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Diversity of Four *Lupinus* L. Taxa Based on Seed Protein Electrophoresis and Amino Acid Profile

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ABSTRACT

The aim of the present study is to evaluate diversity among three *Lupinus* species (*Lupinus albus* L., *Lupinus angustifolius* L., and *Lupinus digitatus* Forssk.) using seed protein electrophoresis and amino acid composition in order to elucidate the taxonomic relationships among them. In addition, to compare the *L. albus* var. *albus* with closely related wild relatives in a trial to attain information useful for breeding programs. The overall pattern of seed proteins and amino acid composition within the same species revealed that there is a considerable variation along with the geographical region. The percentage of polymorphism for all the studied taxa was 26% and a total of 18 bands are detected within the studied *Lupinus* taxa, which are characterized by 4 common bands at 38, 36, 20 and 14.4 KDa. The dendrogram constructed from different concentrations of amino acid in the studied *Lupinus* species, demonstrated that the four studied taxa are not fully discriminated even at both different similarity coefficients and different types of sorting. By using seed protein electrophoresis, the *L. albus* var. *albus* (*L. albus* cultivar) is assembled with the wild *L. albus* below the same group. But at the same time the cultivar showed considerable degree of variability from the wild species and separated alone by using both data from seed proteins and amino acid compositions. The cultivar is characterized with both low degree of polymorphism and deficiency in arginine, leucine and methionine which are considered from the vital important amino acids. Contrary, both *L. albus* and *L. angustifolius* have considerable amounts of these amino acids.

Keyword: Lupin, Protein pattern, Amino acid composition, Methionine, Dendrogram, *L. albus* var. *albus*

INTRODUCTION

Lupinus L. is a large and diverse genus, covering a wide range along both the Old and New world. In spite of this diversity, it is regarded as a natural and distinct group by the definition of Polhill (1976). According to Bisby (1981), *Lupinus* is included in the monotypic subtribe Lupininae Hutch. of the tribe Genisteae (Adanson) Bentham. Nevertheless, this tribal position has been disputed (Badr *et al.*, 1994) and the genus origin is under debate (Cristofolini, 1989; Aïnouch and Bayer, 1999). *Lupinus* comprises annual and perennial herbaceous species, as well as a few soft-woody shrubs and small trees (Dunn, 1984; Turner, 1995). The old World species are less diverse and represented by 12 annuals native to the Mediterranean region and Africa (Gladstones, 1974; Amaral Franco and Pinto da Silva, 1978). According to seed coat texture, these species are discriminated into two groups Malacospermae (smooth-seeded) and Scabrispermae (rough-seeded), consequently divided into 7 sections based on the morphological, genetic and serological evidence (Plitmann and Heyn, 1984; Carstairs *et al.*, 1992; Gladstones, 1998; Noganowsker *et al.*, 2003). The first group comprised closely related wild species under 5 sections (Albi, Angustifoli, Lutei, Micranthi and Princei). However, the second group assorted heterogenous wild and cultivars under 2 sections (Atlanticus and Pilosus) (Gladstones, 1974; Gupta *et al.*, 1996).

Since the 1960s, biological macromolecules have occupied an increasingly important role in systematic studies. The seed protein electrophoresis and amino acid composition are considered powerful tools in these taxonomic investigations (Onarici and Sümer, 2003). Other biochemical markers performed on lupins by many workers such as: Libkind (1931); Smirnova (1938); Hansen and Czochanska (1973); Kurlovich and Nazarova (1990); and Sujak *et al.* (2006). The protein value depends upon both contents and proportion of individual amino acids. Therefore, the evaluation of these amino acids assessed under three categories: 1) essential amino acids "vital important" (phenylalanine, isoleucine, leucine, lysine, methionine, threonine, tyrosine, valine), 2) amino acids compulsory for growth of organisms (the first group plus arginine and histidine), and 3) "dispensable" amino acids (glycine, asparagine, alanine, serine, proline) (Babinec *et al.*, 1994).

The main objective of the present study is to assess diversity among three *Lupinus* species (*Lupinus albus* L., Albi; *Lupinus angustifolius* L., Angustifoli; and *Lupinus digitatus* Forssk., Atlanticus) using seed protein electrophoresis and amino acid composition in order to clarify the taxonomic relationships among them. Also, the objective may be extended to compare the *L. albus* var. *albus* (Egyptian cultivar) with closely related wild relatives in a trial to attain information useful for breeding programs.

MATERIALS AND METHODS

Twenty-two accessions representing three *Lupinus* species (*L. albus*, *L. angustifolius*, and *L. digitatus*)

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beside one *L. albus* var. *albus* from the main geographical distribution origin (Mediterranean region) are used. Seeds are obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA) at Alleppo, Syria and the National Plant Germplasm System (NPGS) at Washington University (Table 1). The seeds are kept as voucher specimens in the herbarium of Faculty of Science, Alexandria University (ALEX). These taxa are subjected to two types of analyses; the first is seed protein electrophoresis which was achieved for all studied taxa. While, the second is amino acid compositions, which was carried out on the two widely collected taxa, *L. albus* and *L. angustifolius*, beside the cultivar.

