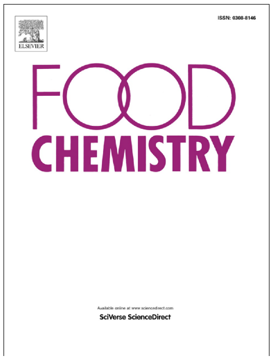

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Targeted Phytochemical Profiling and Functional Evaluation of Bitter and Sweet *Lupinus albus* Genotypes from Diverse Origins

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Abstract

Bitter *Lupinus albus* genotypes, traditionally excluded from food use due to high alkaloid levels, are reassessed as underutilized sources of bioactive phytochemicals. This study conducted a comparative agro-morphological and biochemical analysis of bitter and sweet genotypes. Targeted GC–MS profiling revealed that bitter types exhibited accelerated phenological development and significantly higher levels of stearic, behenic, and α-linolenic acids. They also demonstrated enhanced antioxidant capacity (DPPH IC_{50}: 23.4 ± 2.1 vs. 41.2 ± 3.7 µg/mL) and stronger lipoxygenase-inhibitory activity. Lupanine was the dominant alkaloid in bitter seeds, while erucic acid remained within acceptable dietary limits. Sweet genotypes maintained low alkaloid content and favourable fatty acid ratios. Antifungal assays indicated stronger inhibition of *Colletotrichum acutatum* by bitter types. Principal component analysis highlighted clear genotype-dependent clustering based on phenological, biochemical, and functional traits. These results support the valorisation of bitter *L. albus* for functional food applications and breeding strategies aimed at enhancing nutritional properties.

Keywords *Lupinus albus, Bitter and Sweet chemotypes, Agromorphological traits, Biochemical profiling,GC-MS profiling, Functional foods.*

1. Introduction

The rising global population and demand for sustainable food systems underscore the need for high-yield, nutrient-rich crops. Staple crops such as wheat, rice, and maize, though vital for food security, are often low in essential nutrients, contributing to protein, vitamin, and mineral deficiencies. (Sands et al., 2009, Thudi et al., 2020). This underscores the need to diversify agriculture with nutrient-rich crops that offer sustainable alternatives to animal-based proteins. Leguminous crops, such as *Lupinus albus*, are emerging as ideal candidates due to their superior protein content, rich profiles of essential amino acids, bioactive compounds, and beneficial fatty acids (Pereira et al., 2022). These crops address malnutrition and micronutrient deficiencies while supporting agroecological sustainability through biological nitrogen fixation. (Ayilara et al., 2022, Odeku et al., 2024, Van de Noort, 2024). By integrating such nutrient-dense crops into global food systems, we can enhance dietary diversity, meet rising consumer demands for health-conscious and plant-based foods and contribute to more resilient and sustainable agricultural practices.

In this context, *Lupinus albus* (white lupin) emerges as a compelling candidate due to its superior protein content (35–40% of seed dry weight), balanced amino acid composition, and substantial levels of dietary fiber and health-promoting secondary metabolites, including phenolics, flavonoids, and essential fatty acids (Gresta et al., 2023; Estivi et al., 2023; Pereira et al., 2022). Compared to soybean, *L. albus* not only matches its protein quality but also exhibits a lower concentration of anti-nutritional factors such as trypsin inhibitors. The seeds are particularly rich in polyunsaturated fatty acids, linoleic (C18:2) and alpha-linolenic acids (C18:3), which confer additional cardioprotective and anti-inflammatory benefits (Ferchichi et al., 2021). Moreover, as a nitrogen-fixing legume, *L. albus* contributes significantly to soil fertility, offering agronomic advantages in low-input and sustainable farming systems (Ayilara et al., 2022; Van de Noort, 2024).A unique characteristic of *Lupinus albus* is its capacity to biosynthesize quinolizidine alkaloids (QAs), a class of nitrogen-containing secondary metabolites with dual relevance: they act as natural defence compounds against herbivores and pathogens, and they hold potential for pharmacological applications (Caramona et al., 2024). However, excessive QA accumulation presents toxicity risks, thereby limiting the direct use of unprocessed seeds for human and animal consumption (Osorio & Till, 2022). To overcome this constraint, two major chemotypes have been established: *bitter* lupins defined by QA concentrations exceeding 500 mg/kg DW, and *sweet* lupins, derived through targeted breeding programs to reduce seed QA levels below the regulatory threshold (200 mg/kg DW in the European Union) (Resta et al., 2008; Hama & Strobel, 2020; Tirdil'ová et al., 2022). More recently, breeding initiatives have focused on developing “bitter–sweet” ideotypes, genotypes that preserve elevated QA concentrations in vegetative tissues to confer herbivore and pest resistance, while maintaining seed QA contents within food safety limits (Berger et al., 2014; Otterbach et al., 2019a). This dual-target strategy illustrates a rational and forward-looking approach to reconciling agronomic resilience with nutritional quality and consumer acceptance.

Despite the growing interest in *Lupinus albus* as a sustainable protein crop, the extent of its intraspecific biochemical variability remains insufficiently characterized. This variability, shaped by genetic, environmental and agronomic factors, plays a crucial role in determining the nutritional and functional attributes of lupin seeds (Cabrita et al., 2024; Mimura et al., 2017). While prior studies have broadly examined the nutritional profile of *L. albus* (Valente et al., 2024, Costa et al., 2013), few have dissected the chemotypic variation in key biochemical constituents such as phenolics, flavonoids, alkaloids, and fatty acids. This lack of targeted comparison restricts our understanding of how genetic divergence and alkaloid content influence seed quality and utility in food systems.

In this study, we conducted a comparative analysis of bitter and sweet *Lupinus albus* accessions, all of which are food-grade and derived from diverse geographical origins, to investigate how chemotypic differences shape morphological and biochemical traits relevant to nutrition and functionality. Our primary objective was to characterize and contrast the profiles of key metabolites, including alkaloids, phenolics, flavonoids, and fatty acids, between these two chemotypes. Following this biochemical characterization, we applied Principal Component Analysis (PCA) to explore how accessions cluster according to their geographical origin and compositional variability. Finally, we performed correlation analyses to examine the relationships among morphological and biochemical traits, with a particular focus on identifying trait associations linked to the bitter or sweet chemotypes. This integrative approach enables a nuanced understanding of intraspecific diversity in *L. albus*, highlighting key metabolites and morphological markers that may serve as selection criteria in breeding programs. By providing a comprehensive comparative framework, this work contributes to the valorisation of *Lupinus albus* as a functional food crop and supports the development of ideotypes that align nutritional quality with agro ecological resilience.

2. Material and Methods

2.1. Plant material

The plant material employed in this study comprised eight accessions of cultivated and commercial white lupin (*Lupinus albus* L.), originating from geographically and environmentally diverse regions, including Tunisia, Algeria, Egypt, Italy, and France (Figure 1). This selection was designed to capture a broad spectrum of genetic variability, representative of both Mediterranean and temperate agro ecological conditions. The detailed origin and classification of each accession are provided in Table 1 and illustrated in Figure 1.

2.2. Morphological characteristics

To evaluate the morphological traits of *Lupinus albus* seeds, a controlled experiment was carried out at the Laboratory of Chemistry of Natural Molecules, University of Liège, Gembloux Agro-Bio Tech, Belgium. Seeds were manually scarified to improve germination efficiency. Each accession was cultivated in 3-liter pots with a standardized peat-sand mixture (3:1, v/v). The experimental design included three replicates per genotype, each replicate consisting of 10 plants, resulting in a total of 30 plants per accession (10 plants × 3 replicates). Plants were maintained under controlled conditions (25 ± 2 °C, 16/8 h light/dark photoperiod, 60–70% relative humidity, and a light intensity of 200 µmol m^{-2} s^{-1}) with weekly irrigation of 250 ml per pot. This study focused on 18 traits, including 15 quantitative and 3 qualitative, covering both vegetative (e.g., leaf, stem) and reproductive (e.g., flower, pod) characteristics. Measurements were taken at five stages: vegetative, flowering, growth, green maturity, and full maturity. Observations followed by IBPGR and UPOV *Lupinus* descriptors. A summary of the evaluation data is presented in Table 2.

2.3. Reagents and standards

All reagents were of analytical grade. Methanol (LC–MS grade, ≥99.9%) and acetonitrile (≥99.9%) were obtained from Merck (Darmstadt, Germany). Most solvents and chemicals [n-hexane, chloroform, HCl (37%), NH_4OH, formic acid (≥98%), sodium carbonate, Na_2SO_4, potassium ferricyanide, trichloroacetic acid, ferric chloride, potassium persulfate, linoleic acid, Tween 40, Folin–Ciocalteu reagent, and BF_3/MeOH (14% w/v)] were supplied by Sigma-Aldrich (St. Louis, MO, USA). Analytical standards included a certified FAME mix, individual amino acids, pure alkaloids, phenolics (gallic acid, rutin, quercetin), and antioxidants [Trolox, DPPH, ABTS, β-carotene, BHT], as well as lipoxygenase (Type I-B, soybean), all from Sigma-Aldrich. Nitrogen standards for Kjeldahl analysis were purchased from FOSS (Hillerød, Denmark), Potato Dextrose Agar (PDA) from Condalab (Madrid, Spain), and ultrapure water (18.2 MΩ·cm) was produced with a Milli-Q system (Millipore, Bedford, MA, USA).

2.4 Nutritional traits

2.4.1 Fatty acid analyses

Oil extraction was extracted from 5 g of finely ground lupin seeds using a Soxhlet extractor (VELP Scientifica, Italy) and a hydraulic press (200 MPa, 30 min), following the method of Saha Tchinda et al. (2018). The oil was stored at 4°C, and its content was determined gravimetrically after solvent evaporation under reduced pressure at 40°C using a rotary evaporator (BUCHI R-114). Extraction yield was calculated as:

$$\textbf{Yield (\%)} = \textbf{(Extracted oil mass / Dried powder mass)} \times \textbf{100}$$

Fatty acid methyl esters (FAMEs) were prepared by transesterification of 10 mg of oil using BF_3/methanol and heated at 70°C for 1.5 hours. FAMEs were extracted with n-hexane.

For the profiling, the fatty acids were analysed using gas chromatography with flame ionization detection (GC-FID) and mass spectrometry (GC-MS). An RT-2560 capillary column was used, and identification was based on retention times and mass spectra compared to certified FAME standards and reference libraries (Wiley, Mass-Finder, Adams), as described by Bettaieb et al. (2019) (Figure S1).

2.4.2 Amino acid analysis by HPLC

Proteinogenic amino acids were quantified by acid hydrolysis followed by HPLC separation. Samples were hydrolysed with 6N HCl containing 0.1% phenol at 110°C for 24 hours. After pH adjustment to 2.2, amino acids were separated using a cation-exchange column with a stepwise gradient of pH, ionic strength, and temperature. Ninhydrin post-column derivatization enabled spectrophotometric detection at 570 nm (440 nm for proline). Aspartate and glutamate were converted to their acidic forms, while tryptophan was degraded, requiring alternative methods. Sulfur-containing amino acids were partially degraded, limiting their quantification. Quantification was performed using certified standards of 21 amino acid, with norleucine as an internal standard (Figure S2). Results were expressed a $g \cdot 100\ g^{-1}$ of fresh sample. The procedure followed Henderson et al. (2000).

2.4.3. Protein analysis

Lupin seeds from different varieties were oven-dried at 45°C for 24 hours and subsequently ground using a 1 mm mesh sieve to obtain a uniform powder for analysis. The nitrogen (N) content of the samples was determined using the Kjeldahl method with a FOSS system (FOSS, Denmark), as described by Ezeagu et al. (2002). Crude protein (CP) content was then calculated by multiplying the nitrogen concentration by the conventional conversion factor of 6.25. In addition, the amino acid composition of the samples was evaluated to assess their nutritional profile.

2.5. Phenolic and alkaloid compounds analysis

2.5.1. Extraction of phenolic compound*s*

Phenolic compounds were extracted following Krakowska et al. (2017), with slight modifications. Dried plant material (0.5 g) was macerated in 80:20 methanol solution for 16 hours at room temperature. After centrifugation (5000 rpm, 10 min, 4°C), the supernatant was filtered and evaporated under reduced pressure at 40°C, then reconstituted in methanol to a 20 mg/mL stock solution. Extracts were stored at 4°C in the dark.

