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RESEARCH ARTICLE

Agro-morphological characterization of tarwi (*Lupinus mutabilis* Sweet) accessions using descriptors and spectral metrics derived from UAVs

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Abstract

Tarwi (*Lupinus mutabilis* S.) is a legume native to the Andes, recognized for its high nutritional value, which gives it great potential in food security programs. Therefore, understanding and advancing the conservation of its morphological diversity is essential. In this study, 140 accessions from the national germplasm collection of the National Institute of Agrarian Innovation of Peru were evaluated, along with two cultivars ("INIA 445 Masacanchino" and "Andenes 90"). A traditional agro-morphological characterization was conducted using 16 quantitative and 40 qualitative descriptors, complemented by phenological data obtained from time series of reflectance indices generated by Unmanned Aerial Vehicles (UAVs). Additionally, a principal component analysis (PCA) was applied to select the most relevant variables, and a clustering analysis along with a dendrogram was developed to classify the accessions. The results revealed significant differences between groups ($p < 0.05$) in terms of inflorescence length, number of pods on the main axis, number of primary branches, and yield per plant. Likewise, the morphological groups exhibited variations in phenophases derived from the Normalized Difference Vegetation Index (NDVI). Four morphological groups were identified: group 3 (G3) showed the highest growth rate followed by a decline, while group 4 (G4) stood out for its highest initial growth rate. Furthermore, the observed homogeneous phenological conditions indicated that groups 1 (G1) and 4 (G4) matured earlier, making them promising candidates for selection. These findings demonstrate the wide genetic variability of tarwi, which can be exploited in breeding programs for the development of new cultivars. Thus, the study highlights the importance of morphological characterization in understanding the variability of an understudied crop such as tarwi, contributing to conservation and promoting its protection and sustainability.

Keywords: cultivars; conservation; germplasm; variability; photogrammetry; UAVs.

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1. Introduction

The agro-morphological characterization of phytogenetic resources, essential for global food security, faces significant challenges worldwide (Fiaz et al., 2024). These include limited access to detailed information, inconsistent characterization methods and protocols, budget constraints, and a shortage of trained personnel, as well as the impacts of climate change and environmental degradation (Breman et al., 2021; Schneider, 2023). Recent research confirms that intensifying the agro-morphological characterization of diverse crop collections is essential to mitigate agricultural vulnerability, as these collections reveal high phenotypic variability that is crucial for genetic improvement (Yela et al., 2025). The lack of comprehensive and up-to-date data, along with the absence of standardized protocols for morphological and agronomic data collection and analysis, hampers the understanding of genetic and phenotypic

diversity in cultivated plant species and their wild relatives, limiting the ability to compare and replicate research findings, which is crucial for scientific progress and agricultural application (Hafner et al., 2025).

Morphological description serves as a foundational tool for comprehending the genetic diversity and inherent variability found within plant species. This characterization provides vital, key information necessary for successful genetic improvement programs, biodiversity conservation efforts, and the sustainable management of natural resources (González et al., 2025). Additionally, it allows the identification of accessions with promising agro-morphological traits that can be used to develop varieties with better agronomic attributes (Asma et al., 2022). Agro-morphological attributes are physical and morphological characteristics that determine plant adaptability, productivity, and agronomic value (Itoh & Sato, 2023).

Tarwi, also known as Andean lupin, is a legume native to the Andes, primarily cultivated in Ecuador, Peru, and Bolivia (Chalampuente-Flores et al., 2023; Tapia, 2015). This crop, which has been an integral part of the Andean diet for centuries, has gained attention in recent years due to its high nutritional value (Tello, 1976). It is considered superfood due to its protein content and richness in essential fatty acids like linoleic and oleic acids, which benefit cardiovascular health (Chirinos et al., 2022). Additionally, it has nutraceutical properties due to its alkaloids, with potential for pharmaceutical development (Gabur & Simioniuc, 2023). Tarwi adapts to extreme Andean conditions, growing at altitudes between 3100 and 3850 m a.s.l., being tolerant to low temperatures, with a growth cycle ranging from 240 to 300 days, making it a resilient and versatile crop (Guilengue et al., 2019). These traits make it an important local crop, used for both self-consumption and commercialization in regional markets (Flores et al., 2016). Despite its nutritional and agronomic value, tarwi faces threats that affect its production and morphological diversity (Fernández-García et al., 2023). In this context, phenotypic characterization of its accessions is crucial to understand and utilize its diversity in conservation and sustainable management programs (Cano et al., 2022). This information is key to identifying accessions with desirable traits such as high yield, pest resistance, and tolerance to adverse conditions, facilitating the development of improved varieties that contribute to food security and biodiversity preservation (Salgotra & Chauhan, 2023). However, the lack of detailed information on phenotypic variability limits its use in breeding and conservation programs.

In the context of emerging technologies and methodologies, morphological characterization is essential for understanding tarwi diversity and optimizing its management (Joshi et al., 2023). The integration of UAVs for capturing multispectral images enables the generation of vegetation indices which, when combined with traditional agro-morphological descriptors, have proven to be effective tools in morphological and phenotypic studies (Rodene et al., 2022). Moreover, precision agriculture offers an innovative approach to crop characterization, facilitating large-scale data collection with high accuracy (Jin et al., 2022; Zhang et al., 2023). Advancements in image analysis and machine learning now allow the estimation of phenotypic traits at various scales. Tools like the Normalized Difference Vegetation Index (NDVI) and similar metrics provide crucial information at an intermediate scale. This data allows for the analysis of seasonal trajectories, thresholds, and uncertainties concerning accession phenophases, which in turn enables the assessment of adaptability, productivity, and variability across diverse cultivation environments (Ccopi et al., 2024; Meghraoui et al., 2024).

Aware of the importance of an agro-morphological characterization of tarwi, this study aims to analyze 140 accessions and two cultivars, 'INIA-445 Masacanchino' and 'Andenes 90', preserved in the germplasm bank of the National Institute of Agricultural Innovation (INIA). Through qualitative and quantitative evaluations, along with vegetation index metrics obtained via UAVs, the study seeks to deepen our understanding of the morphology of *Lupinus mutabilis* Sweet.

2. Methodology

2.1. Study area

The Mantaro Valley, the main agricultural area of the Peruvian highlands, stretches 53 km in length with a variable width of 4 to 21 km (Pizarro et al., 2023). At its center is the Santa Ana Agricultural Experimental Station (EEA Santa Ana), belonging to the National Institute of Agricultural Innovation, located in El Tambo, Huancayo, Junín, Peru (75°13'17.60" W, 12°0'42.36" S), at an altitude of 3,303–3,325 m a.s.l. Additionally, the germplasm bank is situated at 3,295 m a.s.l. (75°13'16" W, 12°00'16" S). During the study period, the minimum and maximum temperatures ranged from 5.01°C to 20.42°C, respectively. Relative humidity peaked in December 2022 at approximately 82.07%, with the lowest value recorded in October 2022 at 61.97%. Regarding precipitation, February recorded the highest amount (145.9 mm), while October had the

lowest (11.8 mm).

The agricultural fields of EEA Santa Ana (Figure 1) are equipped with flood irrigation systems and cover a total area of 74.06 hectares, distributed across 30 agricultural plots. It is important to note that the study focused specifically on plot 24, which was previously assigned for research purposes, covering approximately 0.81 hectares. Planting took place between September 15, and harvesting was completed around October 20, 2022, in a clay-loam soil with a slightly acidic pH of 6.6, an electrical conductivity of 5.4 mS/m, and an organic matter content of 3.09%, under irrigation conditions.

2.2. Methodological framework

The methodological framework of the research, structured into five levels. The first level involved a quantitative and qualitative evaluation of the agro-morphological characteristics of *Lupinus mutabilis* S. using 56 descriptors. The second level included the monitoring of 142 plots using an unmanned aerial vehicle (UAV), with 22 photogrammetric flights between October 2022 and July 2023. The third level focused on processing multispectral images to obtain reflectance indices. The fourth level incorporated a principal component analysis to select the most correlated and significant variables, followed by a cluster analysis that identified four morphological groups. The fifth and final level compared the phenophases derived from the NDVI across the four morphological groups.

2.3. Vegetative materials

A total of 140 accessions from the INIA national tarwi germplasm collection was evaluated and characterized, along with two reference cultivars, "INIA 445 Masacanchino" and "Andenes 90," selected for their high adaptability and genetic stability, justified through the year-on-year evaluation work conducted at the INIA germplasm bank.