Seed Protein Electrophoresis (SDS-PAGE)

The extraction of storage seed proteins were performed according to Laemmli (1970), with some modifications as described by Hames and Rickwood (1990), from the dry mature seeds of the different accessions. Sigma standard protein marker of 7 bands was used with the following molecular weights: 14.4, 18.5, 20, 30, 36, 45, 67, 92.5, and 116 KDa. One gram of seeds was grinded with sample buffer (2% SDS, 10% glycerol, 0.002% bromophenol blue, 5% β-mercaptoethanol), mixed with an equal volume of buffer of 0.5 M Tris-HCl (pH 6.8), submitted to heat treatment for 5 minutes in a boiling water bath, and then cooling to room temperature before centrifuged at 12,000 r.p.m. for 5 minutes. An aliquot of 15 µl of supernatants was loaded onto 12% acrylamide gel using Mini-PROTEAN II cell (Bio-Rad) at 75 V through the stacking gel followed by 125 V to the end of the electrophoresis (2 hrs). The gel was incubated in Coomasei Briliant Blue R250 solution overnight and then distained for 12 hrs. The polymorphism percentage was determined according to Bisby (1995) as follows:

$$(\Sigma \text{ accession bands} - \Sigma \text{ common bands for all accessions}) / \Sigma \text{ bands of all accessions}) \times 100$$

Amino Acid Composition

Beckman automatic amino acid analyzer 119 CL was used for the determination of 17 amino acids. The amino acid analysis was performed at the Central Lab. of Faculty of Agriculture, Alexandria University, Alexandria, Egypt. The automatic analyzer was used with hydrolysate system, which was determined for fixed amino acid analysis (after acid hydrolysis). Acid hydrolysis was determined according to Moore and Stein (1958) as follows: 50 mg sample was hydrolysed at 110 ^{o}C for 22 hrs with 5 ml of 6N HCl and mercaptoethanol (5µl/10 ml acid), cool at room temperature and then filtrate. One ml of filtrate was dried in vacuum desiccators in the presence of NaOH and dissolved in 1ml of sodium citrate buffer (pH 2.2),

Table (1): Accession origins, sources and codes of the seed samples of the studied *Lupinus* taxa.

Species	Origin	Sources	Accession codes
Lupinus albus L.	Italy	NPGS	PI 516624
L. albus L.	Spain	NPGS	PI 481559
L. albus L.	Greece	NPGS	PI 457930
L. albus L.	Morocco	ICARDA	IG 110552
L. albus L.	Morocco	NPGS	PI 483073
L. albus L.	France	NPGS	PI 467349
L. albus L.	France	NPGS	PI 467351
L. digitatus Forssk.	Spain	NPGS	W6 11995
L. angustifolius L.	Spain	NPGS	PI 385078
L. angustifolius L.	Spain	NPGS	PI 385111
L. angustifolius L.	Spain	NPGS	PI 385093
L. angustifolius L.	Spain	NPGS	PI 385092
L. angustifolius L.	Spain	NPGS	PI 383249
L. angustifolius L.	Spain	NPGS	PI 385082
L. angustifolius L.	Spain	NPGS	PI 385080
L. angustifolius L.	Spain	NPGS	PI 385100
L. angustifolius L.	Spain	NPGS	PI 385119
L. angustifolius L.	Greece	ICARDA	IG 109143
L. angustifolius L.	Greece	ICARDA	IG 109150
L. angustifolius L.	Cyprus	ICARDA	IG 109139
Lupinus angustifolius L.	Turkey	NPGS	PI 491182
L. albus L. cv. albus	Egypt	Egyptian market	

NPGS: National Plant Germplasm System.
ICARDA: International Center for Agricultural Research in the Dry Areas.

then re-filtrate through 3 MM filter paper. Amino acid concentration (g/100 g sample) is calculated by the following equation:

$$\text{Sample area} \times (\text{standard concentration} / \text{standard area}) \times \text{constant}$$

Where the constant = (2 × dilution (1 ml) × 100) / (10^6 × sample weight (0.005 g) = 0.04

Treatment of Data

Each taxon was considered as an operational taxonomic unit (OTU) for the purpose of classification. The electrogram is documented with Total Lab program (www.Totallab.com) while, PAST program (Hammer and Harper, 2001) was used for dendrogram construction for both seed proteins and amino acids separately and cooperatively. Additionally, One-way Analysis of Variance (ANOVA) was applied to assess the significance of variations in the concentration of different amino acids of twenty-two accessions representing two of the present studied *Lupinus* species (*L. albus*, *L. angustifolius*) and the cultivar. The statistical procedures were performed using STATISTICA 5.0 statistical analysis software manufactured by Stat soft Software Company (www.statsoftsa.co.za).

