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Integrative Agromorphological, Physicochemical, and Microstructural Characterization of Andean Phaseolus Germplasm from Peru

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Abstract

This study characterized 58 *Phaseolus* spp. accessions conserved in the INIA–Amazonas Germplasm Bank (Peru) using an integrated agromorphological, physicochemical, and microstructural approach. Significant variability was observed among vegetative, reproductive, and seed-related traits, reflecting the broad phenotypic diversity of Andean germplasm. Cluster analysis identified groups with contrasting agronomic characteristics, particularly regarding plant height, number of pods per plant, and seed weight. Physicochemical analyses revealed significant differences in colorimetric parameters, phenolic content, and antioxidant activity among accessions. Darker-seeded accessions generally exhibited higher phenolic contents and greater antioxidant capacity. In addition, Fourier-transform infrared (FTIR) spectroscopy, rheological analysis, and scanning electron microscopy (SEM) revealed differences in molecular composition, starch functionality, and granule morphology among accessions. Overall, the evaluated germplasm exhibited substantial phenotypic and biochemical diversity, underscoring its potential value for breeding programs and food-related applications. These findings contribute to the conservation, sustainable utilization, and valorization of native bean genetic resources.

Keywords: *Phaseolus* spp.; germplasm characterization; Andean legumes; antioxidant activity; FTIR spectroscopy; starch functionality; scanning electron microscopy

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1. Introduction

The genus *Phaseolus* (Fabaceae) represents one of the most important plant genetic resources for global food security, providing a substantial source of dietary protein and contributing significantly to human nutrition worldwide [1,2]. Beans are widely cultivated and consumed by approximately 500 million people, highlighting their nutritional and socioeconomic importance [3]. Phaseolus is among the most extensively studied and conserved crop genera maintained in germplasm banks worldwide [4,5]. Wild populations are estimated to have diverged from a common ancestor approximately 165,000 years ago, followed by independent domestication events in Mesoamerica and the Andes around 8000 years ago, resulting in genetically distinct gene pools with contrasting ecogeographical adaptations [6–10].

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Traditionally, bean diversity has been assessed using qualitative traits, such as flower color, seed color, and growth habit, together with quantitative descriptors related to yield, biomass production, and stress tolerance. However, the widespread adoption of high-yielding cultivars has accelerated the replacement of traditional landraces, contributing to genetic erosion and reducing the diversity available for breeding programs [11,12]. In this context, ex situ conservation in germplasm banks remains a key strategy for preserving plant genetic resources and ensuring their long-term availability for research, breeding, and crop improvement [13].

Beyond their agronomic value, beans are recognized for their nutritional and functional properties because of their high contents of proteins, dietary fiber, minerals, starch, and bioactive compounds, including polyphenols and anthocyanins, which are associated with antioxidant activity and potential health benefits [14]. These attributes are particularly relevant for breeding programs aimed at enhancing nutritional quality and adaptability to diverse production environments [15], as well as for the development of value-added products such as functional flours, plant-based beverages, and biobased materials [16].

In Peru, particularly in the Amazonas region, a remarkable diversity of *Phaseolus* germplasm has been maintained through traditional agricultural practices [17]. However, much of this germplasm remains insufficiently characterized, limiting its effective use in breeding programs and its integration into modern food systems [18]. The lack of detailed information on agronomic performance and biochemical composition may contribute to the displacement of native varieties by commercial cultivars. Therefore, characterizing these accessions is essential not only to enhance the value of these genetic resources but also to identify materials with desirable agronomic and functional traits that can support climate-resilient agriculture and help meet increasing food demands [19]. Moreover, conserving these local varieties is critical for maintaining genetic diversity and ensuring the availability of plant genetic resources for future generations [20].

Although Andean bean germplasm is widely recognized for its genetic and nutritional importance, studies integrating agromorphological, physicochemical, and microstructural traits remain limited, particularly in underexplored regions such as the Peruvian Amazon. Consequently, information regarding the relationships among phenotypic variability, biochemical composition, and structural characteristics remains scarce.

Therefore, the present study aimed to characterize *Phaseolus* spp. accessions through a comprehensive approach integrating agromorphological, physicochemical, and microstructural analyses. By linking phenotypic variability with biochemical and structural attributes, this study contributes to identifying germplasm with potential for breeding and functional food applications while supporting the sustainable use and conservation of native genetic resources.

2. Materials and Methods

2.1. Plant Material and Study Site

The plant material consisted of 58 bean accessions (*Phaseolus* spp.) collected from the Amazonas region of Peru. These accessions were obtained from the Germplasm Bank of the National Institute of Agrarian Innovation (INIA–Amazonas). Seeds were randomly selected and evaluated over two consecutive growing seasons (2023–2024).

The experiment was conducted at the Estación Experimental Agraria Amazonas (6°12′20.38″ S, 77°52′34.88″ W; 2347 m a.s.l.). The site is characterized by an average annual precipitation of 760 mm, mean temperatures ranging from 9.8 to 19.6 °C, and an average relative humidity of 78% [21]. The geographic distribution of the 58 bean (*Phaseolus* spp.) accessions collected from the Amazonas region of Peru is shown in Figure 1.

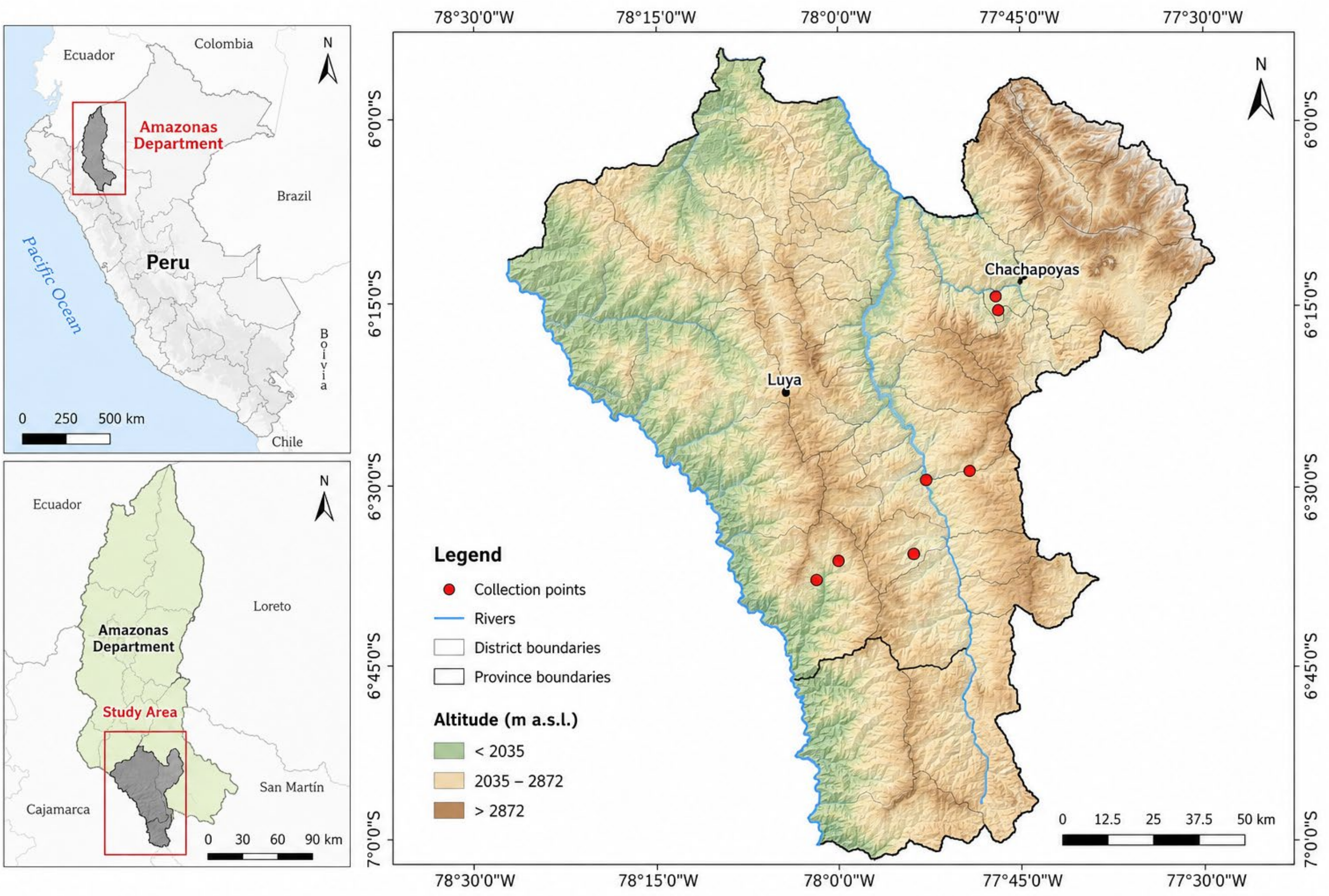

Figure 1. Geographic distribution of 58 bean (*Phaseolus* spp.) accessions collected from the provinces of Luya and Chachapoyas, Amazonas Region, Peru.

2.2. Experimental Design and Agronomic Management Conditions

A completely randomized design with six replicates per accession was implemented. Each experimental unit consisted of a 7.0 × 4.84 m plot containing 18 plants, with a spacing of 2.42 m between rows and 1.0 m between plants.

Soil analyses were performed at the Soil and Water Laboratory (LABISAG) of the National University Toribio Rodríguez de Mendoza. The soil was characterized by a clay texture, alkaline pH (7.8), electrical conductivity of 12.6 $\text{mS}\cdot\text{cm}^{-1}$, organic matter content of 1.9%, nitrogen concentration of 0.6 ppm, phosphorus concentration of 7.39 ppm, and potassium concentration of 208.4 ppm.

Crop management practices included weed control, fertilization based on soil analysis, phytosanitary management, and drip irrigation. Details of the germplasm collection, accession identification, field establishment, and representative floral morphology are provided in Figure S1 (Supplementary Materials).

