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RESEARCH

Genetic diversity, agronomic performance, and bioactive properties, of sweet and bitter white lupin (*Lupinus albus* L.) genotypes

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ABSTRACT

Understanding the agronomic performance and genetic diversity of sweet and bitter lupin genotypes is crucial for developing improved varieties with enhanced yield, nutritional quality, and disease resistance. This study evaluated the agronomic performance and molecular diversity of five sweet (Accessions 1, 2, 3, 4, and 5) and five bitter ('Giza 1', 'Giza 2', 'Giza 3', Accession A, and Accession B) lupin (*Lupinus albus* L.) genotypes across two growing seasons (2021-2022 and 2022-2023). Significant differences were observed among genotypes for phenological, growth, and yield-related traits. Accession-A consistently demonstrated superior performance across most measured traits, including plant height, number of branches and pods per plant, seed number per plant, 100-seed weight, and seed yield. 'Giza-1' and Accession-B also exhibited promising agronomic characteristics. Clustering analysis based on agronomic data categorized genotypes into three groups, with Accession-A forming a distinct group due to its high performance. Principal component analysis revealed strong positive correlations between yield and several agronomic traits, while days to flowering showed a negative association. Molecular analysis using start codon targeted (SCoT) markers revealed polymorphism among the genotypes, with Accession-A and Accession-B exhibiting the highest similarity. Phylogenetic analysis based on SCoT markers clustered the genotypes into four groups, reflecting the genetic diversity among them. Biochemical analysis revealed that sweet lupin (as Accession 5) demonstrated superior total protein content, phenolic, and flavonoid levels. Sweet lupin also had enhanced antioxidant activity, as in Accessions 5 and 3, achieving up to 93% scavenging of DPPH free radicals. Both sweet and bitter lupin displayed significant antibacterial activity, but sweet lupin, as Accessions 5 and 3, showed the highest inhibition zones against *Staphylococcus aureus* and *Escherichia coli*. These findings provide valuable information for lupin breeding programs, particularly for selecting superior genotypes like Accession-A and understanding the genetic relationships among different lupin genotypes.

Key words: Antibacterial activity, antioxidant activity, genetic diversity, lupin genotypes, phylogenetic analysis, polymorphism, SCoT markers.

INTRODUCTION

White lupin (*Lupinus albus* L.), a diploid plant (2*n* = 2*x* = 50) and a member of the Fabaceae family, is among the oldest cultivated legumes (Pereira et al., 2022). Lupin species exhibit varied reproductive strategies, with *L. albus* being predominantly self-pollinated (autogamous), although a certain degree of natural outcrossing can occur (typically 1%-5%, depending on environmental factors and insect activity) (Tessema, 2017). Its cultivation back centuries across East Africa, the Mediterranean basin, and the Atlantic islands of the northern hemisphere (Smýkal et al., 2015). Global lupin cultivation in 2022 reached nearly 10 millions hectares, yielding a total of 1.65 millions tons. The significant producers included Australia, followed by Poland, Russia, Chile, Morocco, and Germany (FAOSTAT, 2024). Lupin cultivation succeeds in diverse conditions, unlike many crops that adapt to harsh environments. Its adaptability, combined with a symbiotic relationship with *Rhizobium* bacteria, allows to fix atmospheric N and enrich soil fertility. This natural soil enrichment enhances agricultural sustainability and productivity. Lupin is a valuable plant with multiple uses, including its seeds and above-ground parts for green manure and medicinal applications (Tessema, 2017). Moreover, lupin seeds are a promising food source due to their high protein abundance and high-quality dietary fiber (Szczepański et al., 2022).

Exploring the genetic diversity of lupin is decisive for developing effective breeding strategies to improve crop performance across diverse environmental conditions. The rich genetic variability within lupin species provides a valuable resource for identifying traits associated with yield enhancement and adaptability to changing climates. By exploring this genetic diversity, breeders can accelerate the selection process, allowing for the rapid development of lupin varieties that are better provided to increase in progressively unpredictable environments (Kamara et al., 2022). This approach enhances crop productivity and strengthens food security by ensuring that lupin can remain a resilient and reliable crop in the face of global climate fluctuations (Mansour et al., 2018). Lupin species show great variety in phenological, morphological and agronomic traits and even genetic makeup. This diversity comes from both natural processes and artificial selection over time. Despite this extensive genetic variation, there has been limited effort to develop improved lupin varieties through breeding programs (Abraham et al., 2019).

Without advanced genetic and molecular tools, exploiting the full potential of lupin genetic diversity remains a challenge. Utilizing advanced tools is crucial for efficient genetic improvement and fully releasing the potential of this promising crop. Start codon targeted (SCoT) markers are a type of molecular marker that can be used to explore genetic diversity in lupin. They are derived from conserved short regions surrounding ATG start codon in plant genes. The SCoT markers are relatively easy to develop and use, and are highly polymorphic, which means they can detect a lot of genetic variation. The SCoT markers have been successfully utilized to explore genetic diversity in different plant species (Essa et al., 2023).

In addition to genetic and agronomic potential, sweet and bitter lupin genotypes are recognized by significant bioactive properties, including antioxidant, antibacterial, and anticancer activities (Gharibzahedi et al., 2024). These properties are primarily attributed to the existence of various phytochemicals, including flavonoids, alkaloids, and phenolic compounds, which play an important role in plant health-promoting effects (Tian et al., 2024). Antioxidant activity in lupin is crucial for reducing free radicals, which can lead to cellular damage and cause chronic diseases (Kamran et al., 2023). Moreover, the antibacterial properties of lupin proteins are of interest due to their potential applications in preventing bacterial infections (Chukwuejim et al., 2024). Exploration of these properties in sweet and bitter lupin genotypes emphasizes their potential as a multifaceted crop, offering benefits beyond traditional agriculture.

This study aimed to explore a comprehensive evaluation of the agronomic, molecular, and biochemical diversity of ten white lupin genotypes. The evaluated genotypes included five bitter and five sweet types, based on their potential value in breeding and functional food applications. The bitter genotypes included three Egyptian cultivars (Giza 1, Giza 2, Giza 3) which are recognized for their adaptability and use in local farming systems. In addition, two bitter accessions (Accession A, and Accession B) were included due to their superior preliminary field performance, particularly in yield-related traits. Moreover, five sweet genotypes (Accessions 1, 2, 3, 4, and 5) were chosen for their low alkaloid content and promising nutritional attributes. This diverse selection provides a solid foundation for identifying genotypes with favorable agronomic and bioactive properties to support future lupin improvement programs.

MATERIALS AND METHODS

Plant materials

Ten white lupin (*Lupinus albus* L.) genotypes were used in this study, comprising five sweet (Accessions 1, 2, 3, 4, and 5) and five bitter genotypes ('Giza 1', 'Giza 2', 'Giza 3', Accession A, and Accession B). The used genotypes were obtained from Food Legumes Research Department, Agricultural Research Center, Egypt. These genotypes were selected to represent both sweet and bitter types, providing a representative subset of white lupin genetic resources relevant to breeding programs focused on improving productivity and functional food qualities. The sweet accessions (1 to 5) represent genotypes with low alkaloid content and promising nutritional. Bitter cultivars (Giza-1, Giza-2, and Giza-3) are used as commercial cultivars with historical agronomic importance and moderate resilience under Egyptian conditions. In addition, other two bitter accessions (Accession-A and Accession-B) were selected for their robust field performance in preliminary trials and demonstrated superior agronomic performance.

Field experiment

Field experiments were conducted over two consecutive growing seasons (2021-2022 and 2022-2023) at the experimental farm of the Faculty of Agriculture, Zagazig University, Zagazig (30°35' N, 31°30' E), Egypt. The soil at the experimental site is characterized as sandy loam (52.95% sand, 27.95% silt, and 19.1% clay) with an alkaline pH of 7.71. A randomized complete block design (RCBD) with three replicates was applied. The ten lupin genotypes were randomly assigned within each block. Sowing was carried out during the first week of November in both seasons, consistent with the recommended planting period for lupin in the region. Each genotype was sown in a 4 m row with inter-row and intra-row spacing of 0.65 and 0.20 m, respectively. Germination rate was monitored post-emergence, with an average germination percentage of 90%-95% across all genotypes, resulting in approximately 18-19 plants for each genotype. This number was adequate for trait evaluation given that data were recorded on ten randomly selected central plants per plot. To mitigate border effects, guard rows were planted around the experimental area. Seeds were inoculated with *Bradyrhizobium lupini* immediately prior to sowing. Basal fertilization consisted of potassium sulfate (48% K_2O) at a rate of 60 kg K_2O ha^{-1} and superphosphate (15.5% P_2O_5) at a rate of 70 kg P_2O_5 ha^{-1}. Nitrogen fertilizer, applied as ammonium sulfate (21% N) at a rate of 40 kg N ha^{-1}, was incorporated at planting. Harvesting was conducted based on the physiological maturity of each genotype. Maturity was determined when 90%-95% of the pods had turned yellow and dried, and seed moisture content was visibly reduced. As a result, the genotypes were harvested on different dates, depending on their phenological development. Harvest time of sweet lupin was around 150 d after sowing, while bitter lupin was around 165 d after sowing.

