary Figures S1 and S2).

3.4.2. Baby Stage

In baby faba beans (Supplementary Figure S1), seed volume showed a moderate negative correlation with Cu ($r = -0.58$) and Zn ($r = -0.71$), and a moderate positive correlation with Mo ($r = 0.53$).

The fresh-to-dry weight ratio (FW/DW), an indicator of relative water content, was positively correlated with K ($r = 0.52$).

The most relevant positive correlations between individual nutrients ($r > 0.7$) were: Cu–Zn ($r = 0.82$), Fe–S ($r = 0.76$), K–Mg ($r = 0.85$), K–Mn ($r = 0.78$), K–S ($r = 0.80$), Mg–Mn ($r = 0.84$), Mg–S ($r = 0.70$), and Mn–S ($r = 0.80$). Additionally, there was a relevant negative correlation between Cu–Mo ($r = -0.72$). More moderate correlations were observed between Ca–K ($r = -0.51$) and Na–Cu ($r = 0.57$).

3.4.3. Tender Stage

In tender beans (Supplementary Figure S2), seed volume was positively correlated with Ni ($r = 0.47$), while no significant negative correlations with mineral content were observed, unlike in baby beans.

The FW/DW ratio also showed positive correlations with specific elements such as K ($r = 0.77$) and S ($r = 0.54$).

The most relevant correlations between individual nutrients ($r > 0.7$) were: K–Mg ($r = 0.81$), K–Mn ($r = 0.82$), K–S ($r = 0.72$), Mg–Mn ($r = 0.93$) and P–S ($r = 0.77$). A lower negative correlation was observed between Na and Ca ($r = -0.48$).

3.5. PCA

Principal Component Analysis (PCA) was conducted on fourteen faba bean genotypes to analyze similarities and discrepancies among landraces based on their physical and qualitative traits. The PCA was developed separately for the baby (Figure 7) and tender stage (Figure 8), as preliminary ANOVA results revealed significant stage effects, indicating distinct data structures for each developmental phase. The distribution of genotypes in each harvest stage is presented according to the most significant principal components.

3.5.1. Baby Stage PCA

The first two PCA components accounted for 64.3% of the total variance in the traits studied (Figure 7A). The first dimension (Dim-1) accounted for 39.9% of the variance (Figure 7B), with key contributing variables including DW, and minerals such as Mg and Fe (Figure 7C). The second dimension (Dim-2) explained 24.4% of the variance (Figure 7D), highlighting minerals like Mo, Cu and Ni.

Cluster analysis revealed three statistically distinct groups of genotypes (Figure 7A): Cluster 1 (landrace H4); Cluster 2 (landraces H17, H20, H21, H22, and H23); and Cluster 3 (commercial variety-Mutxamel and landraces H5, H6, H7, H8, H9, H11, and H12).

Cluster 1, located in the lower left corner of the PCA plot (Figure 7A), presents low P, S, Mn, K, Fe, and Mg content, with slightly higher Ca content.

Cluster 2, located in the upper central-left corner of the PCA plot (Figure 7A), included genotypes with larger and heavier seeds. Additionally, Cluster 2 genotypes exhibited high Mo content.

Cluster 3, located in the central-right corner of the PCA plot (Figure 7A), included genotypes with higher Zn, Cu, and Na content in their seeds.