2.5.1.1 Determination of total phenolic content (TPC)

TPC was determined using the Folin-Ciocalteu method (Odabasoglu et al., 2004). 100 µL of extract was mixed with 1 mL of Folin-Ciocalteu reagent and after 5 minutes, 1 mL of sodium carbonate solution was added. The mixture was incubated for 30 minutes at room temperature, and absorbance was measured at 765 nm, using a UV–Vis spectrophotometer (Specord 200 Plus, Analytik Jena AG, Jena, Germany). Gallic acid was used for the calibration curve (0–100 µg/mL), yielding a regression equation $y = 0.0092x + 0.0254$ and $R^2 = 0.9987$. Results were expressed as mg GAE/g DW. All analyses were done in triplicate.

2.5.1.2 Determination of total flavonoid content (TFC)

Total flavonoid content (TFC) was determined using a modified aluminium chloride method (Stanković, 2011). 500 µL of extract was mixed with 500 µL of 2% $AlCl_3$ solution in methanol and incubated for 30 minutes at room temperature in the dark. Absorbance was measured at 420 nm using a UV-visible spectrophotometer. Rutin was used for the calibration curve (0–100 µg/mL), with the regression equation $y = 0.0125x + 0.0189$ and $R^2 = 0.9978$. TFC was expressed as mg rutin equivalents per gram of dry weight (mg RE/g DW). All experiments were done in triplicate.

2.4.2 Alkaloid extraction

Alkaloid extraction from defatted *Lupinus* seed powder was performed using a modified acid–base protocol derived from classical phytochemical methodologies (Saha Tchinda et al., 2018), optimized to maximize yield and purity. Precisely 10 g of finely ground, defatted seed material were macerated in 100 mL of 1 N hydrochloric acid (HCl) under continuous magnetic stirring for 6 hours at ambient temperature, in the dark, to ensure effective solubilization of alkaloid salts into the aqueous phase. The acidified mixture was centrifuged at 5000 rpm for 10 min at 4 °C using an Eppendorf 5418 R centrifuge (Eppendorf, Hamburg, Germany) to remove particulate matter. The resulting supernatant was carefully collected, and the pH was adjusted to 9.5 using concentrated ammonium hydroxide (NH_4OH), facilitating the conversion of protonated alkaloids into their lipophilic free base forms. The alkaline extract was transferred to a separatory funnel and subjected to liquid–liquid extraction with chloroform (3 × 50 mL). The pooled organic layers were dried over anhydrous sodium sulfate (Na_2SO_4), filtered, and evaporated under reduced pressure at 40 °C using a Rotavapor R-114 (BÜCHI, Switzerland), yielding the crude alkaloid fraction. For chemical profiling and identification, the dried alkaloid residue was redissolved in 1–2 mL of chloroform, filtered through a 0.22 µm PTFE syringe filter, and subjected to gas chromatography–mass spectrometry (GC–MS) analysis. A 1 µL aliquot was injected into a GC system (HP 6890, Agilent Technologies, USA) equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm)**.** Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The oven temperature program was as follows: initial hold at 70 °C for 2 minutes, ramping at 10 °C/min to 280 °C, and held at the final temperature for 10 minutes. Mass detection was performed using a mass selective detector (5972 MSD, Agilent Technologies, USA) operating in electron ionization (EI) mode at 70 eV. Alkaloids were identified based on comparisons of retention times and mass fragmentation patterns with those of standards and reference spectra from the NIST and Wiley mass spectral libraries (Figure S3). Quantification was achieved using external calibration curves prepared from pure alkaloid standards, and results were expressed as a percentage of alkaloid relative to defatted seed powder (%). All extractions and analyses were performed in triplicate to ensure methodological accuracy and reproducibility.

2.5. Biological activities

2.5.1. Antioxidant activities

Antioxidant activity of *Lupinus* seed methanolic extracts was profiled through four complementary in vitro assays.

2.5.1.1. DPPH free radical scavenging

The antioxidant activity of *Lupinus* seed extracts was assessed using the DPPH assay (Sánchez-Moreno et al., 1999). A 0.2 mM DPPH solution was mixed with 80% methanol and 0.1 mM Trolox (standard antioxidant). Test extracts were added, and the mixture was incubated in the dark at room temperature for 30 minutes. Absorbance at 517 nm was measured using a UV–visible spectrophotometer (Specord 210 Plus, Analytik Jena, Germany). Radical scavenging activity was expressed as the percentage of DPPH inhibition, and antioxidant capacity was quantified as Trolox Equivalent Antioxidant Capacity (TEAC) in mg TE/g extract.

2.5.1.2. Ferric reducing antioxidant power (FRAP)

The reducing power of *Lupinus* seed extracts was determined using the FRAP assay (Hseu et al., 2008; Lee et al., 2011). Extracts (25–100 µg/mL) were mixed with phosphate buffer and potassium ferricyanide, incubated at 50°C for 20 minutes, then treated with trichloroacetic acid and centrifuged. The supernatant was mixed with ferric chloride, and absorbance at 700 nm was recorded using a UV-Vis spectrophotometer (Specord 210 Plus, Analytik Jena, Germany). Increased absorbance indicated higher ferric ion reducing capacity. All measurements were performed in triplicate.

2.5.1.3. ABTS Radical Cation Decolorization Assay

The ABTS radical scavenging activity of *Lupinus* seed extracts was evaluated using the method by Loizzo et al. (2019), with Trolox as a reference antioxidant. ABTS radicals were generated by reacting ABTS with potassium persulfate, incubated in the dark for 16 hours. The $ABTS^{+}$ solution was diluted to achieve an absorbance of 0.70–0.75 at 734 nm. Extracts were mixed with the ABTS solution and incubated for 6 minutes at 30°C, and absorbance at 734 nm was measured. Antioxidant activity was expressed as IC_{50} values and converted to Trolox Equivalent Antioxidant Capacity (TEAC) in mg TE/g extract.

2.5.1.4. β-Carotene bleaching assay

The antioxidant capacity of *Lupinus* seed extracts was assessed using the β-carotene–linoleic acid bleaching assay (Kulisic et al., 2004; Tepe et al., 2005). β-carotene, linoleic acid, and Tween 40 were emulsified to create an oil-in-water emulsion. *Lupinus* seed extracts (2 g/L) were added, and the mixture was incubated under light at room temperature for 48 hours. Oxidation was monitored at 490 nm. Antioxidant efficacy was calculated as Relative Antioxidant Activity (RAA), comparing the β-carotene degradation rate to the synthetic antioxidant BHT.

2.5.2. Anti-inflammatory activity: Lipoxygenase inhibition

The anti-inflammatory activity of *Lupinus* seed extracts was assessed by measuring lipoxygenase (LOX) inhibition (Evelyne et al., 2019). Enzyme extracts were prepared by homogenizing lupin seeds in sodium phosphate buffer (pH 6.8) with stabilizing agents, then centrifuged and dialyzed overnight. LOX activity was measured by incubating extracts (25–100 µg/mL) with commercial LOX enzyme and linoleic acid as the substrate. The formation of conjugated dienes was monitored at 234 nm. Inhibition was calculated as the percentage decrease in absorbance compared to the control. Quercetin was used as a positive control, and IC_{50} values were determined for each sample.

2.5.3. Antifungal activity

2.5.3.1. Fungal isolates

The antifungal activity of alkaloid-rich extracts from *Lupinus* seeds was tested against phytopathogenic fungi, including *Alternaria alternata*, *Aspergillus flavus*, *Cladosporium cladosporioides*, *Colletotrichum acutatum*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Fusarium poae*, and *Penicillium expansum*. Fungal isolates were obtained from recognized culture collections, ensuring taxonomic accuracy (De Melo Nazareth et al., 2019; Stracquadanio et al., 2020, 2021; Riolo et al., 2023a, 2023b).

2.5.3.2 Determining the antifungal activity of volatile organic compounds (VOCs) released by alkaloids extract

Antifungal activity of volatile organic compounds (VOCs) from each alkaloid extract was assessed following La Spada et al. (2021) with modifications. A 5 mm agar plug from a 7-day-old fungal culture on PDA was placed at the center of a 9 cm PDA plate. Each extract (100 µL of 0.5% water agar) was applied to a sterile slide positioned in the dish lid; dichloromethane (DCM, 100 µL) served as the control. After incubation, the anti-fungal activity of the VOCs released by each alkaloid extract was evaluated as the percentage of inhibition of mycelial growth, calculated by using the following formula:

$$I(\%) = (D1-D2) / D1 * 100$$

Where I (%) represents the percentage of growth inhibition, D1 is the mean diameter of the fungal colony in the control, and D2 is the mean diameter of the fungal colony grown in the presence of VOCs released by the alkaloid extract. The experiment was repeated three times for each alkaloid extract × fungal pathogen tested.

2.6. Statistical analysis

Statistical analyses were conducted to identify morphological and biochemical traits distinguishing bitter and sweet *Lupinus albus* seeds. One-way ANOVA ($p < 0.05$) assessed trait differences, PCA on significant variables examined accession clustering, and Pearson's correlations evaluated trait associations. All analyses and visualizations were performed in R (v4.2.1; R Core Team, 2022) using Rcmdr, Agricolae, FactoMineR, plotly, factoextra, ade4, ggplot2, corrplot, ggcorrplot, and plotrix for statistical modelling, multivariate analysis, and data visualization.

3. Results & discussion

3.1 Morphometric analysis

The morphometric traits were classified into two categories: natural selection–related traits, which capture ecological adaptations, and domestication-related traits, which reflect human-driven selection during crop improvement.

In terms of natural selection traits, comparative evaluation of phenological traits revealed that bitter (Gba) accessions exhibited accelerated development compared to sweet (GBb) ones. Bitter accessions initiated reproductive growth earlier, with the date of first bud appearance (DFBA) and time to flowering (TF) advancing by approximately 17 and 12 days, respectively, relative to sweet accessions ($p < 0.05$) (Table 3). This shift persisted through development, with TGR and TR occurring nearly four weeks earlier at maturity (Table 3). Such precocity reflects adaptive strategies shaped by natural selection, indicating that bitter lupins have retained adaptive strategies to escape terminal drought and heat stress in Mediterranean and semi-arid environments (kabtni et al., 2020a; Berger & Ludwig 2014; Berger et al., 2012; Annicchiarico et al., 2010; Clements et al., 2005)

In parallel, bitter *Lupinus albus* accessions demonstrated greater vegetative vigor than sweet ones, with increased plant height (HVS, HGR), higher inflorescence insertion (HFS, HIFI), and longer inflorescences (LINF) ($p<0.01$) (Table 3). These results are associated with more rapid canopy establishment, improved light interception and enhanced early biomass accumulation (Ashrei et al., 2018). Such vegetative vigour is consistent with natural selection for photosynthetic efficiency and early growth, although its adaptive value may vary with environmental context and cropping system (Gao et al., 2023; kabtni et al., 2020a).

Bitter lupins showed greater vegetative vigour, with larger leaf area (SF), and elongated leaflets (LS1F), traits that accelerate canopy closure and biomass gain, suggesting a photosynthetic advantage neglected in low-alkaloid breeding. However, the length-to-width ratio of cotyledons (Lslcotyl) did not exhibit a statistically significant difference between bitter and sweet seeds ($p > 0.05$), suggesting that this trait cannot be considered a reliable marker for distinguishing *Lupinus* accessions based on seed taste.

Bitter accessions demonstrated clear reproductive advantages, producing more pods (Npinf) and heavier seeds (G100sw) (Table 3), traits previously identified as key breeding targets in *L. albus* (Pospišil et al., 2022). Pod length (PL) was also slightly greater, while seed morphology alone proved unreliable for predicting alkaloid content (Lucas et al., 2015). While these traits enhance both fitness and agronomic performance, their association with bitterness more likely reflects indirect selection and evolutionary trade-offs than intentional human choice (Abd-Elsamei et al., 2025). This highlights the complexity of *Lupinus* domestication and calls for breeding strategies that integrate biochemical profiling with morphological selection to capture both food safety and adaptive potential.

