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Genetic variation among accessions of *Lathyrus inconspicuous* (L.) as revealed by SDS Polyacrylamide Gel Electrophoresis

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ABSTRACT

Eighteen *L. inconspicuous* accessions collected from different countries were evaluated for variations of seed weight, seed protein content, and electrophoretic patterns of the total seed proteins analyzed under reducing conditions. They exhibited a reasonable genetic variability for the evaluated traits. This genetic variability revealed that improvement through simple selection for these traits is possible. The variation between the seed size of this accessions was attributed to the development process or the life cycle of the plant, and the environmental condition to which the mother plant is exposed. On the other hand, the variation in protein content among the different accessions may be due to genotype and/or seasonal influences. The relationship between protein content and 100 seeds weight in the evaluated accessions was reversible, the accession showed the lowest quantity of the total seed proteins was the accession that exhibited highest weight of 100 seeds and nearly vice versa. Each accession gave a different electrophoretic pattern except the two accessions collected from Iran, exhibited an identical one. The difference in 100 seed weight and total protein content of these accessions indicated that they are not genetically identical. The variation in the electrophoregram of the evaluated accessions located in the bands with molecular weight more than 98 kDa, the heavy subunits of alpha-lathyrin subunits and the region molecular weight around 70 kDa. The results of cluster analysis based of SDS/PAGE under reduction conditions indicated that genetic diversity between Turkish, Syrian, and Iranian and Australian accessions is pronounced, and Turkish accessions are closer to both Syrian and Iranian accessions than the relation between Syrian and Iranian. This suggested that crosses between the Iranian and Syrian accessions could create more genetic variability than crosses with Turkish accessions. The distribution of Turkish and Syrian accessions between more than one clusters revealed that genetic diversity and geographic distribution were independent of each other. PCA showed that all accessions were separated on the first principal component, indicating that the accessions showed a good association, due, probably, to parallel evolution.

Key words: 100 seeds weight, Protein analysis, multivariate analysis, germplasm characterization

IZVLEČEK

ANALIZA GENETSKE VARIABILNOSTI AKCESIJ GRAHORJA (*Lathyrus inconspicuous* (L.) S SDS POLIAKRILAMIDNO GELSKO ELEKTROFOREZO

Osemnajst akcesij grahorja (*L. inconspicuous* L.), zbranih iz različnih držav, je bilo ovrednoteno glede na variabilnost mase semen, vsebnost semenskih proteinov in elektroforetskih vzorcev celokupnih semenskih proteinov analiziranih v reducirajočih razmerah, ob prisotnosti reducenta. Ovrednotene lastnosti so pokazale pričakovano genetsko variabilnost na osnovi katere je možna preprosta selekcija. Variabilnost v velikosti semen med akcesijami je bila odvisna od razvojnih procesov v življenskem ciklu rastlin in okoljskih dejavnikov, katerim je bila izpostavljena materinska rastlina. Po drugi strain so bile razlike v vsebnosti proteinov med različnimi akcesijami odvisne od genotipa in/ali sezonskih okoljskih vplivov. Razmerje med vsebnostjo proteinov in maso 100 semen je bilo med ovrednotenimi akcesijami obratno. Akcesije, ki so imele najmanjšo vsebnost proteinov so imele največjo maso 100 semen in obratno. Vsaka akcesija, z izjemo dveh iz Irana, je imela svojski elektroforetski vzorec. Razlika med maso 100 semen in celokupno vsebnostjo proteinov analiziranih akcesij je pokazala, da akcesije genetsko niso enake. Razlike v elektroforegramih analiziranih akcesij so se pojavljale v elektroforetskih črtah z molekulsko težo večjo od 98 kDa, težjih podenot alfa latrina in v območju elektroforetskih črt z molekulsko težo okrog 70 kDa. Rezultati klasterske analize dobljeni na osnovi SDS/PAGE elektroforeze v reducirajočih razmerah so pokazali, da je genetska raznolikost med turškimi, sirijskimi, iranskimi in

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avstralskimi akcesijami izrazita in da so turške akcesije bližje sirskim in iranskim, kot pa je bližina sirskih in iranskih akcesij med seboj. To nakazuje, da bi dala križanja med sirskimi in iranskimi akcesijami večjo variabilnost kot s turškimi. Porazdelitev turških in sirskih akcesij v več kot en klaster kaže, da sta genetska raznolikost in geografska razširjenost med seboj neodvisni. Analiza glavnih component (PCA) je pokazala, da so se vse akcesije ločile že na prvi glavni komponenti, kar kaže, da so akcesije dobro povezane, verjetno zaradi paralelne evolucije.

Ključne besede: teža 100 semen, analiza proteinov, multivariatna analiza, karakterizacija genskih virov

1 INTRODUCTION

The genus *Lathyrus* is a member of the tribe *Vicieae* (*Fabaceae, Papilionoideae*); which comprises nearly 160 annual and perennial, autogamous and allogamous herbaceous creeping plants. *Lathyrus* species have a broad distribution all over the world in Europe, North America, Asia, tropical East Africa and temperate South America (Goyder, 1986). The main center of genus diversity is the eastern Mediterranean region, with smaller centers in North and South America (Kupicha, 1983).