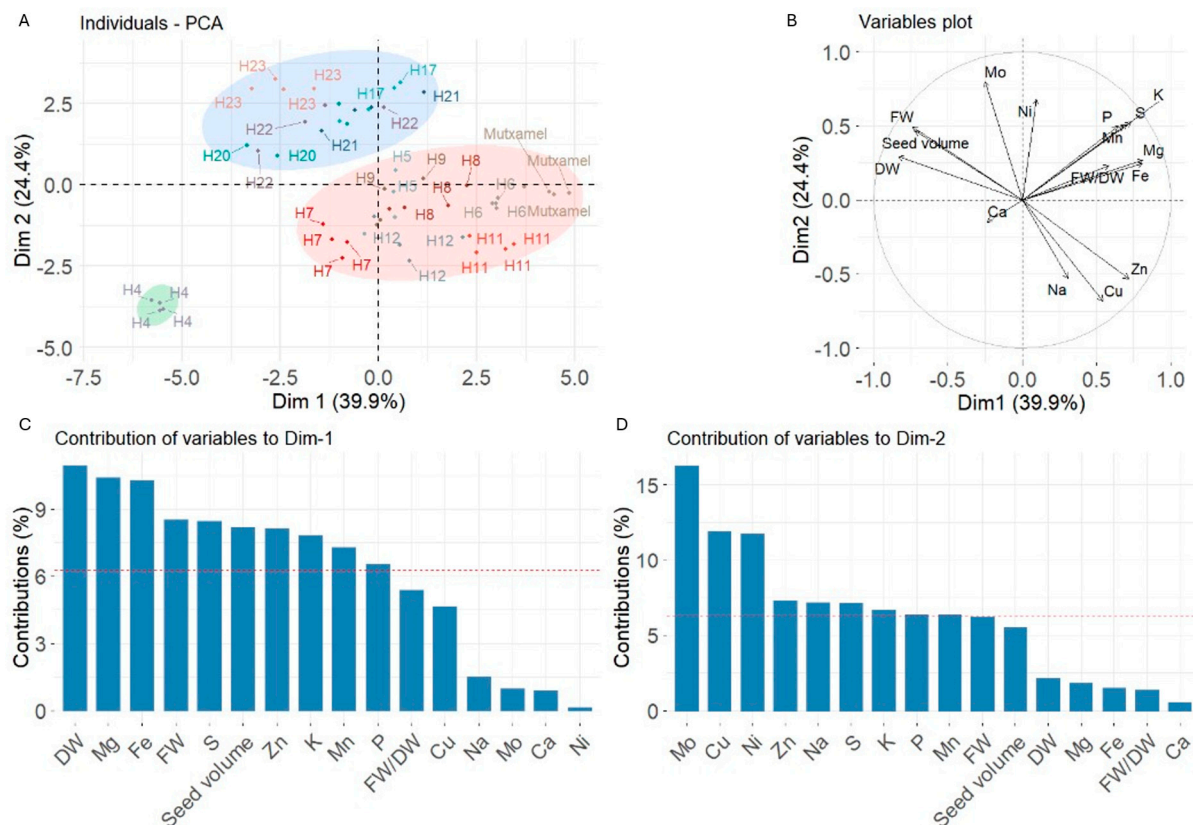


Figure 7. Correlation. PCA projection of all studied variables for the baby data, grouped by types of genotypes. Variables measured in this study and represented here include physical parameters (seed volume, fresh weight (FW) and dry weight (DW)) and mineral composition (Ca, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S, Zn). **(A)** 2D scatter plot of the PCA. Dim1 and Dim2 represent the first and second principal components, respectively, capturing the main patterns in the data by explaining 39.9% and 24.4% of the total variance. Each colored point represents a repetition (from 1 to 4) of a specific accession, labeled with the accession's name or number. The color of the points indicates the genotype of each accession. Colored ellipses represent the three clusters generated by the k-means and silhouette methods: cluster 1 (green), cluster 2 (light blue), and cluster 3 (light red). **(B)** Correlation circle among the studied variables. Each arrow represents the variables studied. Arrows that are close to each other indicate positively correlated variables, while those pointing in opposite directions indicate negative correlations. The position of each arrow reflects its contribution to the principal axes (Dim1 and Dim2) of the PCA. **(C,D)** Contribution of variables to the principal components of the PCA. Panels **(C)** and **(D)** show the contributions of variables to the first (Dim1) and second (Dim2) principal component, respectively. Bars above the red line indicate variables with significant contributions to each component, highlighting those that have greatest impact on the explained variance of the model.

3.5.2. Tender Stage PCA

The first two PCA components accounted for 49.8% of the total variance in the traits studied (Figure 8A). The first dimension (Dim-1) accounted for 31.8% of the variance (Figure 8B), with key contributing variables including minerals such as K, Mn, Mg and

S (Figure 8C). The second dimension (Dim-2) explained 18% of the variance (Figure 8D), highlighting the physical traits, such as FW, DW, and seed volume.