2.3. Morphological and Agronomic Characterization

A total of 42 descriptors (21 qualitative and 21 quantitative) were evaluated according to the IBPGR guidelines [22], including traits related to plant, leaf, flower, pod, and seed characteristics. The qualitative and quantitative descriptors used for the agromorphological characterization of the 58 *Phaseolus* spp. accessions are summarized in Table 1. Each accession was evaluated using six replicates.

Table 1. Qualitative and quantitative descriptors evaluated in 58 bean (*Phaseolus* spp.) accessions from the INIA germplasm collection.

Qualitative Descriptors			Quantitative Descriptors		
N°	**Code**	**Descriptor**	**N°**	**Code**	**Descriptor**
1	VH	Vegetative habit	1	DE	Days to emergence
2	LS	Leaf shape	2	LH	Length of the hypocotyl (cm)
3	AL	Anthocyanin on leaf	3	LTL	Length of the terminal leaflet (cm)
4	CSP	Color of the standard petal	4	PH	Plant height (cm)
5	CFW	Flower wing color	5	NDF	Number of days until flowering
6	FPW	Pod wall fibers	6	DF	Duration of flowering
7	P CP	Position of pod clusters	7	NFB	Number of flower buds
8	PC	Pod curvature	8	LI	Length of inflorescence (cm)
9	OPA	Orientation of the pod apex	9	NDPM	Number of days until pod maturation
10	CPMP	Color at physiological maturity of the pod	10	LP	Length of the pedicel (mm)
11	CSP	Cross-section of the pod	11	PL	Pod length (cm)
12	CDP	Color of the dry pod	12	LPP	Length of the pod pedicel (mm)
13	SDP	Shape of the distal part	13	PW	Pod width (mm)
14	SMLS	Shape of the median longitudinal section	14	LPA	Length of the pod apex (mm)
15	TSS	Transverse seed shape	15	NSP	Number of seeds per pod
16	DSC	Distribution of the secondary color	16	NLP	Number of locules per pod
17	SV	Seed vein	17	NPP	Number of pods per plant
18	SCP	Seed coat pattern	18	WS	Weight of 100 seeds
19	MCS	Main color of the seed	19	SL	Seed length (mm)
20	PSCS	Predominant secondary seed color	20	SW	Seed width (mm)
21	NSC	Number of seed colors	21	ST	Seed thickness (mm)

2.4. Physicochemical, Biochemical, and Structural Analyses of Seeds

2.4.1. Colorimetric Analysis of Seeds

Seed color was measured using a CM-5 colorimeter (Konica Minolta, Osaka, Japan) according to previous studies [23]. The color parameters L* (lightness), a* (red–green axis), and b* (yellow–blue axis) were recorded as described by [24]. The instrument was calibrated using standard reference values (L* = 82.2, a* = 0.32, and b* = 0.33) before analysis. The total color difference (ΔE*) was calculated according to Equation (1):

$$\Delta E^{*} = \sqrt{\left(L^{*} - L_0^{*}\right)^2 + \left(a^{*} - a_0^{*}\right)^2 + \left(b^{*} - b_0^{*}\right)^2} \quad (1)$$

2.4.2. Determination of Phenolic Content and Antioxidant Activity of Seeds

A. Preparation of the methanolic extract

Samples were ground using a grain mill and sieved through an 850 µm mesh (No. 40). Extraction was performed according to the methodology described by Ioannone et al. [25] with slight modifications. Briefly, 2 mg of the finely ground sample was mixed with 10 mL of methanol–water (80:20, *v*/*v*), using a high solvent-to-sample ratio to improve the extraction efficiency of moderately polar bioactive compounds, including phenolics and flavonoids. The mixture was agitated at 300 rpm for 15 min to ensure adequate contact between the solvent and the sample matrix. Figure 2 presents representative seed samples of the 58 *Phaseolus* spp. accession collected from the Amazonas region of Peru.

Figure 2. Seed samples of 58 *Phaseolus* spp. accessions collected in the Amazonas region of Peru.

B. Total phenolic content (TPC)

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method described by [26], with slight modifications. Briefly, 20 µL of the extract was mixed with 40 µL of 10% (v/v) Folin–Ciocalteu reagent and allowed to react for 5 min. Subsequently, 160 µL of 7.5% (w/v) Na_2CO_3 was added, and the mixture was incubated in the dark for 60 min at room temperature (18 ± 2 °C). Absorbance was measured at 765 nm using a Multiskan SkyHigh spectrophotometer (Thermo Fisher Scientific, Vantaa, Finland).

A calibration curve was constructed using gallic acid standard solutions (0–200 mg L^{-1}), and results were expressed as mg of gallic acid equivalents per gram of sample (mg GAE g^{-1}).

C. Evaluation of antioxidant activity using the DPPH method

Antioxidant activity was determined using the DPPH radical scavenging method according to [27], with slight modifications. Briefly, 3.9 mg of DPPH was dissolved in 5 mL of methanol, and the solution was adjusted to an absorbance of 0.5 ± 0.02 at 517 nm. An aliquot of 20 µL of the extract was mixed with 125 µL of DPPH solution (198 µM) and incubated in the dark for 30 min at room temperature. Absorbance was measured at 517 nm using a spectrophotometer (Thermo Fisher Scientific, Vantaa, Finland).

A calibration curve was constructed using Trolox as the standard, and results were expressed as µmol Trolox equivalents per gram of sample (µmol TE g^{-1}). The percentage of inhibition was calculated using the following Equation (2):

$$E\% = [(A_0 - A_1)/A_0] \times 100 \tag{2}$$

where A_0 is the initial absorbance and A_1 is the absorbance in the presence of the extract.

2.4.3. Molecular Characterization by FTIR

The seven accessions selected for FTIR analysis corresponded to Cluster I, identified through hierarchical clustering analysis, which exhibited contrasting agronomic and physicochemical characteristics, particularly with respect to plant height, number of pods per plant, and seed weight. Bean seeds were ground into flour, and 1 g of the sample was used for analysis. Spectra were recorded using a Fourier-transform infrared (FTIR) spectrometer (Nicolet iS50 FT-IR, Thermo Fisher Scientific, Dreieich, Germany). Sample holders with dimensions of 25 × 25 × 2.5 mm were used.

Spectra were collected over the spectral range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1} and 16 scans, following the methodology described in [28]. All measurements were performed in triplicate.

The obtained spectra were preprocessed using baseline correction and normalization prior to analysis. The average spectrum of each accession was used for interpretation to ensure reproducibility and minimize instrumental variability.

2.4.4. Rheological Analysis

The same seven accessions selected for FTIR analysis were also evaluated for rheological properties. Samples were ground and sieved through a No. 100 mesh. A suspension was prepared by mixing 3 g of flour with 25 mL of distilled water.

Viscosity was determined using a modular compact rheometer (Anton Paar, model MCR 302e, Graz, Austria)) equipped with a PP50 parallel-plate geometry with a 1 mm gap. The suspension was initially held at 50 °C for 1 min, heated from 50 to 95 °C at a rate of 10 °C/min, maintained at 95 °C for 2.5 min, cooled from 95 to 50 °C at 10 °C/min, and finally held at 50 °C for 1 min.

The suspension was stirred at 960 rpm for 10 s, followed by 160 rpm for the remainder of the measurement. All measurements were performed in duplicate, following the methodology described in [29].

2.4.5. Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) analyses were performed using a scanning electron microscope (Zeiss V7.05, Cambridge, UK), following the methodology described by [30]. Samples were mounted on aluminum stubs using double-sided carbon tape and coated with a thin layer of gold using a sputter coater to improve conductivity. SEM observations were conducted at an accelerating voltage of 20 kV.

Granule measurements were obtained from multiple fields per sample, and average granule length values were used for statistical comparisons among accessions.

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2.5. Data Analysis

Euclidean distances were calculated for quantitative data, and hierarchical clustering was performed using Ward's method. Qualitative descriptors were analyzed using frequency distributions and visualized through bar charts. The Shapiro–Wilk test for normality and Bartlett's test for homogeneity of variances were performed. After confirming that the assumptions were met, analysis of variance (ANOVA) followed by Tukey's multiple comparison test was conducted at a significance level of 5% ($p < 0.05$). All statistical analyses were performed using RStudio version 2024.04.02 and OriginPro 2018 (OriginLab Corporation, Northampton, MA, USA).

An overview of the experimental workflow used for the agromorphological, physicochemical, and structural characterization of the evaluated *Phaseolus* spp. accessions is presented in Figure 3.