RESULTS

The electrophoretic patterns (using SDS-PAGE), cluster analysis (using PAST program) and percentage of polymorphism of the total seed proteins are represented in Plate (1), Figure (2) and Table (2)

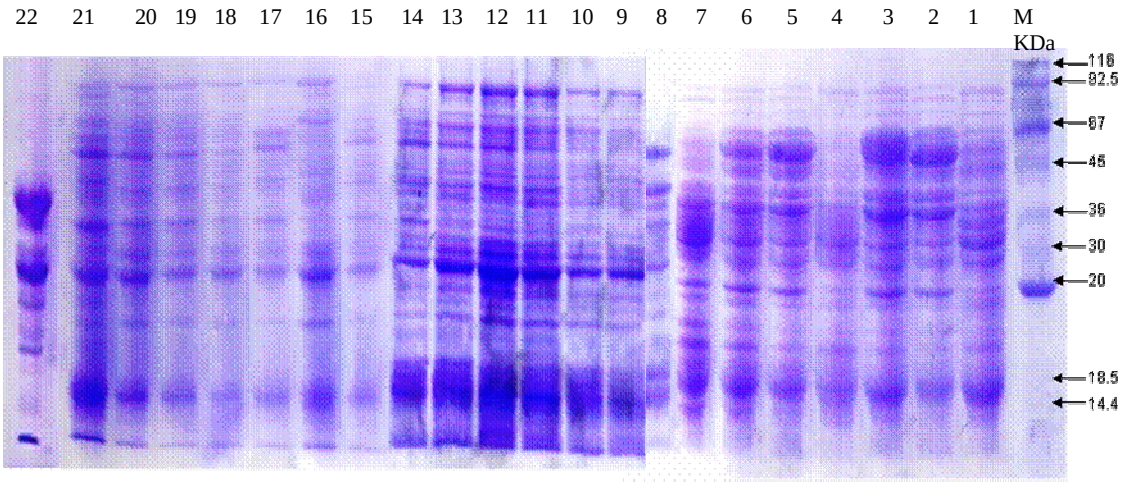

Plate (1): Electrophoretic patterns of seed proteins (SDS-PAGE) of the studied *Lupinus* taxa (1-7, *L. albus*; 8, *L. digitatus*; 9-21 *L. angustifolius*; 22, *L. albus* var. *albus*).

respectively. The present study revealed that the percentage of polymorphism for all the studied taxa was 26% and a total of 18 bands are detected within the studied *Lupinus* taxa, which are characterized by 4 common bands at 38, 36, 20 and 14.4 KDa. Specifically, the four *Lupinus* taxa are discriminated into two delimited groups; the first one aggregated both *L. albus* and the cultivar, while *L. digitatus* and *L. angustifolius* are segregated under the second group. Particularly, *L. albus* and cultivar group shared with one band at 18.7 KDa beside the four common ones for all the studied taxa. The cultivar is characterized by one positive band at 11.8 KDa, and 4 negative ones at 86, 42, 19.8 and 18.5 KDa relative to *L. albus*. On the other hand, *L. albus* accessions are characterized by unique positive band at 18.7 KDa. Within the accessions of *L. albus*, the taxa number 6 and 7 (from France) are segregated from the others at 0.6 similarity coefficient; which have one common positive band at 13 KDa. At 0.7 similarity coefficient, the taxon number 4 (from Moracco) is separated due to the characterization with two negative bands at 60 and 45 KDa, which are present in all studied taxa of *L. albus*. This taxon is characterized by both the minimum number of bands (12) and the lowest percentage of polymorphism (7%). The taxon number 1 (from Turkey) is singly grouped at 0.84 similarity coefficient and is characterized by the unique positive band, compared with the other studied *L. albus* accessions, at 28 KDa.

L. digitatus and *L. angustifolius* taxa were discriminated at relatively low similarity coefficient (0.25). *L. digitatus* is characterized by the lowest number of bands (7 bands) and one negative band at 22.5 KDa compared with all the examined *Lupinus* taxa. Moreover, *L. digitatus* possessed two negative bands at 86 and 42 KDa not in both *L. albus* and *L. angustifolius*. On the other hand, *L. angustifolius* taxa are specified with two bands at 92.5 and 11.8 KDa. These taxa are