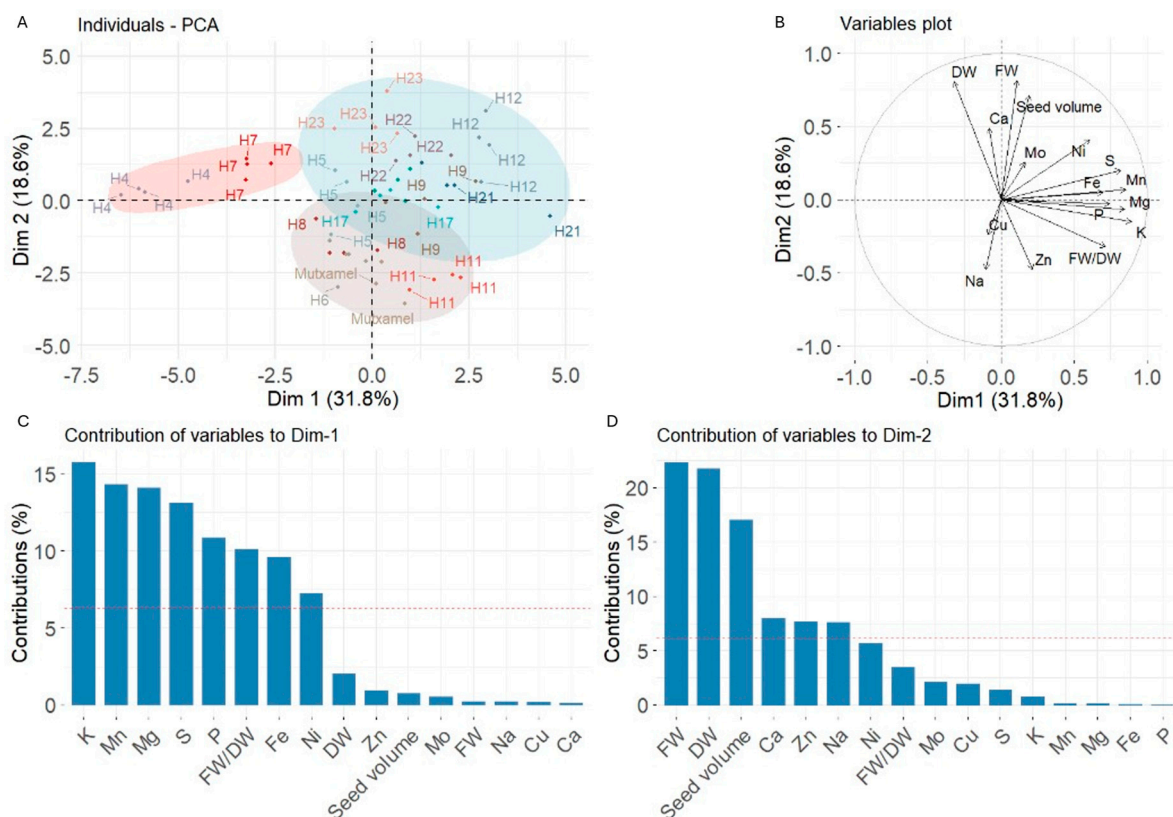


Figure 8. PCA projection of all studied variables for the tender data, grouped by types of genotypes. Variables measured in this study and represented here include physical parameters (seed volume, fresh weight (FW) and dry weight (DW)) and mineral composition (Ca, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S, Zn). (A) 2D scatter plot of the PCA. Dim1 and Dim2 represent the first and second principal components, respectively, capturing the main patterns in the data by explaining 31.8% and 18.6% of the total variance. Each colored point represents a repetition (from 1 to 4) of a specific accession, labeled with the accession's name or number. The color of the points indicates the genotype of each accession. Colored ellipses represent the three clusters generated by the k-means and silhouette methods: cluster 1 (light red), cluster 2 (light blue), and cluster 3 (light brown). (B) Correlation circle among the studied variables. Each arrow represents the variables studied. Arrows that are close to each other indicate positively correlated variables, while those pointing in opposite directions indicate negative correlations. The position of each arrow reflects its contribution to the principal axes (Dim1 and Dim2) of the PCA. (C,D) Contribution of variables to the principal components of the PCA. Panels (C) and (D) show the contributions of variables to the first (Dim1) and second (Dim2) principal component, respectively. Bars above the red line indicate variables with significant contributions to each component, highlighting those that have greatest impact on the explained variance of the model.

Cluster analysis revealed three statistically distinct groups of genotypes: Cluster 1 (landraces H4 and H7); Cluster 2 (landraces H12, H20, H21, H22, and H23); and Cluster 3 (commercial variety Mutxamel and landraces H6, H8, and H11). In addition, three landraces (H5, H9, and H17) showed intermediate positions between Cluster 2 and Cluster 3, indicating a transitional pattern in their traits.

Cluster 1, located in the left side of the PCA plot (Figure 8A), presents low P, K, and Mg content, with a low FW/DW ratio.

