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Assessment of phenotypic diversity of Ñuña, a local common bean (*Phaseolus vulgaris* L.) from the northern Andes in Peru

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Abstract: Ñuña is a local type of common bean (*Phaseolus vulgaris* L.) cultivated in the Andean region of Peru. It stands out for its ability to expand and burst when roasted; however, its phenotypic diversity has not yet been fully explored. This study determined the phenotypic variability of Ñuña conserved in the Germplasm Bank of the National Institute of Agrarian Innovation of Peru. The analysis considered qualitative and quantitative traits, using multivariate statistics and comparison of means. Results revealed high phenotypic variability in both qualitative and quantitative traits. In qualitative traits, Multiple Correspondence Analysis found that the dark and light colours of the seed heads contributed most significantly to the variability of the accessions. Phylogenetic hierarchical analysis formed four clusters, representing 37% (I), 4% (II), 7% (III), and 52% (IV) of the accessions, respectively. For quantitative traits, Principal Component Analysis showed no discrimination between regions of origin but indicated a highly positive correlation between leaf length and width, and between pod length and width, as well as seed length, width, thickness and weight. Hierarchical analysis of quantitative characters also formed four clusters, representing 22% (A), 16% (B), 30% (C), and 31% (D) of the accessions, respectively. These clusters, analyzed for means comparison, showed significant differences ($p < 0.05$) with higher values in cluster B for pod length and width, and seed length, width, thickness and weight. Understanding the variability of the qualitative and quantitative traits of Ñuña is crucial for future genetic improvement studies aimed at achieving cultivars with desirable characteristics.

Keywords: Qualitative traits, quantitative traits, hierarchical analysis, legume, germplasm

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Introduction

Bean (*Phaseolus vulgaris* L.) is the most consumed grain legume worldwide. Its production has spread mainly to developing countries (De Ron *et al*, 2016; Nassary *et al*, 2020; Uebersax *et al*, 2023), for its contribution to dietary protein for more than 300 million people in rural and urban communities in East Africa and Latin America (Petry *et al*, 2015). The areas dedicated to bean production are 36 million hectares worldwide, of which 6 million are located in Latin America (FAOSTAT, 2022).

In Latin America, Ecuador and Peru are considered to be the places of origin of the bean (Kami *et al*, 1995). This gene pool was disseminated through independent

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domestication events, creating diverse landraces (Iwata-Otsubo *et al*, 2016). Within this genetic variability, one particular type is the Ñuña bean (Beem *et al*, 1992; Fernández *et al*, 2014), produced on a small scale by local producers in northern Peru.

The Ñuña plant is characterized by its indeterminate and climbing growth habit (Gamarra, 2021), reaching a height of more than 2 metres at the end of flowering (Gamarra *et al*, 2007). The pods are usually distributed along the entire length of the plant (Melo and Ligarreto, 2010) and contain between five and seven grains. These beans stand out for their protein content, which varies between 18% and 23% (Rodríguez *et al*, 2014). As with common beans, Ñuña presents high levels of other nutrients such as vitamins and minerals (Beem *et al*, 1992; Melo and Ligarreto, 2010).

The main characteristic of the Ñuña kernel when roasted for consumption is to explode and expand like popcorn, acquiring a soft consistency and a pleasant flavour similar to peanuts. This characteristic is associated with the presence of occluded intracellular and intercellular spaces that are forced to expand by the generation of water vapour during roasting (Beem *et al*, 1992).

The Ñuña has diversified under geographical restrictions, with production concentrated continuously in the Andean zone (National Research Council, 1989; Tohme *et al*, 1995; Otálora *et al*, 2006). It develops between the latitudes of 7°30’ S and 19°30’ S, and altitudes ranging from 2,000 to 3,000masl (Pearson *et al*, 2012). Temperatures in this region fluctuate between 10 and 30ºC (Llique, 1993), with a relative humidity of 60 and 75% (Hernández-López *et al*, 2013), annual rainfall of 500 to 3,000mm and a photoperiod of 8 to 14 hours (Llique, 1993).

In Peru, the Ñuña has a wide phenotypic variability that is distributed in the regions of Cusco, Ancash, Huánuco, Apurímac, Ayacucho, La Libertad and Cajamarca. The most significant genotypic variability is found in the latter region (Tohme *et al*, 1995), particularly in the province of Cajabamba, where there is a superior gene pool with varied productivity levels (Debouck, 1986; Franco and Hidalgo, 2003; SantaCruz-Padilla *et al*, 2021).

Despite the existence of morphological and molecular characterization studies of Ñuña (Cruz-Balarezo *et al*, 2009), there are few reports on the morphology of genotypes in northern Peru. Considering that ecogeographic factors influence the phenotypic and genetic characteristics of a species (Knight *et al*, 2005; Herben *et al*, 2012; Díez *et al*, 2013), it is hypothesized that the morphological characteristics of flowers, pods and grains of Ñuña contribute to discriminating the morphological variability of the accessions conserved in the Germplasm Bank of the National Institute for Agrarian Innovation (INIA) in Peru. Furthermore, it is considered that there is phenotypic variability related to the geographical area of origin. Therefore, this research aimed to determine the phenotypic variability of Ñuña conserved in the germplasm bank of INIA in Peru.