Indeed, breeding in *Lupinus* has historically focused on reducing quinolizidine alkaloids to eliminate bitterness, improving edibility but neglecting resilience and key phenological traits including flowering time, maturity, and growth cycle plasticity (Frick et al., 2018). Many of these overlooked traits are intimately connected to the plant’s adaptive capacity and, in some cases, to alkaloid metabolism itself (Osorio & Till, 2022). Breeding should balance quality with resilience by integrating phenology, adaptability, omics, and wild germplasm without reintroducing bitterness. In particular, precocity should be explicitly incorporated into breeding agendas, as early-flowering and early-maturing genotypes offer greater escape from drought and heat stress, enhance yield stability, and increase the suitability of lupins for diverse agroecological contexts. To uncover the hidden trade-offs shaped by past breeding choices, a principal component analysis was performed to clarify the relationships between phenological and morphological traits across accessions.

3.2. Morphometric and intraspecific variability: insights from principal component analysis

A Principal Component Analysis (PCA) was performed on diverse *Lupinus albus* accessions to evaluate morphometric differentiation and intraspecific variability based on seed and pod traits that differed significantly between bitter and sweet genotypes. The first three principal components explained a cumulative variance of 80.18%, with PC1 accounting for 52.74%, PC2 for 19.43%, and PC3 for 8.01% (Figure 2). This dimensional reduction revealed four distinct phenotypic clusters, each defined by specific trait combinations and geographic origins (Figure 2). While taste trait was not a variable in the PCA, the bitter accessions (Clusters 1 and 4) were separated from the sweet accessions (Clusters 2 and 3) along PC2, which discriminated between accessions based on green leaf colour intensity prior to bud emergence (IGR) and flower wing colour (CWw).

Cluster 1 comprised bitter accessions from France, Egypt, and Tunisia, positioned in the negative regions of PC1 and PC2. PC3 further separated two subgroups (Figure 2a1): one of Egyptian and some French accessions (red and blue) with early phenology (TR, TFBA), vigorous growth (SF, HVS), and higher yield potential (Npinf, G100sw), and another of Tunisian and some French accessions (green and blue) associated only with white-violet flower colour (CWwv). This substructuring reflects geographic origins shaping phenotypic diversity even within the same bitter group.

Sweet accessions (Clusters 2 and 3, squares in Figure 2a2–a3) occupied the negative PC1 and positive PC2 axes and were separated along PC3. Cluster 2, comprising Egyptian and Tunisian accessions, formed a compact group characterized by medium green leaves (IGRm) and white flowers (CWw). Cluster 3, consisting of Algerian accessions, was distinguished by taller floral axes (LINF) and earlier phenological development (TR, TGR, TFBA). These patterns indicate that bitter forms were not merely residual landraces but were actively selected for adaptive traits such as precocity and reproductive vigour, showing that domestication involved more than alkaloid reduction.

Cluster 4 consisted of Italian bitter accessions, represented by purple dots, which were distinctly separated from all other groups, located in the positive quadrant of PC1 and the negative quadrant of PC2 (Figure 2a). Similar to Cluster 1, Cluster 4 consists of bitter accessions uniquely characterized by CWwv and IGRD traits, thereby serving as distinctive markers of bitterness. However, cluster 4 exhibited unique morphometric traits, reflecting genetic divergence, local adaptation, or specific breeding history. Key traits contributing to their separation along PC1 included foliar surface area (SF), pod number per plant (Npinf), vegetative height (HVS). Within this heterogeneous cluster, PC3 revealed further structuring (Figure 2, a4), suggesting enhanced vegetative and reproductive development. Cluster 4 represents a potentially valuable genetic resource for future breeding programs due to its specific morphological traits.

PCA of *Lupinus albus* germplasm revealed strong morphometric structuring aligned with geographic origins, driven by vegetative, phenological, and inflorescence traits. Morphological markers, indirectly linked to alkaloid content, effectively separated bitter and sweet accessions. Bitter types showed higher intra-cluster variability, reflecting local adaptation, while sweet accessions were more uniform due to domestication. The distinct Italian bitter cluster represents a valuable genetic resource, combining bitterness markers with favourable agronomic traits. These results underscore the importance of conserving *L. albus* diversity and demonstrate the cost-effectiveness of morphological markers for breeding nutritionally safe accessions.

3.3. Nutritional values analysis

3.3.1 Oil content analysis and fatty acid profile

Comparative analysis of bitter (Gba) and sweet (GBb) *Lupinus albus* accessions indicated that total oil content did not differ significantly between groups, whereas significant differences were detected in their fatty acid composition (Table 4). The bitter accessions exhibited a slightly higher mean oil content than the sweet genotype, indicating that both genotypes possess comparable potential for oil extraction. These results contrast with those of Sbihi et al. (2013), who reported higher oil content in sweet lupin seeds relative to bitter ones. Such discrepancies likely reflect genotypic variation, environmental effects, seed maturity at harvest, or methodological differences in extraction procedures (Cabrita et al., 2024; Aniszewski, 2007; Boschin et al., 2008). This variability underscores the critical importance of assessing genotype–environment interactions when evaluating oil yield potential across *Lupinus* germplasm.

Regarding the qualitative fatty acid profiles, significant differences were identified in the saturated fatty acid (SFA) composition between bitter and sweet *Lupinus albus* accessions (Figure 3a, Table 4). Stearic acid (C18:0) content was significantly higher in bitter seeds compared to sweet seeds ($p = 0.0438$), a difference that may enhance the oxidative stability of the oil (Grundy et al., 2016). Similarly, behenic acid (C22:0) was significantly more abundant in bitter accessions relative to sweet ones ($p = 0.0153$). These long-chain SFAs are particularly valued for industrial applications owing to their favourable rheological properties and high oxidative resistance (Gunstone, 2011). In contrast, although palmitic acid (C16:0), arachidic acid (C20:0), and lignoceric acid (C24:0) contents were slightly elevated in bitter accessions, the differences were not statistically significant (Figure 3a, Tables 4).

Monounsaturated fatty acids (MUFA), particularly oleic acid (C18:1), predominated in both bitter and sweet seeds (Figure 3a), with no significant differences (Tables 4). Oleic acid, recognized for its cardioprotective properties and high thermal stability, reaffirms the nutritional suitability of *Lupinus albus* oil for edible applications (Dumancas et al., 2017). In contrast, erucic acid (C22:1n9), a MUFA of industrial importance but dietary concern, was significantly higher in bitter lupins compared to sweet genotypes (Tables 4, Figure 3a). Although these concentrations remain below internationally accepted safety thresholds (European Food Safety Authority, 2016), the slightly elevated erucic acid levels in sweet accessions suggest that bitter genotypes may be preferable for food applications, particularly for products targeted at vulnerable populations such as infants or individuals with specific health conditions.

Linoleic acid (C18:2) levels were similar between bitter and sweet *Lupinus albus*, whereas α-linolenic acid (C18:3, ω-3) was significantly higher in bitter seeds, improving the ω-3/ω-6 ratio and indicating superior cardioprotective potential (Simopoulos, 2002). This trait supports the use of bitter genotypes in functional foods targeting lipid metabolism and chronic disease prevention. The absence of docosadienoic acid (C22:2) in both types' points to a shared genetic limitation in fatty acid elongation or desaturation (Boschin et al., 2006). These results highlight bitter accessions as reservoirs of allelic variation and biochemical attributes, not suitable for direct consumption, but exploitable as donor germplasm for the development of nutraceutical ingredients and as breeding resources to introduce favourable lipidomic profiles into sweet cultivars. Our results confirm earlier findings by Erbaş et al. (2005) on the predominance of oleic acid in *L. albus* oil, but extend this knowledge by revealing genotype-specific lipid profiles. Beyond differences in alkaloid content, bitter and sweet lupins also diverge in fatty acid composition (Khedr et al., 2024). Bitter accessions, enriched in α-linolenic, stearic, erucic, and behenic acids, are suited for industrial uses such as nutraceuticals, cosmetics, lubricants, and specialty polymers (Cerone & Smith, 2021). In contrast, sweet accessions with low erucic acid levels comply with food safety standards and are therefore more suitable for direct consumption, providing favourable nutritional profiles that support their use in flours, bakery products, dairy analogues (Nazan, 2022), and meat substitutes (Sher et al., 2025).

3.3.2. Assessment of Nitrogen, protein content and amino acid composition

A comparative analysis was carried out between bitter and sweet *Lupinus albus* accessions to evaluate potential differences in nitrogen (%N) and protein (%Protein) seed content. The *ANOVA* revealed no statistically significant differences between the two varietal groups for these basic compositional traits (Table 5). These results indicate that, at least with respect to nitrogen and protein content, bitter and sweet lupine varieties exhibit comparable seed nutritional baselines.

Amino acid profiling of bitter and sweet *Lupinus albus* accessions revealed clear compositional divergence (Table 5). Among the 17 quantified amino acids, alanine (Ala) and valine (Val) were slightly elevated in sweet seeds, while lysine (Lys), a key precursor in quinolizidine alkaloid (QA) biosynthesis, showed a significant increase. These shifts suggest domestication-driven modulation of primary metabolism, selectively altering precursor pools to reduce alkaloid accumulation and improve seed quality. Such compositional adjustments reflect strong selection pressures and reveal metabolic trade-offs underlying lupin domestication.

3.4. Comparative analysis of the alkaloid

The comparative profiling of QAs revealed profound chemotypic divergence between bitter and sweet genotypes (Figure 3b). Expressed as relative proportions (% of total alkaloids), the data demonstrated not only a substantial reduction in total QA content in sweet accessions but also a striking qualitative remodelling of the alkaloid spectrum. This transformation likely reflects the combined effects of targeted breeding, metabolic reprogramming, and underlying genetic polymorphisms in the QA biosynthetic pathway (Kroc et al., 2019).

In bitter accessions, lupanine predominated, representing 65.64 ± 21.93% of the total alkaloid fraction, consistent with previous findings (Kroc et al., 2017). This dominance reflects the canonical lysine-derived QA biosynthetic pathway, wherein lupanine is synthesized through intermediates such as tetrahydroanabasine and 17-oxosparteine, under the catalytic action of lysine decarboxylase (LDC) and 17-oxosparteine synthase (OS) (Cely-Veloza et al., 2023a; Bunsupa et al., 2012; Frick et al., 2017). In addition, sparteine, a neurotoxic tetracyclic QA (Boschin et al., 2008a) accounted for 10.17 ± 6.62% of the total QA pool, further confirming the characteristic toxic chemotype of bitter *L. albus*. Conversely, sweet phenotypes exhibited a dramatic depletion of lupanine (Table 4), accompanied by the near-complete loss of sparteine and multiflorine. These reductions were highly significant ($p < 0.001$) and suggest a concerted suppression of multiple branches of QA biosynthesis. Transcriptomic studies corroborate this view, revealing substantial downregulation of key QA pathway genes in low-alkaloid cultivars (Święcicki et al., 2019; Otterbach et al., 2019b). Similarly, α-isolupanine, an isomer and biosynthetic intermediate of lupanine, was significantly reduced ($p = 0.013$), reinforcing the hypothesis of widespread enzymatic silencing. This pattern reflects a key domestication trait in *L. albus*, as human selection has historically targeted low-alkaloid (sweet) chemotypes to improve seed edibility and nutritional quality through the introgression of recessive alleles such as pauper (Rychel & Kasiążkiewicz, 2019). A particularly remarkable feature of sweet accessions was the hyperaccumulation of 11,12-seco-12,13-didehydromultiflorine, which constituted 93.16 ± 3.84% of their alkaloid profile. This seco-alkaloid, likely originating from oxidative cleavage or spontaneous degradation of multiflorine or related precursors, was minimally present in bitter accessions. Its disproportionate abundance suggests a strategic metabolic rerouting favouring the detoxification of classical QAs (Kroc et al., 2017). However, the precise biosynthetic origin, chemical stability, and toxicological relevance of 11,12-seco-12,13-didehydromultiflorine remain poorly understood, warranting urgent further investigation.