The accessions were initially collected from different regions of Peru between 1979 and 1981 and have been periodically regenerated and renewed, maintaining a minimum viability of 65% to date. The collection site locations were determined based on passport data recorded in the INIA national tarwi collection at the Germplasm Bank, located at the EEA Santa Ana.

Detailed information on the origin of each accession, along with its unique international identifier, is available in the Supplementary Material (Table S1). It is important to highlight that this research was carried out in an ex-situ conservation system, based on the core genetic reservoir, with the future goal of integrating these accessions into genetic improvement programs.

2.4. Study design

The sowing of 140 tarwi accessions and two cultivars was carried out in two stages. The first stage included 85 samples, which were sown on September 15, 2022, while the second stage, consisting of the remaining 57 samples, was sown on October 21, 2022. The germplasm area comprised 142 regenerative plots, each covering an area of 20 m^2. Three seeds were sown per hill in each accession plot, which consisted of four rows. Each row contained eight hills, resulting in 24 plants per row. In total, considering the four rows, 96 plants were obtained per accession (replicates).

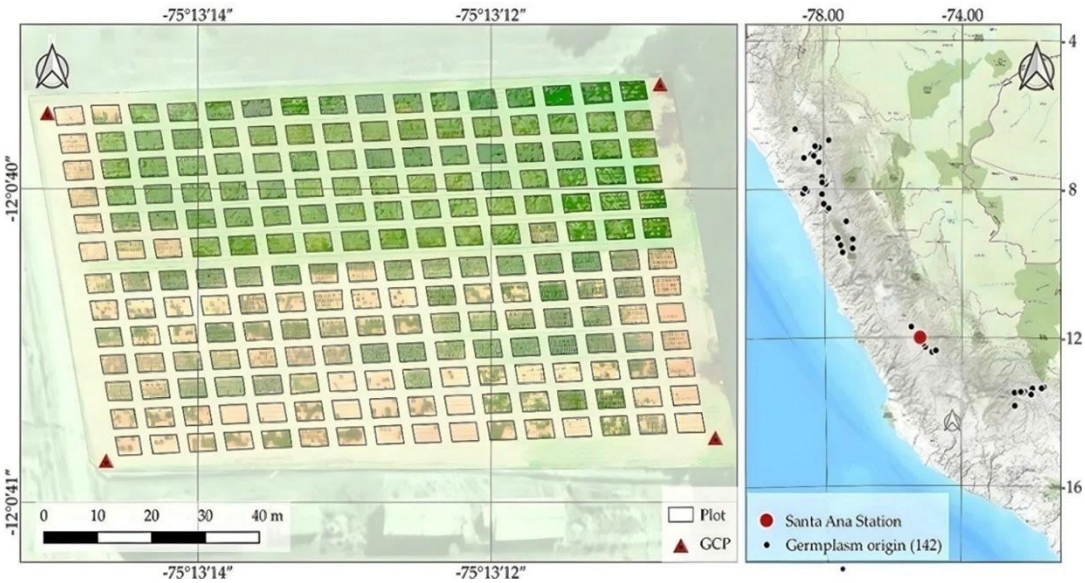

Figure 1. (Left) Distribution of the plot's tarwi germplasm bank at EEA-Santa Ana. (Right) Distribution of the origin and location of the tarwi germplasm bank.

From these, five plants were randomly selected and labeled for further analysis. Additionally, the plants were spaced 0.80 m apart, and 1 m between rows. The alleys between plots were 2 m wide, where maize (*Zea mays*) was planted to facilitate evaluation and ensure the genetic representativeness of each accession within the national tarwi collection. Agronomic management included seed disinfection with Vitavax (Carboxin + Captan) at a 0.5% concentration to prevent diseases. Manual weeding was performed at 30 and 45 days after sowing. To ensure proper plant establishment, four flood irrigations were applied. In later stages, soil moisture was monitored, taking advantage of seasonal rainfall. Additionally, nutritional amendments were applied in two stages: 50% at sowing and the remaining 50% during hilling. The applied proportions were 20-80-40 (N, P_2O_5, and K_2O, respectively), ensuring an adequate nutrient supply for optimal crop development.

Furthermore, five individual plants (replicates) per plot or accession were labeled for agro-morphological characterization, following the methodology of the Genetic Resources Subdirectorate (SDGR) of EEA Santa Ana. The total allocated area was 8,130 m².

2.5. Morphological analysis

The morphological characterization data were obtained based on the descriptors of *Lupinus* spp. used by IBPGR, (1981), with some modifications. Initially, a total of 75 morphological descriptors were evaluated, of which 19 qualitative descriptors were monomorphic and were excluded from the study as they did not contribute to the morphological variability. Consequently, the morphological analysis focused on 56 descriptors, which were examined through a principal component analysis (PCA) using 16 quantitative and 40 qualitative descriptors (complete characterization information available in the Supplementary Material, Table S1, S2, S3, S4 and Figure S1, to capture the maximum possible variability in the data and identify uncorrelated components.

2.6. Field measurements

Field evaluation started at the seedling stage when 50% of cotyledons emerged, continued at the flowering stage when 50% of plants had flowers, and ended at the maturation stage when over 50% of plants had mature pods.

Agro-morphological characterization focused on plant, leaf, flower, inflorescence, pods, and seeds. Plant evaluation considered growth type, size, stem formation, pubescence, color intensity, waxiness, branching, primary branches, days to flowering (50%), and days to maturity (50% dry pods). Leaf evaluation assessed leaflet shape, apex, pubescence, number of leaflets, color, stipule color, and petiole color. Flower evaluation included bud color before flowering, wing and keel color, marginal band, central spot, and intermediate region color, both before flowering and before wilting. Flower insertion and inflorescence length were also measured. Evaluations followed IPGRI methodology (IBPGR, 1981) with modifications.

2.7. Statistical analysis

The agro-morphological data were analyzed using R software (The R Foundation, 2025). A database with both qualitative and quantitative variables was created, averaging five plants per accession. For qualitative descriptors, a frequency-based descriptive analysis and multiple correspondence analysis were conducted. For quantitative descriptors, summary measures such as mean, coefficient of variation, and range were calculated. Principal Component Analysis (PCA) was applied to the quantitative data, and Mixed Data Factor Analysis (MDFA) identified variable associations. Divisive cluster analysis and silhouette analysis were used for accession grouping, resulting in four groups represented in a phylogenetic dendrogram.

2.8. Acquisition and processing of multispectral imagery

Figure 2 illustrates the system and equipment used to capture multispectral images in the evaluation plot. The study was conducted with a MicaSense RedEdge M multispectral camera (Inc., Seattle, USA), mounted on a DJI Matrice 300 RTK UAV (DJI Technology Co., Ltd., Shenzhen, China). This camera is able to take 12-bit multispectral photographs in five spectral bands: blue (475 ± 32 nm), green (560 ± 27 nm), red (668 ± 14 nm), RE (717 ± 12 nm) and NIR (840 ± 57 nm) and with a spatial resolution of 1.6 megapixels (1456 x 1088 pixels).

Moreover, a GNSS DJI D-RTK 2 receiver (DJI Technology Co., Ltd., Shenzhen, China) with horizontal accuracy of 1 cm + 1 ppm (RMS) and vertical accuracy of 2 cm + 1 ppm (RMS) was used for real-time kinematic (RTK) positioning of the UAV. The flights were conducted around noon, at a height of 40 m above ground, taking photographs every 2.0 seconds with a 75% overlap both frontally and laterally. In total, 22 flights were performed weekly, with each flight taking 4 minutes and 15 seconds to cover the entire area (Figure 1).