Days to flowering were recorded at the plot level as the number of days from sowing to the appearance of the first flower on at least 50% of the plants in each plot. For the evaluation of growth and yield-related traits, ten representative plants were randomly selected from the central plants of each row in every replicate to avoid border effects. To mitigate border effects, the first and last plants in each plot were excluded from data collection. The collected plants were used to record traits including plant height, height to the first pod, number of branches per plant, number of pods per plant, number of seeds per pod, 100-seed weight, number of seeds per plant, and seed yield per plant.

DNA extraction

Genomic DNA was extracted from the leaves of 3-wk-old lupin plants using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle, 1991). The DNA quantity and quality were assessed using a spectrophotometer (NanoDrop, Thermo Scientific, Waltham, Massachusetts, USA).

Start codon targeted (SCoT) PCR amplification

The SCoT markers are considered a dominant marker system. This means they typically detect the presence or absence of an amplified DNA fragment. The SCoT-PCR was performed in a 25 µL reaction volume containing 10 µL GoTaq Green Master Mix, 1 µL template DNA, 1 µL SCoT primer (Table 1), and nuclease-free water to adjust the final volume. Amplification was carried out in a thermal cycler (Applied Biosystems, Foster City, California, USA) using the following cycling conditions: Initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 1 min.

Table 1. Start codon targeted (SCoT) primers with melting temperature (Tm), nucleotide sequences, primer length, molecular weight, and GC content.

Primer	Sequences (5′-3′)	Tm	Molecular weight	Primer length	GC content
		°C	g mol^{-1}	nt	%
SCoT1	ACCATGGCTACCACCGGC	67.6	5429.6	18	66.67
SCoT2	CAATGGCTACCACTAGCG	60.6	5468.6	18	55.56
SCoT3	CAATGGCTACCACTACAG	57.6	5452.6	18	50.00
SCoT4	ACAATGGCTACCACTGCC	62.9	5428.6	18	55.56
SCoT5	ACCATGGCTACCACGGCA	66.9	5453.6	18	61.11
SCoT6	ACCATGGCTACCAGCGCG	68.0	5469.6	18	66.67
SCoT7	CCATGGCTACCACCGCAG	65.8	5429.6	18	66.67

Gel electrophoresis

The SCoT-PCR amplicons were separated by electrophoresis on 1% and 2% agarose gels prepared in tris-borate-EDTA (TBE) buffer. Gels were stained with ethidium bromide and visualized under UV light. A 100 bp DNA ladder (Goddard, Irvine, California, USA) was used for molecular weight estimation.

Isolation of total protein

Lupin bean seeds were ground into a fine powder using a mixer (Type 716, Moulinex, Ecully, France) and then defatted with a chloroform-methanol mixture in a Soxhlet apparatus. The defatted flour was stored at a low temperature (4 °C) until further analysis. Protein isolate was obtained from the defatted flour employing a modified method outlined by Fan and Sosulski (1974). The flour was dispersed in water, adjusted to a pH 8.0 and stirred. After centrifugation, the supernatant was altered to pH 4.5 to precipitate the protein. The precipitated protein was redispersed in water, homogenized, and adjusted to a pH 7.0.

Characterization of sweet and bitter lupin protein

Total protein content and normalization. Total N content was determined using the micro-Kjeldahl method. Total protein content (%) was then calculated by multiplying the total N value by a conversion factor of 6.25. The total N was measured according to Silva et al. (2016).

For subsequent biochemical assays, protein extracts were prepared, and their concentrations were determined with bovine serum albumin (BSA) as the standard. Prior to performing antioxidant and antimicrobial activity assays, samples were normalized by loading a consistent amount of total protein (100 µg) per assay.

Total phenolics and flavonoids compounds of bitter and sweet lupin protein extract. To determine the total phenolics (TP) and total flavonoids (TF) content of bitter and sweet lupin samples, a microtiter plate reader was employed. For TP analysis, 50 µL aliquots of the extract were incubated with a mixture of sodium carbonate and Folin-Ciocalteu reagent. The resulting blue color, indicative of polyphenol-reducing properties, was measured spectrophotometrically at 750 nm. The TP concentrations were calculated and stated as mg gallic acid equivalents (GAE) per g sample.

For TF quantification, 50 µL aliquots of the extracts were reacted with ethanolic aluminum chloride. The formation of a yellow-orange complex, characteristic of flavonoid compounds, was monitored at 430 nm. The TF levels were quantified and reported as mg quercetin equivalents (QE) per g sample (Pourmorad et al., 2006).

Antioxidant activity. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of sweet and bitter lupin was estimated according to (Abdel-Moneim et al., 2022); 0.5 mL ethanolic DPPH was added to 1 mL bitter and sweet lupin protein extract samples in triplicate. The mixture was then incubated for 30 min in the dark, the absorbance of the resulting color was measured at 517 nm using spectrophotometer. The absorbance (Abs) was incorporated into the following equation; IC_{50} estimates correspond to minimum concentrations required to scavenge 50% DPPH radical (El-Saadony et al., 2022).

$$\% \text{ DPPH scavenging activity} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \text{ x } 100 \quad (1)$$

Antimicrobial activity. The antimicrobial activity of sweet and bitter lupin protein extracts was evaluated against *Staphylococcus aureus* and *Escherichia coli* in triplicate. The microbial strains were maintained at 4 °C through routine subculturing on nutrient agar slants. Antimicrobial activity was assessed using a modified agar well-diffusion method, as described by El-Saadony et al. (2021b). Briefly, 50 mL melted Mueller-Hinton agar (MHA) were poured into Petri dishes. Once solidified, each plate was surface-inoculated with a loopful of bacterial inoculum. Wells (8 mm diameter) were then punched into the agar, and 6 mm sterile discs saturated with 50 µL lupin protein extract at varying concentrations (100, 150, and 200 µg mL^{-1}) were carefully introduced into the wells. Discs saturated with sterile water served as a negative control. The MHA plates were incubated at 37 °C for 24-48 h (El-Saadony et al., 2019). Antibacterial activity was determined by measuring the diameters of the resulting inhibition zones (mm) (El-Saadony et al., 2021a).

Data analysis

The data from the two growing seasons were subjected to normality testing using the Shapiro-Wilk test, and homogeneity of variance was assessed using Levene's test to confirm the assumptions required for parametric analysis. Then, ANOVA was conducted to determine the significance of differences among genotypes. Following a significant F-test, the means were compared using the least significant difference (LSD) test at a 5% probability level ($P < 0.05$) to separate genotype means for each trait. All statistical analyses and visualizations including ANOVA, principal component analysis (PCA), dendrogram construction (for both agronomic and molecular data), and heatmap generation were performed using R software version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria). The following R packages were used: agricolae for ANOVA and LSD mean separation tests, factoextra and FactoMineR for principal component analysis (PCA), pheatmap for heatmap visualization, and cluster for hierarchical clustering.

For molecular data analysis, SCoT-PCR products were scored as either present (1) or absent (0) for each locus. Polymorphism was calculated as the percentage of polymorphic loci out of the total number of scored loci. Genetic similarity among genotypes was calculated using Dice's coefficient (Dice, 1945) with SPSS software (Norušis, 1993). Cluster analysis, based on Dice's similarity coefficient, was used to construct a dendrogram illustrating phylogenetic relationships among the genotypes (Rokach and Maimon, 2005).

RESULTS

Agronomic performance

Phenological and growth characteristics. Significant differences in days to flowering were observed among the sweet and bitter lupin genotypes across both growing seasons (2021-2022 and 2022-2023) (Table 2). Accession-4 exhibited the latest flowering time, requiring 76.11 d in the first season and 81.64 d in the second. Accession-B also flowered relatively late (68.33 d in the first season and 63.28 d in the second). Accessions 1, 2, and 3 displayed mid-range flowering times, while 'Giza-1', 'Giza-2', and 'Giza-3' flowered significantly earlier. Flowering time was generally consistent across seasons for most genotypes, although minor variations were observed, likely due to seasonal environmental differences.