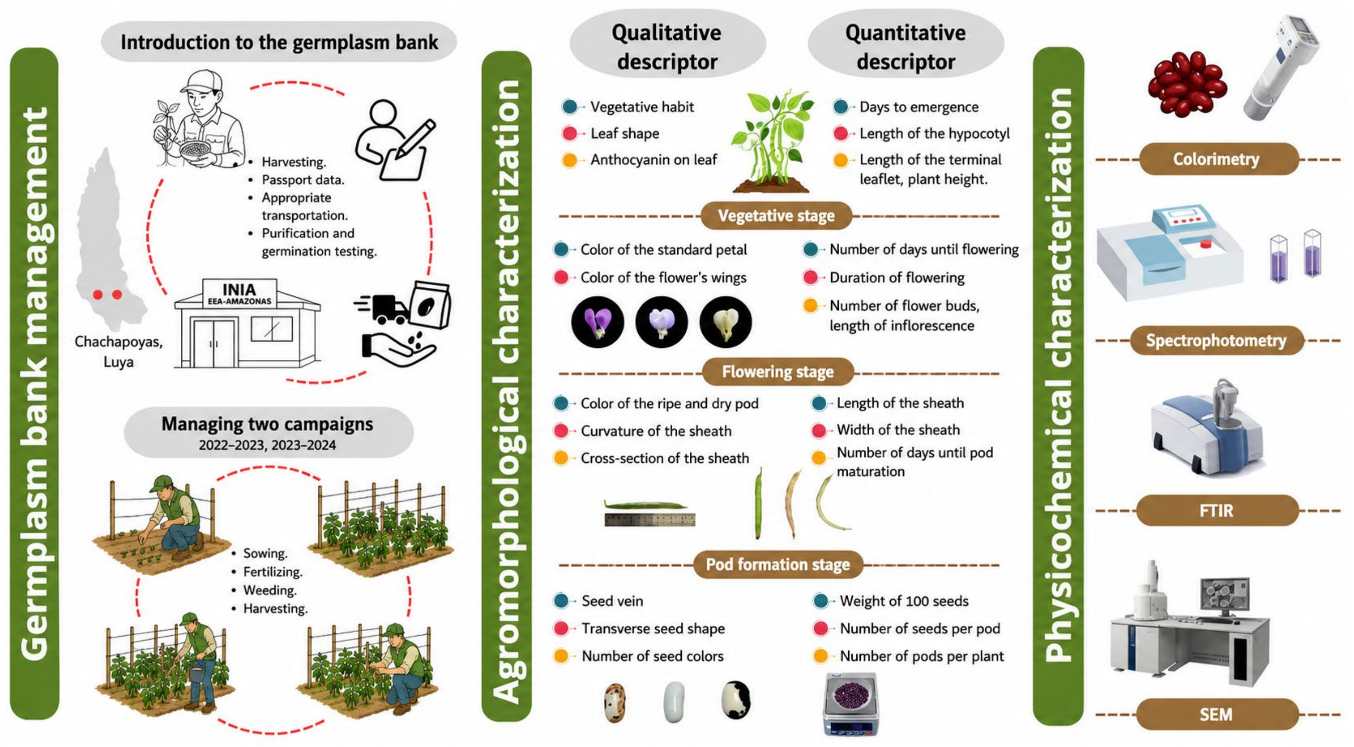

Figure 3. Overview of the experimental workflow used for the agromorphological, physicochemical, and structural characterization of *Phaseolus* spp. accessions.

3. Results

3.1. Variation and Cluster Analysis of Quantitative Agromorphological Traits

The frequency distributions of the 21 quantitative agromorphological descriptors evaluated in 58 *Phaseolus* spp. accessions revealed pronounced variation among phenological, vegetative, reproductive, and seed-related traits (Figure 4).

Days to emergence (DE) ranged from 6 to 13 days, with a mean value of 10.18 days. Similarly, days to flowering (NDF) and days to pod maturation (NDPM) averaged 121.4 and 166.4 days, respectively, indicating substantial variation in crop cycle duration among accessions. Plant height (PH) averaged 132 cm, reflecting differences in vegetative growth among accessions under the evaluated field conditions.

Reproductive traits also showed considerable variation. The number of flower buds (NFB) had a mean value of 5.1, whereas the number of pods per plant (NPP) exhibited a wide range, reaching a maximum of 220 pods and an average of 125.5. Pod-related traits, including pod length (PL), pod width (PW), and pod pedicel length (LPP), also varied markedly among accessions, reflecting differences in reproductive architecture.

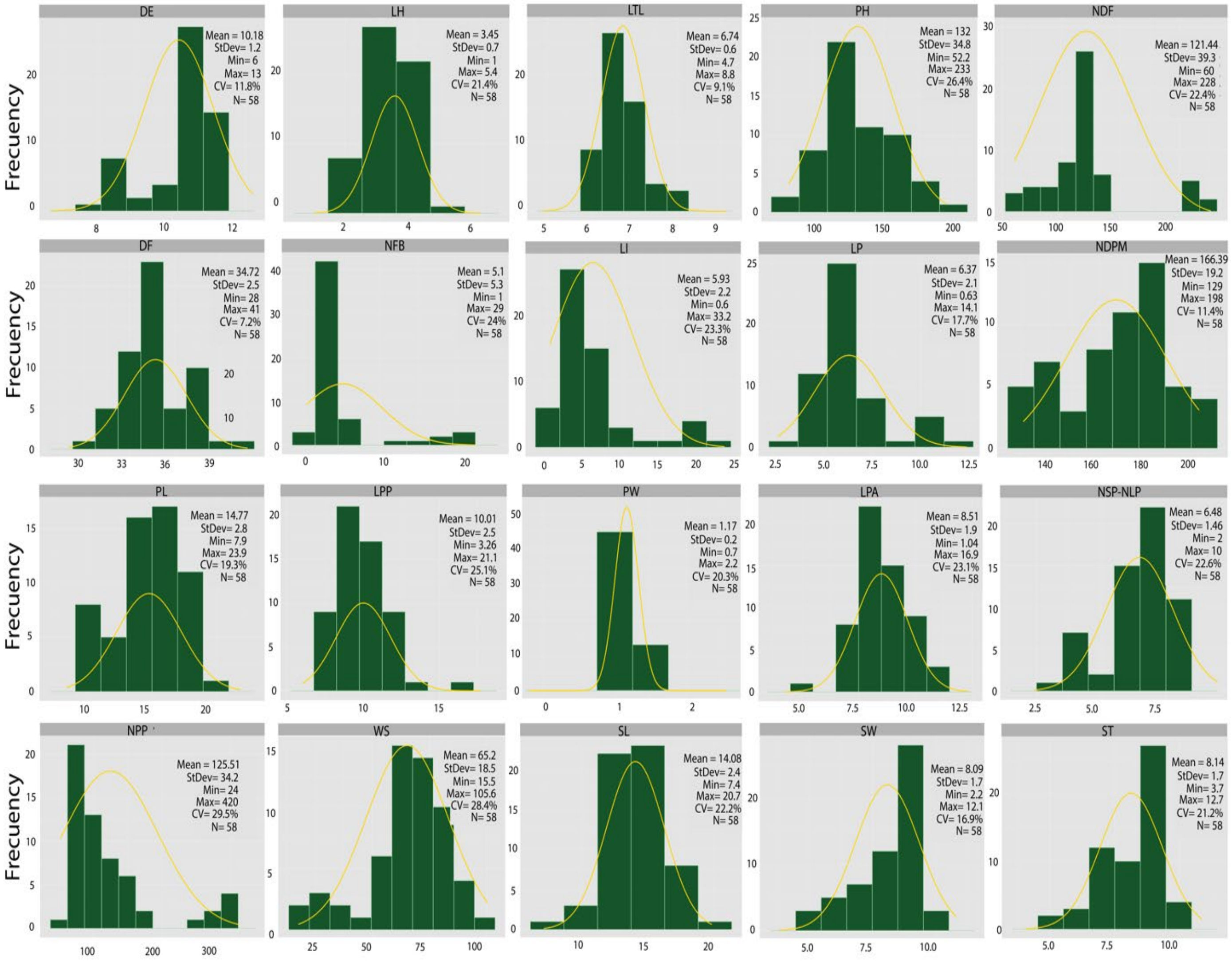

Figure 4. Frequency distributions of 21 quantitative agromorphological descriptors evaluated in 58 *Phaseolus* spp. accessions.

Substantial variation was also observed in seed-related traits. The weight of 100 seeds (WS) ranged from 25.0 to 105.6 g, indicating considerable differences in seed size among accessions. Similarly, seed length (SL), seed width (SW), and seed thickness (ST) exhibited broad ranges, further highlighting the morphological diversity conserved within the germplasm collection.

Several descriptors, particularly PH, NPP, and WS, displayed positively skewed distributions, suggesting the presence of a limited number of accessions with comparatively high values for these traits. Moreover, the high coefficients of variation observed for NPP and WS indicate substantial phenotypic diversity that may be valuable for future selection and breeding programs.

Hierarchical clustering analysis (Figure 5) grouped the accessions into distinct clusters based on their agromorphological characteristics. Cluster I, comprising accessions PER1003513, PER1003514, PER1003528, PER1003530, PER1003531, PER1003532, and PER1003537, was characterized by higher values of plant height, number of pods per plant, days to maturity, and 100-seed weight than the other clusters. These traits distinguished Cluster I from the remaining groups, defining a unique agromorphological profile.

In contrast, the other clusters exhibited intermediate or lower values for these descriptors, reflecting differences in plant development and yield-related traits among accessions.