Material and methods

Origin of the Ñuña accessions

The Ñuña samples were collected from plots belonging to producers located in eight districts within the regions of La Libertad, Cajamarca, and Ancash in Peru, as detailed in Table 1 and illustrated in Figure 1. Most of the Ñuña germplasm collected in northern Peru was from the Cajamarca region (88 accessions), followed by the La Libertad region with 31 accessions and the Ancash region with 3 accessions (Table 1).

Location of the study

The study covered the period from December 2019 to July 2020 and was carried out at the Cochamarca Experimental Annex of the Baños del Inca Agricultural Experimental Station, Cajamarca (7.2756 S, 78.2186 W, 2,820masl, Figure 1). The study area exhibits a climatic classification corresponding to the tropical low montane dry forest (bs-MBT) category, determined according to the methodology of Holdridge (1947). Throughout the research period, mean, minimum and maximum temperatures were 14.3°C, 7.6°C and 21°C respectively, with a rainfall of 117.4mm (SENHAMI, 2020).

Soil chemical properties

A composite sample was extracted from the experimental area using an auger at a 0 to 30cm depth. This sample was treated at the Baños del Inca - INIA Soil, Water and Foliar Laboratory, where it underwent a process of air drying, followed by grinding and sieving through a 2mm mesh. Subsequently, organic matter was determined using the Walkley and Black (1934) method, while phosphorus (P) was evaluated according to the Olsen *et al* (1954) protocol, and potassium (K) was determined using the silver thiourea method. Soil analysis revealed a pH of 6.5, with a 1% organic matter content, 3.82ppm P and 295ppm K.

Soil preparation

The experimental area covered 0.16 hectares, where soil preparation was carried out to a depth of 30cm using a disc plough coupled to an agricultural tractor (New Holland, 110 HP, Model: TS6.110). To correct soil fertility, amendments such as island guano (1,450kg/ha), diammonium phosphate (150kg/ha) and potassium chloride (100kg/ha) were applied. Subsequently, plots of $6m^2$ (6m × 1m) were demarcated, resulting in a total of 122 plots of one row each, corresponding to the 122 Ñuña accessions. Planting was carried out in December 2019, with an arrangement of plants at distances of 0.5m between plants and 1.0m between rows, totalling 12 plants per row, equivalent to 20,000 plants per hectare.

Table 1. Geographical origin and coding of 122 accessions of Ñuña beans from the INIA Germplasm Bank, Cajamarca, Peru.

District, Province, Region	Location	Latitude (S)	Longitude (W)	Altitude (masl)	No. of accessions	Accession code
Sanagoran, Sánchez Carrión, La Libertad	Angasmarquilla	7.72510	78.15520	3,130	9	PER002014, PER002015, PER002016, PER002017, PER002018, PER002019, PER002020, PER002021, PER002022
	Yanac	7.78020	77.95590	2,989	12	PER002023, PER002024, PER002025, PER002026, PER002027, PER002028, PER002029, PER002030, PER002031, PER002032, PER002033, PER002071
Huamachuco, Sánchez Carrión, La Libertad	Olichoco	7.81480	78.05000	3,183	10	PER002034, PER002035, PER002064, PER002065, PER002066, PER002067, PER002068, PER002036, PER002037, PER002038
Cajabamba, Cajabamba, Cajamarca	Chanshapamba	7.66750	78.05180	2,889	13	PER002039, PER002040, PER002041, PER002042, PER002043, PER002044, PER002045, PER002046, PER002047, PER002048, PER002049, PER002050, PER002051
	Chanshapampa	7.63040	78.02699	3,069	9	PER017536, PER017538, PER017548, PER017557, PER017558, PER017559, PER017571, PER017577, PER017593
	Shitabamba	7.67480	78.03730	2,821	10	PER002059, PER002060, PER002061, PER002062, PER002063, PER002072, PER017545, PER017578, PER017580, PER017583
	Churgapampa	7.65645	78.06936	2,828	1	PER017547
	Huanza	7.66693	8.07693	2,596	11	PER017537, PER017539, PER017549, PER017550, PER017553, PER017564, PER017565, PER017587, PER017588, PER017589, PER017590
	Callash	7.63970	78.06299	2,756	5	PER017540, PER017551, PER017552, PER017594, PER017595
	Cajabamba	7.62190	78.04450	2,685	2	PER002069, PER002070
	Colcabamba	7.64897	78.03058	2,899	1	PER017561
	Chanshe	7.67867	78.06359	2,660	2	PER017562, PER017563
Sitacocha, Cajabamba, Cajamarca	Lluchubamba	7.52039	77.96881	3,023	13	PER017541, PER017543, PER017568, PER017574, PER017581, PER017582, PER017591, PER017592, PER017546, PER017570, PER017573, PER017584, PER017576