Plant height varied significantly among genotypes (Table 2). Accession-A was the tallest (182.31 and 166.67 cm in the first and second seasons, respectively). 'Giza-1', 'Giza-2', 'Giza-3', and Accession-B exhibited moderate plant heights, while Accessions 1, 2, 3, 4, and 5 were the shortest. Plant height fluctuated slightly across seasons for most genotypes, but 'Giza-1' and Accession-B maintained relatively stable heights.

Height to the first pod also varied among genotypes. Accession-A had the greatest height to the first pod in both seasons (87.75 and 88.25 cm). 'Giza-1', 'Giza-2', 'Giza-3', and Accession-B also showed high values. Accessions 1, 2, 3, and 4 had moderate values, while Accession-5 had the lowest height to the first pod (34.22 and 40.31 cm).

Table 2. Mean performance of the evaluated sweet and bitter lupin genotypes in two seasons. Means followed by different letters indicate a significant difference according to LSD, $p < 0.05$. DF: Days to flowering; PH: plant height; HFP: height to first pod; NBP: number of branches per plant; NPP: number of pods per plant; NSPod: number of seeds per pod; NSPlant: number of seeds per plant; WHS: weight of 100 seeds; SYP: seed yield per plant.

Genotype	DF	PH	HFP	NBP	NPP	NSPod	NSPlant	WHS	SYP
	d	cm	cm	nr plant^{-1}	nr plant^{-1}	nr pod^{-1}	nr plant^{-1}	g	g plant^{-1}
					First season				
Accession 1	64.42^{c}	90.28^{f}	52.72^{f}	4.28^{d}	13.97^{d}	4.28^{ab}	50.06^{d}	29.92^{d}	20.44^{de}
Accession 2	63.33^{cd}	93.33^{e}	51.17^{g}	4.22^{d}	15.72^{c}	3.81^{ef}	50.50^{d}	24.86^{g}	12.98^{h}
Accession 3	63.74^{cd}	93.78^{e}	49.75^{h}	3.94^{e}	14.00^{d}	4.33^{a}	61.72^{bc}	29.20^{e}	22.58^{c}
Accession 4	76.11^{a}	89.47^{f}	49.33^{i}	3.78^{e}	12.22^{e}	4.19^{c}	42.50^{e}	22.49^{h}	17.39^{g}
Accession 5	62.03^{de}	75.47^{g}	34.22^{j}	3.31^{f}	10.72^{f}	4.00^{d}	42.89^{e}	28.48^{f}	18.45^{fg}
Giza 1	60.14^{e}	135.61^{c}	67.42^{c}	4.31^{d}	11.06^{f}	3.61^{g}	42.86^{e}	31.39^{b}	25.21^{b}
Giza 2	60.31^{e}	142.42^{b}	70.33^{b}	5.14^{bc}	12.50^{e}	4.31^{a}	51.08^{d}	30.66^{c}	19.64^{ef}
Giza 3	60.31^{e}	140.72^{b}	63.86^{e}	5.19^{b}	23.22^{b}	3.86^{e}	59.44^{c}	28.19^{f}	19.59^{ef}
Accession A	64.22^{cd}	182.31^{a}	87.75^{a}	14.44^{a}	43.17^{a}	4.22^{bc}	190.44^{a}	36.11^{a}	80.52^{a}
Accession B	68.33^{b}	122.19^{d}	64.64^{d}	4.94^{c}	15.61^{c}	3.78^{f}	65.19^{b}	29.32^{e}	21.39^{cd}
					Second season				
Accession 1	61.25^{bc}	81.83^{e}	48.31^{e}	4.08^{e}	8.75^{gh}	3.58^{f}	31.06^{h}	26.93^{e}	15.04^{g}
Accession 2	62.69^{bc}	70.39^{f}	39.86^{g}	3.58^{f}	9.81^{f}	4.00^{d}	31.56^{h}	26.29^{g}	15.05^{g}
Accession 3	61.14^{bc}	81.36^{e}	45.22^{f}	3.58^{f}	9.25^{fg}	4.33^{a}	38.19^{f}	24.71^{i}	15.16^{g}
Accession 4	81.64^{a}	82.69^{e}	48.14^{e}	3.11^{g}	8.25^{h}	4.19^{b}	29.50^{h}	25.30^{h}	11.63^{f}
Accession 5	56.75^{d}	73.11^{f}	40.31^{g}	4.06^{e}	11.17^{e}	3.33^{h}	34.47^{g}	26.64^{f}	15.23^{e}
Giza 1	50.33^{e}	136.31^{b}	71.97^{d}	6.33^{c}	21.14^{c}	3.75^{e}	70.78^{c}	34.25^{b}	31.26^{b}
Giza 2	49.14^{e}	123.31^{d}	72.89^{c}	5.44^{d}	18.31^{d}	4.06^{c}	50.14^{e}	28.60^{c}	20.34^{d}
Giza 3	54.19^{d}	130.25^{c}	76.31^{b}	7.28^{b}	28.58^{b}	3.50^{g}	60.26^{d}	27.22^{d}	25.40^{c}
Accession A	60.36^{c}	166.67^{a}	88.25^{a}	12.42^{a}	39.61^{a}	4.22^{b}	175.58^{a}	38.63^{a}	77.25^{a}
Accession B	63.28^{b}	136.31^{b}	72.14^{d}	6.42^{c}	21.08^{c}	4.03^{cd}	80.36^{b}	27.07^{de}	28.80^{b}

Yield contributing traits. The number of branches per plant differed significantly among genotypes (Table 2). Accession-A had the highest number of branches (14.44 and 12.42 in the first and second seasons, respectively). 'Giza-1', 'Giza-2', 'Giza-3', and Accession-B had moderate numbers of branches, while Accessions 1, 2, 3, 4, and 5 had the lowest. 'Giza-2' and Accession-1 showed relatively stable branching across seasons.

The number of pods per plant was also highest for Accession-A (43.17 and 39.61) (Table 2). 'Giza-3' also performed well (23.22 and 28.58 pods). Accessions 1, 2, 3, 4, and 5 had considerably fewer pods per plant and showed greater variability across seasons.

Accession-3 had the highest number of seeds per pod (averaging 4.33). Accession-4 and Accession-A also had relatively high and stable seed numbers per pod. 'Giza-2' averaged 4.31 seeds per pod in the first season and 4.06 in the second. Accession-1 showed some seasonal variation, while Accession-2 and 'Giza-1' had moderate performance, and 'Giza-3' and Accession-5 had lower performance.

Accession-A produced significantly more seeds per plant (190.44 and 175.58) than all other genotypes (Table 2). Accession-B also performed well (65.19 and 80.36 seeds). 'Giza-3' (59.44 and 60.26 seeds) and 'Giza-2' (51.08 and 50.14 seeds) showed consistent performance across seasons. Accessions 2, 3, and 'Giza-1' performed moderately well in one season but declined in the other. Accessions 1, 4, and 5 had lower and less consistent seed numbers per plant.

Accession-A had the highest 100-seed weight (36.11 and 38.63 g). 'Giza-1' also performed well (31.39 and 34.25 g), followed by 'Giza-2' (30.66 and 28.60 g). Accessions 3, 4, and 5 had the lowest 100-seed weights. Accession-A had the highest seed yield per plant (80.52 and 77.25 g) (Table 2). 'Giza-1' was the second highest (25.21 and 31.26 g), followed by Accession-B (21.39 and 28.80 g). Accessions 1, 2, 3, 4, and 5 had the lowest seed yields.

Genotypic classification based on agronomic traits. Hierarchical clustering based on agronomic performance classified the genotypes into three groups (Figure 1). Group A consisted solely of Accession-A, reflecting its superior performance. Group B included 'Giza-3', 'Giza-2', 'Giza-1', and Accession-B, characterized by intermediate agronomic traits. Group C comprised Accessions 4, 5, 1, 2, and 3, which exhibited lower agronomic performance.