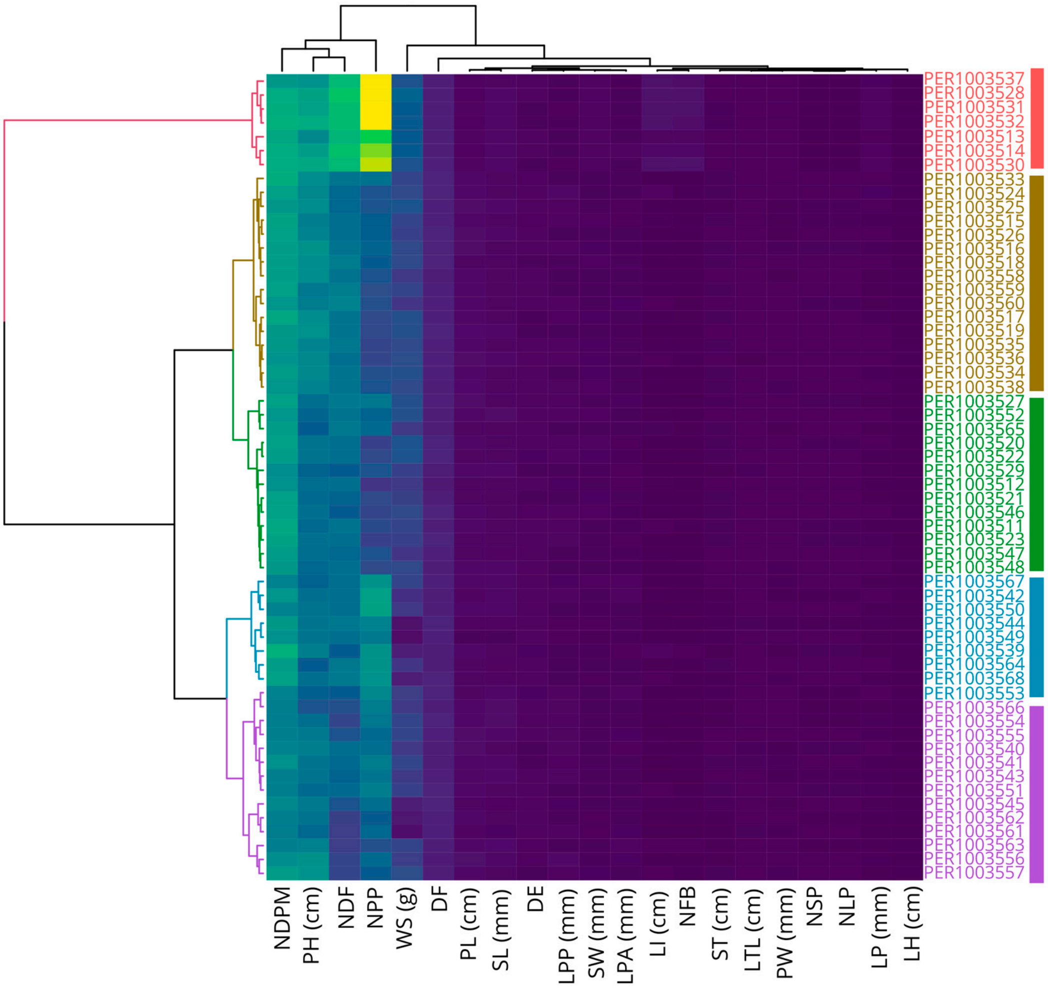

Figure 5. Heatmap and hierarchical clustering of 58 *Phaseolus* spp. accessions based on 21 agromorphological traits using Ward's method and Euclidean distance. Accessions were grouped into five main clusters. Lighter shades represent higher values of the evaluated traits, whereas darker shades represent lower values.

3.2. Correlation and Principal Component Analysis

Principal component analysis (PCA) explained 51.4% of the total variance, with PC1 and PC2 accounting for 35.9% and 15.5%, respectively. PC1 was primarily associated with plant growth and yield-related traits, including plant height, number of pods per plant, and 100-seed weight, whereas PC2 captured variation in pod and reproductive traits such as number of seeds per pod, pod length, and pedicel length. Accessions located on the positive side of PC1 (PER1003530, PER1003531, and PER1003537) exhibited higher values for several agronomically important traits and longer growth cycles, whereas accessions with negative PC1 scores were associated with earlier flowering and smaller plant stature. The distribution of accessions across the PCA space highlights the agromorphological diversity conserved in the INIA–Amazonas Germplasm Bank. Although the first two principal components explained a substantial proportion of the total variance, additional variation was captured by subsequent components.

Pearson correlation analysis (Figure 6b) revealed significant associations among several quantitative descriptors. Strong positive correlations were observed between plant height (PH) and flowering duration (DF, r = 0.582), number of flower buds (NFB, r = 0.680), and inflorescence length (LI, r = 0.632), indicating positive relationships among these traits. Plant height was also positively correlated with the number of pods per plant (NPP, r = 0.478) and 100-seed weight (WS, r = 0.492), suggesting associations between vegetative growth and yield-related traits. Likewise, pod-related traits such as pod length (PL), pedicel length (PPL), and number of locules per pod (NLP) showed strong positive correlations (r > 0.6), reflecting strong relationships among these descriptors. The number of seeds per pod (NSP) was positively associated with both PL and PPL, indicating associations between pod morphology and reproductive performance. In contrast, days to emergence (DE) showed a negative correlation with plant height (PH, r = −0.368), suggesting that accessions with earlier emergence tended to attain greater plant height. Seed morphological traits were also positively associated, particularly seed length (SL) with seed width (SW, r = 0.509) and seed thickness (ST, r = 0.214), reflecting consistency in seed morphology among accessions.

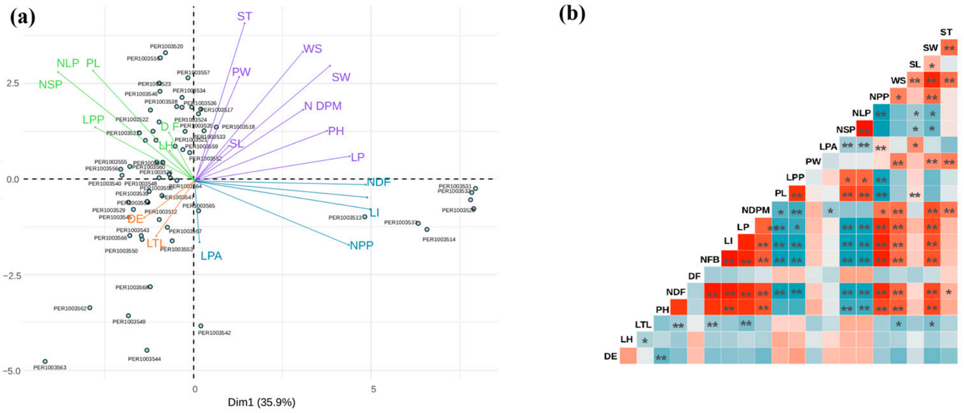

Figure 6. Principal component analysis (PCA) and correlation analysis of quantitative agromorphological traits in 58 *Phaseolus* spp. accessions. (**a**) PCA biplot showing the distribution of accessions based on 21 quantitative traits. (**b**) Correlation matrix displaying the relationships among quantitative descriptors (* $p < 0.05$; ** $p < 0.01$). DE = Days to emergence, LH = Length of the hypocotyl (cm), LTL = Length of the terminal leaflet (cm), PH = Plant height (cm), NDF = Number of days until flowering, DF = Duration of flowering, NFB = Number of flower buds, LI = Length of inflorescence, LP = Length of the pedicel, NDPM = Number of days until pod maturation, PL = Pod length, LPP = Length of the pod pedicel, PW = Pod width, LPA = Length of the pod apex (mm), NSP = Number of seeds per pod, NLP = Number of locules per pod, NPP = Number of pods per plant, WS = Weight of 100 seeds, SL = Seed length (mm), SW = Seed width (mm), ST = Seed thickness (mm).

Overall, PCA and correlation analyses (Figure 6) identified two major groups of interrelated variables: one associated with vegetative growth and yield-related traits (PH, DF, NPP, WS) and another associated with pod morphology and reproductive characteristics (PL, LPP, NLP, NSP). These findings highlight key agromorphological traits that may support germplasm characterization and future breeding efforts.

3.3. Assessment of Qualitative Morphological Characteristics

Figure 7 presents the frequency distribution of qualitative descriptors related to plant and flower characteristics. An indeterminate vegetative habit (VH) was predominant, occurring in 55 accessions, whereas only three accessions exhibited a determinate shrub-like habit (Figure 7A). Anthocyanin pigmentation in leaves (AL) was observed in seven accessions, while the remaining 51 lacked this characteristic (Figure 7B). All accessions exhibited an ovate leaf shape (Figure 7C).

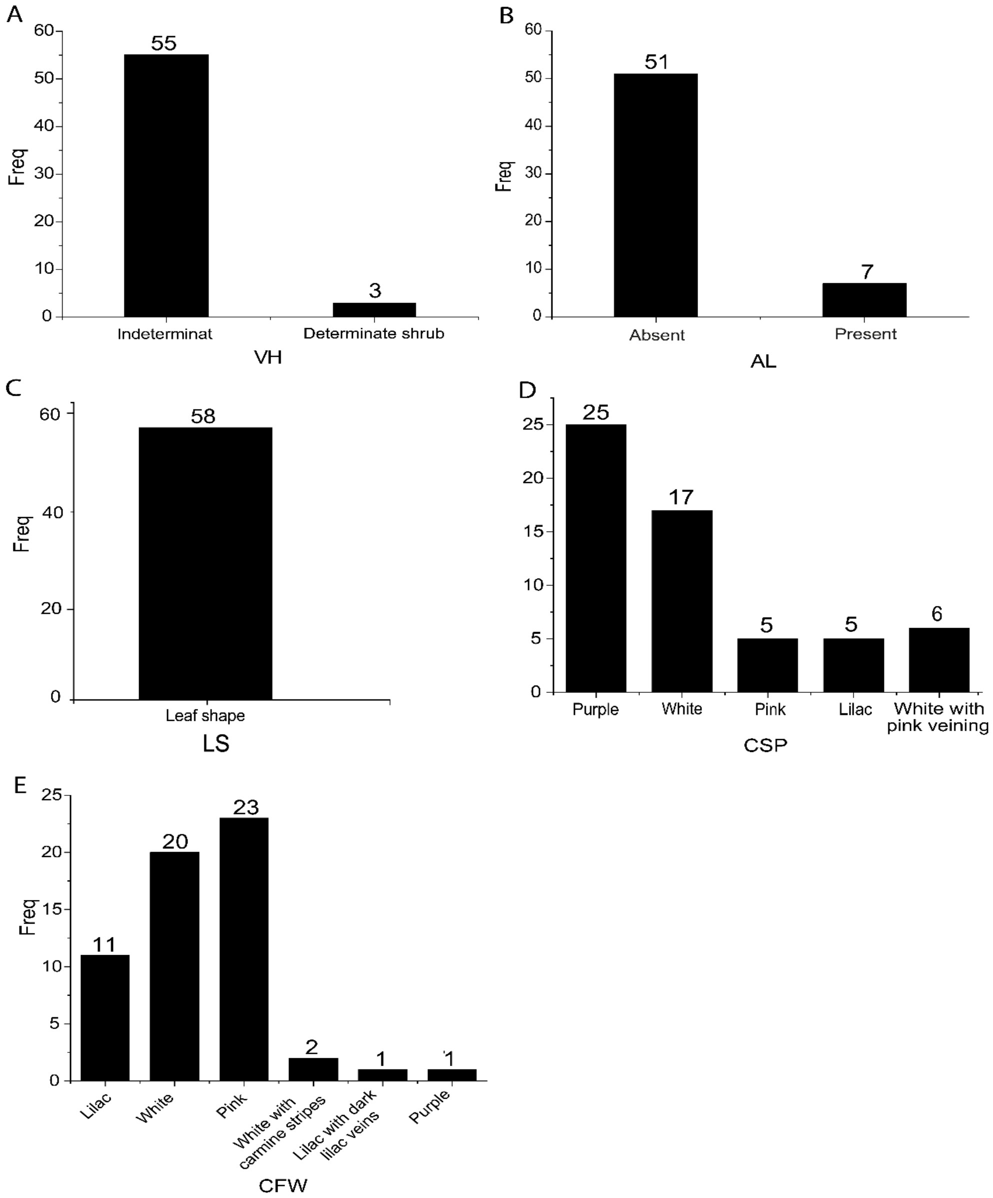

Figure 7. Frequency distributions of plant (**A**–**C**) and flower (**D**,**E**) qualitative traits in 58 *Phaseolus* spp. accessions.