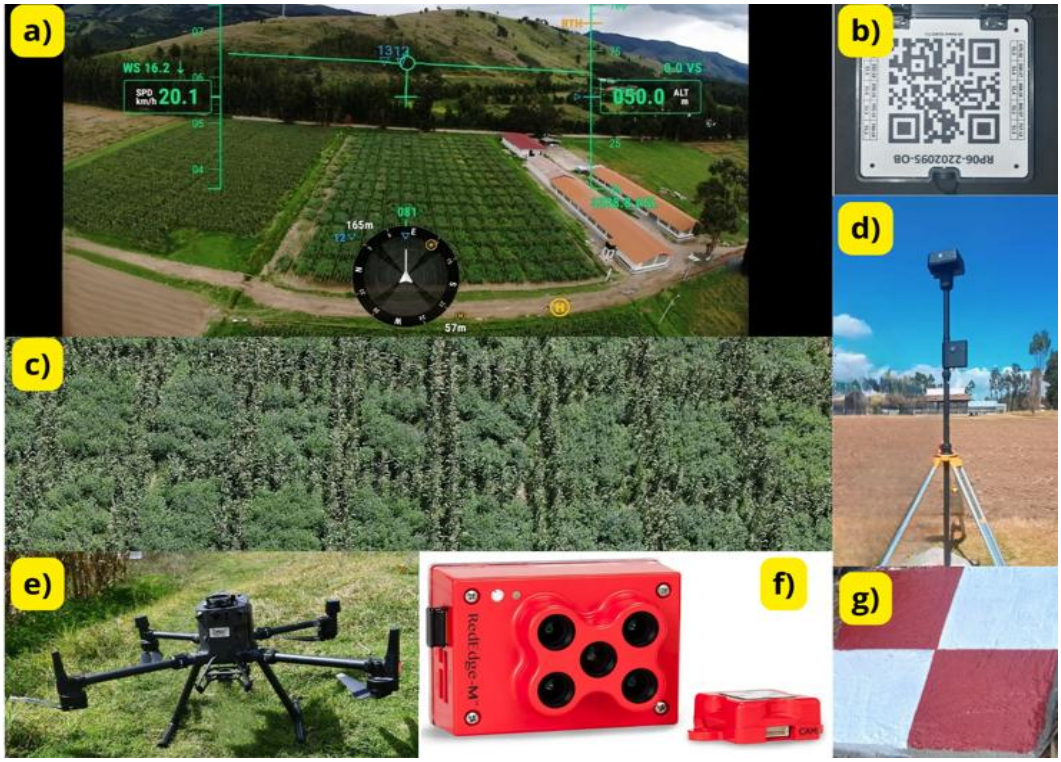

Figure 2. (a) Flight plan, (b) Control Reference Panel, (c) Evaluation plot, (d) DJI Real Time Kinetic (RTK) 2 Global Navigation Satellite System (GNSS), (e) UAV Matrice 300, (f) Micaense RedEdge-M camera, (h) Geodesic Control Point (GCP).

Photogrammetric processing was carried out using Pix4D Mapper Pro software (Prilly, Switzerland). The relative variation between the initial and optimized internal parameters was minimal (0.48%), confirming the suitability of the initial parameters for orthomosaic generation. Four ground control points were established with a Leica FlexLine TS06 plus total station, referenced to a geodetic benchmark, enhancing the topographic accuracy of both the point cloud and reflectance bands. The final ground sampling distance (GSD) was 2.5 cm.

2.8.1. Extraction of Normalized Differential Vegetation Index (NDVI)

The reflectance of each multispectral band was computed using a white reference panel (MicaSense Inc., Seattle, WA, USA). The orthomosaics were obtained using the Pix4D Mapper Pro software. R software (R Core Team) was used for the analysis and handling of the acquired geospatial data, including image processing and the extraction of spectral indices such as NDVI. Additionally, Quantum Geographical Information System (QGIS, 3.30 version) software (QGIS Development Team, Raleigh, NC, USA) was employed for image vectorization, allowing a more detailed analysis of the study plots.

2.8.2 Phenological metrics from NDVI

Several phenological stages were analyzed, such as the calculation of the start of season (SOS), length of season (LOS), and end of season (EOS), among other significant ones. These were obtained by extracting NDVI metrics from 10 months of continuous monitoring of tarwi accessions using the Greenbrown package by Forkel et al. (2015) in R studio software. These were also included in subsequent processes and statistical analyses. The phenophases or phenological metrics allowed us to understand the growth and development of the four tarwi groups. The phenological metrics of NDVI start with the beginning of the season (SOS), which marks the onset of vegetative growth and is determined when NDVI begins to increase significantly. It represents the point at which vegetation initiates its active development (Forkel et al., 2013). The length of the season (LOS) refers to the total period during which vegetation remains active, from the start of the season (SOS) to its end (EOS), providing a measure of the duration of growth (Forkel et al., 2013). The end of the season (EOS) is established when NDVI begins to decrease significantly, indicating the conclusion of vegetative development (Prodhan et al., 2021). The maximum seasonal peak (MSP) represents the highest NDVI value recorded during the growing season, reflecting the greatest amount of green biomass reached in the phenological cycle (Bao et al., 2019). The rate of increase toward the seasonal peak (RSP) measures how quickly NDVI reaches its maximum value in a season, indicating the speed at

which vegetation attains its peak growth (Forkel et al., 2015). The peak activity date (PEAK) refers to the specific moment within the phenological cycle when NDVI reaches its highest value, similar to the point of maximum growth (Forkel et al., 2013). Finally, the mid-growing season (MGS) is calculated as the intermediate date between the start (SOS) and the end of the season (EOS), providing a reference for the midpoint of the phenological development (Yuke & Yuke, 2020).

3. Results and discussion

3.1. Morphological variability

3.1.1. Quantitative variables

In this study, 16 quantitative variables were evaluated (Díaz et al., 2002), revealing that 4 showed the greatest variation: inflorescence length (LI) with a CV of 13.55% (17.0 – 42.8 cm), number of pods on the main axis (NPCA) with a CV of 14.05% (11.80 – 24.60 pods), and number of primary branches (NPB) with a CV of 23.96% (5.80 – 27.50 branches). Yield ranged from 11.5 to 2217 kg/Ha, within the range reported by Sierra y Selva Exportadora (2020).

On the other hand, the Andenes 90 cultivar reported a yield of 450.5 kg/Ha, values higher than those reported by Monroy-Guerrero et al. (2022), therefore the CV reported a high value of 69.30%, values similar to those reported by Huaringa-Joaquin et al. (2023). Variables with a CV below 20% showed less variability, including seed length, width, and thickness (CV = 5.91%, 5.40%, and 6.75%, respectively), as well as days to flowering (DF) and days to harvest (DH) (CV = 14.03% and 6.36%, respectively). The complete details of these variables, including the mean, standard deviation, coefficient of variation (CV), and minimum and maximum values, are shown in Table 1.

Pod size analysis revealed maximum values of 1.88 cm in width and 11.18 cm in length in two accessions, suggesting genetic influence. Yield per plant showed high variability, ranging from 10.8 to 249.2 g, with an average of 94.01 g. Seed size had low variability, with average dimensions of 1.03 cm in length, 0.85 cm in width, and 0.533 cm in thickness. The number of pods per plant varied from 26.20 to 228, indicating genetic diversity. The weight of 100 seeds ranged from 18.2 to 37.8 g, highlighting significant variability in tarwi seed characteristics.

3.1.2. Qualitative variables

Within the characterized tarwi collection, 42.3% of the accessions have plants with prominent stems, a value lower than the nearly 90% reported by Huaringa-Joaquin et al. (2023). This difference may be due to the variations between the cultivated accessions. Regarding leaf-related descriptors, 95.1% of the accessions have acuminate central leaflets, while 4.9% have non-acuminate central leaflets (available in Supplementary Material, Table S2).

3.1.3 Multivariate analysis for qualitative variables

The qualitative variables (Figure 3) contributing most to genetic diversity are flower wing color before opening (FWCJBO), flower wing color before wilting (FWCJBW), and the marginal band color of the flower standard before wilting (MBCSFJBW). Flower keel color before wilting (FKCJBW) also contributes, though to a lesser extent. FWCJBO is the most significant, dividing the 140 accessions and 2 cultivars into four groups based on flower color: White, Yellow, Orange, Pink, Red, Green, Blue, Purple, Brown, and Lilac. Blue or multicolored flowers are dominant, while white is recessive for other authors (Camarena Mayta et al., 2012; Chalampuente-Flores et al., 2023). These traits are highly heritable and consistently expressed.