Multiflorine was significantly ($p < 0.001$) undetectable in sweet seed, suggesting strong selective pressure against its biosynthesis, potentially via mutations or deletions in pathway-specific genes. Sparteine, a tetracyclic QA known for its neurotoxicity (Flores-Soto et al., 2006), showed a similar trend: it accounted for 10.17 ± 6.62% of the total QA fraction in bitter *Lupinus albus* accessions but was nearly absent in sweets phenotypes (Figure 3b, Table 4), confirming the success of breeding strategies aimed at reducing toxic alkaloid levels. In contrast, minor QAs such as oxylupanine, 11,12-dehydrolupanine, and 13-hydroxyl-oxylupanine displayed low and statistically non-significant differences between genotypes, likely reflecting minimal biosynthetic flux or stochastic metabolic variation. A particularly striking and unexpected biochemical feature of the sweet phenotypes was the hyperaccumulation of 11,12-seco-12,13-didehydromultiflorine, which constituted the dominant alkaloid in sweet accessions (93.15 ± 3.84%), while its presence remained minimal in bitter phenotypes (Figure 3b), a difference that was statistically highly significant (Table 4). This seco-alkaloid likely arises from oxidative cleavage or spontaneous degradation of multiflorine or related precursors. Its disproportionate abundance in sweet seeds suggests a metabolic redirection away from toxic alkaloids, possibly serving as a detoxification mechanism (Kroc et al., 2017). These findings align with previous studies by Boschin et al. (2008b) and Kordan et al. (2012), which demonstrated that bitter *L. albus* accessions accumulate high QA concentrations dominated by lupanine, whereas sweet varieties undergo substantial phytochemical remodelling, replacing classical QAs with structurally modified derivatives like 11,12-seco-12,13-didehydromultiflorine. This metabolic reprogramming illustrates the profound impact of genetic selection on the secondary metabolite landscape of *L. albus*, with significant implications for food safety and the breeding of cultivars adapted for human and animal consumption.

Sweet *Lupinus albus* accessions showed a strong reduction in total quinolizidine alkaloids (QAs) and a marked qualitative shift in alkaloid composition (Figure 3b), reflecting the profound effect of domestication on secondary metabolism. While selection for low QA content improved seed palatability and food safety, secondary metabolism was not eliminated but reshaped to balance agronomic goals with ecological defence (Klčová et al., 2024; Hama & Strobel, 2020). The pauper locus on chromosome 18 is linked to low alkaloid content, yet its molecular basis remains unresolved, and QA regulation appears polygenic with epistatic influences (Rychel & Książkiewicz, 2019; Cely-Veloza et al., 2023a; Osorio & Till, 2022). These findings highlight the need for integrative breeding approaches leveraging genomic, pangenomic, and transcriptomic data to achieve stable low-QA phenotypes across environments.

3.5. Comparison of total phenolic and flavonoid content

Total phenolic content (TPC) was significantly higher in sweet *L. albus* genotypes (165.74 ± 76.12 mg GAE·100 g^{-1} DW) than in bitter ones (94.77 ± 35.36 mg GAE·100 g^{-1} DW) ($p < 0.05$; Figure 4), indicating a nearly twofold enrichment. This pattern suggests a metabolic trade-off: bitter accessions, enriched in QAs (Rahim et al., 2023), likely channel resources toward alkaloid biosynthesis, whereas sweet chemotypes, with reduced QA levels, appear to redirect flux into the phenylpropanoid pathway, enhancing phenolic accumulation and antioxidant potential (Mancinotti et al., 2022). Beyond their secondary role in defence, phenolic compounds enhance stress tolerance through the regulation of redox homeostasis. (Hilal et al., 2024; Oomah et al., 2006). Reported TPC values, however, vary widely across germplasm and studies (Wang & Clements, 2008; Karamać et al., 2018), reflecting genetic background, environment, developmental stage, and methodological differences in extraction and quantification (Siger et al., 2012, kabtni et al., 2020b).

Similarly, total flavonoid content (TFC) was significantly higher ($p < 0.001$) in sweet seed extracts relative to bitter ones (Figure 4), indicating enhanced phenylpropanoid flux and providing alternative defences in low-alkaloid genotypes, while also contributing antioxidant and health-promoting properties (Pinheiro et al., 2004; Karamać et al., 2018). Antagonistic regulation between phenylpropanoid and alkaloid pathways, also reported in other legumes (Agati et al., 2012; Cheynier et al., 2013), suggests a conserved metabolic trade-off. Comparable patterns have been observed in other legumes; for example, *Glycine soja* (wild soybean) accessions consistently display substantially higher flavonoid and phenolic concentrations than their domesticated counterpart *G. max* (Chen et al., 2021), reflecting a domestication-driven shift that favours agronomic traits over secondary metabolite diversity. Similarly, wild chickpea (*Cicer reticulatum*) genotypes exhibit greater antioxidant capacity compared to cultivated *C. arietinum* forms (Kaur et al., 2019). These cross-species comparisons reinforce the hypothesis that selection for reduced antinutritional compounds during crop domestication may have inadvertently constrained metabolic investment in defence-related phenylpropanoids, while breeding for low-alkaloid lupins appears to have maintained or even enhanced phenolic defences.

3.6. Principal component analysis of biochemical traits in *Lupinus albu*s accessions

To explore the biochemical differentiation among sweet and bitter *Lupinus albus* accessions, a Principal Component Analysis (PCA) was conducted. The first three principal components accounted for 85.03% of the total biochemical variance, reflecting a strong dimensionality reduction and efficient capture of the major axes of biochemical variation. Specifically, PC1 explained 49.73% of the variance, PC2 captured 19.94%, and PC3 contributed 15.36% (Figure 5).

Principal Component Analysis (PCA) revealed four distinct biochemical clusters, reflecting the underlying chemotypic diversity among the analysed accessions (Figure 5). Clusters 1 and 2 predominantly comprised bitter genotypes (represented by circles), whereas Clusters 3 and 4 consisted exclusively of sweet genotypes (represented by squares). The primary axis of chemotypic discrimination was aligned with the first principal component (PC1), which exhibited strong positive loadings for lupanine, stearic acid (C18:0), linolenic acid (C18:3), erucic acid (C22:1n9), and docosanoic acid (C22:0). Accordingly, bitter accessions were positioned on the positive side of PC1, characterized by elevated lipid and alkaloid concentrations (Figure 5). In contrast, sweet accessions clustered on the negative side of PC1, associated with higher levels of total flavonoid content (TFC), 11,12-seco-12,13-didehydromultiflorine, and the amino acid valine. These segregation patterns highlight the central role of lipid remodelling and alkaloid biosynthesis in mediating the observed chemotypic divergence between bitter and sweet phenotypes, while also reflecting the impact of breeder-driven selection in shaping these contrasting metabolic profiles

Cluster 1, comprising bitter *Lupinus albus* accessions from France and Italy, was positioned in the positive quadrants of PC1, PC2, and PC3 (Figure 5, b1, b2). These accessions exhibited distinctly elevated levels of long-chain saturated and unsaturated fatty acids—including stearic acid (C18:0), linolenic acid (C18:3), behenic acid (C22:0), and erucic acid (C22:1n9)—as well as major alkaloids such as lupanine and sparteine, and the amino acids lysine and alanine (Figure 5). This distinctive biochemical signature suggests a metabolically robust chemotype shaped by extensive domestication and targeted breeding in France and Italy (Magalhães et al., 2017; Bunsupa et al., 2012; Boschin et al., 2007), where efforts have focused on optimizing lipid composition and alkaloid profiles. Such selection has enhanced both agronomic performance and adaptability, while simultaneously increasing the potential of these genotypes for nutritional and industrial applications, particularly in the food sector.

Cluster 2, in contrast, encompassed bitter accessions from Tunisia and Egypt and was primarily segregated along PC2. Accessions within this cluster occupied the positive side of PC1 and the negative side of PC2 (Figure 5), and were characterized by higher concentrations of isolupanine and multiflorine, distinguishing them from Cluster 1. Intriguingly, Cluster 2 exhibited a sub-structuring pattern along PC3, reflecting geographic differentiation (Figure 5, b2). The Egyptian subgroup, positioned on the negative side of PC3, was distinguished by increased total phenolic content (TPC). Conversely, the Tunisian subgroup, located on the positive side of PC3, was associated with elevated concentrations of alanine and lysine. This biochemical divergence suggests distinct metabolic adaptations potentially driven by environmental selection pressures or underlying genetic differentiation between North African *L. albus* populations (Siger et al., 2012; Karamać et al., 2018; Abraham et al., 2019).

Clusters 3 and 4, corresponding to the sweet-seed chemotypes, were clearly separated from the bitter clusters along the negative axis of PC1 and the positive axis of PC2 (Figure 5). This positioning reflects their lower total alkaloid and oil content but elevated levels of antioxidant and amino acids. Both clusters were distinguished from bitter clusters by significantly higher total flavonoid content (TFC), elevated level of valine (Val), and the alkaloid derivative 11,12-seco-12,13-didehydromultiflorine, suggesting a metabolic shift toward antioxidant-rich and nutritionally favourable profiles in sweet *L. albus* accessions (Figure 5). Further differentiation among sweet accessions was revealed along PC3 (Figure 5, b3,b4). Cluster 3, comprising sweet accessions from Egypt and Tunisia, was positioned in the negative region of PC3 (Figure 5, b3,b4). This subgroup was characterized from the other sweet group (Cluster 4) by high levels of total flavonoids, indicating an antioxidant-enriched chemotype. These adaptive strategies not only enhanced agronomic performance and environmental resilience but also illustrate the expression of active and adaptive plasticity in response to selective pressures

(Abraham et al., 2019). In contrast, Cluster 4, formed exclusively by Algerian sweet accessions, was located on the positive side of PC3 and exhibited a distinct biochemical fingerprint. This group was enriched in lysine and alanine, indicating a divergence toward a slightly more amino acid profile (Figure 5, b3,b4). In comparison to Cluster 3, comprising *L. albus* accessions from Egypt and Tunisia, the higher lupanine levels detected in Algerian genotypes indicate that some may retain intermediate alkaloid concentrations (Figure 5, b3,b4). This chemotypic profile may be of particular interest for breeding programs aiming to develop dual-purpose varieties with both nutritional value and enhanced pest resistance (Zafeiriou et al., 2021). This structuring of sweet-seed accessions into two metabolically distinct subgroups reinforces the influence of geographic origin and genetic background in shaping the biochemical landscape of *Lupinus albus* (Tosoroni et al., 2025). It also highlights the value of PC3 constructed by TPC, Alanine, and Lysine levels in uncovering subtle intra-chemotype variations that are otherwise obscured by dominant PC1 traits, such as alkaloids and fatty acids.

The orthogonality of morphometric and biochemical differentiation underscores the value of integrating both trait types in core collection design and breeding. Although sweetness was not included in the PCA, its alignment with biochemical clustering, particularly lower alkaloid and phenolic variation, suggests potential co-regulation or trade-offs in secondary metabolism. This warrants validation through molecular and transcriptomic studies.

3.7. Biological activities

3.7.1 Antioxidant activities

Despite significant differences in total phenolic content (TPC) between sweet and bitter *Lupinus albus* genotypes, only the FRAP assay revealed a statistically significant variation ($p < 0.05$) (Figure 4), with values of 4.60 ± 0.48 mg TE/g extract for the bitter genotype and 2.97 ± 0.47 mg TE/g extract for the sweet genotype. This suggests that the bitter seeds exhibit moderately higher reducing power. In contrast, ABTS, DPPH, and β-carotene bleaching assays showed comparable results across varieties (Table 5) reflecting the fact that different assays probe distinct antioxidant mechanisms (electron transfer vs. radical scavenging vs. lipid peroxidation). This dissociation highlights that TPC alone is an unreliable proxy for antioxidant capacity, as antioxidant efficacy is determined not merely by the overall abundance of phenolics but by their structural diversity, redox potential, and stability under physiological conditions (Deepa et al., 2013). Moreover, the bioavailability of individual compounds and their synergistic or antagonistic interactions with non-phenolic antioxidants (e.g., carotenoids, alkaloids, peptides) play decisive roles in shaping functional outcomes (Prior et al., 2005; Dai & Mumper, 2010). These considerations suggest that an over-reliance on bulk TPC measures risks oversimplifying antioxidant potential and obscuring the mechanistic basis of dietary benefits.

ABTS and DPPH assays revealed comparable radical scavenging activity between genotypes, suggesting that non-phenolic antioxidants such as flavonoids, tocopherols, and carotenoids may offset differences in TPC (Brand-Williams et al., 1995; Kotha et al., 2022; Fiedor & Burda, 2014). This aligns with previous reports of strong antioxidant activity in lupin seeds, though results across studies remain inconsistent (Tsaliki et al., 1999; Król et al., 2018). Such discrepancies are often methodological, driven by extraction protocols, solvent polarity, and analytical standards underscoring the need for harmonized assays to separate biological variation from artefacts. These findings highlight the multifactorial basis of antioxidant capacity and the limitations of using bulk indices such as TPC as proxies for functional quality (Kabtni et al., 2020b). Integrating targeted and untargeted metabolomics would provide a more comprehensive and mechanistic assessment of antioxidant potential in *L. albus*.