Continued on next page

Table 1 continued

District, Province, Region	**Location**	**Latitude (S)**	**Longitude (W)**	**Altitude (masl)**	**No. of accessions**	**Accession code**
Condebamba, Cajabamba, Cajamarca	Cauday	7.57435	78.07074	2,815	7	PER017535, PER017556, PER017560, SC_7280, PER017572, PER017575, PER017586
	Ogosgon	7.56360	78.09330	2,697	8	PER002052, PER002053, PER002054, PER002055, PER002056, PER002057, PER002058, PER017555
	Huarasullo	7.57815	78.10208	2,588	2	PER017566, PER017567
Cachachi, Cajabamba, Cajamarca	El aliso	7.44810	78.26905	3,233	3	PER017542, PER017544, PER017554
Llacanora, Cajamarca, Cajamarca	La paccha	7.19240	78.42650	2,629	1	PER002073
Bambas, Corongo, Ancash	Bambas	8.60247	77.99639	2,931	3	PER018011, PER018012, PER018027

Cultivation treatments

Manual weeding was carried out 45 days after planting (DAP). Subsequently, trellising was carried out at 50 DAP by installing 2.5m high posts at the ends of the plots. On these posts, a galvanized wire (N° 16) was stretched along 6m, which supported the plants. A phytosanitary control against boring larvae (Lepidoptera: Noctuidae) was also implemented, using alphacypermethrin (25ml/20L of water).

Determination of variables

Evaluations were carried out from the beginning of flowering until harvest, recording data from 10 plants per accession. Descriptors adapted from the International Plant Genetic Resources Institute for *Phaseolus vulgaris* (IPGRI, 2001) were studied (Table 2). The Royal Horticultural Society Colour Chart (RHS, 2001) was used to assign the colours of flowers, pods and seeds. The study included consideration of 12 qualitative descriptors (Table 2) and 9 quantitative descriptors (Table 3).

Statistical analysis

Characterization information was subjected to descriptive and multivariate statistical analysis. Multivariate statistics included multiple correspondence analysis (MCA) for qualitative traits and principal component analysis (PCA) for quantitative traits. In both cases, decision trees were constructed using hierarchical and phylogenetic dendrograms. The Euclidean distance and the Ward.D2 (Ward, 1963) method were used as a similarity measure to carry for grouping between accessions. For the clusters of the quantitative traits, a comparison of means was carried out using Tukey's test ($p < 0.05$). The analyses were carried out with the packages Factoextra (Kassambara and Mundt, 2020) and FactorMiner (Lê *et al*, 2008) for MCA and PCA. The dendrograms were elaborated with the cluster (Maechler *et al*, 2021) and circlize (Gu *et al*, 2014) packages, while the visualization of the results was performed with ggplot2 (Wickham, 2016). Comparison of means was run with the AgroR package (Shimizu *et al*, 2023). All analyses were performed using RStudio statistical software (R Core Team, 2023).

Results

The phenotypic data for 12 qualitative and 9 quantitative descriptors collected on 122 accessions of Ñuña beans from the INIA collection are summarized in Supplemental Table 1 and were used in statistical analyses to assess and describe their genetic diversity.

Multiple correspondence analysis of qualitative characters

The qualitative characters evaluated were subjected to MCA, the results of which are presented in Figure 2. A marked association was observed between several characters, such as darker colour of seeds (DCS) and lighter colour of seeds (LCS) which presented the highest contributions to the variability of the 122 accessions of Ñuña. Seed coat pattern (SCP), flower wing colour (WIC) also associated and had similar contributions to the clustering of the accessions. There was a joint association between standard colour (STC), dry pod colour (DPC) and colour of immature pods (CIP). This pattern suggests that certain characteristics share discernible similarities, while others show a less prominent relationship. These findings provide further insight into the interrelationships between the qualitative variables assessed.

Hierarchical analysis of qualitative characteristics

The analysis of the phylogenetic hierarchical tree of the 122 accessions of Ñuña is shown in Figure 3. It is possible to observe the formation of four morphological clusters, grouped according to their most similar characters. Cluster I represents 37% of the accessions (45 accessions), which showed similarity in seven characters associated with different morphological stages: darker colour of seeds, between white-tinged black, grey-brown and greyish purple-tinged white to brown; lighter colour of seeds between white tinged purple, yellow, greyish orange, brown, purple to absent; seed coat pattern between mottled, spotted, around the hilum; standard colour between green yellow and yellow green pigmented; wing colour between white, white pigmented violet to violet blue; colour of immature pods between green, yellowish green and green pigmented; and dry pod colour between yellow and orange. Cluster II represents 4% of the accessions (five accessions), which showed similarity in nine characters associated with different phenological stages: darker colour of seeds and lighter colour of seeds dyed purple; striped seed coat pattern; purple colour of immature pods; pod curvature curved; seed shine matt; oval seed shape; intermediate leaf persistence and absent vein in seeds. Cluster III represents 7% of the accessions (nine accessions), which showed similarity in eight characters associated with different phenological stages: in the presence of a dark colour stripe moving from the hilum towards the top of the grain, the standard colour between yellow-green pigmented with purple to purple violet; the darker colour of seeds between purple with white lateral stripe and violet blue with orange-white lateral stripe; lighter colour of seeds between greyish orange with white lateral stripe to greyish purple with white lateral stripe; long-stripes seed coat pattern; wing colour between purple to purple violet; colour of immature pods between green and yellowish green; pod curvature between slightly curved to curved; dry pod colour between yellow and orange. Cluster IV represents 52% of the accessions (63 accessions), which showed similarity in seven characters associated with different phenological stages: darker colour of seeds between greyish orange, greyish red,