Table 1
Descriptive statistics of quantitative variables

Acronym	Descriptor	Min. value	Max. value	Mean	*SD	**CV%
ST	Stem thickness	5.48	9.50	7.14	0.80	11.24
NPB	Number of primary branches	5.80	27.50	13.38	3.20	23.96
LNS	Leaflets number per sheet	7	9.40	8.58	0.38	4.48
LI	Length of inflorescences	17	42.80	31.45	4.26	13.55
NPCA	Number of pods per central axis	11.80	24.60	17.91	2.51	14.05
TNPP	Total number of pods per plant	26.20	228	102.07	34.77	34.06
PLMS	Pod length on the main stem	7.60	11.18	9.53	0.57	6.07
PWMS	Pod width on the main stem	1.32	1.88	1.57	0.09	5.86
TSWHS	Total seed weight in hundred seeds	18.20	37.80	30.01	8.29	227.65
YPLA	Yield per plant	10.80	249.20	94.01	55.23	58.75
YPLO	Yield per plot (Kg/Ha)	11.5	2217	757.80	525.18	69.30
SLE	Seed length (cm)	0.83	1.18	1.03	0.61	5.91
SWI	Seed width (cm)	0.71	0.98	0.85	0.46	5.40
STH	Seed thickness (cm)	0.46	0.63	0.53	0.36	6.75
DF	Days to flowering	111	187	125.71	17.64	14.03
DH	Days to harvest	211	296	244.55	15.56	6.36

*SD= Standard deviation, **CV= Coefficient of variability.

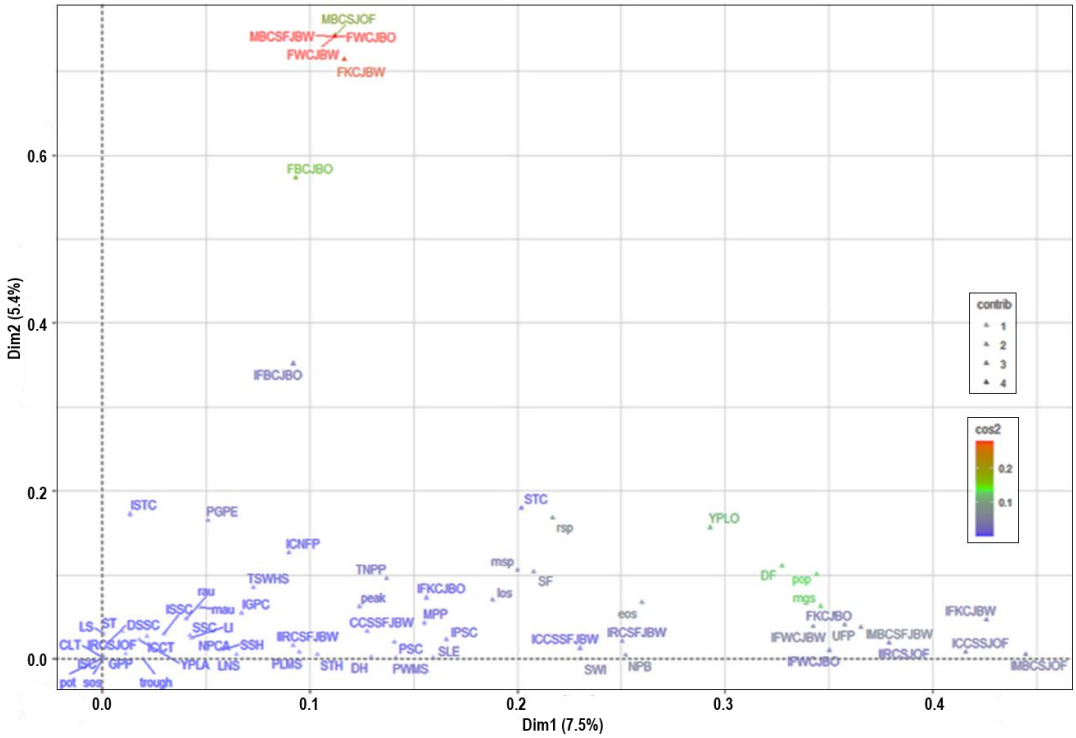

Figure 3. Contribution of variables in the multiple correspondence analysis.

3.1.4. Quantitative variables in correspondence analysis

Figure 4 shows the quantitative variables that have the greatest influence on the phenotypic diversity of the 140 accessions and 2 tarwi cultivars evaluated.

The days to flowering (DF), which vary between 111 and 187 days, are particularly noteworthy. These values are higher than those reported by (Guilengue et al., 2019) who mention values between 80.8 - 103.4 days. This difference may be attributed to the place of cultivation and the difference in cultivars, which in the cited study were *Lupinus albus* and two accessions "Misak" and "Mihai". Additionally, it was identified that yield characters, such as yield per plot (YPLO), are related to the total number of pods per plant (TNPP).

The variables most positively linked to the first axis are the total weight of one hundred seeds (TSWHS), pod width (PWMS) and seed width (SWI). Negatively, the variable days to harvest (DH) is linked. The variables most positive linked to the second axis are days to flowering (DF), number of primary branches (NPB), inflorescence length (LI) and number of leaflets per leaf (LNS). In a negative sense, yield per plant (YPLA), number of pods per central axis (NPCA), total number of pods per plant (TNPP) and yield per plot (YPLO) are linked. In addition, there is a correlation between inflorescence length (LI) and number of leaflets per leaf (LNS).

3.1.4 Grouped cluster analysis

In the previous analysis, descriptors with greater discriminant power (10 quantitative and 16 qualitative) were selected. Through group analysis, four groups were identified, which are shown in different colors in Figure 5 through a cluster analysis. Each of the PER CODE nomenclatures is available in data availability statement.

The groups were assigned colors: Group 1 (G1, orange), Group 2 (G2, fuchsia), Group 3 (G3, blue), and Group 4 (G4, yellow). In that sense G1 (22.53%) includes 32 accessions with blue plants, 93.8 pods per plant, and a yield of 712.28 kg/ha. G2 (39.43%) has 56 accessions, also blue plants, 94.37 pods per plant, and a yield of 579.05 kg/ha. G3 (35.91%) consists of 51 accessions, mostly blue plants, with 115.65 pods per plant and the highest yield (985.33 kg/ha). G4 (2.11%) includes 3 accessions with pink plants, 103.33 pods per plant, and a yield of 712.0 kg/ha. These findings differ from (Huaringa-Joaquin et al., 2023), who grouped 89 accessions into two clusters, likely due to agro-morphological traits. A hierarchical clustering dendrogram in the Supplementary Material (Figure S1) illustrates similarity relationships among accessions.

The horizontal axis of the dendrogram indicates the dissimilarity distance, reflecting the degree of difference among the analyzed accessions. The values 0.4 and 0.2 on the horizontal scale represent these levels of dissimilarity. A 0.4 value suggests a

greater difference between groups, indicating that branches merging at this level share lower similarity. In contrast, a 0.2 value indicates lower dissimilarity, meaning that accessions grouped within this range share more characteristics in common.

The dendrogram displays color-differentiated groups, highlighting the presence of clear patterns in the genetic variability of the analyzed accessions. Accessions positioned closer in the dendrogram, with merges toward the left, exhibit higher similarity and belong to the same group or cluster. Conversely, accessions with more distant mergers show greater genetic or phenotypic variability, indicating significant differences in their characteristics.

3.1.5 Morphological variability of quantitative variables at the group level

Yield per plot (YPLO) and yield per plant (YPLA) show a CV of 69.30% and 58.75% respectively, making them the variables with the greatest dispersion. These values were higher than reported by Guilengue et al. (2019), who found the highest CVs for the number of pods in primary branches and the number of seeds in primary branches (29.18% - 37.68%). Conversely, the variables days to flowering (DF) and days to harvest (DH) show the least dispersion with a CV of 14.03% and 6.37% respectively, making them the most homogeneous. The analysis of variance revealed that two variables, PWMS and YPLO, presented significant differences (p-value ≤ 0.001) and highlighted the difference between groups. Additionally, it was found that these variables were also significant (p-value ≤ 0.001) for differentiation between groups (Table 2).

3.1.6 Morphological variability of qualitative variables at the group level

Table 3 shows the morphological variability concerning qualitative variables, which were evaluated using contingency tables to determine if the association between the categories of each categorical variable and the groups was significant (p ≤ 0.001). Qualitative variables with a value of p-value < 0.05 are also shown, indicating that these variables contribute to the high genetic diversity of tarwi, potentially related to cross-pollination as mentioned in studies by Huaringa-Joaquin et al. (2023).