Cluster 2, located in the upper central-right corner of the PCA plot (Figure 8A), included genotypes with larger and heavier seeds. Additionally, Cluster 2 genotypes

exhibited higher Mg, Ni, S, Fe, Mn, and Ca content. Cluster 3, located in the central-lower part of the PCA plot (Figure 8A), included genotypes with higher Zn, Cu, and Na content in their seeds, along with smaller and lighter seeds.

3.6. Hierarchical Heatmap Visualization

To obtain an integrative overview of the relationships among traits and genotypes, a hierarchical heatmap was generated including all measured physical and mineral variables across the fourteen faba bean genotypes and the two developmental stages (baby and tender) (Supplementary Figure S3). Data were standardized (z-scores) prior to clustering, and both traits (rows) and genotype–stage combinations (columns) were hierarchically grouped using Euclidean distance and complete linkage.

The heatmap revealed a heterogeneous distribution of trait intensities, reflecting the wide variability among genotypes and developmental stages. Although no strict separation between baby and tender beans was observed, several accessions exhibited partial stage-dependent clustering. For example, genotypes H5 and H17 grouped their tender-stage profiles closely together, whereas others, such as H4, H11, and H22, showed greater similarity between stages. In contrast, most genotypes—including the commercial cultivar Mutxamel as well as accessions H6, H8, and H9—displayed marked separation between developmental stages. Overall, the clustering pattern highlighted substantial diversity in the combined physical and mineral trait profiles among the evaluated genotypes.

On the trait axis, distinct groups were formed. Potassium (K), magnesium (Mg), and manganese (Mn) clustered together with the fresh weight/dry weight ratio (FW/DW), phosphorus (P), and sulfur (S), indicating a consistent association among these variables. A larger cluster included sodium (Na), copper (Cu), and zinc (Zn), along with molybdenum (Mo), iron (Fe), nickel (Ni), calcium (Ca), and silicon (Si), whereas the remaining physical traits—seed volume, fresh weight (FW), and dry weight (DW)—were positioned in an intermediate block.

In summary, the hierarchical heatmap provided a global view of the relationships among genotypes, developmental stages, and traits, revealing coordinated patterns among several mineral elements and distinguishing groups of variables with shared variation trends across the evaluated faba bean accessions.

4. Discussion

The findings of this study provide a comprehensive characterization of the diversity and developmental changes in physical and mineral traits among 14 faba bean genotypes harvested at two early developmental stages: baby and tender. The results highlight pronounced genotype $\times$ harvest stage interactions for both physical and mineral traits, underscoring the complexity of seed developmental processes [37]. This outcome is consistent with previous reports documenting substantial genotypic diversity in faba bean, particularly for seed mineral composition traits such as Mg, Ca, Mn, K, and S, which often exhibit high heritability and are therefore valuable for breeding programs targeting nutritional quality [7].

The dual-stage evaluation adopted in this study provides relevant information on how nutrient accumulation evolves during seed development and allows the identification of genotypes combining nutritional quality and developmental stability. Since faba beans are consumed at both “baby” and “tender” stages, assessing mineral and physical traits across these harvest points offers insight into consumer-relevant nutritional traits and breeding targets. Previous studies have shown that the timing of harvest has a major influence on the concentration of mineral and metabolic compounds in legumes and other crops,

underscoring the importance of stage-specific evaluation in biofortification and quality improvement [19,20].

At the multivariate level, the hierarchical heatmap revealed partial grouping by developmental stage and wide between-genotype variability, supporting the multidimensional nature of mineral and physical traits in immature faba beans. This heterogeneity, in turn, underscores the potential of local germplasm—such as Valencian landraces—to contribute to nutritional enhancement and fresh-market quality, a dimension rarely explored in studies focused mainly on improved or international accessions.

The progressive increase in seed volume from the baby to tender stage observed across all accessions reflects classical grain-filling dynamics, where water influx and assimilate partitioning drive seed cell expansion and dry matter accumulation [38,39]. However, the magnitude of this increase varied markedly among genotypes, with H12 exhibiting the most pronounced volumetric gain (+280%), followed by H9 (+147.6%) and the commercial variety Mutxamel (+120%). In contrast, accessions like H20 (+22.2%) and H4 (+52.4%) showed more modest growth. This heterogeneity likely stems from genotype-specific differences in assimilate allocation, sink strength, water uptake capacity, and cell expansion rates during seed development [39,40]. Notably, smaller-seeded genotypes such as H11 and Mutxamel kept consistently low volumes across both stages, suggesting a genetic constraint on seed size that may be independent of developmental timing. Importantly, seed size is a trait of commercial relevance in faba beans, as varieties are often classified according to seed weight and larger seeds are generally preferred in certain markets [10]. This reinforces the need to evaluate both yield-related traits and nutritional profiles when considering landraces for fresh-market purposes.