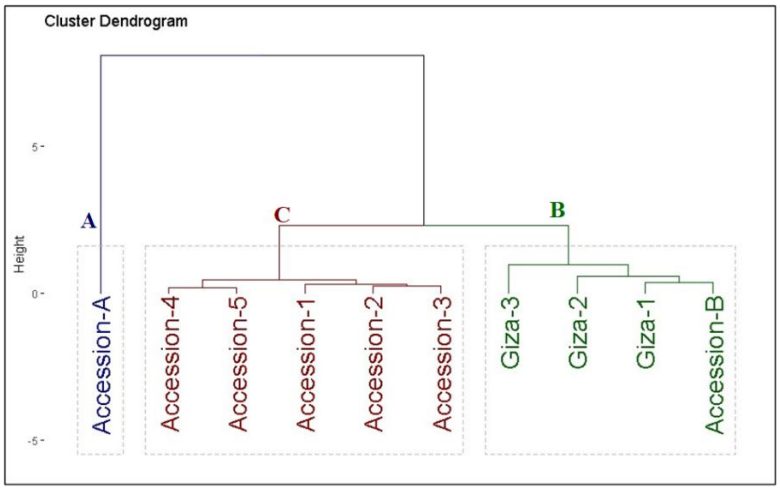

Figure 1. Dendrogram of the distances among 10 sweet and bitter lupin genotypes based on agro-morphological traits.

Association among genotypes agronomic traits. Principal component analysis (PCA) revealed relationships among genotypes and agronomic traits (Figure 2). The first two principal components (PCs) explained 74.09% of the total variance (PC1 = 62.81%, PC2 = 11.28%). The PC1 differentiated genotypes into three groups, aligning with the clustering results. Accession-A, located on the positive axis of PC1, was associated with higher agronomic performance. Accessions 4, 3, 2, 1, and 5, on the negative axis, exhibited lower performance. 'Giza-3', 'Giza-2', 'Giza-1', and Accession-B occupied an intermediate position. Number of pods per plant, branches per plant, seeds per plant, height to the first pod, 100-seed weight, and plant height were positively correlated with seed yield per plant. Days to flowering showed a negative association with seed yield.

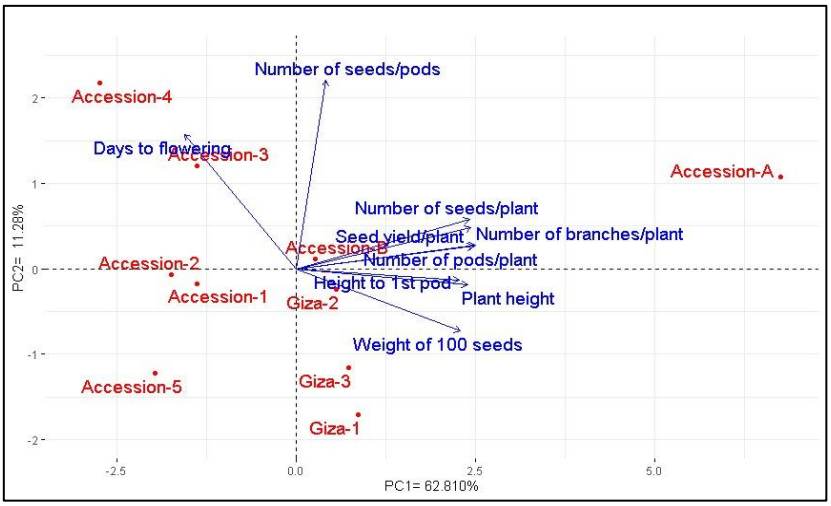

Figure 2. Principal component biplot for the 10 sweet and bitter lupin genotypes according to the agromorphological traits.

A heatmap further illustrated the relationships between genotypes and agronomic attributes (Figure 3). Accession-A displayed high values (blue) for all agronomic traits. Accessions 2, 1, and 5 had the lowest values (red), while Accession-B, 'Giza-3', 'Giza-1', and 'Giza-2' showed intermediate performance.

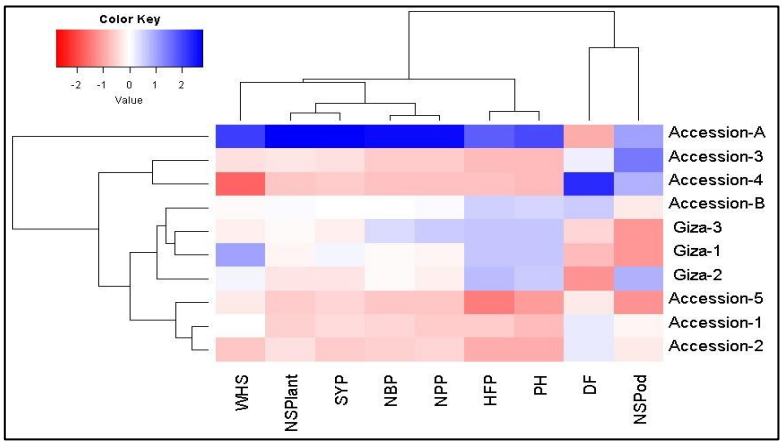

Figure 3. Heatmap categorizing 10 sweet and bitter lupin genotypes into clusters according to agronomic traits. WHS: Weight of 100 seeds; NSPlant: number of seeds per plant; SYP: seed yield per plant; NBP: number of branches per plant; NPP: number of pods per plant; HFP: height to first pod; PH: plant height; DF: days to flowering; and NSPod: number of seeds per pod.

Molecular analyses

The SCoT analysis using seven primers revealed molecular diversity among the genotypes (Figure 4). Fifty-three loci were detected (Table 3), with an average of 7.57 fragments per primer. Of these, 38 were polymorphic (5.43 per primer) and 15 were monomorphic (2.14 per primer), with polymorphism ranging from 14.29% to 100% (average 71.25%). The lowest genetic distance (2) was observed between Accession-A and Accession-B, indicating close genetic similarity (Table 4). The highest genetic distance (4.58) was between 'Giza-2' and Accession-4, suggesting greater divergence. Dice's coefficient analysis (Table 5) showed the highest similarity between Accession-A and Accession-B (0.944) and the lowest between 'Giza-2' and Accession-4 (0.734).

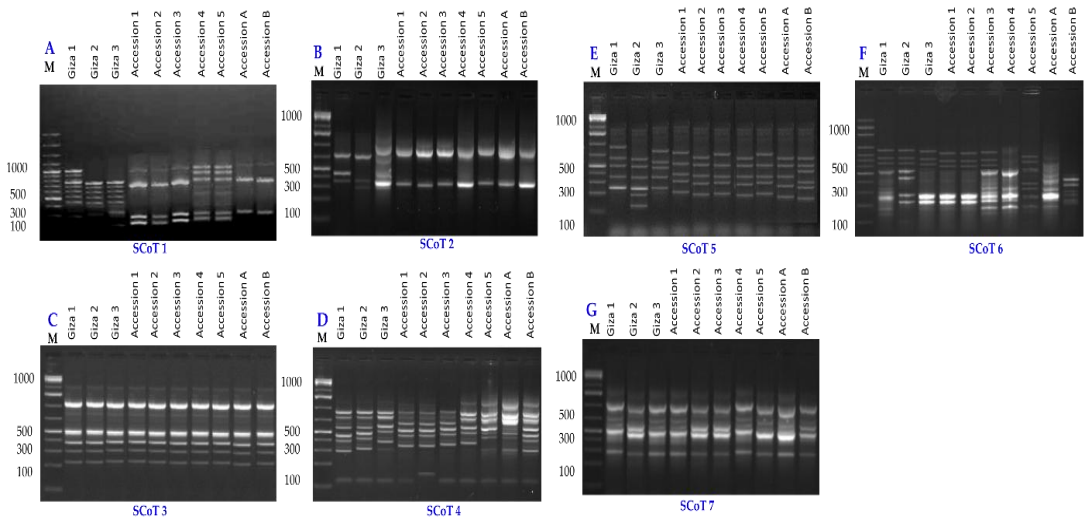

Figure 4. Start codon targeted (SCoT)-PCR amplification patterns of 10 sweet and bitter lupin genotypes using seven SCoT primers (A-G). M: 100 bp DNA ladder.

Table 3. Number of bands, monomorphic, polymorphic, and unique, detected by seven start codon targeted (SCoT) primers in 10 sweet and bitter lupin genotypes and the associated polymorphism (%).

Primer	Bands	Monomorphic bands	Polymorphic bands	Unique bands	Polymorphism (%)
SCoT1	9	1	8	0	88.89
SCoT2	6	0	6	1	100.00
SCoT3	7	6	1	0	14.29
SCoT4	10	3	7	0	70.00
SCoT5	7	2	5	1	71.43
SCoT6	8	1	7	0	87.50
SCoT7	6	2	4	0	66.67
Total	53	15	38	2	
Average	7.57	2.14	5.43	0.29	71.25

Table 4. Genetic distance among the 10 sweet and bitter lupin genotypes based on start codon targeted (SCoT) banding profiles.