3.7.2. Anti-Inflammatory activities

Although the enzymatic activity of lipoxygenase (LOX) in lupins has been well characterized (Stephany et al., 2015; Yoshie-Stark & Wäsche, 2004), their potential as natural LOX inhibitors remains largely explored. In this study, LOX inhibition assays revealed a statistically significant difference ($p = 0.0144$) between the two chemotypes (Figure 4), highlighting genotype-specific variability in their capacity to interfere with lipid peroxidation pathways. The bitter genotype exhibited higher LOX inhibitory activity (408.46 ± 236.94 units/mg extractable protein) compared to the sweet genotype (191.11 ± 48.57 units/mg extractable protein). Despite the significance difference, the large standard deviation observed in bitter seeds indicates substantial intra-genotypic variability, highlighting the need for further investigation into the genetic regulation and phenotypic stability of this trait (García-Lafuente et al., 2009). These findings align with prior reports in other *Lupinus* species, such as *L. angustifolius* and *L. mutabilis* (Millan-Linares et al., 2014; Gamarra-Castillo et al., 2006), and reinforce the potential of *L. albus* as a promising source of functional food ingredients with anti-inflammatory properties. Future research should employ targeted metabolomic profiling combined with activity-guided fractionation to isolate and characterize the specific bioactive constituents responsible for LOX inhibition and validate their efficacy through in vivo models.

3.7.3. Antifungal potential of *Lupinus albus* extracts against pathogenic fungi

The antifungal bioassay conducted with alkaloid-rich extracts from bitter and sweet accessions of *Lupinus albus* revealed a markedly higher percentage of mycelial growth inhibition by the bitter variety across all tested fungal pathogens (Figure 6). The inhibitory effects were substantial, for instance, *Fusarium oxysporum* (57.93% ± 6.11 in bitter vs. 16.14% ± 1.41 in sweet), with comparisons showed highly significant differences ($p < 0.001$). Regarding F. *poae* (57.80 ±12.57% in bitter vs. 12.07±1.28 % in sweet). *F. oxysporum* and F. *poae* were moderately sensitive, with growth inhibition correlating with extract concentration. This is notable given their soilborne or grain-associated niches and involvement in systemic or toxigenic disease. The inhibition of *F. poae* is particularly relevant due to its role in contaminating cereals with trichothecenes such as T-2 toxin, which pose regulatory challenges in food safety (Witte et al.,2024; Wachowska et al.,2017; Ferrigo et al., 2016).

Comparative analysis between the two chemotypes revealed highly significant differences ($p < 0.001$) in antifungal activity against *Aspergillus flavus* (59.73 ± 8.64% inhibition with bitter seed extract vs. 15.69 ± 1.23% with sweet seed extract) and *Penicillium expansum* (45.39 ± 19.86% vs. 6.61 ± 0.73%, respectively) (Figure 6). Both fungal species, well known for their prolific mycotoxin production, aflatoxins by *A. flavus* and patulin by *P. expansum,* demonstrated moderate to high intrinsic resistance, consistent with previous reports attributing such tolerance to effective oxidative stress response systems and detoxification enzymes (Navale et al., 2021). Despite this inherent resilience, the bitter *L.albus* extracts elicited substantial inhibition, highlighting their potential utility as natural antifungal agents. These findings support the possible application of bitter chemotype-derived alkaloids in postharvest disease management, particularly as components of integrated or hurdle-based preservation strategies aimed at reducing fungal spoilage and mycotoxin contamination in food chains

Colletotrichum acutatum (58.33 ± 11.56% inhibition with bitter seed extract vs. 21.21 ± 2.55% with sweet extract) and *Cladosporium cladosporioides* (59.16 ± 10.09% vs. 15.69 ± 1.23%, respectively) displayed markedly higher antifungal susceptibility to the bitter *Lupinus albus* extract (Figure 6). Statistical comparisons between the two chemotypes confirmed highly significant differences in antifungal activity ($p < 0.001$). Although both fungal species are typically classified as causing intermediate levels of infection and postharvest spoilage (Saleh & Al-Thani (2019). The stronger inhibition observed with bitter seed extracts suggests a chemotype-dependent effect. In particular, the hemibiotrophic lifestyle of *C. acutatum* may render it more vulnerable to lupanine-type alkaloids, which are hypothesized to interfere with key infection stages such as appressorium development and early host colonization (Damm et al., 2012). These findings support the need for further cytological and transcriptomic analyses to elucidate the underlying antifungal mechanisms

Alternaria alternata (55.08 ± 11.3% inhibition with bitter seed extract vs. 23.01 ± 2.73% with sweet extract) exhibited the highest sensitivity among all tested fungal species (Figure 6). This pronounced susceptibility may be attributed to its relatively thin cell wall and limited intrinsic resistance to membrane-disrupting phytochemicals, such as quinolizidine alkaloids (Cely-Veloza et al., 2023b). The strong antifungal activity observed is particularly significant given the agronomic importance of *A. alternata* as a pathogen of vegetables and fruit crops, where it causes black mold and leaf spot diseases that severely compromise product quality, marketability, and postharvest shelf life (Leyva Salas et al., 2017). These results suggest a promising application of *Lupinus albus* bitter seed extracts in integrated disease management strategies targeting *Alternaria*-induced disorders

This differential antifungal efficacy highlights a distinct chemotype-dependent response, potentially linked to both the higher total alkaloid concentration and the unique profile of bioactive quinolizidine alkaloids in the bitter variety as reported by Sbihi et al. (2013). Our results suggest that specific alkaloids or their synergistic combinations play a pivotal role in disrupting fungal growth via interference with membrane integrity, enzymatic inhibition, or oxidative stress induction (Sequin et al., 2023). These findings underscore the role of alkaloid-mediated chemical defence in *L. albus* and reinforce the potential of the bitter chemotype as a source of potent natural antifungal agents for biocontrol applications in agriculture and postharvest management (Dozio et al., 2025a). These findings are in agreement with the work of Cely-Veloza et al. (2022) and Romeo et al. (2018), who reported potent antifungal activity in *L. albus* extracts, potentially independent of varietal alkaloid profiles. The enhanced activity of the bitter chemotype highlights its promise as a source of natural antifungal agents. Further phytochemical investigations are necessary to identify and characterize the specific compounds underlying this selective antifungal efficacy, with potential applications in sustainable crop protection and food preservation strategies.

3.8 Integrated correlation analysis of biochemical, morphological and bioactive traits

3.8.1. Seed biomass reflects coordinated lipid and secondary metabolite biosynthesis

Correlation analysis revealed that seed weight (G100sw) was positively and strongly associated with several biochemical traits linked to oil quality and metabolic vigour (Figure 7). A strong negative correlation was observed between seed weight (G100sw) and phenological traits related to seed and flowering timing, namely time of green ripening (TGR; $r = –0.89$), time of first bud appearance (TFBA; $r = –0.63$), time of ripening (TR; $r = –0.83$), and time of flowering (TF; $r = –0.77$). Negative associations indicate that early-flowering, early-maturing genotypes produce heavier seeds, likely due to greater assimilate accumulation under favourable grain-filling conditions (Jansen et al., 2009; Ullah et al., 2024). Therefore, early phenological development appears to be a critical trait linked to superior seed quality and agronomic performance. Notably, G100sw was highly correlated with C18:3 (r = 0.74), C18:0 (r = 0.69), and C22:1n9 (r = 0.67), suggesting that seed mass is tightly coupled to lipid metabolism, particularly unsaturated fatty acid accumulation. The positive correlation with FRAP (r = 0.47) and lupanine (r = 0.60) also suggests that heavier seeds tend to accumulate higher levels of antioxidants and alkaloid defence compounds, possibly reflecting superior physiological maturity and storage capacity.

3.8.2. Coordinated regulation of early vegetative growth and specialized metabolism in *Lupinus albus*

Height at vegetative stage (HVS) exhibited a strong positive correlation with lupanine content (*r* = 0.75), while lupanine also correlated moderately with central leaflet shape (LslF, *r* = 0.67). These associations suggest a developmental co-regulation rather than a direct functional link. During the vegetative phase, metabolic resources are predominantly allocated to primary growth processes, cell proliferation, organ expansion, and vascular differentiation, while alkaloid biosynthesis remains secondary and largely confined to autotrophic tissues (Glenn et al., 2013). The observed correlations likely reflect the action of shared transcriptional regulators or hormonal networks (e.g., auxin, jasmonate) that simultaneously influence both growth traits and specialized metabolism (Kućko et al., 2020). Similarly, lupanine levels may reflect general vegetative vigor rather than being causally involved in it. Thus, while lupanine may serve as an indirect marker of architectural traits in *Lupinus albus*, its physiological role during vegetative development appears limited. Its predictive value for breeding should therefore be interpreted in light of broader developmental and regulatory contexts (Bunsupa et al., 2012).

3.8.3. Key metabolites driving antifungal potency in lupin

Antifungal activity in bitter *Lupinus* seed extracts was strongly correlated with lupanine (*C. acutatum*, r = 0.86; *A. alternata*, r = 0.72) and antioxidant capacity (FRAP, r = 0.68), suggesting that both alkaloid-mediated toxicity and redox-active metabolites contribute to efficacy. -chanistic interpretation that membrane disruption underlies antifungal potency remains speculative. This limitation is not trivial: as highlighted by Dozio et al. (2025 b), the development of natural or nature-derived alkaloid fungicides increasingly depends on rigorous mechanistic validation to translate correlations into actionable leads for crop protection. In this light, lupanine and associated metabolites in *Lupinus* may represent promising candidates, but their advancement as eco-friendly antifungal agents will require experimental strategies that directly test causality and optimize structure–activity relationships.

α-Linolenic acid (C18:3) and erucic acid (C22:1n9) correlated positively with antifungal activity against *F. oxysporum* (r = 0.61, 0.64), while C18:3 was also strongly associated with inhibition of *A. alternata* (r = 0.72), *A. flous* (r = 0.76), and *C. acutatum* (r = 0.77), indicating its potential as a biochemical marker for antifungal resistance. These findings align with metabolomic studies linking unsaturated fatty acids to stress responses (Ahmed et al., 2016) and warrant targeted validation.

The strong negative correlation between total flavonoid content and antifungal activity (*F. oxysporum–TFC*, r = –0.82) further suggests that extracts dominated by alkaloids are more antifungally potent than those with phenolic-rich profiles (Romeo et al., 2018). These findings suggest that antifungal potency in *Lupinus* is driven by a metabolic axis centred on lupanine, unsaturated fatty acids, and redox-active compounds, with trade-offs indicating prioritization of alkaloid-based defence. Targeted validation using microscopy, membrane assays, and activity-guided fractionation is needed to confirm these relationships and establish lupanine as a key biochemical marker of antifungal activity.

3.8.4. Metabolic Trade-Offs Between Bitter and Sweet Chemotypes

Bitter seeds exhibited strong positive correlations with alkaloids such as Lupanine (r = 0.89), Multiflorine (r = 0.62), and Sparteine (r = 0.64), as well as the fatty acid C22:1n9 (r = 0.69), and antioxidant capacity measured via FRAP (r = 0.79) (Figure 7). In contrast, sweet seeds displayed strong negative correlations with phenolic and flavonoid metabolites, including TFC (r = –0.95), TFBA (r = –0.73), and 11,12-seco-12,13-didehydromultiflorine (r = –0.997) (Figure 7). The strong negative correlation between bitter and sweet seeds suggests that these metabolites represent mutually exclusive metabolic states in *Lupinus albus*. This antagonism may result from metabolic competition for shared precursors or differential pathway regulation by environmental or developmental cues (Wink, 2008; Mithöfer & Boland, 2012). The strong positive association of GBa with major alkaloids (lupanine, multiflorine, sparteine) highlights its role in secondary metabolite biosynthesis and plant defence (Costa et al., 2013), whereas GBd's correlation with phenolics and flavonoids suggests involvement in antioxidative and signalling pathways (Agati et al., 2012; Harborne & Williams, 2000).