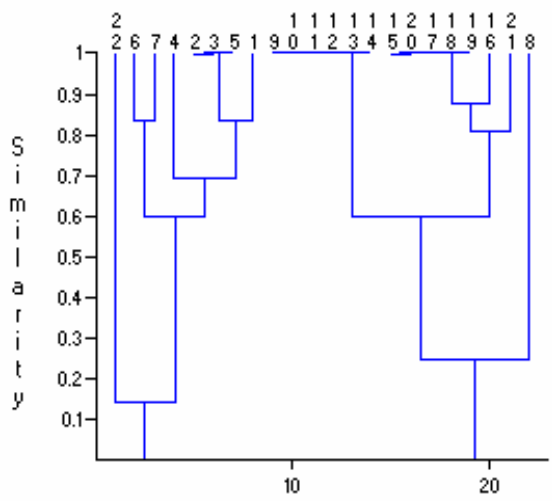

Figure (2): Dendrogram constructed from the data of seed protein electrophoresis using PAST program by paried group and correlation similarity coefficient (1-7, *L. albus*; 8, *L. digitatus*; 9-21 *L. angustifolius*; 22, *L. albus* var. *albus*).

separated into two subgroups at 0.6 similarity coefficient. The first subgroup distinguished the accessions from 9 to 14 (From Spain) from the others. This cluster was highly homogenous and shared 2 bands at 19.8 and 18.5 KDa. While, the second subgroup divaricated into two clusters at 0.8 similarity coefficient; under one of them the taxon number 21 (from Turkey) is segregated. This taxon is distinct with the positive band at 19 KDa, not in the taxa assorted within the same subgroup. At 0.86 similarity coefficient, taxon number 16 is separated and distinguished by one negative band at 60 KDa compared with all studied taxa of *L. angustifolius*.

The different concentrations of amino acids in the studied *Lupinus* species are represented in Table (3), while the constructed dendrogram (Fig. 3) demonstrated that the four studied taxa are not fully discriminated even

Table (2): Total number of bands, number of both positive and negative bands and percentage of polymorphism (P) of the studied *Lupinus* taxa. 1-7, *L. albus*; 8, *L. digitatus*; 9-21 *L. angustifolius*; 22, *L. albus* var. *albus*.

Taxa	Total no. of bands	No. of positive bands	No. of negative bands	P (%)
1	15	1 (28 KDa)		27
2	14			20
3	14			20
4	12		2 (60 and 45 KDa)	7
5	14			20
6	15			27
7	14		1 (76 KDa)	20
8	7		2 (86 and 42 KDa) relative to *L.albus* and *L. angustifolius* and 1 (22.5 KDa) relative to all studied taxa	43
9	16			25
10	16			25
11	16			25
12	16			25
13	16			25
14	16			25
15	13			6
16	12		1 (60 KDa) relative to *L. angustifolius*	0
17	13			6
18	13			6
19	13			6
20	13			6
21	14			7
22	8	1 (11.8 KDa) relative to *L. albus*	4 (86, 42,19.8 and 18.5 KDa) relative to *L. albus*	50

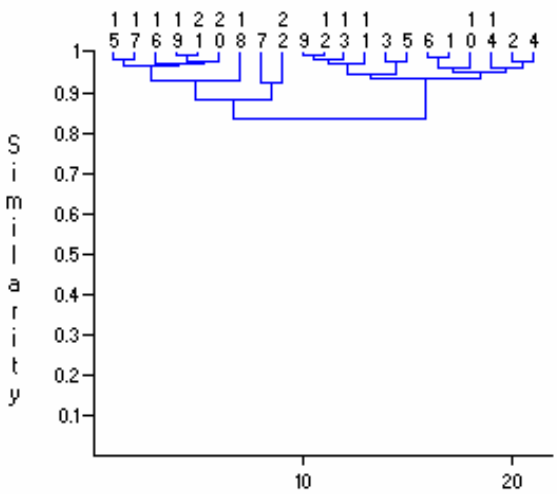

Figure (3): Dendrogram constructed from the data of amino acid composition using PAST program by paried group and Rho similarity coefficient (1-7, *L. albus*; 9-21 *L. angustifolius*; 22, *L. albus* var. *albus*).

at both different similarity coefficients and different types of sorting.

As witnessed by the present data, the concentration of amino acids differed significantly ($p < 0.05$) with the different taxa (Fig. 5) and geographic origin. Generally, the cultivar attained the highest concentration for all the studied amino acids except for glutamine, methionine, leucine, lysine and arginine. Whereas, the highest concentrations of glutamine (8.25 g $100g^{-1}$), leucine (2.72 g $100g^{-}1$) lysine (1.89 g $100g^{-}1$) and arginine (4.29 g $100g^{-1}$) are attained by *L. angustifolius*, while that of methionine (0.36 g $100g^{-1}$) was achieved by *L. albus*. The variation was highly significant ($p < 0.05$) between the two taxa and the cultivar for asparagine, serine, threonine, proline, valine, isoleucine, leucine, tyrosine and histidine. On the other hand, the variation in amino acids; glycine, phenylalanine, lysine and cystine was not significant between the studied taxa. To go throughout with this, arginine in *L. angustifolius* differs significantly with that of *L. albus* and cultivar, methionine in *L. albus* with that of *L. angustifolius* and cultivar, glutamine and alanine in the cultivar with that of *L. albus* and *L. angustifolius*. It is worth to mention that, different taxa exhibit different ranking except for the three first amino acids according to their concentrations. The ranking was glutamine > asparagine > arginine > leucine > isoleucine > proline > threonine & lysine > glycine > tyrosine > alanine > phenylalanine >serine > histidine > valine & methionine >cystine for *L. albus*, glutamine > asparagine > arginine > leucine > threonine > isoleucine > lysine > glycine > serine > proline > valine > phenylalanine > alanine > tyrosine > histidine > methionine > cystine for *L. angustifolius* and glutamine > asparagine > arginine > threonine > leucine > isoleucine > tyrosine > proline > serine > lysine > phenylalanine > glycine > valine > alanine > histidine > methionine > cystine for the cultivar. Once again the dendrogram resulted from both seed proteins and amino acid concentrations in *L. albus*, *L. angustifolius* and the cultivar is presented in Figure (4). This dendrogram was highly concordance with that resulted from the usage of seed proteins only, except for the cultivar which is separated from the other taxa at 0.74 similarity coefficient.