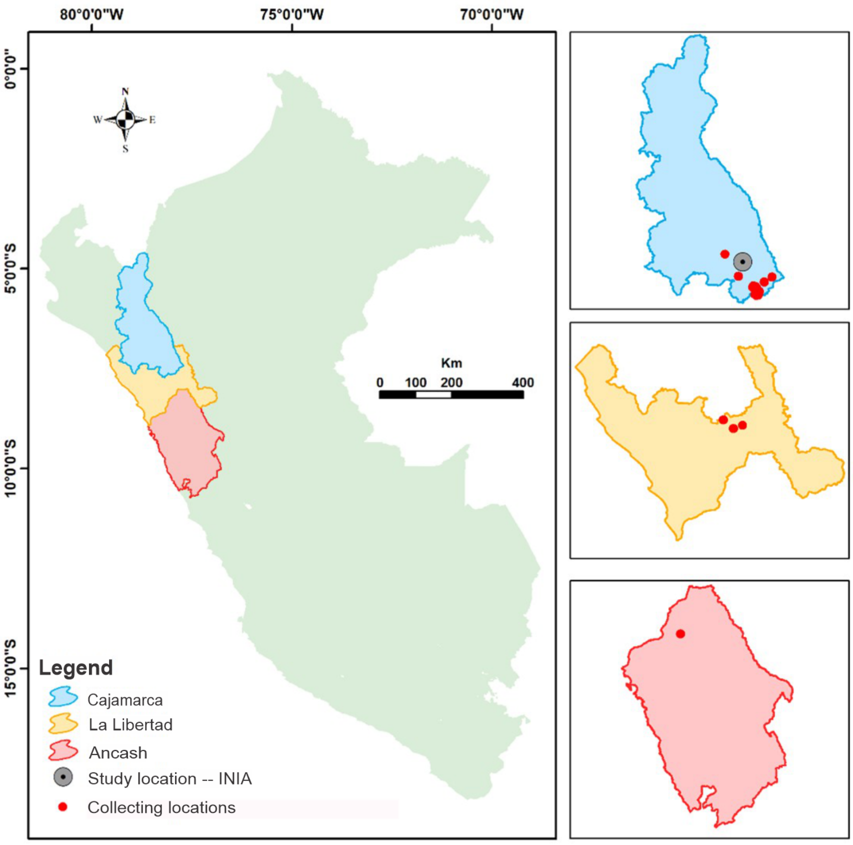

Figure 1. Map of collecting locations of 122 accessions of Ñuña beans collected in three regions in Peru and conserved in the INIA Germplasm Bank.

Table 2. Qualitative morphological characteristics assessed, codes and period of assessment. IPGRI descriptor code adapted from *Descritores de Phaseolus vulgaris* (IPGRI, 2001).

IPGRI descriptor code	Characteristic	Codes	Period of assessment
4.2.4	Standard colour	STC	Flowering
4.2.5	Wing colour	WIC	Flowering
4.2.6	Colour of immature pods	CIP	Immature pods expanded
4.2.9	Pod curvature	POC	Immature pods expanded
6.2.17	Dry pod colour	DPC	Harvest
6.1.8	Leaf persistence	LEP	When 90% of pods are dry
4.3.2	Darker colour of seeds	DCS	Grain dry
4.3.3	Lighter colour of seeds	LCS	Grain dry
4.3.1	Seed coat pattern	SCP	Grain dry
4.3.4	Seed shine	SSH	Grain dry
6.3.2	Veins in seeds	VIS	Grain dry
4.3.5	Seed shape	SES	Grain dry

grey brown, purple, violet-blue and black; lighter colour of seeds between greyish orange, brown grey, greyish purple, purple, purple or greyish red and green yellow; seed coat pattern between absent, striped, to pattern around the hilum; standard colour between purple, purple violet and yellow-green pigmented with purple violet; wing colour between white to purple violet; colour of immature pods between yellowish green and green pigmented and dry pod colour between yellow, orange and purple.