The divergence between GBa and GBd pathways suggests a metabolic trade-off, reflecting strategic allocation between alkaloid-based chemical defences and phenolic antioxidant systems (Caretto et al., 2015; Ncube et al., 2016). This metabolic plasticity underscores the dynamic stress-responsive strategies of *Lupinus albus* under biotic and abiotic stresses. These findings highlight the need for metabolomic and transcriptomic studies to elucidate the biosynthetic origins of GBa and GBd and their regulatory interplay. Deciphering these networks could inform breeding and biotechnological strategies to enhance *Lupinus albus* resilience and optimize defence trade-offs under biotic and abiotic stress (Mithöfer & Boland, 2012; Orozco-Ávila et al., 2017).

4. Conclusion

This study demonstrates the potential of *Lupinus albus* as a valuable crop for functional food development. Morphological traits essential for agronomic performance were largely independent of seed alkaloid content, suggesting that marker-assisted and genome-assisted selection can transfer favourable growth traits from bitter to sweet genotypes without compromising food safety. Although sweet seeds accumulated higher total phenolics than bitter genotypes, antioxidant activity depended on the qualitative composition, highlighting the need to enrich targeted bioactive with validated health functions. The marked divergence in alkaloid composition further supports breeding efforts to reduce toxicity while improving nutritional quality. Integrating metabolomic, transcriptomic, and epigenetic analyses with advanced molecular breeding and functional assays will enable precision tailoring of bioactive profiles. Overall, this work provides a framework for advancing *L. albus* as a sustainable source of plant-based proteins and health-promoting compounds, bridging agronomic improvement with nutritional innovation.

Data Availability Statement: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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CRediT authorship contribution statement

Imen Akremi: Conceptualization, Formal analysis, Investigation, Methodology,Writing – original draft,Writing – review and editing. **Souhir Kabtni:** Conceptualization,Formal analysis,Methodology, Visualization, Writing – original draft, Writing – review and editing. **Hajer Ben Ammar:** Conceptualization, Visualization, Writing – original draft, Writing – review and editing. **Manon Genva:** Investigation**. Mario Riolo:** Investigation**. Slim Rouz:**Resources **. Safia El-Bok:**Resources. **Santa Olga Cacciola:**Supervision**. Marie-Laure Fauconnier:** Validation, Supervision**. Sonia Marghali:**Conceptualization, Writing – review and editing, Validation, Supervision, Funding acquisition.

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Figure captions:

Figure 1. Distribution Map *of Lupinus albus* Accessions Sampled Across the Mediterranean and Neighboring Regions.

Figure 2. Principal Component Analysis (PCA) of *Lupinus albus* accessions based on morphometric variation, highlighting differentiation by geographical origin and seeds taste. Bitter seeds are represented by dots and sweet seeds are represented by squares. (**a1**) Zoom on Cluster 1 (bitter

accessions from Egypt, France, and Tunisia). (**a2**) Zoom on Clusters 2 and 3 (sweet accessions). (**a3**) Zoom on Cluster 4 (Italian bitter accessions). Trait loadings are indicated by vectors.

Figure 3. Pie chart illustrating the relative proportions of fatty acids and alkaloids in bitter and sweet *Lupinus albus* seeds. (**a**) Fatty Acid Composition of seed Oil . (**b**) Alkaloid profile in bitter and sweet *L. albus* accessions. All experiments were performed in triplicate.

Figure 4. Box Plots of Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Antioxidant Activity (FRAP), and Lipoxygenase Activity in Bitter and Sweet *Lupinus albus* Accessions. Asterisks indicate statistically significant differences between groups according to the following thresholds: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (***). All experiments were performed in triplicate.

Figure 5. Principal Component Analysis (PCA) of *Lupinus albus* accessions based on biochemical traits. Bitter seeds are represented by dots and sweet seeds are represented by squares. (**b**) Four clusters were identified. (**b1, b2**) Zoom on Cluster 1 and 2 (bitter accessions from Egypt, France, and Tunisia). (**b3, b4**) Zoom on Cluster 3 and 4 (sweet accessions from Egypt, Algeria, and Tunisia). All experiments were performed in triplicate.

Figure 6. Box Plot of Antifungal Activity in Bitter and Sweet *Lupinus albus* Accessions**.** Asterisks indicate statistically significant differences between groups according to the following thresholds: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****). All experiments were performed in triplicate.

Figure 7. Pearson’s Correlation among morphometric, biochemical traits, and biological activities in *Lupinus albus* seeds. Positive and negative correlations coefficients are indicated by color intensity

Table1: Geographical origins of Lupinus albus accessions in this study.

Code	**Seeds Taste**	**Geographic Origin**	**Sources**
LaT	Sweet	Tunisia	Landrace Tborba
LaK	mixed	Tunisia	Landrace Shabna
LaS	Bitter	Tunisia	Landrace Sejnene
LaE1A	Bitter	Egypt	Commercial cultivar
LaE2D	Sweet	Egypt	Commercial cultivar
LaF	Bitter	France	Commercial cultivar
LaAl	Sweet	Alger	Commercial cultivar
LaI	Bitter	Italie	Commercial cultivar

Table 2: The agro-morphological parameters assessed, selected from the UPOV guidelines for Lupinus

	Quantitative traits	**Qualitative traits**
Phenological Development	Date of first bud apparence (DFBA)	
	Height at beginning of flowering (HDF)	
	Time of flowering (TF)	
	Time of green ripening (TGR)	
	height at green ripening (HGR)	
	Time of ripening (TR)	
Vegetative Growth Characteristics	Cotyledons: length/width (LSl cotyl)	Green color intensity of Leaf prior to bud emergence (IGR): medium (IGRm) or dark (IGRdG)
	Central leaflet: length/ width (LslF)	
	Height at flowering (HFS)	
	central leaflet: area (SF)	
	Height at vegetative stage (HVS)	
Reproductive Development and Inflorescence	height of insertion of first inflorescence at green ripening (from ground level to insertion of first inflorescence) (HIFI)	Flowers: color of wings (CW): white (CWw) or white/violet (CWwy)
	Length of inflorescence (LINF)	
Pod and seed	Pod: length (PL)	Seed: sweet (SBd) or bitter (SBa)
	Pod number per plant (Npinf)	
	Grain: 100 seed weight (G100sw)	

Table 3: Means value and standard deviation of the morphological traits measured in *Lupinus* albus seeds: ***P value***: the significance probability value, Df: degrees of freedom.

	Bitter	**Sweet**	**Df**	**Anova test** ***P-value***
Numbers of replication	134	106		

Agro-morphological traits	**TR**	201.84±13.17	217.16±4.55	1	<2e-16 ***
	TGR	169.67±27.68	195.83±8.51	1	<2e-16 ***
	TFBA	62.41±7.07	79.74±11.38	1	<2e-16 ***
	TF	85.88±11.56	97.45±11.16	1	1.71e-13 ***
	SF	8.88±2.97	6.911±2.36	1	0.0000124 ***
	PL	7.77±1.11	7.37±0.48	1	0.000623 ***
	Npinf	3.93±0.69	3.64±0.48	1	0.000389 ***
	LslF	3.31±0.82	2.62±0.34	1	0.00000000121 ***
	Lslcotyl	1.22±0.22	1.221±0.19	1	0.615
	LINF	18.36±6.26	19.93±2.11	1	0.0138 *
	HVS	23.10±6.60	16.18±4.56	1	<2e-16 ***
	HIFI	48.13±11.26	43.20±3.49	1	0.0000207 ***
	HGR	66.74±16.65	63.14±4.57	1	0.0309 *
	HFS	36.51±13.82	33.97±6.28	1	0.0813 .
	G100sw	42.56±20.76	35.08±5.13	1	0.000355 ***

Signifiant. codes: 0 ‘*’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1**

Table 4: Comparative Analysis of Oil Content, Fatty Acid Composition and Alkaloids composition in *Lupinus albus* seeds. Values represent the mean ± standard deviation (SD) of the percentage of total fatty acids. *ANOVA* results indicate highly significant differences (P< 0.001) among accessions for all measured traits.

	Oil content	**Fatty acid**										**Alkaloid**						
Anova test		(C16:0)	(C18:0)	(C18:1)	(C18:2)	(C18:3)	(C20:0)	(C20:1)	(C22-1n9)	(C22)	(C24)	Sparteine	**α**_isolupan	Lupanine	11,12-	Multiflorine	11,12-	Oxylupani
Df	1	1										1						
P-value	0.24	0.252	0.0438 *	0.229	0.944	0.00351 **	0.119	0.4	0.000321 ***	0.0153 *	0.23	0.000255 ***	0.013 *	0.0000000572 ***	<2e-16 ***	0.000709 ***	0.166	0.325

Asterisks indicate levels of statistical significance: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

Table 5: Nutritional Profiling of Lupinus albus seeds: Insights into Protein and Amino Acid Composition. This table compares the mean ± standard deviation for key nutritional parameters (%N, %MS, %Protein) and amino acid content (g/100g), highlighting significant differences identified through *ANOVA* analysis

Anova test	**N**	**Antioxidant activity**			**Amino Acid**																	**% N**	**%Protéin**
		ABTS	**DPPH**	**carotene**	**Asp**	**Thr**	**Ser**	**Glu**	**Pro**	**Gly**	**Ala**	**Cys-Cys**	**Val**	**Met**	**Ile**	**Leu**	**Tyr**	**Phe**	**His**	**Lys**	**Arg**		
Bitter	134	15.64±1.69	1.79±0.19	0,23±0,015	3.97±0.16	1.26±0.02	1.82±0.09	9.12±0.78	1.54±0.07	1.46±0.05	1.19±0.00	0.42±0.01	1.50±0.14	0.20±0.04	1.70±0.02	2.60±0.06	1.48±0.08	1.38±0.04	0.79±0.02	1.650.01	3.57±0.21	5.82±0,26	36.37±1,63

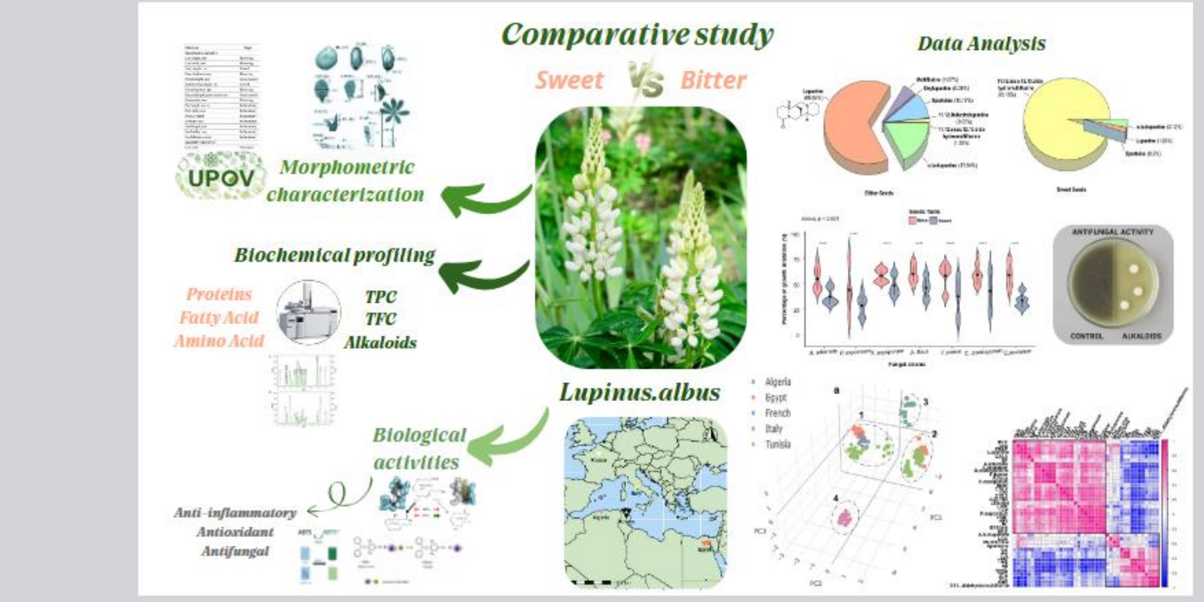
Sweet	106	16.03±0.72	1.83±0.08	0,24±0,015	4.04±0.18	1.29±0.03	1.83±0.07	9.15±0.60	1.55±0.06	1.49±0.06	1.22±0.05	0.43±0.01	1.51±0.016	0.21±0.02	1.67±0.05	2.63±0.07	1.47±0.048	1.40±0.05	0.81±0.03	1.70±0.06	3.58±0.16	6.02±0,38	37.64±2.40
Df		1			1																	1	1
p-value		0.533	0.525	0.57	0.369	0.101	0.913	0.936	0.711	0.253	0.0329 *	0.552	0.0283 *	0.649	0.115	0.367	0.798	0.317	0.156	0.0148 *	0.853	0.166	0.166

Declaration of interest statement

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical abstract



Highlights

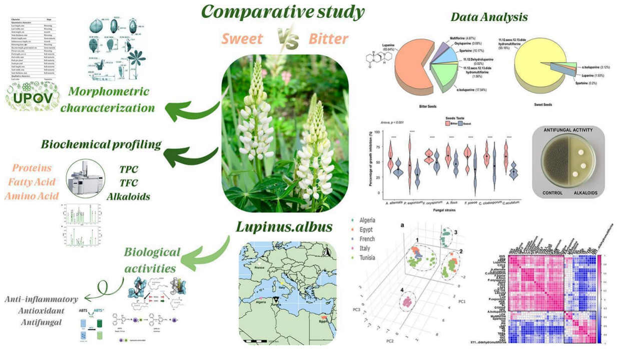
-Bitter *Lupinus albus* evaluated as a source of bioactive phytochemical compounds.