Discussion

The major physiological and biochemical features of *Lupinus* are the capability to synthesize plenty of protein. Among the rich specific diversity of lupin there are species, varieties and forms, which accumulate large amounts of protein, oil and other useful substances (Kurlovich, 1998). The present study demonstrated that the rough-seeded *Lupinus digitatus* is characterized by the lowest number of seed protein bands and also with unique both positive and negative bands. These protein results are highly congruent with the pattern of relationships indicated by many other tools as leaf flavonoids (William *et al.*, 1983), alkaloids (Aïnouch *et al.*, 1996) and seed coat texture (Marzouk, 2006) that rough-seeded group represented a separate clade.

One remarkable result emerging from this study is that *L. digitatus* aggregated with *L. angustifolius* below

Table (3): Variation in the concentration of 17 amino acids in *Lupinus albus* (1-7; 7 accessions), *Lupinus angustifolius* (9-21; 13 accessions) and Egyptian cultivar (22, one accession).

TAXON	ASP	THR	SER	GLU	PRO	GLY	ALA	CYS	VAL	MET	ISO	LEU	TYR	PHE	HIS	LYS	ARG
1	2.86^{c}	1.80^{a}	1.34^{a}	5.61^{a}	1.54^{e}	1.61^{bcd}	1.35^{cd}	0.14^{gh}	1.69^{de}	0.34^{abc}	1.81^{de}	2.17^{a}	1.38^{d}	1.25^{ab}	0.95^{a}	1.74^{bcd}	3.39^{c}
2	3.34^{de}	1.80^{a}	1.34^{a}	6.65^{c}	1.79^{f}	1.55^{abc}	1.57^{fgh}	0.12^{efg}	1.7^{de}	0.52^{cd}	1.66^{bc}	2.2^{ab}	1.52^{f}	1.5^{ab}	0.92^{a}	1.87^{cdef}	3.1^{b}
3	4.14^{i}	1.80^{a}	1.34^{a}	10.77^{l}	2.18^{j}	2.11^{h}	1.76^{i}	0.07^{abcd}	2.13^{f}	0.44^{bcd}	2.07^{gh}	2.77^{de}	1.84^{j}	1.73^{ab}	1.07^{b}	1.98^{efg}	4.56^{i}
4	3.66^{f}	1.80^{a}	1.34^{a}	8.46^{h}	1.92^{g}	1.74^{de}	1.6^{gh}	0.17^{h}	1.88^{e}	0.55^{d}	1.73^{cd}	2.37^{bc}	1.51^{f}	1.43^{ab}	0.94^{a}	1.77^{cd}	3.56^{d}
5	3.93^{h}	1.80^{a}	1.34^{a}	9.62^{m}	1.92^{g}	1.95^{fg}	1.55^{fgh}	0.05^{abc}	1.9^{e}	0.29^{abc}	1.88^{ef}	2.45^{c}	1.35^{d}	1.49^{ab}	0.92^{a}	1.6^{b}	3.31^{c}
6	3.67^{f}	1.80^{a}	1.34^{a}	8.29^{h}	1.77**f**	1.71^{de}	1.36^{cd}	0.09^{cdef}	1.79^{de}	0.24^{ab}	1.95^{f}	2.35^{bc}	1.66^{h}	1.48^{ab}	0.97^{a}	1.81^{cde}	3.38^{c}
7	3.29^{d}	1.80^{a}	1.34^{a}	8.33^{h}	1.56^{e}	1.71^{de}	1.35^{cd}	0.07^{abcd}	1.25^{ab}	0.18^{ab}	2.05^{g}	2.72^{d}	1.59^{g}	1.38^{ab}	1.08^{bc}	1.83^{cde}	3.31^{c}