Principal component analysis (PCA) of quantitative traits

The PCA of quantitative traits explained 71.6% of the total variability and is presented in Figure 4. In this analysis, the accessions did not show differentiation of their quantitative traits according to their origin. Leaflet length and leaflet width showed a high positive correlation, as indicated by the direction of the arrows in Figure 4. The same, but to a lesser degree, occured for the characters: immature pod length, pod width, pod

Table 3. Quantitative characteristics assessed, codes and period of assessment. IPGRI descriptor code adapted from *Descritores de Phaseolus vulgaris* (IPGRI, 2001). n/a, not available in descriptor list.

IPGRI descriptor code	Characteristics	Codes	Period of assessment	Units
4.2.7	Immature pod length	IPL	Fully expanded immature pods	cm
6.2.13	Pod width	PWI	Fully expanded immature pods	mm
6.2.14	Pod beak length	PBL	Fully expanded immature pods	mm
6.3.3	Seed weight (100 units)	SEW	In dry grain, 12 to 14% moisture content	g
6.3.5.1	Seed length	SEL	In dry grain, 12 to 14% moisture content	mm
6.3.5.2	Seed width	SW	In dry grain, 12 to 14% moisture content	mm
6.3.5.3	Seed height	SEH	In dry grain, 12 to 14% moisture content	mm
4.1.1	Leaflet length	LL	At 50% flowering, on the third trifoliate leaf	cm
n/a	Leaflet width	LW	At 50% flowering, on the third trifoliate leaf	cm

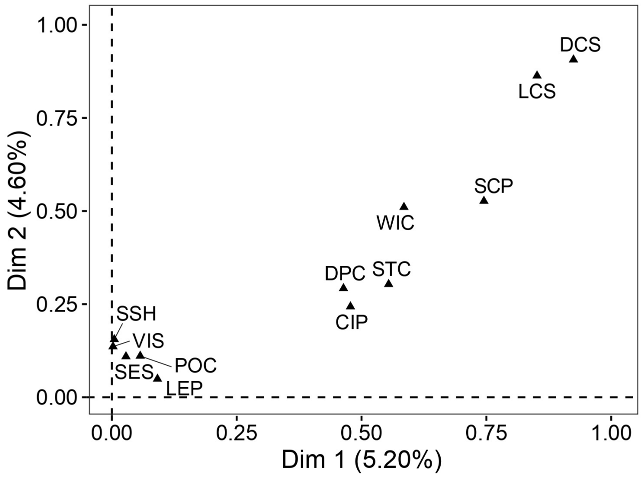

Figure 2. Multiple correspondence analysis of qualitative variables of the germplasm of Ñuña (*Phaseolus vulgaris*). DCS, darker colour of seeds; LCS, lighter colour of seeds; SCP, seed coat pattern; WIC, wing colour; STC, standard colour; DPC, dry pod colour; CIP, colour of immature pods; SSH, seed shine; VIS, seed veins; POC, pod curvature; SES, seed shape and LEP, leaf persistence.

beak length, hundred seed weight, seed length, seed width and seed height, respectively.

Hierarchical analysis of quantitative characteristics

The hierarchical cluster analysis based on the quantitative traits (Figure 5) revealed the formation of four clusters, established according to their most similar characteristics. Table 4 shows the mean values of the characters of Clusters A, B, C and D, highlighting significant differences ($p < 0.05$) between clusters. Cluster A, representing 22% of the accessions (27 in total), was characterized by lower values for immature pod length, pod width, pod beak length, hundred seed weight, seed length, seed width and seed height compared to the rest of the clusters (Table 4). Cluster B, comprising 16% of the accessions (20 in total), was significantly superior to the other clusters showing higher values for immature pod length, pod width, pod beak length, hundred seed weight, seed length, seed width and seed height. However, Cluster B had lower values for leaflet length and leaflet width compared to the other clusters. Cluster C, comprising 30% of the accessions (37 in total), showed higher mean values than Cluster A, but lower than Clusters B and D for the character's immature pod length, pod beak length, hundred seed weight, seed length, seed width and seed height. For width of immature pod trait, Cluster C had a higher mean than Clusters A and D, but lower than Cluster B. Cluster C had a lower mean for the leaflet length trait, than the rest of the clusters. For the leaflet width character, the mean of Cluster C was lower than Clusters A and D, but higher than the mean of Cluster B. Finally, Cluster D, comprising 31% of the accessions (38 in total), was characterized by higher values than Clusters A and C for immature pod length, pod beak length, hundred seed weight, seed length, seed width and seed height. As for pod width, its mean value was higher than that of Cluster A, but lower than the means of Clusters B and C. Cluster D stood out with higher values for leaflet length and leaflet width compared to Clusters A, B and C. There was a marked diversity in the distances between clusters, particularly between Cluster A and Clusters C and D. This suggests that the accessions in these clusters show significant morphological differences.