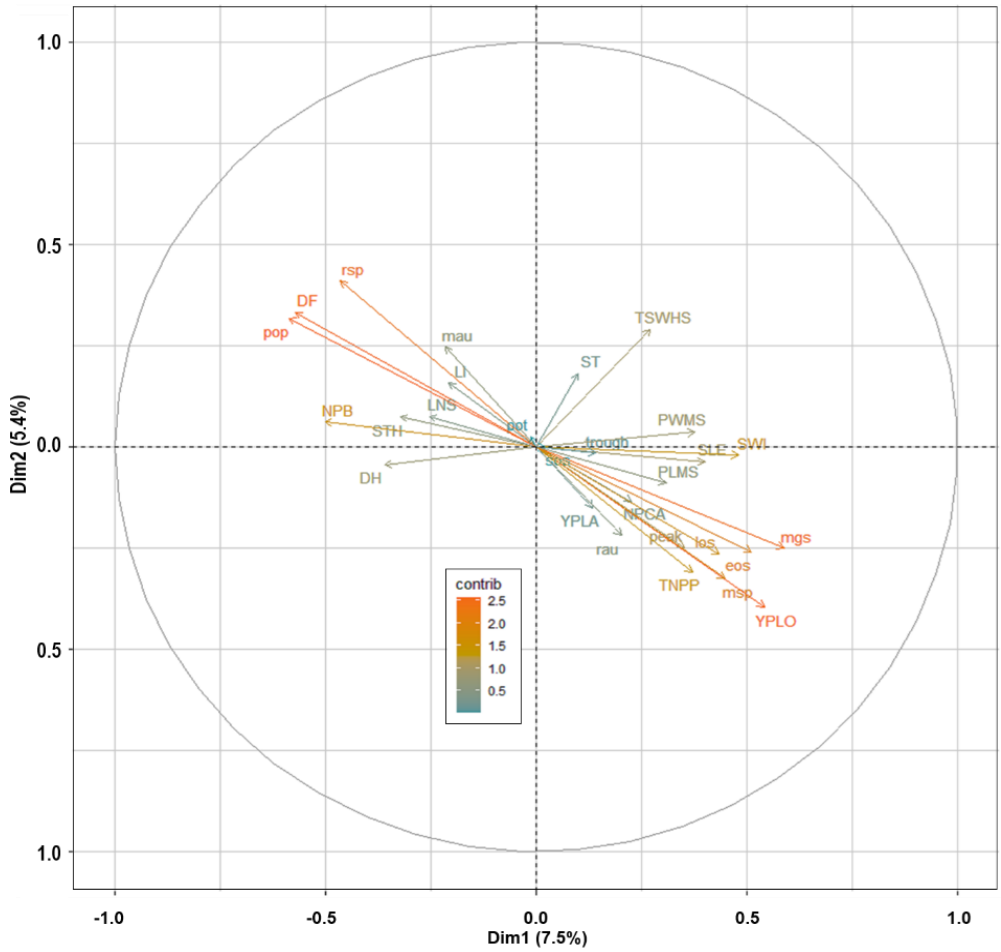

Figure 4. Contribution of quantitative variables in the correspondence analysis.

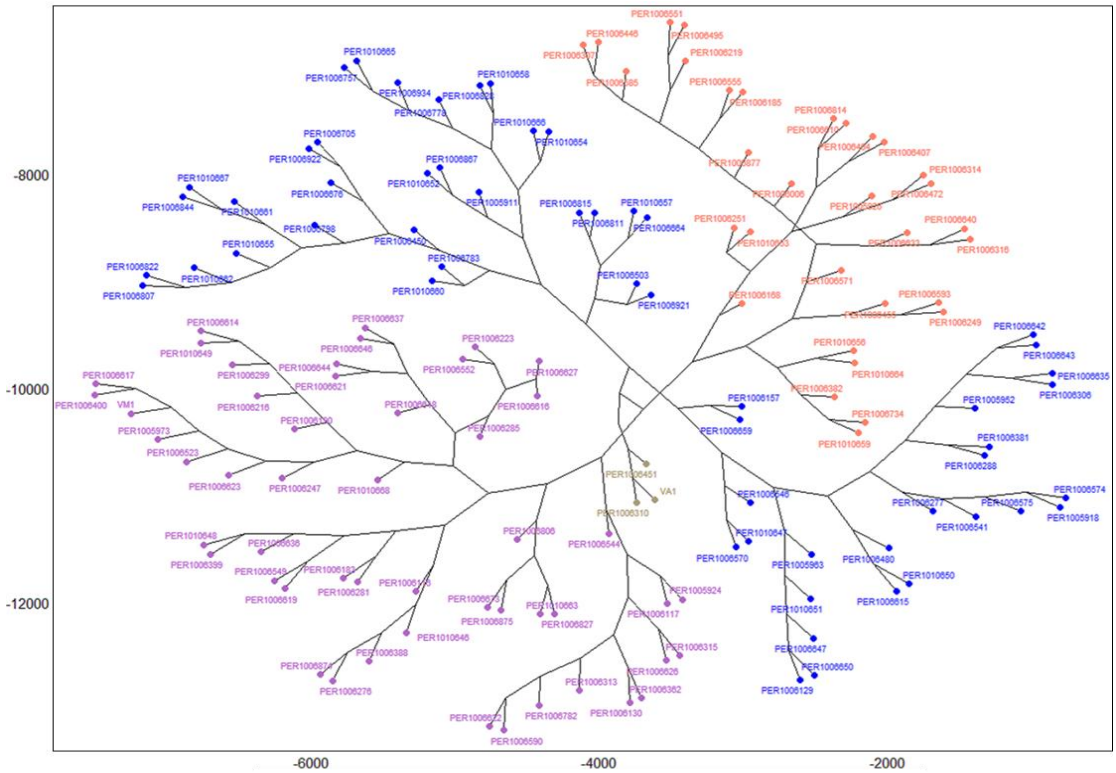

Figure 5. Cluster for qualitative and quantitative variables of the 140 tarwi accessions and 2 cultivars, where group 1 is assigned the orange color, group 2 is assigned the fuchsia color, group 3 is assigned blue color and group 4 is assigned yellow color.

Table 2
Statistics of quantitative characters organized in four groups

Characteristic	G1, N = 32[1]	G2, N = 56[1]	G3, N = 51[1]	G4, N = 3[1]	p-value[2]*
DF	127.16 (19.80) [111.00, 187.00]	131.41 (17.03) [111.00, 173.00]	119.37 (15.20) [111.00, 179.00]	119.33 (9.07) [111.00, 129.00]	0.002
DH	237.97 (19.10) [211.00, 282.00]	248.34 (14.71) [211.00, 295.00]	245.14 (12.33) [211.00, 296.00]	234.33 (20.23) [211.00, 247.00]	0.001
NPB	12.24 (2.64) [7.60, 20.80]	14.58 (3.81) [5.80, 27.50]	12.91 (2.40) [9.00, 21.00]	11.40 (1.04) [10.80, 12.60]	0.002
TNPP	93.80 (30.83) [26.20, 149.40]	94.37 (36.33) [34.00, 202.00]	115.65 (31.77) [55.40, 228.00]	103.33 (38.88) [58.60, 129.00]	0.005
PLMS	9.69 (0.59) [7.60, 10.72]	9.37 (0.59) [8.00, 11.18]	9.62 (0.54) [8.48, 11.00]	9.44 (0.26) [9.14, 9.62]	0.011
PWMS	1.55 (0.10) [1.32, 1.72]	1.54 (0.09) [1.40, 1.76]	1.60 (0.08) [1.46, 1.84]	1.73 (0.13) [1.62, 1.88]	<0.001
TSWHS	30.36 (3.77) [23.34, 39.75]	29.39 (3.62) [21.84, 36.86]	30.46 (3.54) [23.85, 39.46]	39.80 (2.89) [36.91, 42.68]	0.017
YPLO	1,424.57 (953.68) [27.36, 3,291.00]	1,158.11 (1,010.95) [23.00, 4,434.00]	1,970.67 (1,012.44) [252.00, 4,241.00]	1,424.00 (973.31) [824.00, 2,547.00]	<0.001
SWI	10.36 (0.74) [8.33, 11.90]	10.09 (0.60) [8.37, 11.65]	10.46 (0.44) [9.66, 11.53]	11.05 (0.58) [10.67, 11.72]	0.001
SLE	8.66 (0.56) [7.72, 9.82]	8.37 (0.44) [7.11, 9.75]	8.61 (0.37) [7.97, 9.50]	8.87 (0.36) [8.49, 9.20]	0.006
MGS	0.81 (0.06) [0.68, 0.89]	0.78 (0.07) [0.61, 0.87]	0.84 (0.03) [0.72, 0.87]	0.83 (0.03) [0.79, 0.86]	<0.001
RSP	0.02 (0.00) [0.01, 0.03]	0.02 (0.01) [0.01, 0.04]	0.02 (0.00) [0.01, 0.03]	0.02 (0.01) [0.01, 0.04]	0.002
PEAK	0.91 (0.03) [0.85, 0.95]	0.91 (0.02) [0.81, 0.94]	0.92 (0.02) [0.84, 0.94]	0.91 (0.03) [0.88, 0.94]	0.022
MSP	0.66 (0.09) [0.40, 0.79]	0.64 (0.08) [0.47, 0.77]	0.68 (0.06) [0.55, 0.81]	0.66 (0.10) [0.55, 0.75]	0.044

[1] Mean (SD) [Range]; n / N (%); [2] Kruskal-Wallis rank sum test; Fisher's exact test; G= Group.
* Note: Non-significant quantitative descriptors were omitted.