Concurrently, all accessions showed a decline in the fresh-to-dry ratio (FW/DW) as seeds transitioned from baby to tender stage, indicating decreasing relative water content during maturation—a well-known hallmark of seed desiccation and reserve deposition [38]. Landraces H5, H6, and H7 exhibited the strongest declines (12–15%), whereas H21 and H20 retained higher relative hydration (−3.5%), suggesting differential regulation of water retention mechanisms. From an agronomic perspective, higher moisture retention may be advantageous for fresh-market beans, since tissue hydration contributes to tenderness and texture, which are critical traits for consumer acceptance [10,12]. Importantly, several landraces (e.g., H9 and H11) achieved FW/DW ratios comparable to the commercial cultivar Mutxamel, indicating that traditional germplasm can meet the same consumer-oriented standards of texture and tenderness. This underscores the relevance of local accessions for fresh-market quality, adding value beyond their role as reservoirs of genetic diversity.

In contrast, landraces such as H4 and H7, with consistently low FW/DW ratios, may be less suitable for fresh-market purposes. These genotype-specific patterns echo findings in other legumes, where rapid volumetric expansion is often coupled with pronounced dehydration [41], reinforcing the importance of considering both seed size and hydration status in varietal selection.

Building on these physical differences, mineral concentrations followed clear stage-dependent trajectories. Some accessions showed similar profiles across stages while others exhibited clear separation, reflecting differential developmental responses. Consistent with the classical “dilution effect”, a developmental pattern described in legumes and other species [42,43], most accessions in this study exhibited a general decline in mineral concentrations from baby to tender seeds. This outcome reflects the common developmental pattern in which tissue expansion during seed filling outpaces nutrient accumulation [44]. Nevertheless, the extent of this dilution varied substantially across genotypes. The commercial cultivar Mutxamel, along with H6 and H8, showed some of the most pronounced decreases ($\approx -12\%$), indicating that its widespread cultivation for the fresh market does

not necessarily guarantee superior mineral retention. By contrast, several landraces (H9, H12, H20, H21, and H22) maintained stable mineral levels between stages, suggesting an enhanced capacity for sustaining nutrient influx or remobilization during maturation [45]. These landraces therefore emerge as particularly promising candidates for biofortification initiatives, as they combine consumer-oriented traits with a superior ability to buffer against mineral dilution.

In agreement with this dilution trend, macronutrients including K, Mg, P, and S showed higher concentrations in baby seeds and lower at the tender stage. This pattern is well documented in legumes, where tissue expansion during seed filling exceeds the rate of mineral accumulation, leading to a dilution of nutrient concentrations [44,46,47]. The commercial cultivar Mutxamel exemplified this phenomenon, showing particularly pronounced declines in Mg (−26.7%) and P (−15.1%). By contrast, several landraces (notably H9, H12, H20, H21, and H22) maintained more stable levels of K, Mg, P, and S, indicating contrasting physiological regulation among genotypes. However, two minerals deviated from the general pattern: Na and Mo tended to increase during development.

Notably, Na showed the most pronounced accumulation in the landrace H21 (+55.3%), illustrating genotype-specific variation in elemental partitioning, which may be related to their specific physiological roles in osmotic adjustment, ionic balance, and redox metabolism during seed filling and desiccation [48,49]. Such differences may also be influenced by environmental factors, including soil mineral availability, irrigation water composition, and temperature during seed filling, which can further modulate nutrient uptake and allocation among developing seeds [50–53]. Molybdenum (Mo), a micronutrient essential as a cofactor in nitrogen metabolism, consistently increased from the baby to the tender stage in nearly all accessions, with H17 showing the strongest rise (+57.7%), followed by H20, H22, and H23. Similar increases in Mo concentrations have been reported in legumes and other crops, where seed Mo content positively correlated with subsequent seedling vigor and nitrogen use-efficiency [54,55]. This reinforces the idea that Mo dynamics in developing seeds are tightly linked to physiological assimilation rather than passive dilution. In contrast, sodium (Na) showed a more genotype-dependent behavior, increasing in about half of the landraces (e.g., H5, H6, H9, H12, H20, H21, and H23), which may indicate a partial reconcentration associated with osmotic regulation during late seed development [48,49]. Indeed, Na accumulation or redistribution in seed has been associated with osmotic adjustments mechanisms and ionic homeostasis in legumes exposed to variable water or nutrient regimes [56,57].