-GC-MS profiling reveals distinct alkaloid, phenolic, and fatty acid compositions.

-Bitter and sweet genotypes show differential alkaloid and lipid accumulation.

-Fatty acid patterns support chemotypic differentiation among lupin varieties.

-Integrated metabolite profiling enhances selection for food safety improvement.



Graphics Abstract

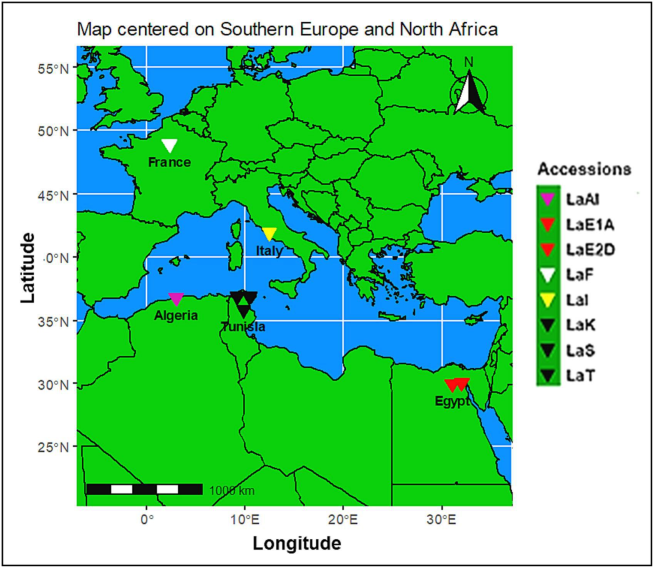
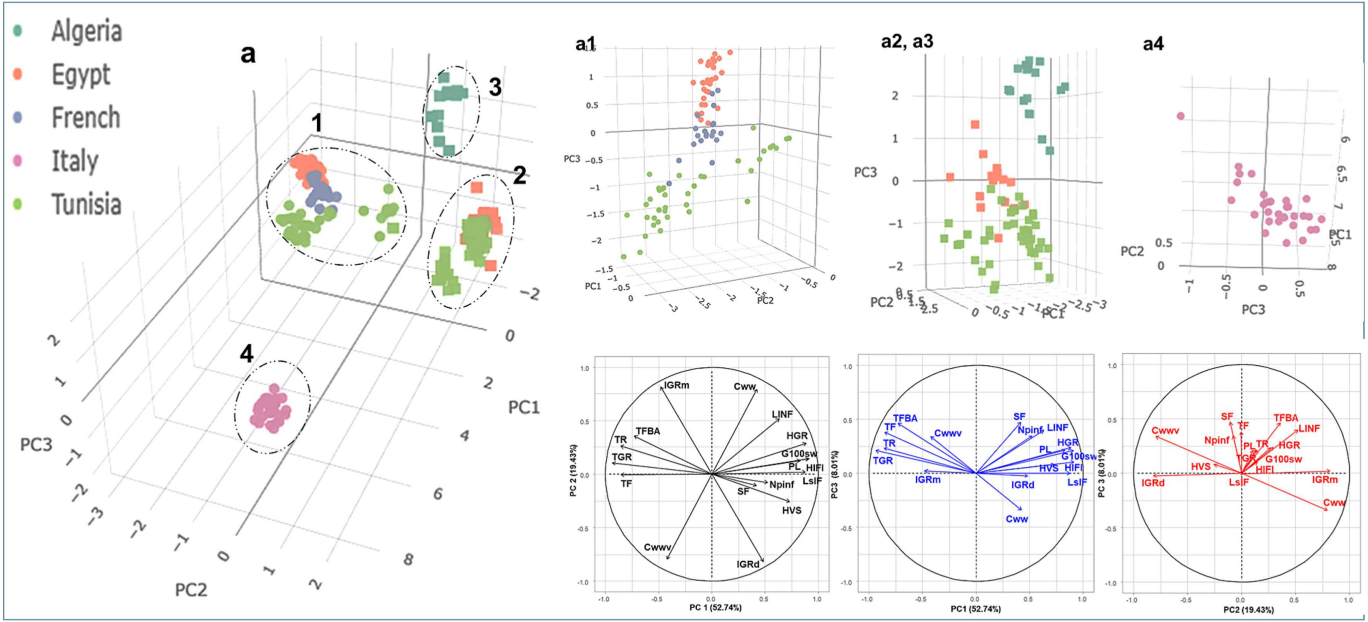

Map centered on Southern Europe and North Africa
55°N
50°N
45°N
40°N
35°N
30°N
25°N
Latitude
0°
10°E
20°E
30°E
Longitude
N
France
Italy
Algeria
Tunisia
Egypt
1000 km
Accessions
LaAl
LaE1A
LaE2D
LaF
LaI
LaK
LaS
LaT

Figure 1



Algeria
Egypt
French
Italy
Tunisia
a
1
2
3
4
PC1
PC2
PC3
a1
a2, a3
a4
PC 1 (52.74%)
PC 2 (19.43%)
PC3 (8.01%)
IGRm
Cww
LINF
HGR
G100sw
PL
HIFI
LsIF
Npinf
SF
HVS
IGRd
Cwwv
TF
TGR
TR
TFBA

Figure 2

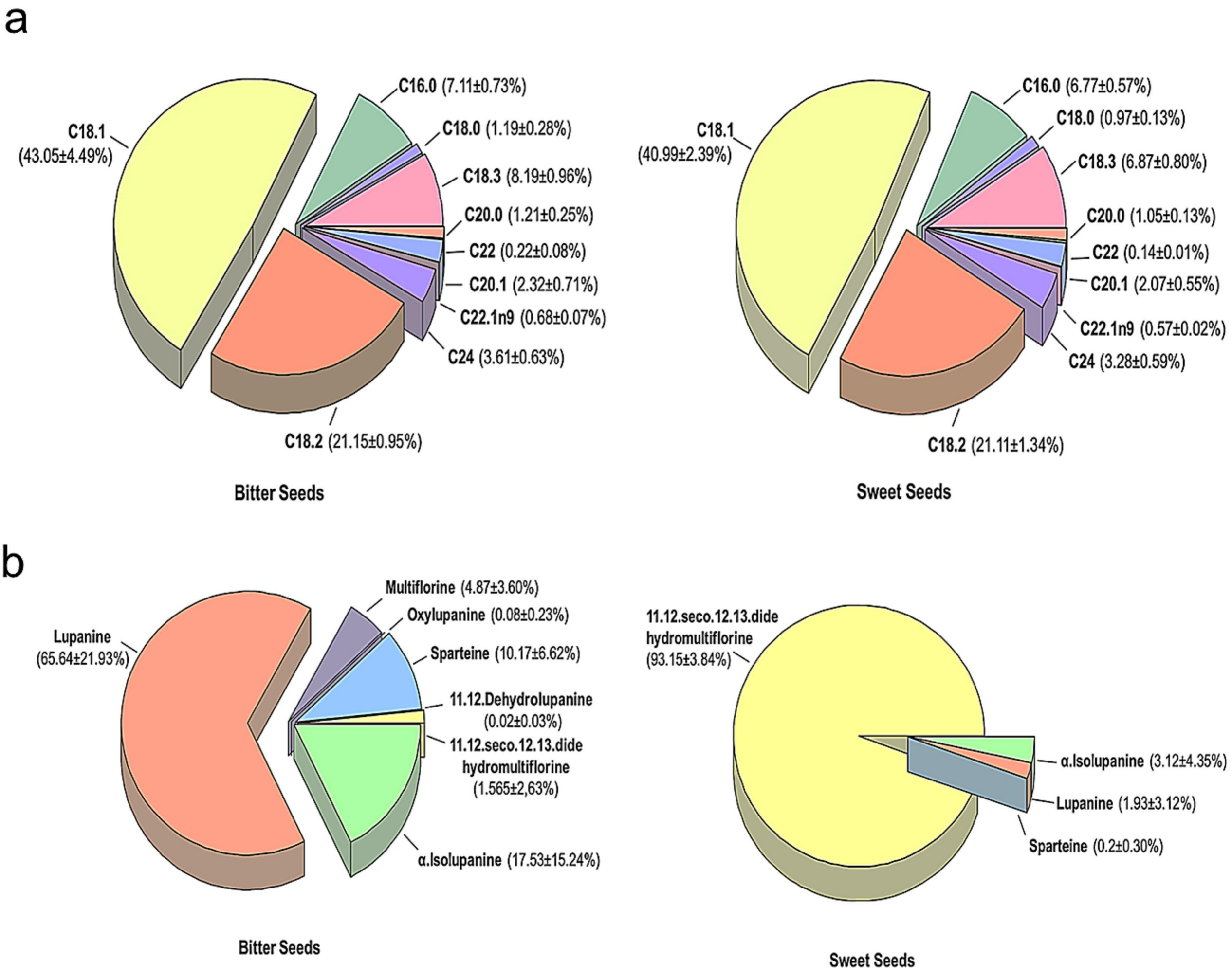

a
C18.1 (43.05±4.49%)
C16.0 (7.11±0.73%)
C18.0 (1.19±0.28%)
C18.3 (8.19±0.96%)
C20.0 (1.21±0.25%)
C22 (0.22±0.08%)
C20.1 (2.32±0.71%)
C22.1n9 (0.68±0.07%)
C24 (3.61±0.63%)
C18.2 (21.15±0.95%)
Bitter Seeds
C18.1 (40.99±2.39%)
C16.0 (6.77±0.57%)
C18.0 (0.97±0.13%)
C18.3 (6.87±0.80%)
C20.0 (1.05±0.13%)
C22 (0.14±0.01%)
C20.1 (2.07±0.55%)
C22.1n9 (0.57±0.02%)
C24 (3.28±0.59%)
C18.2 (21.11±1.34%)
Sweet Seeds
b
Lupanine (65.64±21.93%)
Multiflorine (4.87±3.60%)
Oxylupanine (0.08±0.23%)
Sparteine (10.17±6.62%)
11.12.Dehydrolupanine (0.02±0.03%)
11.12.seco.12.13.dide hydromultiflorine (1.565±2,63%)
α.Isolupanine (17.53±15.24%)
Bitter Seeds
11.12.seco.12.13.dide hydromultiflorine (93.15±3.84%)
α.Isolupanine (3.12±4.35%)
Lupanine (1.93±3.12%)
Sparteine (0.2±0.30%)
Sweet Seeds