Regarding flower color, purple (*n* = 25) and white (*n* = 17) were the most common categories, whereas pink, lilac, and white with pink veins were less frequent (*n* = 5–6) (Figure 7D). Floral wing color was predominantly pink (*n* = 23), followed by white (*n* = 20) and lilac (*n* = 11). Combinations such as white with crimson lines, lilac with dark veins, and purple were rarely observed (*n* = 1–2) (Figure 7E).

Overall, the evaluated accessions exhibited high uniformity in vegetative traits and moderate diversity in floral characteristics.

Figure 8 presents the morphological characteristics of pods in the 58 accessions. The pod cross-section was predominantly pyriform (*n* = 56), whereas only one accession exhibited a highly flattened elliptical shape and another displayed a rounded elliptical form (Figure 8A). Pod curvature was mainly slight (*n* = 35), followed by straight (*n* = 20) and curved (*n* = 3) forms (Figure 8B). Green was the predominant pod color (*n* = 32), followed by green with purple stripes (*n* = 17) (Figure 8C), whereas dry pod color was primarily golden (*n* = 24), followed by golden with purple stripes (*n* = 17) and yellow (*n* = 17) (Figure 8D). Pod wall texture was predominantly leathery (*n* = 38) (Figure 8F). The distal part of the pod was mainly truncated (*n* = 29) or sharply truncated (*n* = 25), while only a few accessions exhibited a pointed shape (*n* = 4) (Figure 8G). In addition, all accessions exhibited a uniform distribution of pods on the plant (Figure 8H).

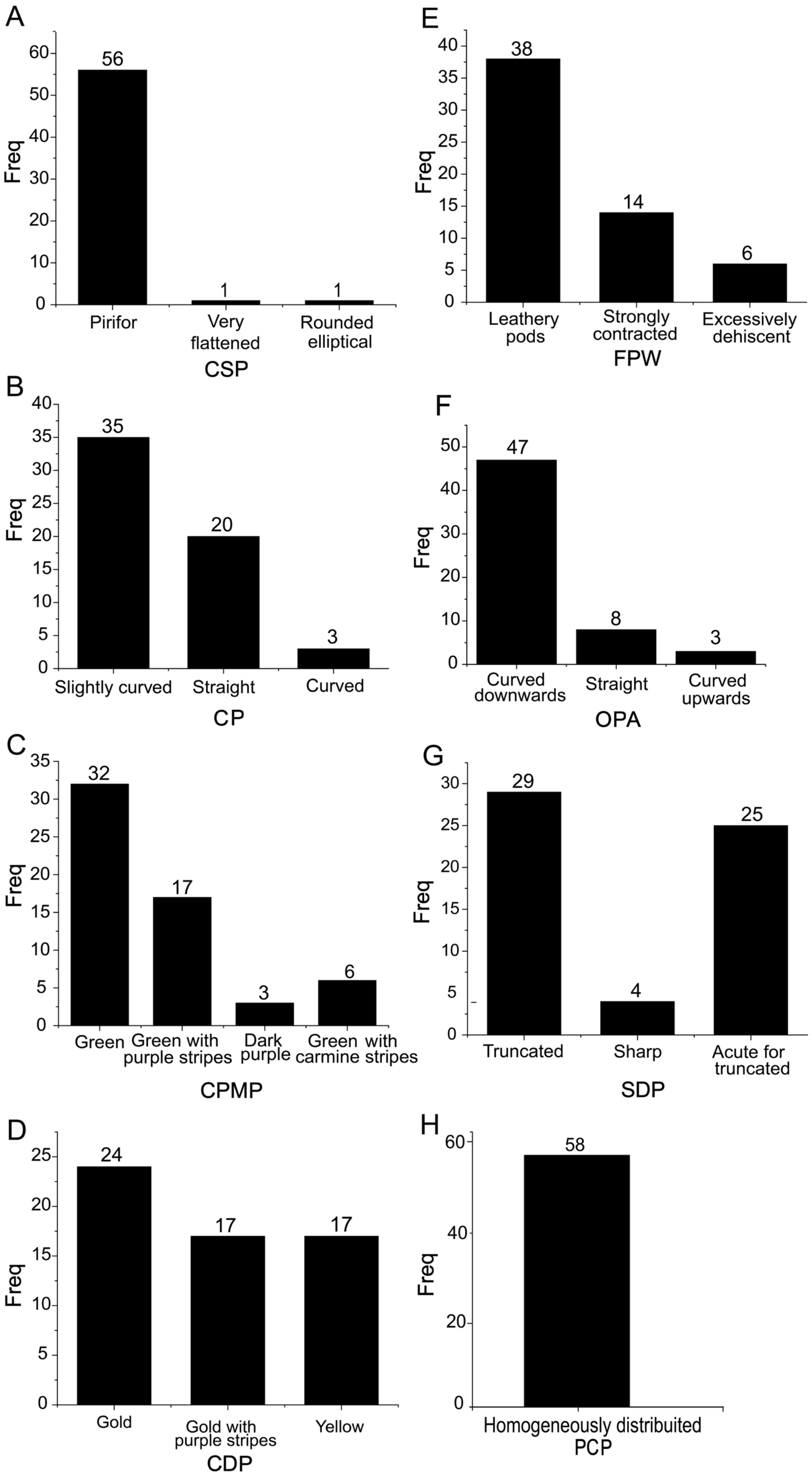

Figure 8. Frequency distributions of pod qualitative traits (**A**–**H**) in 58 *Phaseolus* spp. accessions.

Overall, the evaluated accessions exhibited high uniformity in pod distribution and wall texture, combined with moderate variation in pod shape and coloration.

Figure 9 illustrates the morphological characterization of seeds from the 58 accessions. Regarding transverse shape, flattened (*n* = 20) and narrow elliptical (*n* = 19) seeds were the most common categories, followed by circular forms (*n* = 15) (Figure 9A). In longitudinal view, oval (*n* = 14), kidney-shaped (*n* = 13), and rectangular (*n* = 13) forms predominated (Figure 9B). The number of seed colors was most frequently two (*n* = 39), followed by one color (*n* = 18) and more than two colors (*n* = 1) (Figure 9C).

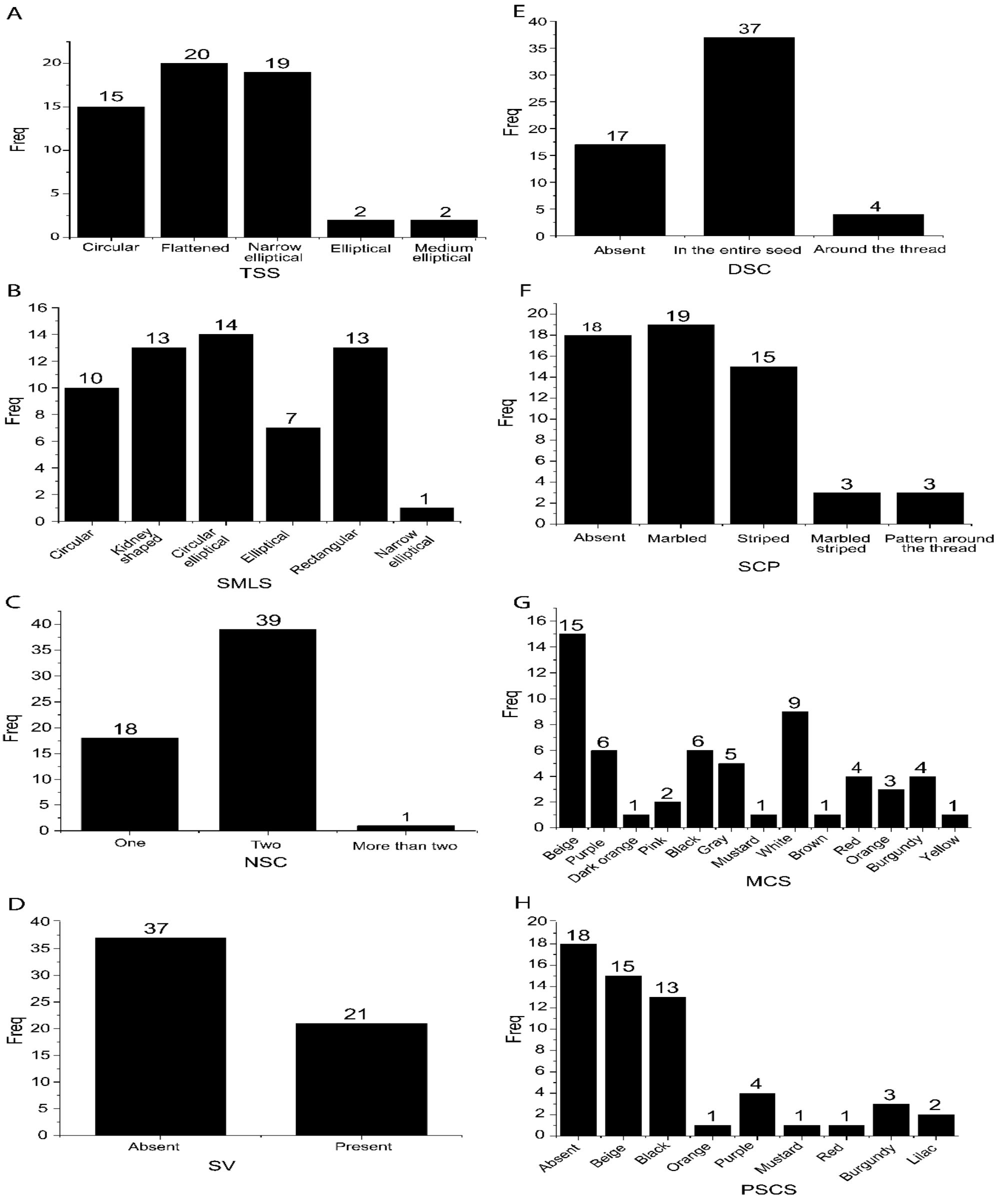

Figure 9. Frequency distributions of seed qualitative traits (**A**–**H**) in 58 *Phaseolus* spp. accessions.