Micronutrients (Cu, Fe, Mn, Ni, and Zn) showed genotype-dependent patterns, with both dilution and reconcentration occurring. The dilution effect pre-dominated for most elements due to biomass accumulation outpacing nutrient uptake (e.g., young seeds expanding faster than minerals can be added) [44].

At the multivariate scale, Ca, Fe and Mo contrasted with the K–Mg–Mn group, illustrating their distinct accumulation trajectories. This suggests that the changes in mineral composition during seed development are not solely attributable to dilution but also reflect element-specific physiological functions [7,45].

At the varietal level, H4 consistently showed the lowest mineral concentrations across most elements and stages, while H6, H8, H11, H17, H21, and Mutxamel were notable for their elevated mineral content at the baby stage. By the tender stage, H9, H11, and H21 emerged with the highest overall mineral concentrations. Particularly noteworthy are landraces like H21, H9, and H11 that maintained high mineral levels at both developmental stages. This stability across stages is a valuable breeding trait, as it ensures consistent nutritional quality regardless of the harvest moment for fresh consumption. Collectively, these observations highlight genetic potential beyond commercial plant material, marking

them as exceptional candidates for breeding programs targeting both fresh consumption and nutritional quality [16].

To further explore these patterns, pairwise associations among elements were examined at the multivariate level. Several moderate to high positive associations [36] were identified between different nutrient pairs, including zinc and copper ($r = 0.69$), aligning with several reports [5,58–60]. A similarly high correlation was observed between potassium and manganese ($r = 0.86$), consistent with Baloch et al. [58]. Among all elements, magnesium and manganese exhibited the highest correlation ($r = 0.89$), a trend previously reported in peanut [59] and in *Arabidopsis thaliana* seeds [61]. Phosphorus and sulfur were also positively correlated ($r = 0.77$), as documented by Wang et al. [59] and Branch and Gaines [60]. Furthermore, manganese, magnesium, phosphorus, and potassium showed significant correlations with sulfur ($r = 0.71$, $r = 0.72$, $r = 0.77$ and $r = 0.80$, respectively), corroborating findings by Wang et al. [59]. Moderate correlations such as K–P ($r = 0.64$) and Mg–P ($r = 0.59$), further support this pattern. Taken together, these consistent relationships suggest shared metabolic pathways and regulatory processes, including metal co-transport, enzymatic cofactor requirements, and synergistic roles in storage compounds like phytate or sulfur-containing metabolites [62–64], and may indicate coordinated uptake or translocation pathways, likely linked to their shared roles in osmotic regulation, phloem loading, and energy metabolism [39,62,65].

By contrast, negative correlations such as Na–Mo ($r = -0.49$), Cu–Mo ($r = -0.54$), and K–Ca ($r = -0.32$) indicate possible antagonistic relationships, possibly reflecting ionic competition during uptake or transport. Such antagonisms are well documented in legumes and cereals, where excessive Ca can interfere with K uptake through competition for cation transporters [65] and high Na availability may restrict the assimilation of divalent cations or trace elements including Mg and Mo [49,66]. In faba bean specifically, Garcia & Grusak [47] noted that nutrient imbalances at the root level could alter the partitioning of several macro- and micronutrients during pod filling, reinforcing the need to consider these competitive dynamics in breeding.

Mineral status was also strongly shaped by physical seed traits. Seed volume showed significant ($p < 0.05$) low [36] negative correlations with most macronutrients (P, $r = -0.32$; S, $r = -0.29$; K, $r = -0.31$; Mg, $r = -0.27$), confirming the dilution effect previously described for this species. Similar relationships have been documented in soybean, chickpea, and *Medicago truncatula*, where rapid tissue expansion during seed filling dilutes nutrient concentrations [44,46,47,67]. For instance, Egli [44] reported an inverse association between seed size and mineral density in soybean, and the same phenomenon was later confirmed in chickpea [68] and in primrose [69]. In our dataset, this trade-off was evident across accessions, with genotypes producing larger tender seeds generally showing sharper declines in mineral density.

Conversely, the FW/DW ratio showed moderate positive correlations with several macronutrients, especially with K ($r = 0.69$), S ($r = 0.62$), Mg ($r = 0.55$), and P ($r = 0.53$), indicating that seeds with higher relative water content tend to accumulate more minerals. This relationship reflects the role of hydration in enhancing phloem unloading and solute transport during early seed development, when metabolic activity and assimilate flow are maximal [39,47,70].