Discussion

Qualitative characterization

Qualitative analysis revealed that characters associated with seed colour showed the greatest morphological variability, followed by seed coat pattern and, to a lesser degree, flower-related characters (Figure 2). This indicates that seed-related characters are fundamental for the identification and differentiation of Ñuña bean accessions, establishing associations between seed morphology and the phenotypic diversity observed among them. This result is consistent with previous studies by Martirena-Ramírez *et al* (2017) and Espinosa-Pérez *et al* (2015), who highlighted that flower colour and seed colour are essential to assessing phenotypic variability of common bean. Similarly, Morales-Morales *et al* (2019) have determined the same for cowpea

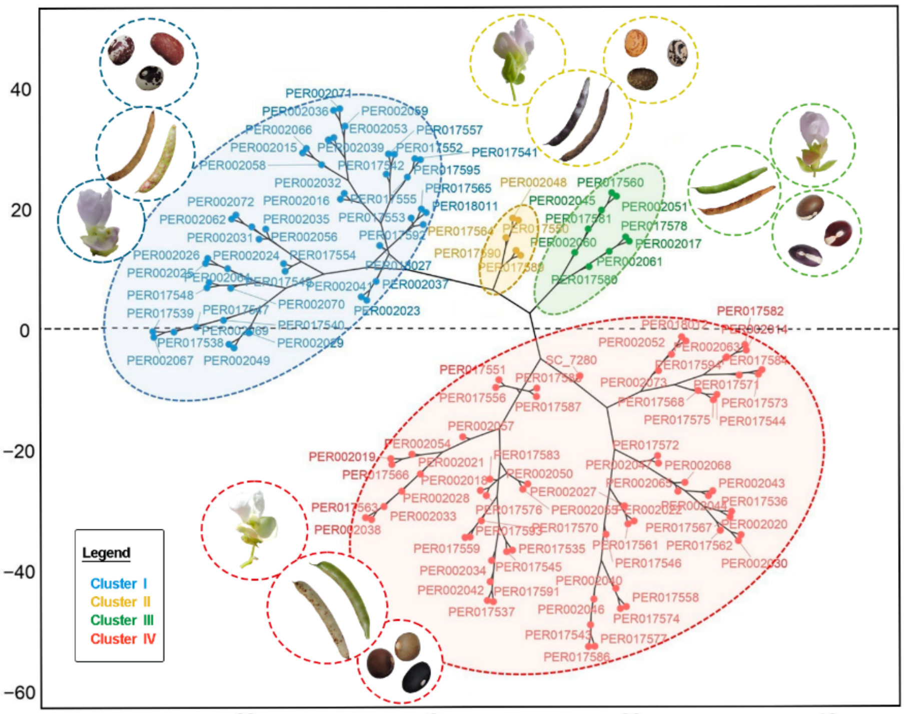

Figure 3. Hierarchical clustering based on multiple correspondence analysis of qualitative variables of the germplasm of Ñuña (*Phaseolus vulgaris*). DCS, darker colour of seeds; LCS, lighter colour of seeds; SCP, seed coat pattern; WIC, wing colour; STC, standard colour; DPC, dry pod colour; CIP, colour of immature pods; SSH, seed shine; VIS, veins in seeds; POC, pod curvature; SES, seed shape and LEP, leaf persistence. The photographs inserted next to each group show the representative qualitative traits of each group.

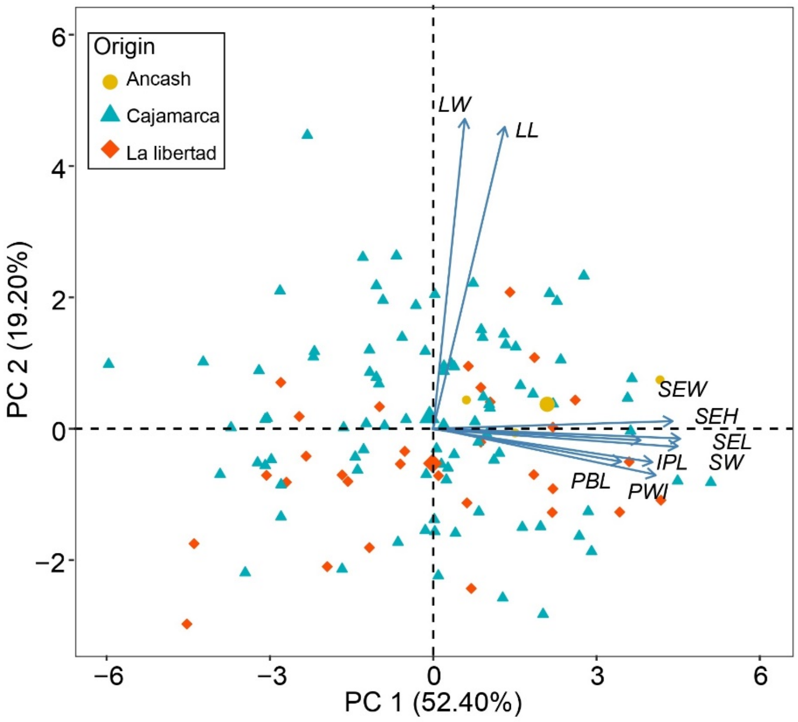

Figure 4. Projection of nine quantitative characters in the first two dimensions of the Principal Component Analysis of 122 Ñuña (*Phaseolus vulgaris*) accessions from the germplasm collection of INIA, Estación Experimental Agraria Baños del Inca, Cajamarca, Peru. Accessions are colour coded by origin regions. The arrows indicate the inertia of the contribution of the characters IPL, immature pod length; PWI, pod width; PBL, pod beak length; SEW, seed weight; SEL, seed length; SW, seed width; SEH, seed height; LL, leaflet length; and LW, leaflet width. And the distance between them implies their correlation.