Seed venation was present in 21 accessions (Figure 9D), and a secondary color was observed in 39 accessions, whereas 19 accessions lacked this characteristic (Figure 9E). Seed patterns were mainly mottled (*n* = 19) or speckled (*n* = 15), whereas striped seeds (*n* = 3) and patterns concentrated around the hilum (*n* = 3) were less frequent (Figure 9F). Light tones (*n* = 15) represented the predominant primary seed color category, whereas beige (*n* = 18) and black (*n* = 15) were the most frequent secondary colors (Figure 9G,H).

Overall, the evaluated accessions exhibited moderate diversity in seed morphology and coloration.

3.4. Physicochemical Characteristics of Bean Accessions

3.4.1. Color, Total Phenolic Content, and Antioxidant Capacity

Color analysis of the 58 *Phaseolus* spp. accessions (Figure 10) revealed substantial variation among accessions. This variation was particularly evident in lightness (L*) values, where accessions such as PER1003551 (18.82 ± 7.71) and PER1003563 (24.55 ± 1.32) exhibited darker seed coloration, whereas PER1003549 (82.91 ± 1.52) and PER1003528 (81.75 ± 1.26) showed the highest lightness values.

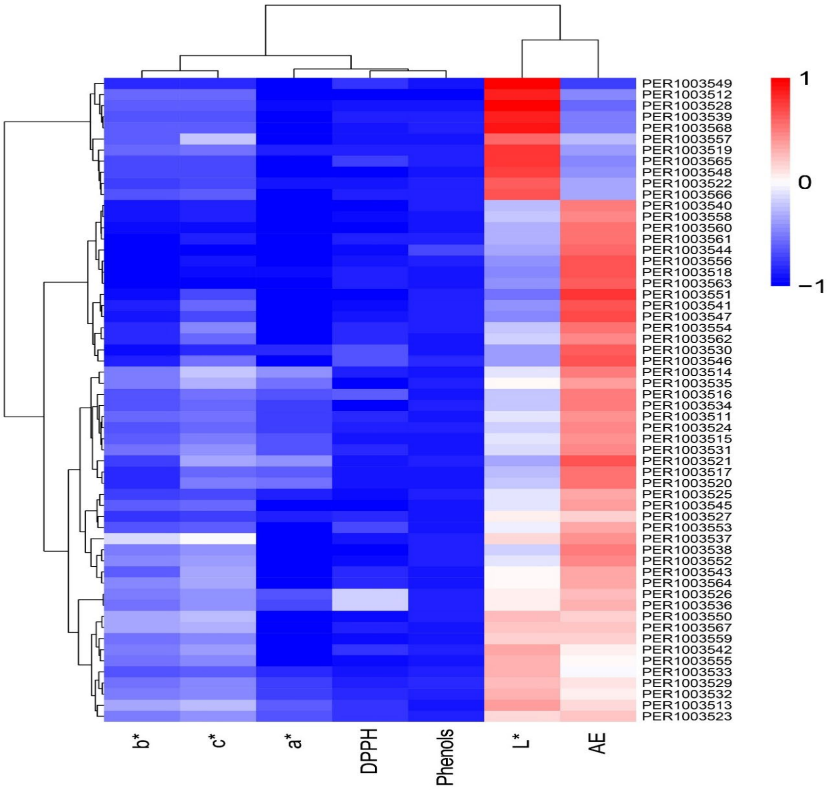

Figure 10. Heatmap with hierarchical clustering of 58 *Phaseolus* spp. accessions, constructed using colorimetric parameters (L*, a*, b*, C*, ΔE), total phenolic content, and antioxidant capacity (DPPH). The color gradient represents the normalized intensity of each variable, with blue indicating lower values and red indicating higher values. This visualization highlights the phenotypic variability among accessions and facilitates the identification of groups with similar biochemical and color profiles.

Hierarchical clustering based on physicochemical traits identified groups of accessions with similar physicochemical profiles. Several accessions with lower L* values tended to exhibit higher total phenolic content and antioxidant activity (DPPH), although this trend was not consistent across all accessions.

Accessions belonging to Cluster I, previously identified based on agromorphological traits, also displayed distinctive colorimetric and bioactivity profiles. For example, PER1003521 (a* = 24.57 ± 2.11) and PER1003543 (a* = 21.35 ± 1.41) exhibited the highest a* values, indicating a stronger red color component, whereas PER1003537 exhibited the highest chroma value (C* = 40.42 ± 1.72), indicating greater color saturation.

Significant differences in total phenolic content were observed among accessions, with values ranging from 0.61 to 12.05 mg GAE g^{-1}. PER1003542 exhibited the highest phenolic content and was also characterized by darker coloration and higher antioxidant activity. Antioxidant activity, determined by the DPPH assay, also varied among accessions, with PER1003526 and PER1003536 exhibiting the highest values, reaching up to 33.32 μmol TE g^{-1}.

3.4.2. Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR spectral analysis was performed on seven bean accessions selected based on contrasting agronomic and physicochemical characteristics. Eight major absorption bands were identified in Figure 11, with prominent bands observed at 3281 and 2929 cm^{-1}. These bands were attributed to O–H and C–H stretching vibrations commonly associated with carbohydrates and lipid-containing compounds. The band observed at 1633 cm^{-1} was associated with amide-related functional groups linked to protein constituents, whereas peaks at 1480 and 995 cm^{-1} corresponded to carbohydrate-related structures, including starch and other polysaccharides.

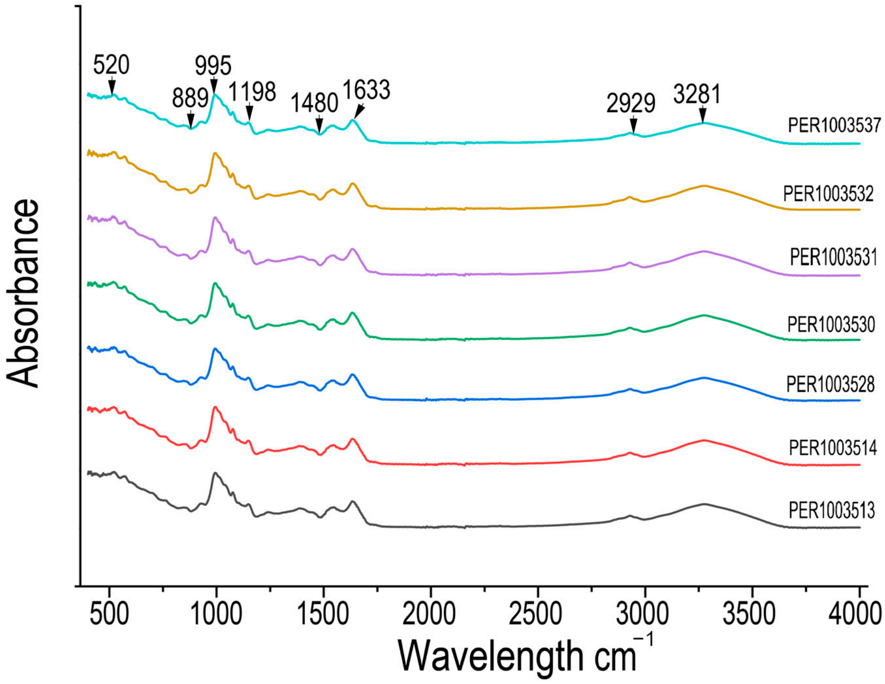

Figure 11. FTIR spectra of seven bean accessions from the INIA–Amazonas Germplasm Bank.

Differences in band intensity and sharpness were observed among accessions, particularly in the regions around 995 and 1198 cm^{-1}, suggesting variation in the relative abundance of chemical constituents. These spectral differences were more evident among accessions with darker seed coats.

3.4.3. Rheological Properties

Table 2 summarizes the rheological behavior of seven *Phaseolus* spp. accessions, revealing differences in peak viscosity. Although most values fell within a relatively narrow range, accession PER1003531 exhibited the highest peak viscosity (5.26 Pa), indicating a greater tendency for starch swelling and paste development during the pasting process. In contrast, accession PER1003513 exhibited lower peak viscosity values, indicating reduced viscosity development under the evaluated conditions.

Table 2. Rheological properties of seven bean accessions.

Accession	Peak Viscosity (Pa)	Peak Temperature (°C)	Gelatinization Temperature (°C)
PER1003537	2.56 ± 0.05 d	95.8 ± 2.32 a	68.02 ± 2.16 a
PER1003532	2.89 ± 0.23 d	95.3 ± 0.40 a	67.7 ± 2.36 b
PER1003531	5.26 ± 0.23 a	95.2 ± 1.23 a	68.1 ± 5.36 a
PER1003530	4.23 ± 2.36 b	94.9 ± 2.35 a	69.3 ± 6.35 a

Table 2. *Cont.*

Accession	Peak Viscosity (Pa)	Peak Temperature (°C)	Gelatinization Temperature (°C)
PER1003528	4.20 ± 1.23 b	95.2 ± 1.36 a	67.5 ± 3.16 b
PER1003514	4.12 ± 2.3 b	95.01 ± 1.02 a	66.5 ± 5.30 b
PER1003513	3.20 ± 2.3 c	95.01 ± 2.34 a	68.10 ± 4.52 a

Values followed by different letters (a–d) within the same column are significantly different according to Tukey's test ($p < 0.05$).

Differences in rheological behavior were also observed among accessions. A similar trend was observed in the SEM images, where accessions with larger starch granules tended to exhibit higher peak viscosity values. However, additional analyses would be required to confirm this association.