Focusing on developmental stages, the correlation between seed volume and mineral content was significant at the baby stage but disappeared at the tender stage, confirming that the overall trend was driven mainly by developmental differences rather than genotypic variation in seed size. In contrast, FW/DW remained a consistent predictor of mineral accumulation in both stages, particularly for elements with high phloem mobility such as K and Mg, and for S, [65,71,72].

Consistently, Principal Component Analysis (PCA) provided an integrated view of mineral and physical traits, revealing partially consistent genotype groupings across developmental stages. The observed clustering patterns highlight the interplay between seed size, hydration, and mineral composition, suggesting that both genetic background and developmental timing influence nutrient accumulation in faba beans. These PCA patterns align with the correlation trends described earlier: clusters rich in K, Mg, and S likely represent genotypes with superior phloem loading capacity for mobile ions, whereas those characterized by higher seed volume and weight may prioritize carbon deposition over mineral concentration. Therefore, the PCA supports the multifactorial control of seed quality and helps define two contrasting breeding ideotypes—one focused on “mineral-density” (stable, nutrient-rich genotypes) and another on “high-yield” (large, biomass-oriented genotypes)—revealing a potential trade-off to consider in faba bean improvement.

Complementing the PCA, heatmap analysis further illustrated how physical parameters (seed volume, FW, and DW) co-varied with mineral traits across genotypes and stages, while also displaying distinct clustering trends. In particular, FW and DW grouped closely with K, Mg, Mn, P, and S, indicating consistent co-variation between hydration status and major mineral elements. Together, these multivariate analyses provide a coherent picture of coordinated yet partially independent variation, emphasizing that both morphological and nutritional traits are shaped by genotype and developmental stage.

Similar stage-dependent reorganizations of genotype groupings have been reported in other legumes, where early developmental phases tend to accentuate genotypic differentiation in mineral profiles that later converge during maturation [7,68,73]. Our results are consistent with these trends, reinforcing that developmental plasticity influences nutrient partitioning and can modulate apparent genotypic contrasts over time. This highlights the value of analyzing immature stages, where genotypic variation in mineral accumulation is more evident and thus more informative for breeding programs.

Within this framework, some landraces—particularly H21, H9, and H11—consistently exhibited high mineral density and favorable physical traits across both stages, suggesting strong and stable genetic control of nutrient accumulation. Conversely, other genotypes such as H5, H9, and H17 showed greater positional changes between stages, indicating a flexible regulation of solute transport or remobilization. Together, these findings reveal untapped potential within Mediterranean landraces for combining nutritional quality and consumer-oriented characteristics, aligning with global goals of dietary diversification and biofortification [16].

The mineral concentrations observed in our study were generally consistent with previous reports. Hacisalihoglu [5] found similar values, although potassium and manganese were notably higher in our genotypes (2.184 vs. 1.25 g 100 g⁻¹ and 27.64 vs. 17.92 mg kg⁻¹, respectively). Khazaei & Vandenberg [7] reported comparable overall values, yet our genotypes exhibited substantially higher molybdenum (12.13 vs. 2.23 mg kg⁻¹), manganese (27.64 vs. 14.09 mg kg⁻¹), copper (16.64 vs. 8.203 mg kg⁻¹), and potassium (2.184 vs. 1.131 g 100 g⁻¹). Baloch et al. [58] observed similar mineral profiles in Turkish landraces, but our genotypes showed even higher potassium (2.184 vs. 1.30 g 100 g⁻¹), phosphorus (0.654 vs. 0.297 g 100 g⁻¹), and zinc (53.53 vs. 24.2 mg kg⁻¹), with copper slightly lower (16.64 vs. 18.9 mg kg⁻¹). Similarly, Etemadi [10] measured multiple minerals in faba beans and reported profiles comparable to ours; however, our genotypes had higher potassium (2.184 vs. 1.78 g 100 g⁻¹), whereas their calcium concentration was lower (0.134 vs. 0.19 g 100 g⁻¹).