9	3.65^{f}	1.80^{a}	1.34^{a}	9.10^{k}	2.05^{h}	2.00^{g}	1.64^{h}	0.21^{i}	1.9^{e}	0.3^{bbc}	2.19^{ij}	2.8^{de}	1.51^{f}	1.75^{ab}	1.13^{bc}	2.03^{fg}	4.84^{g}
10	2.57^{a}	1.80^{a}	1.34^{a}	7.53^{e}	1.41^{d}	1.52^{ab}	1.05^{a}	0.03^{a}	1.63^{cd}	0.1^{a}	1.49^{a}	2.12^{a}	1.14^{b}	1.37^{ab}	0.9^{a}	1.88^{cdef}	3.56^{d}
11	3.84^{g}	1.80^{a}	1.34^{a}	9.00^{jk}	2.17^{j}	1.90^{fg}	1.51^{efg}	0.08^{bcde}	1.56^{cd}	0.24^{ab}	2.26^{jk}	2.76^{de}	1.25^{c}	1.48^{ab}	1.38^{d}	1.89^{cdefg}	5.28^{k}
12	3.98^{h}	1.80^{a}	1.34^{a}	9.30^{l}	2.56^{k}	1.98^{g}	1.38^{cd}	0.12^{efg}	1.8^{de}	0.3^{abc}	2.12^{ghi}	2.51^{c}	1.52^{f}	1.7^{ab}	1.16^{bc}	2.06g	4.45^{hi}
13	3.41^{e}	1.80^{a}	1.34^{a}	9.30^{l}	2.05^{h}	1.63^{bcd}	1.14^{b}	0.13^{fgh}	1.46^{bc}	0.26^{ab}	1.89^{ef}	2.42^{c}	1.22^{c}	1.51^{ab}	0.99^{a}	1.81^{cde}	4.23^{g}
14	3.42^{e}	1.80^{a}	1.34^{a}	8.09^{g}	2.04^{h}	1.68^{cde}	1.44^{de}	0.1^{defg}	1.63^{cd}	0.24^{ab}	1.79^{de}	2.23^{ab}	1.25^{c}	1.43^{ab}	1.16^{bc}	2.33h	4.38^{h}
15	2.66^{b}	2.52^{b}	2.06^{b}	5.88^{b}	0.72^{a}	1.48^{a}	1.33^{c}	0.07^{abcd}	1.23^{a}	0.25^{ab}	1.59^{b}	2.22^{ab}	1.09^{a}	1.18^{a}	1.11^{bc}	1.59^{b}	2.97^{a}
16	4.46^{k}	2.52^{b}	2.06^{b}	8.93^{ijk}	1.42^{d}	1.83^{ef}	1.49^{ef}	0.08^{bcde}	1.43^{bc}	0.3^{abc}	2.33^{k}	3.1^{f}	1.64^{h}	1.61^{ab}	1.53^{e}	1.8^{cde}	4.53^{i}
17	4.09^{i}	2.52^{b}	2.06^{b}	7.94^{f}	1.13^{b}	1.67^{cd}	1.59^{fgh}	0.13^{fgh}	1.44^{bc}	0.3^{abc}	1.95^{f}	2.91^{e}	1.45^{e}	1.32^{ab}	1.35^{d}	1.71^{bc}	3.65^{de}
18	3.7^{f}	2.52^{b}	2.06^{b}	7.04^{d}	1.42^{d}	1.72^{de}	1.66^{h}	0.13^{fgh}	1.8^{de}	0.17^{ab}	2.35^{k}	2.72^{d}	1.81^{j}	1.9^{ab}	1.88^{f}	2.32^{h}	5.7l^{g}
19	3.82^{g}	2.52^{b}	2.06^{b}	7.48^{e}	1.34^{c}	1.69^{cde}	1.39^{cd}	0.03^{ab}	1.28^{ab}	0.2^{ab}	2.15^{hi}	2.79^{de}	1.47^{e}	1.37^{ab}	1.17^{bc}	1.4^{a}	3.3^{c}
22	4.71^{l}	2.52^{b}	2.06^{b}	8.9^{ij}	1.45^{d}	1.88^{fg}	1.92^{j}	0.05^{abc}	1.71^{de}	0.25^{ab}	2.54^{l}	3.33^{g}	1.83^{j}	1.73^{ab}	1.54^{e}	1.92^{defg}	4.76^{j}
21	4.79^{m}	2.52^{b}	2.06^{b}	8.78^{i}	1.45^{d}	1.99^{g}	1.76^{i}	0.1^{defg}	1.58^{cd}	0.25^{ab}	2.64^{m}	3.47^{h}	1.76^{i}	1.68^{ab}	1.43^{d}	1.92^{defg}	4.09^{f}
22	4.34^{j}	2.52^{b}	2.06^{b}	7.13^{d}	2.13^{i}	1.74^{de}	1.65^{h}	0.13^{fgh}	1.66^{cde}	0.25^{ab}	2.3^{k}	2.37^{bc}	2.24^{k}	1.81^{b}	1.18c	1.84^{cde}	3.71^{e}