Genotype	Giza 1	Giza 2	Giza 3	Accession 1	Accession 2	Accession 3	Accession 4	Accession 5	Accession A	Accession B
Giza 1	0.00									
Giza 2	3.61	0.00								
Giza 3	3.32	3.46	0.00							
Accession 1	3.87	4.47	3.16	0.00						
Accession 2	4.24	4.12	3.61	3.00	0.00					
Accession 3	4.36	4.47	4.47	3.74	3.00	0.00				
Accession 4	4.24	4.58	4.36	3.61	4.00	3.87	0.00			
Accession 5	4.00	4.36	4.36	3.87	4.24	3.87	3.74	0.00		
Accession A	4.47	4.12	4.36	3.87	3.46	3.61	3.46	2.83	0.00	
Accession B	4.00	4.12	4.12	3.61	3.74	3.61	3.16	2.45	2.00	0.00

Table 5. Dice measurement for similarity coefficient of the 10 sweet and bitter lupin genotypes based on start codon targeted (SCoT) banding profiles.

Genotype	Giza 1	Giza 2	Giza 3	Accession 1	Accession 2	Accession 3	Accession 4	Accession 5	Accession A	Accession B
Giza 1	1.00									
Giza 2	0.847	1.00								
Giza 3	0.874	0.857	1.00							
Accession 1	0.819	0.750	0.878	1.00						
Accession 2	0.775	0.779	0.835	0.880	1.00					
Accession 3	0.765	0.744	0.750	0.816	0.877	1.00				
Accession 4	0.780	0.734	0.765	0.831	0.784	0.800	1.00			
Accession 5	0.805	0.759	0.765	0.805	0.757	0.800	0.816	1.00		
Accession A	0.744	0.773	0.753	0.795	0.829	0.817	0.833	0.889	1.00	
Accession B	0.805	0.785	0.790	0.831	0.811	0.827	0.868	0.921	0.944	1.00

Phylogeny analysis

Clustering analysis based on SCoT banding profiles grouped the genotypes into four clusters (Figure 5). Cluster A contained only Accession-4. Cluster B comprised Accessions A, B, and 5. Cluster C included Accessions 1, 2, 3, and 'Giza-3'. Cluster D consisted of 'Giza-1' and 'Giza-2'.

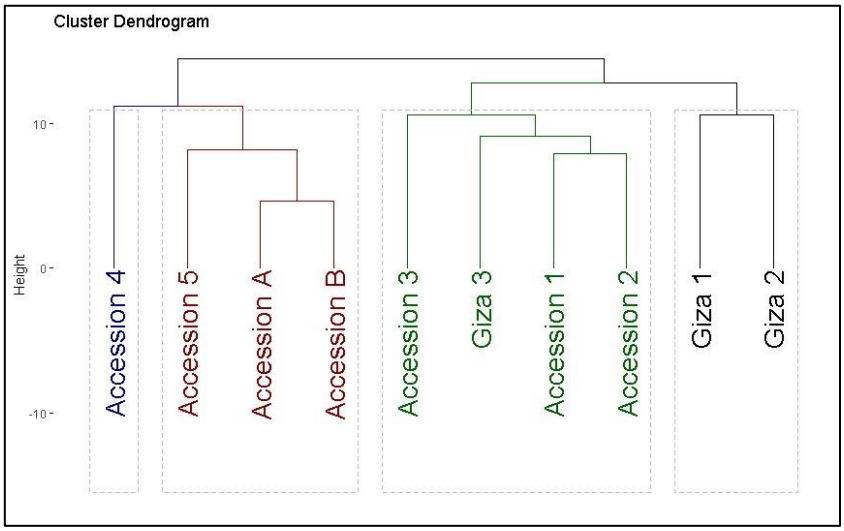

Figure 5. Phylogenetic tree of 10 sweet and bitter lupin genotypes revealed based on start codon targeted (SCoT) banding profiles.

Total protein, flavonoids, and total phenolics contents

Table 6 shows total phenolics and protein contents of sweet and bitter lupin genotypes. The sweet lupin showed superior protein content than bitter lupin. In bitter lupin, 'Giza 1' showed the highest total phenolic content (52 mg g^{-1}), total flavonoid content (27 mg g^{-1}), and total protein (24 mg g^{-1}). Meanwhile, in sweet, Accession 5 showed the highest total phenolics content (55 mg g^{-1}), total flavonoids content (27 mg g^{-1}), and total protein (25 mg g^{-1}).

Table 6. Total flavonoids content, total phenolics content, and total protein of different sweet and bitter lupin genotypes. Means followed by different letters indicate a significant difference according to LSD, $p < 0.05$.

Samples	Total flavonoids content	Total phenolics content	Total protein
	mg g^{-1}	mg g^{-1}	mg g^{-1}
Giza 1	27 ± 0.8^{b}	52 ± 0.9^{b}	24 ± 1.2^{a}
Giza 2	21 ± 0.7^{d}	46 ± 0.5^{d}	15 ± 0.2^{e}
Giza 3	25 ± 0.5^{bc}	49 ± 0.2^{c}	18 ± 0.1^{bc}
Accession A	11 ± 0.1^{f}	41 ± 1.1^{ef}	10 ± 0.5^{g}
Accession B	17 ± 0.3^{e}	42 ± 0.5^{e}	11 ± 0.4^{g}
Accession 1	10 ± 0.5^{f}	41 ± 0.8^{ef}	13 ± 0.7^{f}
Accession 2	21 ± 0.9^{d}	39 ± 0.7^{f}	17 ± 0.2^{cd}
Accession 3	24 ± 0.1^{c}	50 ± 0.5^{bc}	19 ± 0.3^{b}
Accession 4	16 ± 0.2^{e}	40 ± 0.7^{ef}	16 ± 0.1^{de}
Accession 5	30 ± 0.6^{a}	55 ± 0.2^{a}	25 ± 0.2^{a}

Biological activities

Antioxidant activities. Figure 6 shows the scavenging activity of bitter and sweet lupin genotypes against DPPH free radicals. 'Giza 1' significantly scavenged 92% of DPPH free radicals. Meanwhile, Accessions 5 and 3 showed the highest antioxidant activity against DPPH at 93% and 90%, respectively (Figure 6).

Antibacterial activity. Table 7 shows the bactericidal activity of sweet and bitter lupin genotypes against Gram negative *Escherichia coli* (EC) and Gram positive bacteria *Staphylococcus aureus* (SA). The results indicated that lupin protein has considerable antibacterial activity against the tested pathogens. 'Giza 1' showed the uppermost inhibition zone against *S. aureus* (35 mm) and *E. coli* (25 mm) in bitter lupin. Meanwhile, in sweet lupin, Accessions 5 and 3 showed the highest inhibition zones against *S. aureus* (36 and 34 mm) and *E. coli* (26 and 23 mm), respectively.

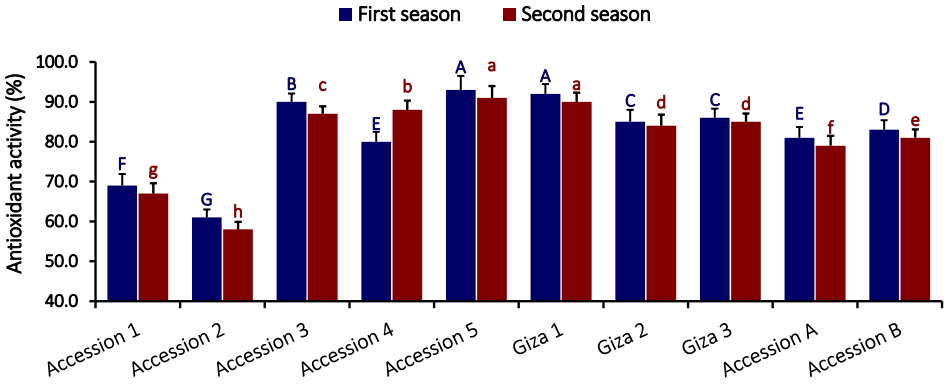

Figure 6. Percentage of antioxidant activity of five bitter and sweet lupin genotypes against DPPH radicals. Bars at top of columns indicate standard error. Columns marked with different letters indicate a significant difference according to LSD, $p < 0.01$. Uppercase letters denote the first season, while lowercase letters correspond to the second season. DPPH: 2,2-Diphenyl-1-picrylhydrazyl.