The comparison with Dhull et al. [74] is particularly revealing regarding the potential of immature seeds. They reported that mature seeds (low sodium: 0.013 g 100 g⁻¹; high potassium: 1.062 g 100 g⁻¹) were more suitable for low-sodium diets than immature seeds (0.05 g 100 g⁻¹ sodium and 0.250 g 100 g⁻¹ potassium). However, our results indicate that

this pattern is genotype-dependent. The potassium content of our immature seeds not only matches but exceeds the mature seed values reported by Dhull et al. [74], ranging from 1.602 to 2.494 g 100 g⁻¹. For sodium, while some genotypes (e.g., H6 and H8) reached 0.05 g 100 g⁻¹, the rest were much lower, and several (H17, H20, H22, H23) showed extremely low levels, in some cases below 0.010 g 100 g⁻¹. These findings underscore the high potassium content of Valencian genotypes and reveal the potential to accumulate beneficial potassium levels early in seed development while maintaining low sodium concentrations. This supports the suitability of certain genotypes of immature faba bean seeds for inclusion in low-sodium dietary strategies. Overall, these comparisons confirm that landraces from the Mediterranean basin are valuable reservoirs of mineral-rich germplasm and should be prioritized in conservation and breeding strategies.

Collectively, these consistencies with prior literature reinforce the importance of Mediterranean landraces as strategic genetic resources for improving both the nutritional value and market potential of faba beans. Their ability to maintain high mineral concentrations across early harvest stages highlights their suitability for biofortification programs and for developing cultivars that meet the dual goals of nutritional enhancement and fresh-market quality.

5. Conclusions

This study highlights the complex interplay between physical development and mineral nutrition in immature faba bean seeds, showing that both harvest stage and genotype decisively shape seed quality traits. Baby beans consistently contained higher mineral concentrations, reflecting the dilution effect during tissue expansion, whereas tender beans provided greater yield potential through larger size and fresh weight.

Notably, the commercial cultivar Mutxamel did not systematically outperform local landraces, as traditional genotypes often matched or exceeded it in mineral retention and hydration traits, reinforcing the value of conserving Mediterranean germplasm as reservoirs of nutritional quality.

By assessing mineral and physical traits at two edible stages, this study also provides practical insights for breeding programs. Understanding how nutritional traits evolve between the “baby” and “tender” stages helps identify genotypes that combine stable mineral composition with desirable consumer attributes. This dual-stage evaluation adds value for both fresh-market selection and early breeding decisions focused on nutritional enhancement.

Altogether, these findings emphasize the dual opportunity of selecting genotypes that combine consumer-relevant traits for the fresh market with enhanced nutritional value. Future research should validate these genotype-specific patterns under diverse field conditions and across broader genetic backgrounds. Integrating metabolomic and transcriptomic approaches may further uncover the regulatory networks and source–sink dynamics underlying mineral accumulation, supporting precision breeding strategies that maximize both yield potential and nutritional quality. Future work should also assess anti-nutritional factors (e.g., phytate) that affect mineral bioavailability, providing a more comprehensive evaluation of nutritional quality in faba beans.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae11121411/s1>, Figure S1: Pearson correlation matrix among all the variables measured in this study for the baby data. Red indicated positive correlation while blue corresponds to negative correlation. Only significant correlations (p -value < 0.05) are shown. Circle size increases with higher significant levels. The coloured bar on the right shows the correspondence between the p -value and the different colours. Variables measured in this study and represented here are physical parameters (seed volume, fresh weight (FW), dry weight (DW))

and the ratios (FW/DW)) and mineral composition (Ca, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S, Zn).; Figure S2: Pearson correlation matrix plot among all the variables measured in this study for the tender data. Red indicated positive correlation while blue corresponds to negative correlation. Only significant correlations (p -value < 0.05) are shown. Circle size increases with higher significant levels. The coloured bar on the right shows the correspondence between the p -value and the different colours. Variables measured in this study and represented here are physical parameters (seed volume, fresh weight (FW), dry weight (DW) and the ratios (FW/DW)) and mineral composition (Ca, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S, Zn). Figure S3: Hierarchical heatmap showing standardized (z-score) values of physical and mineral traits across fourteen faba bean genotypes and two developmental stages (baby and tender). Both traits (rows) and genotype $\times$ stage combinations (columns) were clustered using Euclidean distance and the complete linkage method. Color intensity represents relative standardized values, with red indicating higher and blue lower values.

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Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

FW	Fresh weight
DW	Dry weight
FW/DW	Fresh-to-dry weight ratio
Ca	Calcium
K	Potassium
Mg	Magnesium
Na	Sodium
P	Phosphorus
S	Sulfur
Cu	Copper
Fe	Iron
Mn	Manganese
Mo	Molybdenum
Ni	Nickel
Zn	Zinc
SE	Standard error

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