Peak temperature remained relatively constant among accessions, ranging from 94.9 °C to 95.8 °C. Greater variation was observed in gelatinization temperature. Accessions PER1003514 (66.5 °C), PER1003528 (67.5 °C), and PER1003532 (67.7 °C) exhibited the lowest values, indicating earlier gelatinization during heating. In contrast, accessions PER1003530 and PER1003531 exhibited higher gelatinization temperatures, suggesting greater thermal resistance of starch granules under the evaluated conditions.

3.4.4. Microscopy (SEM)

Scanning electron micrographs (SEM) presented in Figure 12 show the starch granule morphology of seven *Phaseolus* spp. accessions (Figure 12A–G) at a scale of 10 µm. In general, the granules exhibited an oval shape with smooth surfaces and well-defined edges; however, notable morphological differences were observed among accessions. Panels D–G generally exhibited larger granules than the other accessions and were frequently accompanied by amorphous structures and fibrous fragments adhering to their surfaces, likely representing residual plant matrix material. Some granules also exhibited slight deformations and surface irregularities. In contrast, accessions D and E showed a more uniform granule distribution, characterized by relatively consistent size and intact surfaces.

Figure 12H revealed significant differences ($p < 0.05$) in starch granule length among accessions. PER1003532 and PER1003537 exhibited the largest granules, whereas PER1003513 and PER1003514 showed the smallest granules. These results highlight variation in starch granule size among the evaluated accessions. Differences in granule morphology were also reflected in the rheological behavior of the accessions, although the relationship was not consistent across all samples.

To further examine the relationships between starch granule morphology and rheological properties, a Pearson correlation analysis was performed (Figure 13). Grain length and grain width showed a strong positive correlation ($r = 0.96$, $p < 0.001$), indicating a high degree of consistency in granule dimensions among accessions. In contrast, maximum viscosity exhibited moderate negative correlations with grain length ($r = -0.47$) and grain width ($r = -0.50$), suggesting that larger granules were not necessarily associated with higher viscosity values in the evaluated accessions. Peak temperature was positively correlated with grain width ($r = 0.46$) and grain length ($r = 0.33$), whereas gelatinization temperature showed weak correlations with both granule dimensions and rheological parameters. Overall, these results indicate that starch granule size alone does not fully explain the rheological behavior of the evaluated accessions, suggesting that other compositional factors may also contribute to viscosity development.

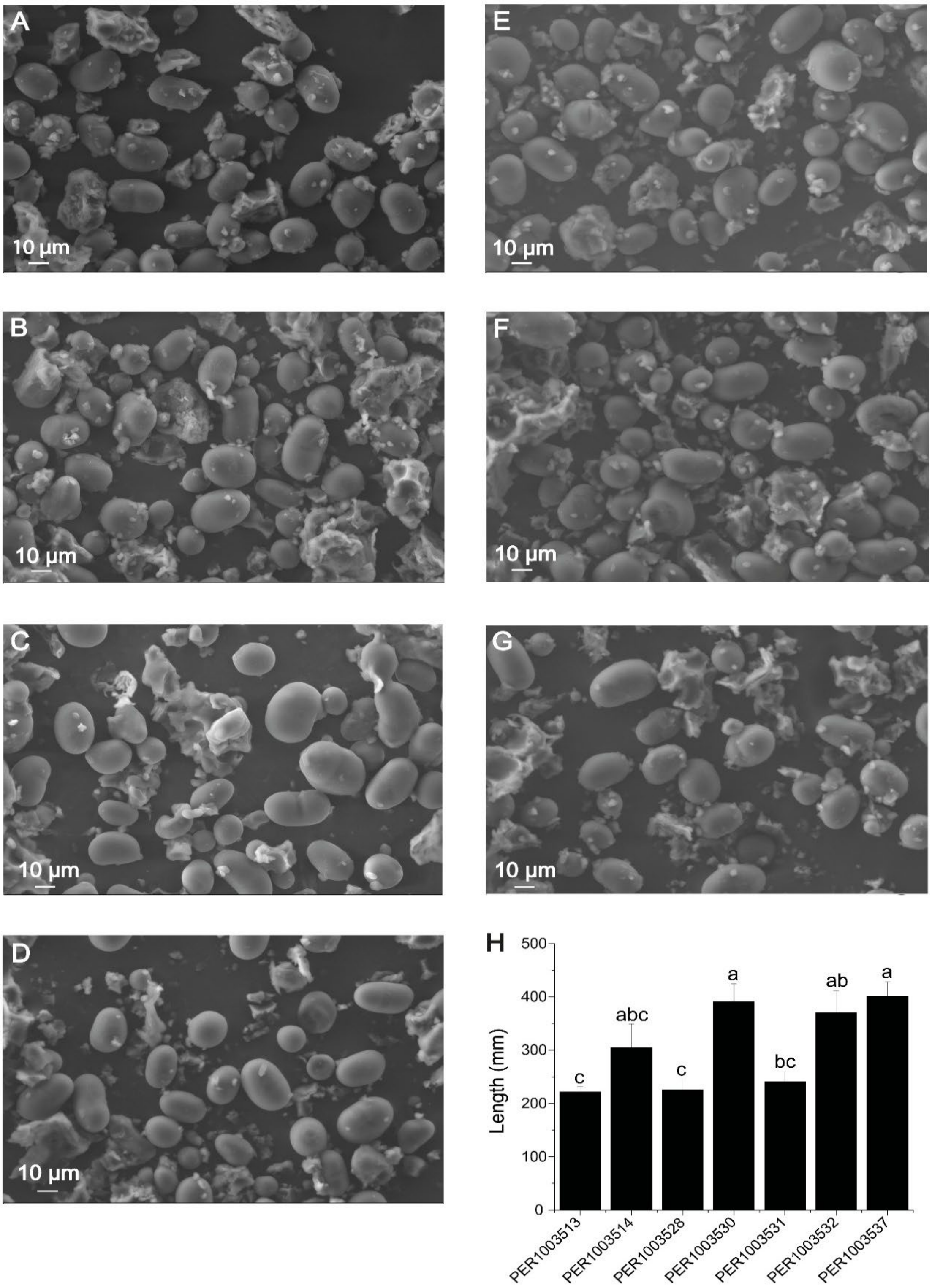

Figure 12. Micrographs obtained by scanning electron microscopy (SEM) of starch granules isolated from seven accessions of *Phaseolus* spp.: (**A**) PER1003513, (**B**) PER1003514, (**C**) PER1003528, (**D**) PER1003530, (**E**) PER1003531, (**F**) PER1003532, and (**G**) PER1003537. (**H**) Average length of starch granules. Different letters above the bars indicate statistically significant differences between accessions ($p < 0.05$).

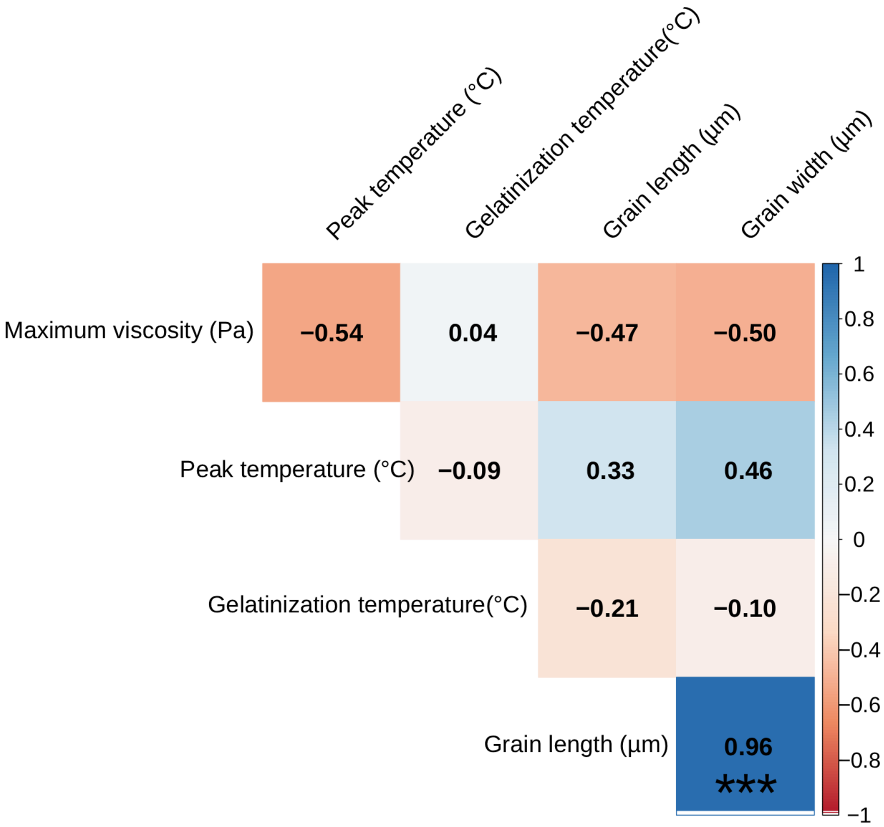

Figure 13. Pearson correlation matrix among peak viscosity, peak temperature, gelatinization temperature, and starch granule dimensions in seven *Phaseolus* spp. accessions. Asterisks indicate significance levels (*** $p < 0.001$).

4. Discussion

4.1. Patterns of Agromorphological Variation in Quantitative and Qualitative Descriptors

The evaluated *Phaseolus* spp. accessions exhibited substantial variation in both quantitative and qualitative traits, reflecting the combined influence of phenotypic diversity and environmental conditions. Similar levels of variation have been reported in previous studies [31], highlighting the importance of Andean germplasm as a valuable reservoir of phenotypic diversity. This variability is likely associated with phenotypic plasticity, which enables certain accessions to adapt to heterogeneous environments, particularly under the low-input agricultural conditions commonly found in Andean regions. In several cases, the agronomic values recorded in the present study were comparable to or higher than those previously reported for common bean germplasm [32–34], underscoring the potential of these accessions for breeding purposes.