Table 7. Antibacterial activity of different sweet and bitter lupin genotypes estimated by inhibition zones diameters. Means followed by different letters indicate a significant difference according to LSD, $p < 0.05$.

Samples	Inhibition zone diameter (mm)	
	Escherichia coli	*Staphylococcus aureus*
Giza 1	25 ± 0.2^{a}	35 ± 1.2^{ab}
Giza 2	20 ± 0.4^{de}	30 ± 1.5^{cd}
Giza 3	21 ± 0.3^{cd}	31 ± 2.1^{c}
Accession A	22 ± 0.6^{bc}	26 ± 0.9^{f}
Accession B	19 ± 0.8^{ef}	29 ± 0.8^{de}
Accession 1	15 ± 0.4^{g}	25 ± 0.4^{f}
Accession 2	13 ± 0.7^{h}	22 ± 0.9^{g}
Accession 3	23 ± 0.9^{b}	34 ± 0.4^{b}
Accession 4	18 ± 0.2^{f}	28 ± 0.1^{e}
Accession 5	26 ± 0.6^{a}	36 ± 0.4^{a}

DISCUSSION

Exploring genetic diversity in lupin is crucial for enhancing yield and adaptability, especially under current climate change. The present study employed field phenotyping over two growing seasons and SCoT marker profiling providing detailed insights into lupin genetic variations. Integrating phenotypic and molecular data is essential for accelerating breeding programs and ensuring that lupin remains a valuable and adaptable crop in

the face of global climate fluctuations. The observed significant differences in phenological performance, growth characteristics, and yield-attributing traits among the assessed sweet and bitter lupin genotypes indicate their considerable genetic diversity and highlight their potential for breeding programs. The consistency of several agronomic traits across two growing seasons for most genotypes emphasizes their stability and adaptability to be exploited in lupin breeding programs. The variation in days to flowering is noteworthy, as it reflects the adaptability of different genotypes to different climatic conditions. Accession-4, with its extended time to flowering, is well-suited for environments where a longer growing season is beneficial. Conversely, earlier flowering genotypes like 'Giza-1', 'Giza-2', and 'Giza-3' are likely more appropriate for regions with shorter growing seasons or where an early harvest is desired. This diversity in flowering times is critical for optimizing lupin production across different regions. The variability in plant height among the genotypes also indicates their differing growth habits, which can be employed to meet specific agronomic needs. Accession-A, with its significantly taller plants, could be advantageous in systems where plant height correlates with higher yield or where taller plants are preferred. The moderate heights of 'Giza-1', 'Giza-2', 'Giza-3', and Accession-B suggest a balance between growth vigor and practical agricultural applications, making these genotypes useful across various farming practices. In contrast, the shorter plant heights observed in Accessions 1, 2, 3, 4, and 5 might be less desirable for biomass production but could be beneficial in conditions where shorter stature reduces the risk of lodging, such as in windy regions. The height of the first pod is another critical trait, particularly for mechanical harvesting. Accession-A, which consistently had the highest first pod height, presents a distinct advantage for mechanized agriculture, minimizing the risk of yield loss during harvest. In contrast, the lower pod heights of Accession-5 and similar genotypes may necessitate more careful harvesting techniques to avoid losses, particularly in non-mechanized systems. In this context, Gulisano et al. (2023) reported that lupin genotypes vary considerably in flowering time, pod development, maturation rates, plant height, and biomass accumulation which are critical factors influencing adaptation to different environmental conditions and their yield potential.

Number of branches per plant is a vital yield-contributing trait, and the substantial differences observed among the genotypes reflect their potential productivity. With its consistently high number of branches, Accession-A demonstrates promise as a high-yielding genotype due to its capacity for more extensive reproductive structures. The moderate branching observed in 'Giza-1', 'Giza-2', 'Giza-3', and Accession-B offers a balanced approach between reproductive and vegetative growth, which may be advantageous under less favorable conditions. In contrast, the low branching in Accessions 1, 2, 3, 4, and 5 suggests that these genotypes may require further selection or breeding to enhance their branching potential and seed yield. Number of pods per plant further supports superior performance of Accession-A, which significantly surpassed other genotypes. The consistent performance of 'Giza-3' also highlights its potential as a stable and productive genotype. However, the lower and less stable pod numbers in Accessions 1, 2, 3, 4, and 5 suggest these genotypes might be more sensitive to environmental conditions or less genetically optimized for pod production, indicating a need for further improvement. Regarding number of seeds per pod, Accession-3 emerged as the most consistent and highest-performing genotype. The trait stability observed across seasons for Accession-3, Accession-4, and 'Giza-2' suggests that these genotypes are well-suited for breeding programs to improve number of seeds. On the other hand, the variability observed in 'Giza-3' and Accession-5 may indicate a greater sensitivity to environmental fluctuations. Accession-A dominance is further evident in number of seeds per plant, underscoring its genetic potential for high seed production. The strong performance of Accession-B and 'Giza-3' also supports their inclusion in breeding programs aimed at increasing seed yield. Conversely, the lower and more variable seed production in Accessions 1, 4, and 5 suggests these genotypes may be less suited to high-yield environments or may require more specific environmental conditions to perform optimally. Weight of 100 seeds is a critical indicator of seed quality and market value. Accession-A consistent superiority in this trait across both seasons highlights its potential for producing high-quality seeds. 'Giza-1' strong performance also suggests it is a valuable genotype in terms of seed size and weight, which are important factors for both yield and marketability. The lower seed weights in Accessions 2, 3, 4, and 5 indicate that these genotypes may need further improvement to enhance seed quality. Seed yield per plant confirms Accession-A position as the most promising genotype, with the highest yields recorded across both seasons. This superior performance suggests that Accession-A has a robust genetic makeup that maintains high productivity under varying conditions. 'Giza-1' and Accession-B, which also performed well, are promising candidates for environments

where consistent yield is crucial. Similarly, Tadele and Berhanu (2024) documented significant differences in yield-attributing traits such as pod number, seed size, and harvest index, demonstrating that certain genotypes consistently outperform others under specific environmental conditions. These findings highlight the critical role of careful genotype selection in lupin breeding programs, aiming to maximize yield and enhance adaptability across diverse agroecological zones.

The results of the PCA and heatmap analysis provide valuable perspectives into the relationships among the evaluated lupin genotypes and their agronomic traits (Soliman et al., 2024). Genotypes on positive axis of PC1, particularly Accession A, were correlated with higher agronomic performance. This suggests that Accession-A possessed favorable characteristics such as more branches, pods, and seeds per plant, along with greater plant height and weight. These traits are crucial for achieving higher seed yields, as evidenced by their robust positive correlation with seed yield per plant. The clustering of these high-performing traits on the positive axis of PC1 highlights the potential of Accession A as a candidate for breeding programs to enhance yield in lupin. Conversely, genotypes positioned on the negative side of PC1, including Accessions 1, 2, 3, 4, and 5, exhibited lower performance across the measured agronomic traits. These genotypes displayed lower values in traits critical for yield, which may indicate a lack of adaptation to the environmental conditions or inherent genetic limitations. The negative correlation between days to flowering and seed yield per plant further suggests that delayed flowering may be detrimental to yield, particularly under stress conditions where early maturity is often advantageous. The genotypes 'Giza 3', 'Giza 2', 'Giza 1', and Accession B occupied an intermediate position between the high and low-performing groups. This suggests that these genotypes exhibit a balance of traits, leading to moderate performance. Their intermediate position indicates potential stability and adaptability, making them suitable for environments where consistency across seasons is desirable. The heatmap analysis offered a visual representation of the agronomic performance of each genotype, further validating the results of the PCA (Swailam et al., 2021). Using a color scale allowed for easy identification of high and low values across the genotypes. Accession A, which consistently appeared in favorable colors on the heatmap, stood out for its superior performance across all measured traits, reinforcing its potential as a high-yielding genotype. In contrast, Accessions 2, 1, and 5, marked by less favorable colors, confirmed their lower performance. The intermediate performance of Accession B, 'Giza 3', 'Giza 1', and 'Giza 2', represented by varying shades between extremes, further corroborated their positioning in the PCA, highlighting their potential for stability and adaptability across different environments.