ASP: asparagines, **THR:** threonine, **SER:** serine, **GLU:** glutamine, **PRO:**praline, **GLY:** glycine, **ALA:** alanine, **CYS:** cystine, **VAL:** valine**, MET:** methionine, **ISO:** isoleucine, **LEU:** leucine, **TYR:** tyrosine, **PHE:** phenylalanine, **HIS:** histidine, **LYS:** lysine, and **ARG:** arginine.
Different letters indicate a significant difference at the 0.05 level of probability as evaluated by ANOVA tst.

the same group. According to Noganowsker *et al.* (2003), the Malacospermae species (to which *L. angustifolius* belonged) might be resulted from a several independent evolutionary lineages from the ancient rough-seeded stock. The protein and amino acid similarities between the two examined species supported this point of view.

The overall pattern of seed proteins and amino acid composition within the same species revealed that there is a considerable variation along with the geographical region. This is in accordance with Kittelson and Maron (2001) who stated that across long spatial scales, plants often exhibit genetically based differentiation in traits that allow adaptation to local sites. On the other hand, Ana *et al.* (2004) examined Portuguese *L. albus* cultivars by seed protein electrophoresis and indicated that the polypeptide patterns could reflect microclimatic specificities related with altitude and temperature. Contrary, Aïnouch and Bayer (1999) recorded that in

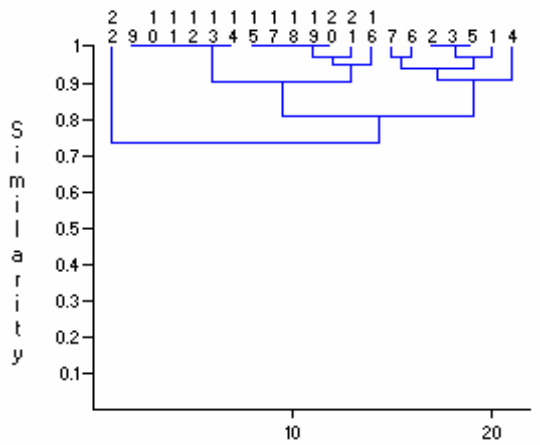

Figure (4): Dendrogram constructed from the data of both seed protein electrophoresis amino acid composition using PAST program by paried group and Jaccard similarity coefficient (1-7, *L. albus*; 9-21 *L. angustifolius*; 22, *L. albus* var. *albus*).