Plant height varied considerably among accessions and was positively associated with reproductive traits such as the number of flower buds, inflorescence length, and seed size. These relationships indicate a close link between vegetative vigor and reproductive performance. However, the influence of plant height on yield may vary depending on environmental conditions and crop management practices [35,36]. The grouping of accessions into different clusters also provides useful information for identifying phenotypically divergent materials with potential use in breeding programs focused on heterosis and adaptability [37].

Multivariate analyses supported these patterns and indicated that yield performance in Phaseolus is influenced by the interaction between vegetative growth and reproductive efficiency. Positive correlations among plant height, number of pods per plant, and seed weight indicate coordinated developmental processes; similar observations have been reported previously [38,39]. Likewise, the association among pod-related traits reflects efficient assimilate allocation during reproductive stages. In contrast, the negative correlation between days to emergence and plant height suggests that rapid establishment may favor early vigor and competitiveness. These findings suggest coordinated relationships among several agronomic traits, consistent with previous reports.

PCA summarized the overall grouping patterns among accessions; however, the first two components explained only 51.4% of the total variance. The remaining variation was distributed across subsequent dimensions. Although PCA is widely used to summarize complex multivariate datasets, reduced-dimensional analyses do not always capture all relationships among accessions, particularly when quantitative traits are influenced by both phenotypic and environmental factors [40]. Similar findings have been reported in common bean germplasm studies, where genotype × environment interactions strongly influence phenotypic expression [41]. Therefore, relationships among some accessions should be interpreted cautiously, since similarities identified in the reduced-dimensional space may not fully reflect multidimensional differences among accessions. Environmental effects and unmeasured traits may also have contributed to the unexplained variation detected in the present study.

Despite these limitations, the combined PCA and correlation analyses revealed consistent associations among agronomic traits and accession performance. These findings reinforce the usefulness of multivariate approaches for identifying traits associated with yield stability and environmental adaptability in breeding programs [42,43].

The predominance of indeterminate growth habit among the evaluated accessions indicates adaptation to traditional production systems, where continuous vegetative growth and prolonged pod production may provide resilience under variable environmental conditions [44]. In contrast, determinate genotypes are generally preferred in mechanized agricultural systems [45]. The low frequency of anthocyanin presence in leaves may be associated with differences in genetic background and environmental adaptation, considering the role of these compounds in abiotic stress tolerance [46,47].

The uniform ovate leaf shape observed across accessions may reflect similar ecological pressures and adaptive responses to Amazonian environments. Leaf morphology plays an important role in light interception, photosynthetic efficiency, and microclimate regulation [48], making it an important trait for breeding programs focused on resilience to climate variability.

Variation in flower color and pigmentation reflected both phenotypic diversity and ecological influences. The predominance of purple and white flowers, together with pink wing coloration, **may reflect** local adaptation and selection pressures. Floral pigmentation has been reported to influence pollinator behavior and reproductive success [49,50].

Pod morphology and coloration are useful descriptors for identifying and classifying *Phaseolus* types [51,52]. In the present study, a broad diversity of these traits was observed among accessions from the Amazonas region, consistent with previous reports in Andean germplasm collections [53]. Pod traits are influenced by both phenotypic variability and environmental conditions [54,55], whereas coloration is likely associated with differences in anthocyanin accumulation under varying agroclimatic conditions [56]. In addition, farmer selection based on pod appearance, taste, and storage characteristics may have contributed to the phenotypic diversity observed.

Seed morphology also revealed substantial morphological and phenotypic variability among accessions. The variability in shape and color patterns agrees with previous studies describing high diversity in common bean germplasm [57]. These traits are associated with pigment-related genes such as P, T, G, and B [58–60], whose expression may be influenced by environmental conditions [61].

Overall, the agromorphological variability observed in *Phaseolus* spp. is associated with the combined influence of genetic diversity and environmental conditions such as soil, altitude, and climate [62]. The combined evaluation of qualitative and quantitative traits provides valuable information for understanding agronomic performance and ecological adaptation. In addition, qualitative descriptors may serve as phenotypic markers associated with quantitative traits, supporting breeding and selection strategies for locally adapted varieties.

Taken together, these findings indicate that the diversity identified among the evaluated accessions represents an important resource for breeding programs and for maintaining agroecological resilience in traditional Andean farming systems. The wide variation observed in traits such as plant height, number of pods per plant, and seed weight highlights the potential of these accessions as parental material for breeding programs. Likewise, the identification of contrasting agronomic groups provides a useful basis for selecting accessions with complementary traits and broad environmental adaptability.

4.2. Functional and Biochemical Variability Among Phaseolus *spp. Accessions*

The observed physicochemical variability among *Phaseolus* spp. accessions reflects a strong interplay between phenotypic traits and biochemical composition. The broad range of colorimetric parameters (L*, a*, C*) highlights the influence of pigment composition, mainly anthocyanins and flavonoids, which determine seed coat coloration and contribute to antioxidant potential [63].

The observed association between lower luminosity and higher phenolic content indicates that darker accessions tend to contain higher levels of bioactive compounds, likely due to increased concentrations of flavonoids and anthocyanins, consistent with previous findings in legumes [64–66].

The high phenolic content observed in PER1003542 and the strong antioxidant activity of PER1003526 and PER1003536 indicate that color intensity may provide a useful preliminary indicator of antioxidant potential, although this relationship may be influenced by accession-related and environmental factors [67,68]. These results are consistent with previous reports indicating that darker seed coats tend to accumulate higher levels of secondary metabolites, thereby enhancing their nutraceutical value.

FTIR spectral features observed in the evaluated accessions are consistent with the presence of major biochemical constituents such as lipids, proteins, and carbohydrates, as reported in previous studies [69,70]. Bands corresponding to CH_2 and CH_3 stretching vibrations suggest the presence of aliphatic chains typical of lipid compounds, while signals associated with C=O stretching are likely related to amide groups in proteins. Likewise, absorption bands associated with C–N and C–O–C bonds indicate the presence of structural carbohydrates, including starch, which are commonly detected in legume seeds by FTIR analysis [71,72]. Variations in band intensity and sharpness, particularly in regions around 995 and 1198 cm^{-1}, suggest differences in chemical composition among accessions. These differences were more pronounced in darker seeds, in agreement with the observations that pigmentation intensity is associated with biochemical composition [73,74]. Similar studies have demonstrated that multivariate approaches such as principal component analysis (PCA) applied to FTIR spectra can improve the discrimination of legume accessions according to their biochemical and phenolic profiles [75]. Although PCA was not performed in the present study, these spectral differences collectively indicate compositional differentiation among the evaluated accessions. In this context, FTIR may represent a useful complementary tool for linking visual traits such as seed color with molecular composition relevant to food quality and functional applications [76].

Rheological analysis highlighted accession-related variation in starch functionality. Accessions with higher viscosity (e.g., PER1003531 and PER1003530) may reflect differences in starch-related properties among accessions, contributing to differences in gelation behavior and structural stability [77]. These rheological characteristics may be relevant for food formulations requiring different textures and gelatinization properties.

SEM analysis corroborated these functional patterns at the microstructural level. Previous studies have reported that larger starch granules are often associated with higher swelling capacity and viscosity, whereas smaller granules (e.g., PER1003513 and PER1003514) have been reported to exhibit greater thermal stability and slower enzymatic degradation [78]. The observed relationship between granule morphology and rheological behavior suggests that structural characteristics of starch granules may be related to differences in pasting performance among accessions; similar studies have reported that starch granule size and organization can influence viscosity properties, hydration behavior, and thermal characteristics in legumes and other starch-rich matrices [79].

The correlation analysis further supported the complexity of the relationship between starch granule morphology and rheological behavior. Although granule dimensions exhibited coordinated variation among accessions, their associations with rheological parameters were generally weak to moderate. Similar observations have been reported in legumes and other starch-rich materials, where starch functionality is influenced by multiple structural and compositional factors rather than by granule size alone [80]. These findings suggest that the physicochemical differences observed among the evaluated accessions likely reflect the combined influence of granule morphology and other starch-related characteristics.

Taken together, these results demonstrate that colorimetric, biochemical, rheological, and microstructural differences among *Phaseolus* accessions reflect underlying phenotypic and biochemical diversity, although environmental conditions may modulate their expression. This integrative understanding provides valuable insights for the development of functional foods, targeted breeding programs, and strategies aimed at enhancing the nutritional and industrial value of germplasm conserved in the INIA–Amazonas collection.

5. Conclusions

This study demonstrated that the 58 *Phaseolus* spp. accessions conserved in the INIA–Amazonas Germplasm Bank exhibit substantial agromorphological, biochemical, and functional diversity, reflecting the broad phenotypic variability preserved within the evaluated germplasm. The identification of accessions with favorable agronomic characteristics, particularly in terms of plant height, number of pods per plant, and seed weight, highlights their potential for breeding programs aimed at improving yield and environmental adaptability.

The integration of physicochemical analyses revealed considerable variation in seed color, total phenolic content, and antioxidant capacity, with darker-seeded accessions generally exhibiting higher levels of bioactive compounds. These findings support the potential use of colorimetric traits as rapid indicators of nutraceutical value. Furthermore, FTIR, rheological, and SEM analyses provided complementary evidence of differences in molecular composition, starch functionality, and microstructural characteristics, suggesting that phenotypic variation among accessions is associated with differences in nutritional quality and technological properties.

Overall, the combined evaluation of agromorphological, biochemical, and structural traits proved effective for identifying promising germplasm with potential for agricultural and food-related applications. These results contribute to the conservation and sustainable use of native bean genetic resources while supporting their incorporation into breeding programs, functional food development, and climate-resilient production systems. Collectively, these findings highlight the importance of Andean germplasm as a valuable resource for food security and sustainable agricultural development.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d18060372/s1, Figure S1: Andean legume germplasm collection from the INIA Germplasm Bank.

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Data Availability Statement: The data presented in this study are available on reasonable request from the corresponding author. The data are not publicly available due to institutional restrictions related to the management and conservation of genetic resources maintained by INIA.

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