The SCoT marker profiling indicated a moderate level of genetic diversity among the assessed genotypes with an average polymorphism rate of 36.36%. The clustering analysis divided the genotypes into four distinct groups, suggesting varying levels of genetic relatedness. Pairwise genetic distances and similarities revealed close relationships between Accession A and Accession B which belong to the same lupin species, and greater divergence between 'Giza-2' and Accession-4 which belong to different lupin species. These findings offered valuable insights into the genetic structure of the lupin germplasm studied. Identification of genetically diverse groups can guide the selection of parents for breeding programs to enhance genetic diversity in offspring (Al-Khayri et al., 2023). The polymorphic SCoT markers can be used to develop marker-assisted selection strategies for desirable traits in lupin (Rychel-Bielska et al., 2024).

Lupin seeds face nutritional limitations due to deficiencies in essential amino acids required for human and monogastric animal diets. To improve the nutritional profile of lupin, targeted breeding programs aim to develop high-yielding genotypes with enhanced protein content and favorable agronomic traits, offer a promising and cost-effective solution. Therefore, exploring the genetic diversity and bioactive properties of various lupin genotypes is crucial for advancing these breeding efforts and enhancing the overall nutritional value of lupin seeds. The electropherogram analysis revealed distinct differences in the protein profiles of bitter and sweet lupin genotypes. Sweet lupin exhibited a more diverse range of protein bands than bitter ones, suggesting richer protein composition. Accessions 2 and 3 showed 26 and 27 bands with a broad molecular weight range of 13-93 kDa. This range suggested a wider variety of proteins in sweet lupin, which could be attributed to their higher genetic and phenotypic variability. Conversely, bitter lupin genotypes, such as 'Giza 1', 'Giza 2', and 'Giza 3', showed fewer bands (10-12 bands) within a narrower molecular weight range (8-47 kDa). This narrower range could reflect limited diversity in protein types or lower protein complexity than sweet lupin. These differences in protein profiles are crucial as they can influence the functional properties of the lupin, including their nutritional and bioactive attributes. Similar results were depicted by Singla et al. (2017);

who identified distinct protein profiles in lupin seeds through electrophoretic analysis of total soluble proteins and globulin fractions. Also, Spina et al. (2022) reported significant variation among lupine genotypes in protein content and identified four lupin lines with highest protein content compared to the other assessed genotypes.

The total protein, flavonoids, and phenolics level analysis further highlighted the nutritional and biochemical differences between sweet and bitter lupin. Sweet lupin exhibited higher protein content than their bitter counterparts, with Accession 5 showing the highest protein content. This higher protein content in sweet lupin may contribute to their enhanced functional properties, making them more suitable for applications requiring high protein content. Otherwise, as 'Giza 1', bitter lupin demonstrated the uppermost total flavonoids and phenolics contents among the bitter genotypes. Phenolics and flavonoids are known for their antioxidant properties, and their higher levels in 'Giza 1' suggest potential health benefits and applications in functional foods. Previous studies elucidated variations of phytochemical composition in lupin genotypes, including total phenolics compounds, phenolics acids, flavonoids, and antioxidant activity (Vollmannova et al., 2021).

Both bitter and sweet lupin genotypes demonstrated significant antioxidant activity against DPPH free radicals, indicating their potential to reduce harmful reactive oxygen species. 'Giza 1' exhibited 92% scavenging activity, while Accessions 5 and 3 from the sweet lupin demonstrated higher activities at 93% and 90%, respectively. This could be related to the presence of specific antioxidant compounds or proteins that are more abundant in these genotypes. The antioxidant capacity of lupin seeds is correlated with their phenolic content. Ben Hassine et al. (2021) conducted a thorough phytochemical analysis on seeds from three *Lupinus* species, comprising two wild accessions (*L. luteus* and *L. cosentinii*) and one cultivar (*L. albus*). The scavenging activity of DPPH radical varied between 0.39 and 3.50 mg TE g^{-1}, while FRAP measurements ranged from 4.11 to 5.75 mg TE g^{-1}. The cupric reducing antioxidant capacity (CUPRAC) values for lupin seeds varied from 7.20 to 8.95 mg TE g^{-1}, with the highest values recorded for *L. cosentinii*.

Lupin protein, from both bitter and sweet genotypes, displayed remarkable antibacterial activity against both Gram-negative and Gram-positive bacteria. However, bitter lupin 'Giza 1' showed the uppermost inhibition zones against *E. coli* and *S. aureus*, indicating strong antibacterial potential. Similarly, sweet lupin, Accessions 5 and 3, exhibited significant antibacterial activity, with Accession 5 displaying the largest inhibition zones. This suggests the presence of antimicrobial compounds or peptides within the lupin protein extracts. These fractions exhibited potent antibacterial activity against a panel of seven pathogenic bacteria. The minimum inhibitory concentrations (MICs) of the bioactive fraction were significantly lower than those of the 11S globulin component, demonstrating its superior antimicrobial efficacy.

CONCLUSIONS

Phenotypic evaluation revealed Accession-A as a superior lupin genotype with high yield potential, while molecular analysis using start codon targeted (SCoT) markers confirmed significant genetic diversity among sweet (Accessions 1, 2, 3, 4, and 5) and bitter ('Giza 1', 'Giza 2', 'Giza 3', Accession A, and Accession B) lupin varieties, highlighting Accession-A and Accession-B's close relationship and Giza-2 and Accession-4's divergence. Furthermore, biochemical analysis demonstrated that sweet lupin genotypes possessed higher protein content, superior antioxidant and antibacterial activities, and enhanced anticancer potential, suggesting their suitability for functional food development and breeding programs focused on improving yield, adaptability, and bioactive properties.

Author contributions

Conceptualization: A.H.A., E.I.M., H.A.W., A.A.H. Methodology, Software, Validation, Formal analysis, Investigation, Investigation, Resources, Data curation, Writing-original draft preparation, Writing-review and editing, and Visualization: A.H.A., E.I.M., H.A.W., N.M.A., J.M.AL-K., D.A., T.A.F., M.A., E.F., A.A.S.A., H.A., F.H., U.B.A., S.M., A.A.H. Supervision: A.H.A., E.I.M., H.A.W., A.A.H. Project administration: A.H.A., E.I.M., H.A.W., A.A.H. Funding acquisition: A.H.A., E.I.M., H.A.W., A.A.H., U.B.A., J.M.AL-K. All authors have read and agreed to the published version of the manuscript.

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References

Abdel-Moneim, A.E., El-Saadony, M.T., Shehata, A.M., Saad, A.M., Aldhumri, S.A., Ouda, S.M., et al. 2022. Antioxidant and antimicrobial activities of *Spirulina platensis* extracts and biogenic selenium nanoparticles against selected pathogenic bacteria and fungi. Saudi Journal of Biological Sciences 29(2):1197-1209. doi:10.1016/j.sjbs.2021.09.046.

Abraham, E.M., Ganopoulos, I., Madesis, P., Mavromatis, A., Mylona, P., Nianiou-Obeidat, I., et al. 2019. The use of lupin as a source of protein in animal feeding: Genomic tools and breeding approaches. International Journal of Molecular Sciences 20(4):851. doi:10.3390/ijms20040851.

Al-Khayri, J.-M., Alshamrani, S.M., Rezk, A.-A., Shehata, W.-F., Almaghasla, M.-I., Shalaby, T.A., et al. 2023. Pre-Breeding genetic diversity assessment of tomato (*Solanum lycopersicum* L.) cultivars based on molecular, morphological and physicochemical parameters. Phyton-International Journal of Experimental Botany 92(5):1493-1512. doi:10.32604/phyton.2023.027375.

Ben Hassine, A., Rocchetti, G., Zhang, L., Senizza, B., Zengin, G., Mahomoodally, M.F., et al. 2021. Untargeted phytochemical profile, antioxidant capacity and enzyme inhibitory activity of cultivated and wild lupin seeds from Tunisia. Molecules 26(11):3452. doi:10.3390/molecules26113452.

Chukwuejim, S., Utioh, A., Choi, T.D., Aluko, R.E. 2024. Lupin seed proteins: A comprehensive review of composition, extraction technologies, food functionality, and health benefits. Food Reviews International 40(2):691-714. doi:10.1080/87559129.2023.2191701.

Dice, L.R. 1945. Measures of the amount of ecologic association between species. Ecology 26(3):297-302. doi:10.2307/1932409.

Doyle, J. 1991. DNA protocols for plants. p. 283-293. In Hewitt, G.M., Johnston, A.W.B., Young, J.P.W. (eds.) Molecular techniques in taxonomy. Springer, Berlin, Heidelberg, Germany. doi:10.1007/978-3-642-83962-7_18.