The contingency analysis identified six qualitative variables as the most discriminant among 140 accessions and two tarwi cultivars: MBCSFJBW, FWCJBO, FWCJBW, FKCJBW, MBCSJOB, and FBCJBO. Morphological variability revealed that all accessions in G1 and G2 had blue flowers, while G3 had 98% blue and 2% lilac flowers. G4 exhibited 100% pink flowers. Three dominant flower colors were identified: blue, pink, and lilac (Figure 6 a).

The coloration of the flowers varies from the beginning of their formation to maturation, as mentioned by Camarena Mayta et al. (2012). Other authors (Annicchiarico et al., 2019) report that the most common flower colors in tarwi are blue, violet and white, while cream, pink and yellow are less frequent. This color variation is due to anthocyanins and flavonoids. Regarding the color of the keel before flowering (FKCJBW), as shown in Figure 7 b,

100% of G4 are white, 100% of G2 are purple, and in G1 and G3 mostly present a purple color, with green to a lesser extent. On the other hand, Figure 7 c shows that the color of the flower buds just before opening (FBCJBO) is white and yellow for G1, G2 and G3, while G4 presents white and pink colors. Figure 6 d shows the morphological details of the four groups.

The seed shape (SSH) was one of the variables that was not significant (p-value > 0.05). In groups G1, G2, and G3, various seed shapes were clustered (flattened spherical or lenticular, oval, and flattened oval), with oval shape being the most representative. Group G4, on the other hand, represented 100% oval-shaped seeds (Figure 7 d).

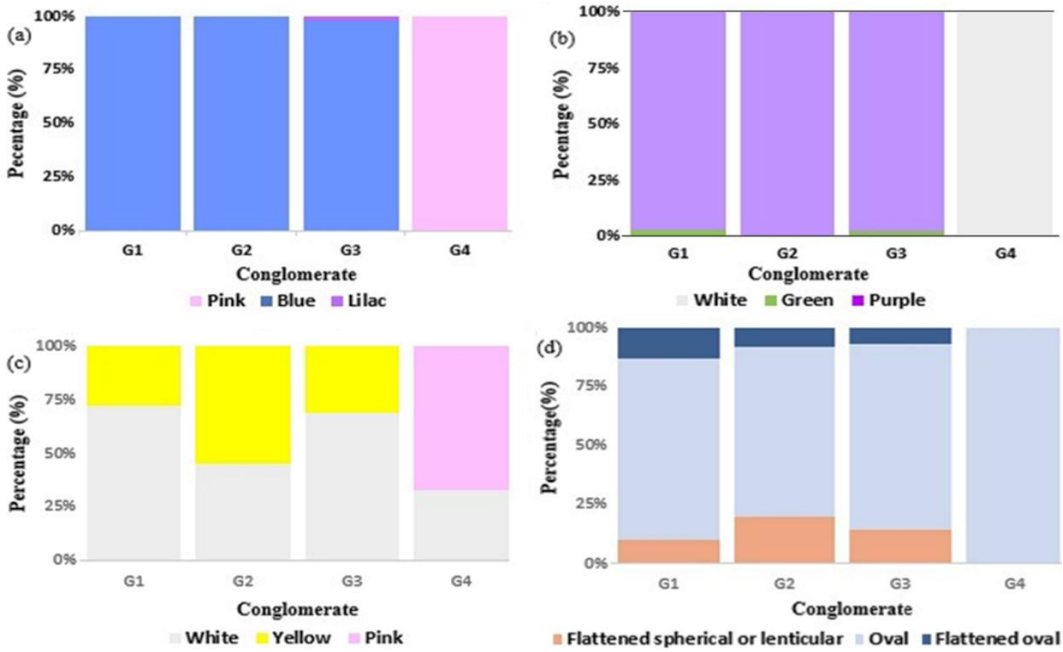

Figure 6. Frequencies of accessions in the tarwi collection, according to the state of qualitative characteristics. (a) Describe the color of the flower's wing just before opening (FWJCBO) for each group. (b) Describe the flower keel color just before wilting (FKCJNW) for each group. (c) Describe the flower bud color just before opening (FBCJBO) for each group. (d) Describe the shapes of the seeds (SSH) of the four groups.

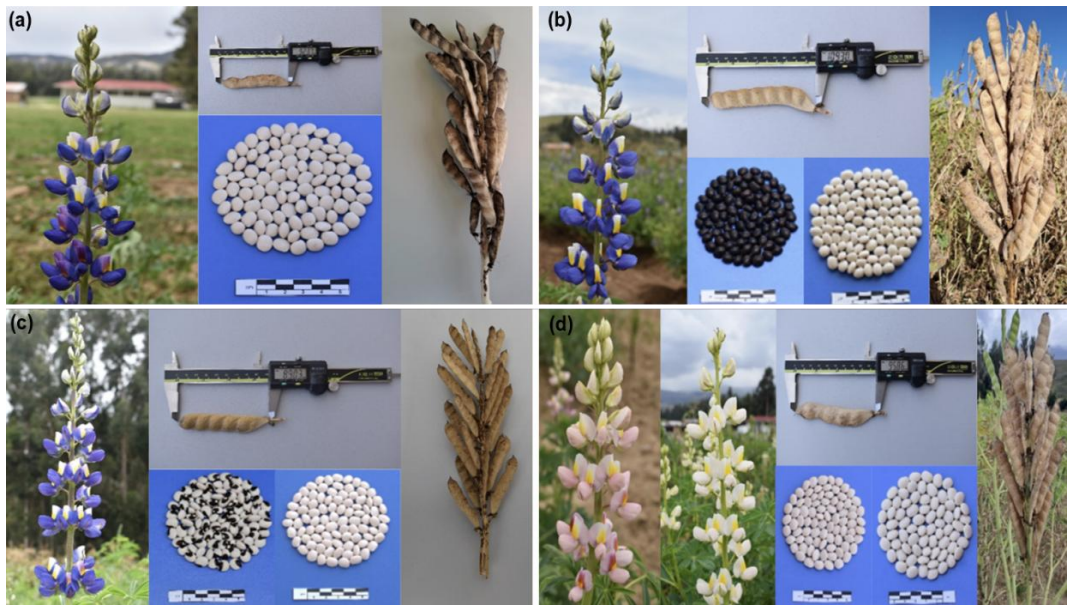

Figure 7. The phenotypic variability of *Lupinus mutabilis* S. includes: (a) Group 1 with green stem, blue wings, white central spot, yellow intermediate region, white seeds, and pod length of 7.60 - 10.72 cm; (b) Group 2 with green stem, blue wings, white central spot, yellow intermediate region, white and black seeds, and pod length of 8.00 - 11.18 cm; (c) Group 3 with green stem, blue wings, white central spot, yellow intermediate region, white seeds with black secondary color in a half-moon distribution, and pod length of 8.48 - 11.00 cm; (d) Group 4 with green stem, pink wings, white central spot, yellow intermediate region, white seeds, and pod length of 9.14 - 9.62 cm.