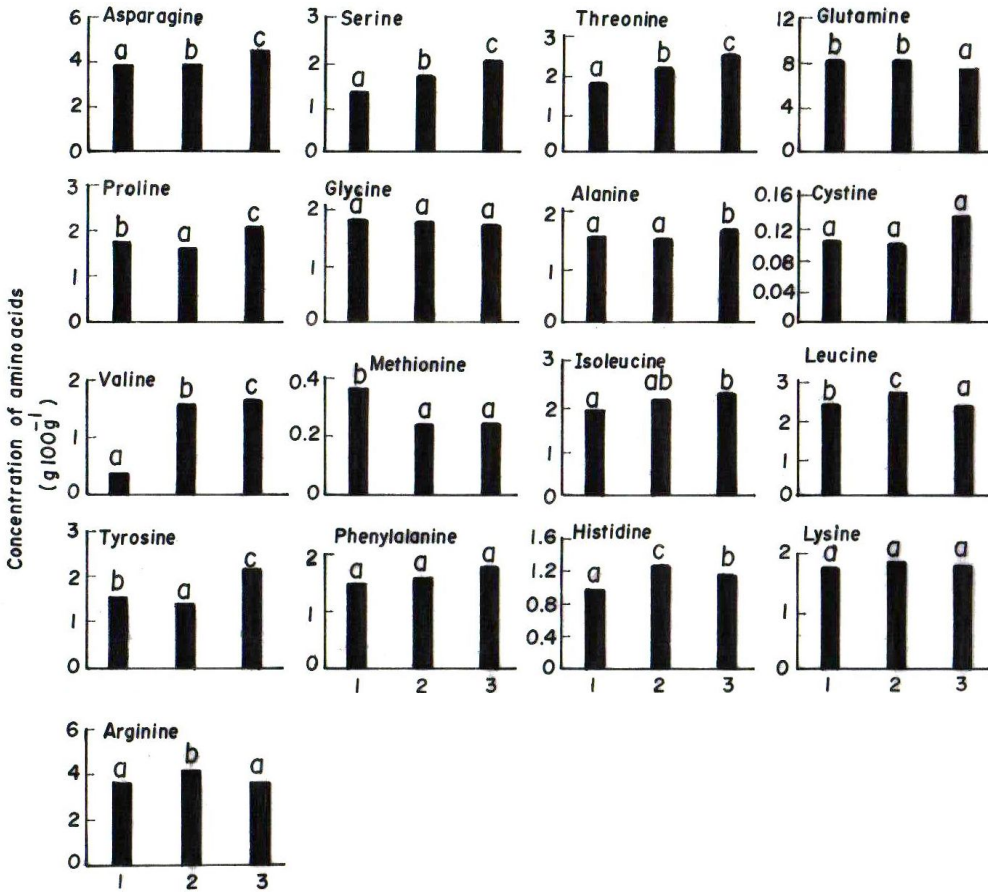

Figure (5): Variation in the mean concentration (g $100g^{-1}$) of different amino acids in **1:** *Lupinus albus*, **2:** *Lupinus angustifolius* and **3:** *L. albus* var. *albus*. Different letters at top of each bar indicate a significant difference at 0.05 level of probability as evaluated by ANOVA test.

smooth-seeded sections, no ITS nucleotide differences were found among the members of the same species.

The present results demonstrated that the *L. albus* var. *albus* (*L. albus* cultivar) is assembled with the wild *L. albus* below the same group, as by seed proteins. But at the same time the cultivar showed considerable degree of variability from the wild species and separated alone by using both data from seed proteins and amino acid compositions. These variations may be regarded to domestication which leads to gradual genetic exchanges brought by the changed selection pressures. The domestication syndrome in crop plants were examined by Schwanitz (1966), Harlan (1975) and Hawkes (1983) who suggested the biochemical changes from these syndromes. The cultivar is characterized with a low number of seed protein bands which reflect the low degree of both polymorphism and genetic diversity. As suggested by Karam (1994), Bisby (1995) and Bidak and Marzouk (2005), the decrease in genetic diversity may lead to extinction and consequently the loss of *Lupinus* cultivar. Meanwhile, the cultivar is characterized by the deficiency in arginine, leucine and methionine which are considered from the vital important amino acids (Babinec *et al.*, 1994). On the other hand, both *L. albus* and *L. angustifolius* possessed considerable amount of these amino acids (methionine in *L. albus* and both arginine and leucine in *L. angustifolius*). *Lupinus albus* accessions are characterized by the highest methionine concentration which ranged from 0.18 to 0.52 g $100g^{-1}$, while Kurlovich (1998) recorded a range from 0.177 to 0.32 g $100g^{-1}$. The methionine is one of the most limiting amino acids in Leguminosae and this variability among *L. albus* accessions serves an opportunity for its effective utilization in future breeding. On the other side, *Lupinus angustifolius* is distinguished by its wide range of variability for both seed proteins and amino acid compositions, compared with other studied taxa.

In conclusion, seed protein electrophoresis and amino acid composition could be used successfully to distinguish between studied *Lupinus* species and the *L. albus* var. *albus* for taxonomic analysis or seed identification. The present study also recommended the intensification of breeding programs between the cultivar and its wild relatives to enrich its biological diversity.

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التنوع بين أربعة أنواع من الترمس إعتمادًا على التفرد الكهربى لبروتين البذور وتركيبه من الأحماض الأمينية

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الملخص العربـــى

تهدف الدراسة الحالية إلي تقييم التنوع بين ثلاثة أنواع من الترمس (*Lupinus albus* L., *Lupinus angustifolius* L. *Lupinus digitatus* Forssk.) باستخدام التفرد الكهربي لبروتين البذور وتركيباته من الأحماض الأمينية وذلك بغرض بيان العلاقات التصنيفية فيما بينهم ومقارنتها بالنوع المنزرع بمصر ومدي تشابهه بأقاربه البرية كمحاولة لاستخدامهم في برامج التهجين المختلفة. وقد أظهرت الدراسة تباينًا واضحًا بين كل من بروتين البذور والأحماض الأمينية داخل النوع الواحد مع اختلاف البيئات الجغرافية. وكانت النسبة المئوية للتباين بين الأنواع 26%.

وقد تم تحديد عدد 18 شريط بين أنواع الترمس المختلفة والتي تميزت بأربعة شرائط مشتركة وذلك عند كل من 38, 36, 20 and 14.4 KDa. وقد أظهر dendrogram المبني علي التركيزات المختلفة للأحماض الأمينية التمييز التام بين الأربعة أنواع وذلك باستخدام عوامل تشابهات مختلفة. بينما أوضح التفرد الكهربي لبروتين البذور تجمع النوع المنزرع في مصر مع النوع البري *L. albus* تحت مجموعة واحدة، ولكن باستخدام كل من التفرد الكهربي للبروتين وتركيزات الأحماض الأمينية المختلفة تم فصل النوع المنزرع منفردًا. وقد تميز هذا النوع المنزرع بدرجة منخفضة من التنوع وأيضًا بنقص في arginine, leucine methionine وهم من الأحماض الأمينية الحيوية، وذلك علي العكس من *L. albus* و*L. angustifolius* واللذين كان لهما تركيزات مرتفعة من هذه الأحماض.