El-Saadony, M.T., Alkhatib, F.M., Alzahrani, S.O., Shafi, M.E., Abdel-Hamid, S.E., Taha, T.F., et al. 2021a. Impact of mycogenic zinc nanoparticles on performance, behavior, immune response, and microbial load in *Oreochromis niloticus*. Saudi Journal of Biological Sciences 28(8):4592-4604. doi:10.1016/j.sjbs.2021.04.066.

El-Saadony, M.T., El-Wafai, N.A., El-Fattah, H.I.A., Mahgoub, S.A. 2019. Biosynthesis, optimization and characterization of silver nanoparticles using a soil isolate of *Bacillus pseudomycoides* MT32 and their antifungal activity against some pathogenic fungi. Advances in Animal and Veterinary Sciences 7(4):238-249.

El-Saadony, M.T., Saad, A.M., Elakkad, H.A., El-Tahan, A.M., Alshahrani, O.A., Alshilawi, M.S., et al. 2022. Flavoring and extending the shelf life of cucumber juice with aroma compounds-rich herbal extracts at 4 °C through controlling chemical and microbial fluctuations. Saudi Journal of Biological Sciences 29(1):346-354. doi:10.1016/j.sjbs.2021.08.092.

El-Saadony, M.T., Saad, A.M., Najjar, A.A., Alzahrani, S.O., Alkhatib, F.M., Shafi, M.E., et al. 2021b. The use of biological selenium nanoparticles to suppress *Triticum aestivum* L. crown and root rot diseases induced by *Fusarium* species and improve yield under drought and heat stress. Saudi Journal of Biological Sciences 28(8):4461-4471.

Essa, S.M., Wafa, H.A., Mahgoub, E.S.I., Hassanin, A.A., Al-Khayri, J.M., Jalal, A.S., et al. 2023. Assessment of eight faba bean (*Vicia faba* L.) cultivars for drought stress tolerance through molecular, morphological, and physiochemical parameters. Sustainability 15(4):3291. doi:10.3390/su15043291.

Fan, T.Y., Sosulski, F.W. 1974. Dispersibility and isolation of proteins from legume flours. Canadian Institute of Food Science and Technology Journal 7(4):256-259. doi:10.1016/S0315-5463(74)73923-2.

FAOSTAT 2024. Statistical database. Food and Agriculture Organization of the United Nations. Available at http://www.fao.org/faostat/en/#data (accessed on 19 August 2024).

Gharibzahedi, S.M.T., Smith, B., Altintas, Z. 2024. Bioactive and health-promoting properties of enzymatic hydrolysates of legume proteins: A review. Critical Reviews in Food Science and Nutrition 64(9):2548-2578. doi:10.1080/10408398.2022.2124399.

Gulisano, A., Lippolis, A., van Loo, E.N., Paulo, M.-J., Trindade, L.M. 2023. A genome-wide association study to dissect the genetic architecture of agronomic traits in Andean lupin (*Lupinus mutabilis*). Frontiers in Plant Science 13:1099293. doi:10.3389/fpls.2022.1099293.

Kamara, M.M., Rehan, M., Mohamed, A.M., El Mantawy, R.F., Kheir, A.M., Abd El-Moneim, D., et al. 2022. Genetic potential and inheritance patterns of physiological, agronomic and quality traits in bread wheat under normal and water deficit conditions. Plants 11(7):952. doi:10.3390/plants11070952.

Kamran, F., Phillips, M., Harman, D.G., Reddy, N. 2023. Antioxidant activities of lupin (*Lupinus angustifolius*) protein hydrolysates and their potential for nutraceutical and functional foods. Food Chemistry Advances 2:100297. doi:10.1016/j.focha.2023.100297.

Mansour, E., Moustafa, E.S., Qabil, N., Abdelsalam, A., Wafa, H.A., El Kenawy, A., et al. 2018. Assessing different barley growth habits under Egyptian conditions for enhancing resilience to climate change. Field Crops Research 224:67-75. doi:10.1016/j.fcr.2018.04.0.

Norušis, M.J. 1993. SPSS for Windows, release 6.0. Prentice Hall (Higher Education Division, Pearson Education), Hoboken, New Jersey, USA.

Pereira, A., Ramos, F., Sanches Silva, A. 2022. Lupin (*Lupinus albus* L.) seeds: Balancing the good and the bad and addressing future challenges. Molecules 27(23):8557. doi:10.3390/molecules27238557.

Pourmorad, F., Hosseinimehr, S., Shahabimajd, N. 2006. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. African Journal of Biotechnology 5(11):1142-1145.

Rokach, L., Maimon, O. (eds.) 2005. Clustering methods. p. 321-352. In Data mining and knowledge discovery handbook. Springer, New York, USA. doi:10.1007/0-387-25465-X_15.

Rychel-Bielska, S., Bielski, W., Surma, A., Annicchiarico, P., Belter, J., Kozak, B., et al. 2024. A GWAS study highlights significant associations between a series of indels in a *FLOWERING LOCUS T* gene promoter and flowering time in white lupin (*Lupinus albus* L.) BMC Plant Biology 24(1):722. doi:10.1186/s12870-024-05438-1.

Silva, T.E.d., Detmann, E., Franco, M.d.O., Palma, M.N.N., Rocha, G.C. 2016. Evaluation of digestion procedures in Kjeldahl method to quantify total nitrogen in analyses applied to animal nutrition. Acta Scientiarum. Animal Sciences 38:45-51. doi:10.4025/actascianimsci.v38i1.29171.

Singla, P., Sharma, S., Singh, S. 2017. Amino acid composition, protein fractions and electrophoretic analysis of seed storage proteins in lupins. Indian Journal of Agricultural Biochemistry 30(1):33-40. doi:10.5958/0974-4479.2017.00005.3.

Smýkal, P., Coyne, C.J., Ambrose, M.J., Maxted, N., Schaefer, H., Blair, M.W., et al. 2015. Legume crops phylogeny and genetic diversity for science and breeding. Critical Reviews in Plant Sciences 34(1-3):43-104. doi:10.1080/07352689.2014.897904.

Soliman, A.A., Ibrahim, M.A., Mousa, M.I., Mansour, E., He, Y., Yu, H. 2024. Genetic potential and inheritance pattern of agronomic traits in faba bean under free and infested *Orobanche* soil conditions. BMC Plant Biology 24(1):301. doi:10.1186/s12870-024-05017-4.

Spina, A., Saletti, R., Fabroni, S., Natalello, A., Cunsolo, V., Scarangella, M., et al. 2022. Multielemental, nutritional, and proteomic characterization of different *Lupinus* spp. genotypes: A source of nutrients for dietary use. Molecules 27(24):8771. doi:10.3390/molecules27248771.

Swailam, M., Mowafy, S., El-Naggar, N., Mansour, E. 2021. Agronomic responses of diverse bread wheat genotypes to phosphorus levels and nitrogen forms in a semiarid environment. SABRAO Journal of Breeding & Genetics 53(4):592-608. doi:10.54910/sabrao2021.53.4.4.

Szczepański, A., Adamek-Urbańska, D., Kasprzak, R., Szudrowicz, H., Śliwiński, J., Kamaszewski, M. 2022. Lupin: A promising alternative protein source for aquaculture feeds? Aquaculture Reports 26:101281. doi:10.1016/j.aqrep.2022.101281.

Tadele, Y., Berhanu, S. 2024. Agronomic performances, yield and nutritional values of lupin grain varieties at Gircha research center, Gamo Highlands. Cogent Food & Agriculture 10(1):2374940. doi:10.1080/23311932.2024.2374940.

Tessema, F. 2017. Biomass yield and nutritive value of sweet lupine in mid altitudes of Lemo District, Hadiya Zone, southern Ethiopia. MSc thesis in Animal Nutrition. Wolaita Sodo University, Wolaita Sodo, Ethiopia. Available at https://hdl.handle.net/10568/103250.

Tian, Y., Cortés-Avendaño, P., Yang, B., Glorio-Paulet, P., Repo-Carrasco-Valencia, R., Suomela, J.P. 2024. Flavonoid diversity in bitter and debittered seeds of Andean lupin (*Lupinus mutabilis* Sweet). Food Chemistry 442:138411. doi:10.1016/j.foodchem.2024.138411.

Vollmannova, A., Lidikova, J., Musilova, J., Snirc, M., Bojnanska, T., Urminska, D., et al. 2021. White Lupin as a promising source of antioxidant phenolics for functional food production. Journal of Food Quality 2021(1):5512236. doi:10.1155/2021/5512236.