Table 3
Frequency analysis of qualitative characteristics for difference groups

Characteristic	G1, N = 32[1]	G2, N = 56[1]	G3, N = 51[1]	G4, N = 3[1]	p-value[2] *
SF					<0.001
Main stem not prominent	19 / 32 (59%)	42 / 56 (75%)	19 / 51 (37%)	2 / 3 (67%)	
Main stem prominent	13 / 32 (41%)	14 / 56 (25%)	32 / 51 (63%)	1 / 3 (33%)	
PGPE					<0.001
Absent	3 / 32 (9.4%)	0 / 56 (0%)	2 / 51 (3.9%)	2 / 3 (67%)	
Present	29 / 32 (91%)	56 / 56 (100%)	49 / 51 (96%)	1 / 3 (33%)	
FBCJBO					<0.001
White	23 / 32 (72%)	25 / 56 (45%)	35 / 51 (69%)	1 / 3 (33%)	
Yellow	9 / 32 (28%)	31 / 56 (55%)	16 / 51 (31%)	0 / 3 (0%)	
Pink	0 / 32 (0%)	0 / 56 (0%)	0 / 51 (0%)	2 / 3 (67%)	
FWCJBO					<0.001
Pink	0 / 32 (0%)	0 / 56 (0%)	0 / 51 (0%)	3 / 3 (100%)	
Blue	32 / 32 (100%)	56 / 56 (100%)	50 / 51 (98%)	0 / 3 (0%)	
Lilac	0 / 32 (0%)	0 / 56 (0%)	1 / 51 (2.0%)	0 / 3 (0%)	
MBCSJOF					<0.001
White	0 / 32 (0%)	0 / 56 (0%)	1 / 51 (2.0%)	0 / 3 (0%)	
Orange	0 / 32 (0%)	0 / 56 (0%)	1 / 51 (2.0%)	0 / 3 (0%)	
Pink	0 / 32 (0%)	0 / 56 (0%)	0 / 51 (0%)	3 / 3 (100%)	
Blue	32 / 32 (100%)	56 / 56 (100%)	48 / 51 (94%)	0 / 3 (0%)	
Lilac	0 / 32 (0%)	0 / 56 (0%)	1 / 51 (2.0%)	0 / 3 (0%)	
IFKCJBO					<0.001
Very light	29 / 32 (91%)	27 / 56 (48%)	29 / 51 (57%)	3 / 3 (100%)	
Light	3 / 32 (9.4%)	8 / 56 (14%)	17 / 51 (33%)	0 / 3 (0%)	
Medium	0 / 32 (0%)	20 / 56 (36%)	5 / 51 (9.8%)	0 / 3 (0%)	
dark	0 / 32 (0%)	1 / 56 (1.8%)	0 / 51 (0%)	0 / 3 (0%)	
FWCJBW					<0.001
Pink	0 / 32 (0%)	0 / 56 (0%)	0 / 51 (0%)	3 / 3 (100%)	
Blue	32 / 32 (100%)	56 / 56 (100%)	50 / 51 (98%)	0 / 3 (0%)	
Lilac	0 / 32 (0%)	0 / 56 (0%)	1 / 51 (2.0%)	0 / 3 (0%)	
IFWCJBW					<0.001
Medium	16 / 32 (50%)	0 / 56 (0%)	0 / 51 (0%)	1 / 3 (33%)	
Dark	14 / 32 (44%)	5 / 56 (8.9%)	33 / 51 (65%)	2 / 3 (67%)	
Very dark	2 / 32 (6.2%)	51 / 56 (91%)	18 / 51 (35%)	0 / 3 (0%)	
MBCSFJBW					<0.001
Pink	0 / 32 (0%)	0 / 56 (0%)	0 / 51 (0%)	3 / 3 (100%)	
Blue	32 / 32 (100%)	56 / 56 (100%)	50 / 51 (98%)	0 / 3 (0%)	
Lilac	0 / 32 (0%)	0 / 56 (0%)	1 / 51 (2.0%)	0 / 3 (0%)	
IMBCSFJBW					<0.001
Medium	21 / 32 (66%)	0 / 56 (0%)	0 / 51 (0%)	1 / 3 (33%)	
Dark	10 / 32 (31%)	5 / 56 (8.9%)	33 / 51 (65%)	2 / 3 (67%)	
Very dark	1 / 32 (3.1%)	51 / 56 (91%)	18 / 51 (35%)	0 / 3 (0%)	
CCSSFJBW					<0.001
White	25 / 32 (78%)	17 / 56 (30%)	32 / 51 (63%)	3 / 3 (100%)	
Purple	6 / 32 (19%)	39 / 56 (70%)	19 / 51 (37%)	0 / 3 (0%)	
Brown	1 / 32 (3.1%)	0 / 56 (0%)	0 / 51 (0%)	0 / 3 (0%)	
IRCSFJBW					<0.001
Yellow	26 / 32 (81%)	10 / 56 (18%)	36 / 51 (71%)	3 / 3 (100%)	
Orange	6 / 32 (19%)	28 / 56 (50%)	13 / 51 (25%)	0 / 3 (0%)	
Brown	0 / 32 (0%)	18 / 56 (32%)	2 / 51 (3.9%)	0 / 3 (0%)	
FKCJBW					<0.001
White	0 / 32 (0%)	0 / 56 (0%)	0 / 51 (0%)	3 / 3 (100%)	
Green	1 / 32 (3.1%)	0 / 56 (0%)	1 / 51 (2.0%)	0 / 3 (0%)	
Purple	31 / 32 (97%)	56 / 56 (100%)	50 / 51 (98%)	0 / 3 (0%)	
UFP					<0.001
Little	3 / 32 (9.4%)	2 / 56 (3.6%)	4 / 51 (7.8%)	0 / 3 (0%)	
Medium	11 / 32 (34%)	44 / 56 (79%)	18 / 51 (35%)	1 / 3 (33%)	
Very much	18 / 32 (56%)	10 / 56 (18%)	29 / 51 (57%)	2 / 3 (67%)	
MPP					0.011
Little	1 / 32 (3.1%)	0 / 56 (0%)	3 / 51 (5.9%)	0 / 3 (0%)	
Medium	22 / 32 (69%)	28 / 56 (50%)	38 / 51 (75%)	2 / 3 (67%)	
Very much	9 / 32 (28%)	28 / 56 (50%)	10 / 51 (20%)	1 / 3 (33%)	
IPSC					0.04
Medium	32 / 32 (100%)	48 / 56 (86%)	49 / 51 (96%)	3 / 3 (100%)	
Dark	0 / 32 (0%)	0 / 56 (0%)	1 / 51 (2.0%)	0 / 3 (0%)	
Very dark	0 / 32 (0%)	8 / 56 (14%)	1 / 51 (2.0%)	0 / 3 (0%)	

[1] Mean (SD) [Range]; n / N (%); [2] Kruskal-Wallis rank sum test; Fisher's exact test.
* Non-significant variables were omitted.

In the case of Peruvian germplasm, 23% of the accessions in the tarwi collection presented a 100-seed weight within the optimal ranges of improved varieties (28 - 30 g), and 46% showed values higher (Velazques Carrera, 1993).

The 100-seed weight of the "INIA 445 Masacanchino" cultivar was 32.4 g, while the Andenes 90 cultivar obtained the highest 100-seed weight of 42.68 g. Moreover, it is suggested that this variable also depends on seed size. For the present study, these variables were not significant (p-value > 0.05) among the 4 groups, implying relatively homogeneous seeds within the tarwi collection.Additionally, the recorded values of seed size (SLE, SWI, and STH) have similar characteristics to improved varieties (Guaytarilla & Falconí, 2014). The potential of a genotype depends on the number of pods and seed weight, which are factors that determine productivity considering that the environment strongly influences these characteristics, so they may or may not express their maximum potential, allowing discrimination between accessions and between groups.

The challenge for plant breeders is to develop pure lines with uniform, heritable colors, along with desirable agronomic traits and high-yield genes. Within the collection, some germplasms exhibit these characteristics, while others, such as accessions from G3 (PER005325 and PER006726), display secondary colors but still show high yields.

Figure 7 details the phenotypic variability in *Lupinus mutabilis* S. Four groups of accessions are presented, showing variability in characteristics such as flower color, wing color, central spot of the standard, color of the intermediate region of the standard, seed color, and pod length range, which were recorded both in the field and laboratory.

The Andenes 90 cultivar, characterized by its pink wings, is in group 4. The existence of genetically diverse germplasms can be included in breeding programs through the selection of characteristics of interest. Other researchers indicate that collection and characterization are necessary to obtain and find additional genetic resources that are not known (Perez et al., 2015).