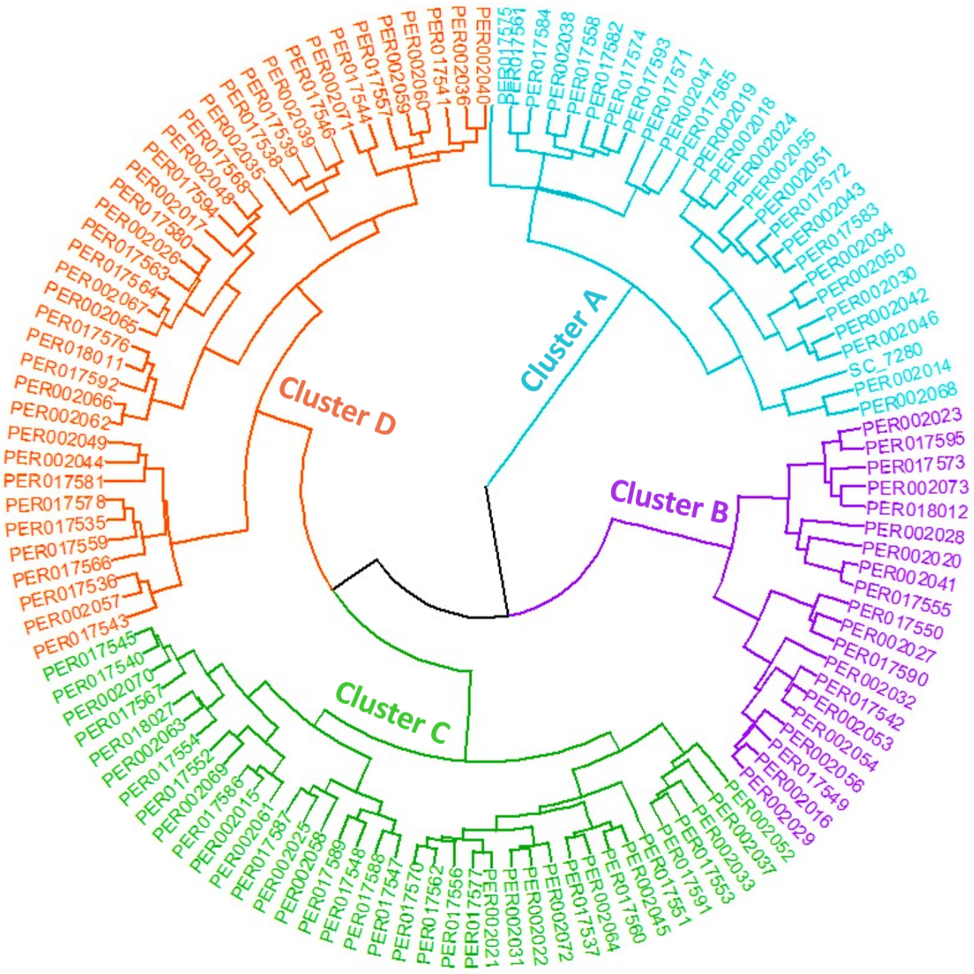

Figure 5. Hierarchical dendrogram of Ñuña (*Phaseolus vulgaris*), Ward.D2 method. Euclidean distance based on nine quantitative characters from the germplasm collection of INIA, Estación Experimental Agraria Baños del Inca, Cajamarca, Peru.

Table 4. Descriptive analysis and comparison of means between clusters for quantitative traits. CV, coefficient of variation; MSD, minimum significant difference; IPL, immature pod length; PWI, pod width; PBL, pod beak length; SEW, seed weight; SEL, seed length; SW, seed width; SEH, seed height; LL, leaflet length; and LW, leaflet width. *Means followed by the same letter in the rows do not differ statistically from each other, according to Tukey's test ($p < 0.05$).

Character	**Average character values ***				**MSD**	**CV(%)**
	Cluster A	**Cluster B**	**Cluster C**	**Cluster D**		
IPL (cm)	10.38 c	13.88 a	11.59 b	12.34 b	0.77949	9.46
PWI (mm)	12.55 c	15.35 a	14.59 b	14.29 b	0.58016	5.93
PBL (mm)	9.34 d	12.55 a	10.59 c	11.55 b	0.91175	12.08
SEW (g)	41.10 c	73.43 a	55.85 b	60.08 b	6.18127	15.77
SEL (mm)	8.99 d	13.40 a	10.24 c	11.53 b	0.74487	9.91
SW (mm)	7.50 c	9.10 a	8.35 b	8.56 b	0.27129	4.71
SEH (mm)	6.76 c	7.97 a	7.45 b	7.55 b	0.27629	5.40
LL (cm)	11.82 b	11.75 b	11.67 b	13.66 a	0.93886	11.02
LW (cm)	8.48 b	7.90 b	8.15 b	9.73 a	0.68477	11.43

(*Vigna unguiculata* L. Walp). Murga-Orrillo *et al* (2024) affirmed that in cowpea seed colours mark the preferences of producers and consumers since the dark colour of the testa presents greater antioxidant activity. From this perspective, a generalized tendency in bean research to use morphological characters of the seed as primary indicators of genetic variability is confirmed, underlining its importance in the classification and differentiation of accessions.