Figure 3

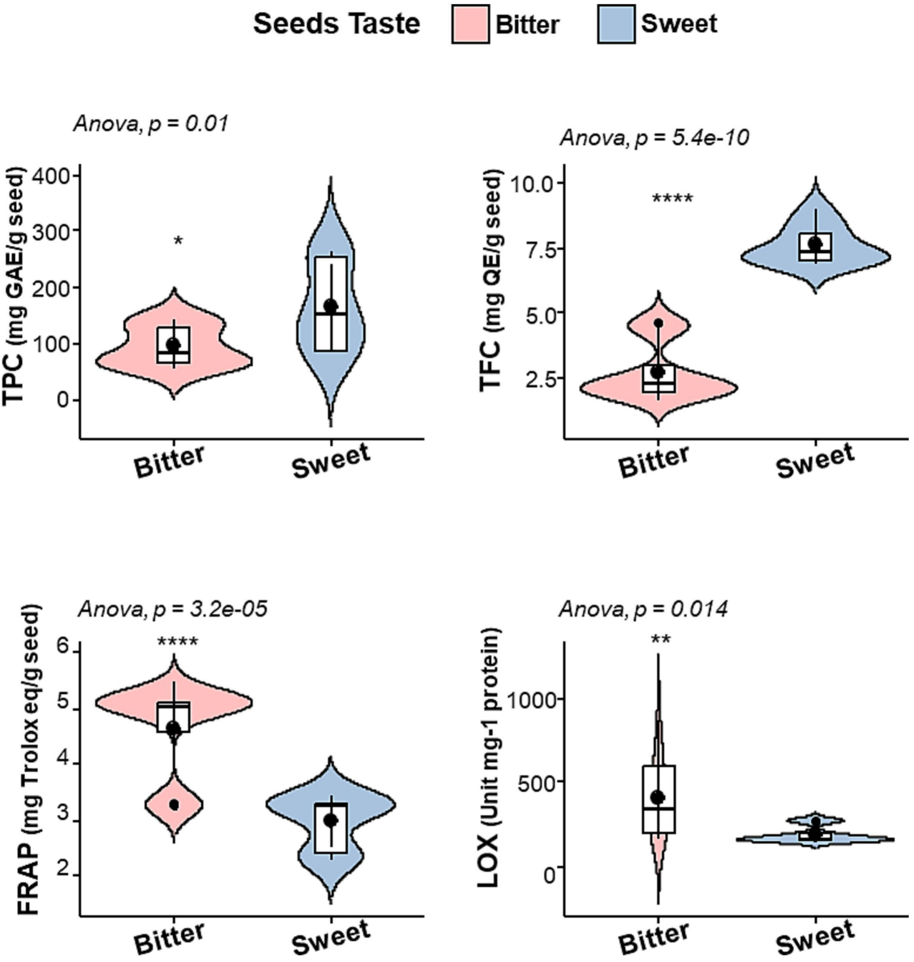

Seeds Taste
Bitter
Sweet
Anova, p = 0.01
TPC (mg GAE/g seed)
400
300
200
100
0
*
Bitter
Sweet
Anova, p = 5.4e-10
TFC (mg QE/g seed)
10.0
7.5
5.0
2.5
****
Bitter
Sweet
Anova, p = 3.2e-05
FRAP (mg Trolox eq/g seed)
6
5
4
3
2
****
Bitter
Sweet
Anova, p = 0.014
LOX (Unit mg-1 protein)
1000
500
0
**
Bitter
Sweet

Figure 4

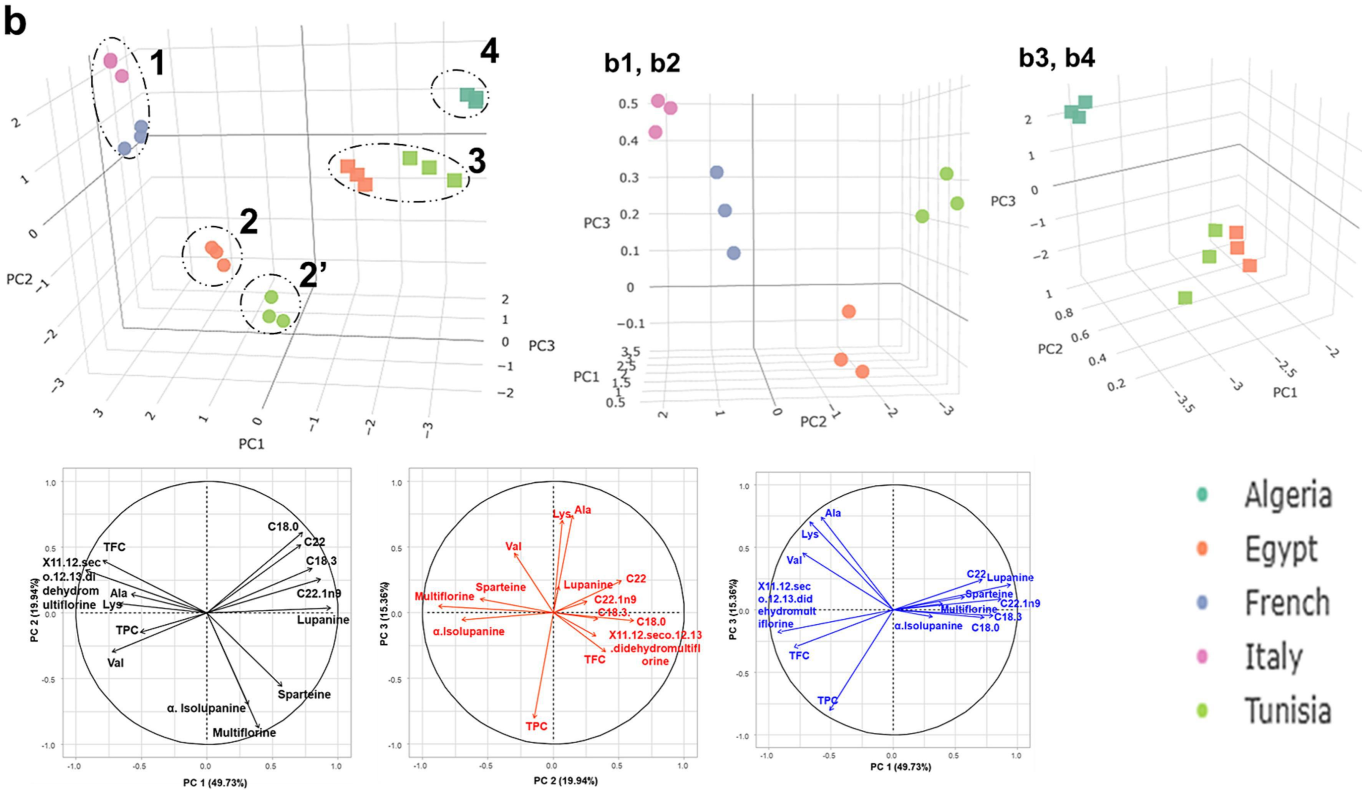

b
1
2
2'
3
4
PC1
PC2
PC3
b1, b2
b3, b4
C18.0
C22
C18.3
C22.1n9
Lupanine
TFC
X11.12.seco.12.13.didehydromultiflorine
Ala
Lys
TPC
Val
Sparteine
α. Isolupanine
Multiflorine
PC 1 (49.73%)
PC 2 (19.94%)
PC 3 (15.36%)
Algeria
Egypt
French
Italy
Tunisia

Figure 5

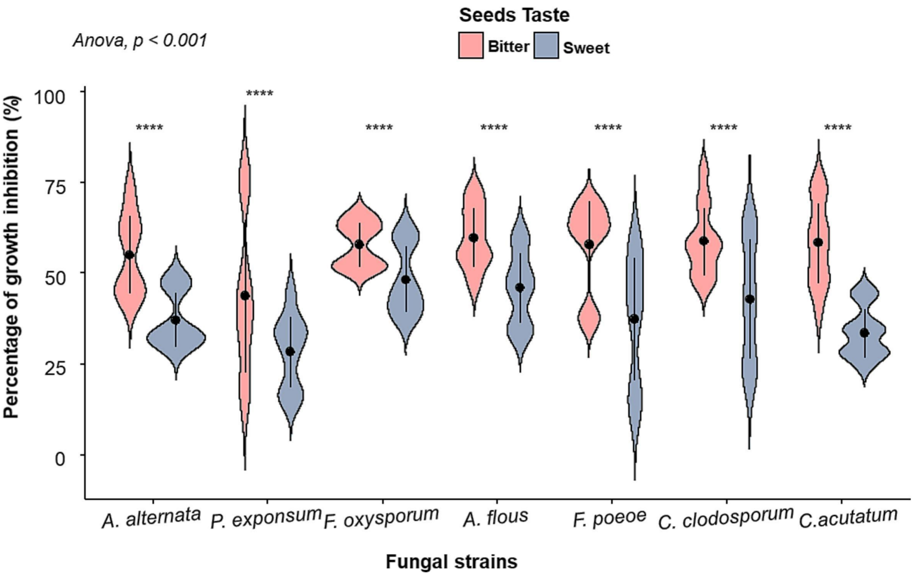

Anova, p < 0.001
Seeds Taste
Bitter
Sweet
Percentage of growth inhibition (%)
100
75
50
25
0
****
****
****
****
****
****
****
A. alternata
P. exponsum
F. oxysporum
A. flous
F. poeoe
C. clodosporum
C.acutatum
Fungal strains

Figure 6

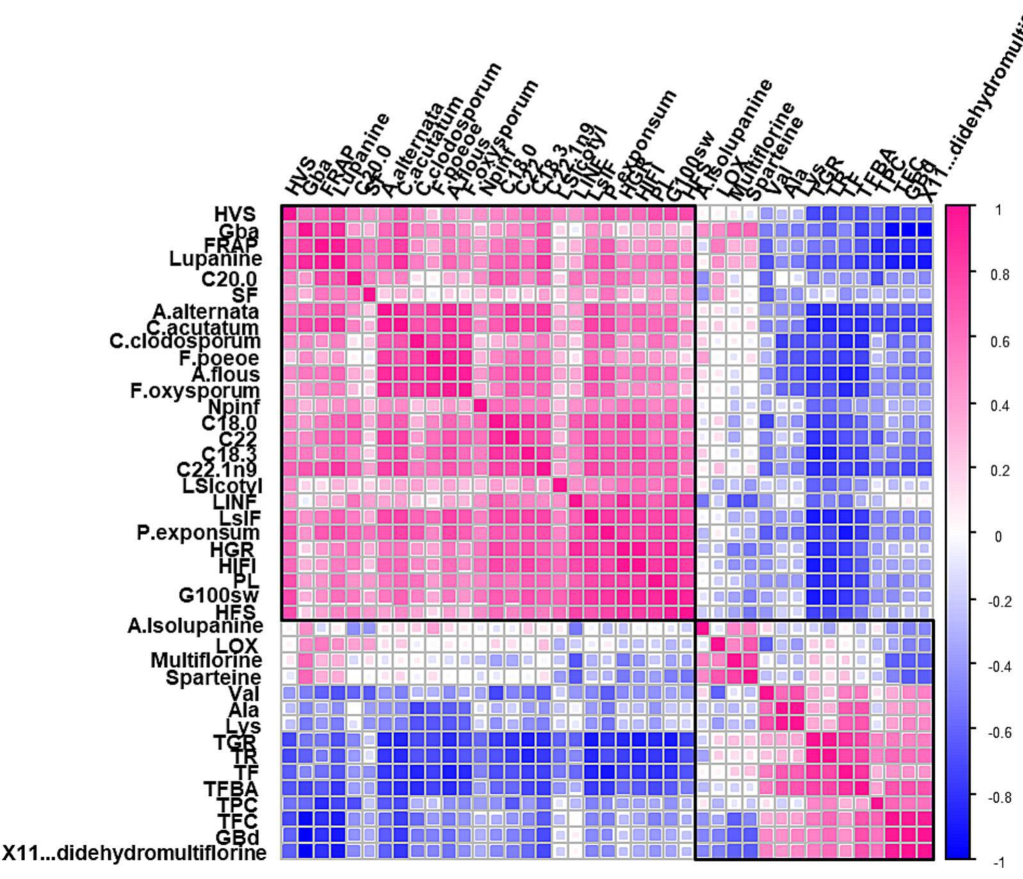

HVS
Gba
FRAP
Lupanine
C20.0
SF
A.alternata
C.acutatum
C.clodosporum
F.poeoe
A.flous
F.oxysporum
Npinf
C18.0
C22
C18.3
C22.1n9
LSIcotyl
LINF
LsIF
P.exponsum
HGR
HIFI
PL
G100sw
HFS
A.Isolupanine
LOX
Multiflorine
Sparteine
Val
Ala
Lys
TGR
TR
TF
TFBA
TPC
TFC
GBd
X11...didehydromultiflorine
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1

Figure 7