In Peru, improved tarwi cultivars have a short vegetative period, requiring 167 to 225 days to harvest. While similar traits exist in the national tarwi collection, progressive maturation of lateral branches limits mechanized harvesting (Peralta et al., 2013). Farmers prefer early varieties harvested in under seven months, though some wish to preserve traditional varieties for cultural and food heritage reasons. Additionally, certain descriptors in tarwi cultivation may enhance vegetative growth but negatively impact yield (Guilengue et al., 2019).

3.3 Phenological trajectories by groups through NDVI

Int the Figure 8 shows the most relevant phenological trajectories of the 4 agro-morphological groups identified for Tarwi (*Lupinus mutabilis* S.). It is observed that groups G1, G2, and G4 present the same start of season (SOS) on day 81, while G3 has a start of the season on day 80. Therefore, the difference in SOS between the groups is not significant.

Likewise, many authors detail that this period largely depends on the geographical area where it is cultivated (Chalampuente-Flores et al., 2023). For example, the *Lupinus mutabilis* S. cultivar "INIA 445 - Masacanchino" is found in an altitudinal range of 3,289 to 3,645 m a.s.l. and has a maturation period of 200 to 208 days, which is why it is considered early (INIA, 2021). In contrast, Andenes 90 cultivar is cultivated in an altitudinal range of 2,500 to 3,800 m a.s.l., has a growth cycle of 200 to 240 days, and is considered a late cultivar (De la Cruz, 2018).

Groups G1 and G4 have a similar end of season (EOS), while G2 and G3 experience a later EOS. G3 shows the maximum growth and the highest final decrease rate (RAU), indicating a faster NDVI drop at the end of the season. In contrast, G4 has the highest initial growth rate (RSP), suggesting a quicker start to the growing season.

Overall, all four groups have similar start and end dates, as well as nearly identical season durations, indicating homogeneous phenological conditions. Groups G1 and G4 are earlier, making them promising candidates for selection. The small variation in the start of vegetative growth aligns with previous reports of slight differences (Ge et al., 2016; Peng et al., 2022; Wu & Xin, 2023). Regarding season length (LOS), G1, G2, and G3 had a duration of 178 days, while G4 lasted 177 days, a minor difference that doesn't significantly affect the phenology interpretation. Some authors report a season duration of 200-300 days for full maturation (Chalampuente-Flores et al., 2023; Rodríguez-Ortega et al., 2023).

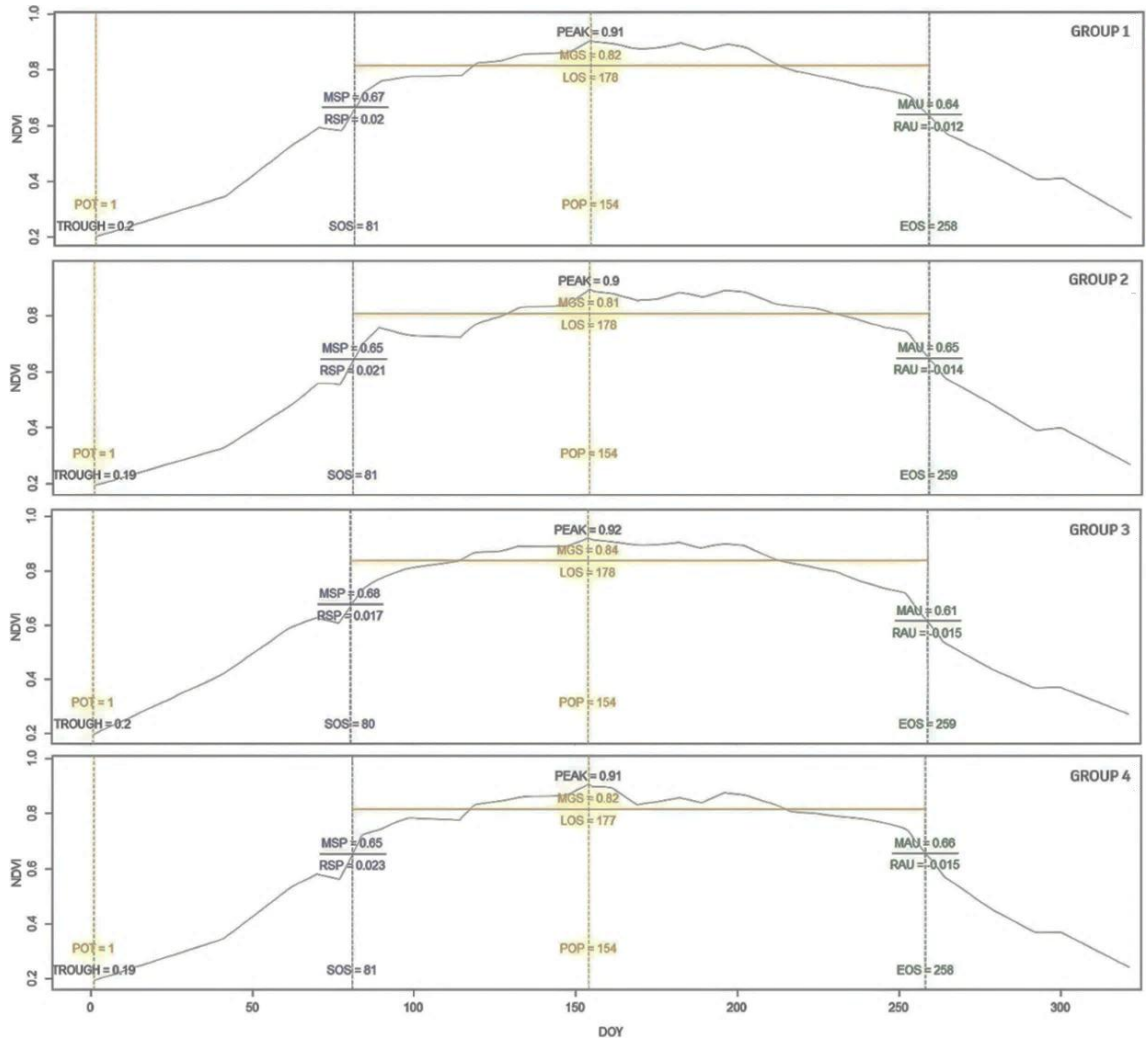

Figure 8. Phenological development of the 4 groups identified from Normalized Differential Vegetation Index (NDVI) phenophase identification, with respect to the days of the year (DOY).

4. Conclusions

The genetic variability of tarwi is manifested in key characteristics related to the plant's productivity and yield, such as the total number of pods per plant and seed weight. Despite the low percentage of seeds with secondary colors, this feature suggests significant genetic variability, possibly attributable to the degree of cross-pollination in this species. The present study has successfully identified promising materials for genetic improvement.

The phenological data analyzed through the phenophases of the four groups show high coherence, suggesting similar phenological conditions in the region, with slight variations. In this context, the G1 and G4 groups, which are earlier maturing, are particularly promising for breeding programs aimed at developing new tarwi cultivars.

Despite the small differences observed, the overall consistency of the data can serve as a baseline for long-term monitoring of phenological changes.

Understanding genetic variability, both within and among populations, is crucial for strengthening both in-situ and ex-situ conservation efforts. The significant productive and nutritional potential of tarwi presents a valuable opportunity to bolster food security and sovereignty in the Andean region. To fully realize this potential, it is essential to promote regional strategies that encourage the consumption of lupine in its diverse forms and colors.

Continued evaluation of germplasm, crop management practices, traditional uses, and innovative approaches is necessary to improve productivity and quality levels, thereby meeting both domestic and international demand.

Authors' contributions

E. Peña-Elme: Conceptualization, investigation, methodology, validation, visualization, writing; **K. Ortega-Quispe:** Investigation, methodology, visualization, writing, review and editing; **L. Enriquez-Pinedo:** Data curation, investigation, visualization, writing; **F. Cerrón-Mercado**: Methodology, visualization, editing, writing; **N. Amaro-Camarena:** Data curation, visualization; **C. Girón-Aguilar:** Conceptualization, investigation, writing; **H. Loayza-Loza**; visualization, editing, writing; **S. Pizarro-Carcausto:** methodology, statistical analysis, writing, investigation.

Conflicts of Interest

The authors do not declare any conflicts of interest.

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