The phylogenetic tree presented in Figure 3 showed that the qualitative characters related to seed, flower and pod are valid phenotypic traits to discriminate morphologically the different accessions of Ñuña; these characteristics, which vary among accessions, could be related to their areas of origin. In addition, it highlighted that there were no duplicate accessions in the studied collection at the phenotypic level. These results are valid for genetic diversity conservation since they indicate that the 122 accessions could represent unique genetic resources, which should be studied in detail to determine the variations of these accessions due to environmental and anthropogenic factors. Also, Cruz-Balarezo *et al* (2009) conducted clustering analyses on 24 accessions of Ñuña, which determined that there was no duplication of germplasm, even in cases where the accessions shared similar characters. On the other hand, in common bean, Vásquez *et al* (2024) identified four distinct morphological groups in 58 accessions with promising characteristics for breeding programmes. These studies highlighted the importance of characterizing Ñuña bean accessions for conservation and breeding initiatives.

Quantitative characterization

Regarding the spatial distribution of the 122 Ñuña accessions, based on quantitative traits, no defined clustering pattern for geographic origin was identified (Figure 4). This could be due, in part, to similar climatic conditions in the Andean region or common agricultural practices (Figure 1). Mishra *et al* (2010) have shown that genetic diversity among bean genotypes did not show a direct relationship between clustering patterns and geographic origin. Consequently, the maintenance of Ñuña bean genetic characteristics from different geographic origins is crucial to preserving its biodiversity and genetic improvement. The PCA presented in Figure 4 shows strong positive correlations between leaf, immature pod and seed traits, demonstrating that these traits are mutually dependent. This could be related to shared developmental processes or the expression of common genes that regulate the size and structure of the mentioned plant organs. In common bean, Lescay-Batista *et al* (2017) and García-Fernández *et al* (2023) found a close association between pod characters and seed weight. Also, in lima bean (*Phaseolus lunatus* L.), a strong correlation was found between yield and stem length, number of seeds per pod, hundred-seed weight, primary leaf length and width, pod weight and pod length (Akande and Balogun, 2007; López-Alcocer *et al*, 2016).

The four clusters identified in Figure 5 were evaluated through a mean comparison test ($p < 0.05$), as detailed in Table 4. Cluster B presents significant differences, with higher means in the traits related to productivity, compared to the means of Clusters A, C and D. The accessions of Cluster B will be valuable in genetic improvement programmes because they could provide higher yields. Also, Pesantes-Vera and Soto (2013) in Ñuña, and Kinhoégbè *et al* (2020) in pigeonpea (*Cajanus cajan* (L.) Huth), used characters related to productive yield in the selection of promising individuals. However, it is essential to conduct further studies on the 122 accessions of Ñuña to identify relevant traits, such as resistance to abiotic and biotic factors, as well as nutritional aspects, in order to enrich future breeding programmes.

Conclusions

In the qualitative traits of Ñuña, MCA determined that the most significant contributions to the variability of the accessions were the seed heads' dark and light colours. In the phylogenetic hierarchical analysis, four clusters were formed, represented by 37% (I), 4% (II), 7% (III) and 52% (IV) of the accessions respectively.

In the quantitative characters of Ñuña, PCA showed no discrimination between regions of origin of the accessions, but a high positive correlation between length and width of leaves, as well as between length and width of pods, and length, width, height and weight of seeds.

In the hierarchical analysis of the quantitative characters, four clusters were also formed, represented by 22% (A), 16% (B), 30% (C) and 31% (D) of the accessions. Subjected to comparative analysis of means, these clusters showed significant differences ($p < 0.05$) with higher values in cluster B for pod length and width, length, width, height and seed weight.

Understanding the variability of qualitative and quantitative traits of Ñuña, and classifying them, is the starting point for subsequent genetic improvement studies aimed at obtaining Ñuña cultivars with better yields and high nutritional value, resistant to biotic and abiotic factors.

Authors contribution

Angel Esteban Santa Cruz Padilla: conceptualization, formal analysis, writing – original, research, data curation, resources, methodology, proofreading and editing.

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Conflict of interest statement

The authors have declared that no competing interests exist.

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Supplemental data

Supplemental Table 1. Agromorphological characterization data of 122 Ñuña accessions from the INIA Germplasm Bank - Peru.

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