Lathyrus has a long history as cultivated plant and it has agronomic importance as forage or fodder such as *Lathyrus inconspicuous, L. ochrus* and *L. articulatus,* as human food such as *L. cicera and L. sativus*, and as ornamental plants, such as *L. odoratus*.

Knowledge of genetic variation is a useful tool in genebank management, helping in the establishment of core collections, facilitating efficient sampling and utilization of germplasm (identifying and/or eliminating duplicates in the gene stock), and selecting of desirable genotypes to be used in breeding programs.

Characterization of germplasm using biochemical techniques (storage proteins and isozymes) has received a great attention in the last decades. This attention was attributed to the increased recognition of germplasm resources in croplants improvement. Genetic markers are useful for screening germplasm with the minimum cost in time and labour (Nakajima, 1994). The qualitative traits of the seed proteins obtained by electrophoresis have been successfully used to assess the genetic variation among the accessions of the wild species (Elham et al., 2010; Vishwanath et al., 2011). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS/PAGE) is among the biochemical technique that is widely used due to its simplicity and effectiveness for describing the genetic structure of the accessions of wild plant species. Seed storage proteins have been used as genetic markers in: (1) identifying variation among the taxa of each species; (2) screening the purity of the ever expanding number of cultivars; (3) establishing genome relationships; (4) exploiting the important traits of landraces and wild relatives to provide increasing crop production and stabilizing yield (Sammour 1991), and (5) using information on genetic diversity to make decisions regarding selection of superior genotypes for improvement yield of plants through breeding. Protein electrophoresis is considered a reliable, practical and reproducible method because seed storage proteins are the third hand copy of genomic DNA and largely independent of environmental fluctuations (Sammour, 1987;Javaid et al., 2004; Iqbal et al., 2005).

SDS/PAGE has not yet been carried out for *Lathyrus inconspicuous*, although it is an important forage or fodder in drought-stricken, rain-fed areas where soil quality is poor and extreme environmental conditions prevail (Palmer *et al*. 1989). “Despite it’s tolerance to drought it is not affected by excessive rainfall and can be grown on land subject to flooding (Kaul et al., 1986; Rathod, 1989; Campbell et al., 1994).

The present study was initiated to study genetic variation in accessions of *L. inconspicuous* on the basis of 100-seeds weight, protein content of the seed meal and SDS-PAGE markers.

2 MATERIALS AND METHODS

Plant material

The designated germplasm of *Lathyrus inconspicuous* that was used in this study included 18 different accessions. They were obtained from the International Center for Agricultural Research in The Dry Areas ICARDA, Aleppo, Syria. Accessions are listed in Table 1.

Table 1: Accession number, origin and total weight of 100 seeds of accessions of *L. inconspicuous.*

Accession number	Accession (IG)	Origin	Wt of 100 seeds (g)	Concentration of total protein in mg/ml	Number of protein bands
A	65037	TUR, Diyarbakir	1.481	130	**30**
B	65038	TUR, Siirt	1.738	123	**28**
C	65048	IRN, Lorestan	1.932	121	**29**
D	65054	IRN, East Azerbaijan	1.433	118	**29**
E	65077	AUS	1.494	125	**27**
F	65282	SYR, Homs	1.702	127	**25**
G	65436	SYR, Alepppo	1.684	139	**25**
H	65508	SYR, Idlib	1.559	140.5	**26**
I	65579	SYR, Sweida	1.345	132	**26**
J	65627	SYR, Damascus	0.885	147	**26**
K	65638	SYR, Tartous	1.828	119	**26**
L	65679	TUR, Ankara	1.296	142	**26**
M	65739	TUR, Antakya	1.995	109	**28**
N	65847	TUR, Izmir	1.896	124	**27**
O	65866	TUR, Gaziantep	1.155	134	**27**
P	65913	TUR, Urfa	0.674	130	**28**
Q	65935	TUR, K.Maras	1.225	132	**26**
R	65951	TUR, Adiyaman	1.880	124	**27**

Seed protein extraction: The seed meal was obtained from a composite sample of 18-20 dehuled seeds for each accession. Each sample was prepared by grinding cotyledons to flour; the total crude proteins were extracted using 0.125 M Tris-Borate pH 8.9 with 2% SDS (Ratio 1:10 w/v).

Protein Analysis: Total seed proteins were quantitatively estimated in each sample by the method of Bradford (1976). The final concentration was adjusted to 20 µg/µl protein in sample buffer. The extracts were denaturated in 2X sample buffer (1M Tris-HCl pH = 6.8, 2% SDS, 20% glycerol, 0.02% BPB, 5% 2-mercapto-ethanol), and heated at 100 ºC for 4 minutes. One dimensional SDS-PAGE was performed according to the method of Lammeli (1970) using 17% polyacrylamide gel. The gel was stained with Coomassie blue and visualized in white fluorescent light. Phosphorylase b (98 kDa), ova albumin (43 kDa), carbonic anhydrase (28.35 kDa) and β-lactoglobulin (18.85 kDa) were used as marker proteins.

Data Analysis: The band identification was based on electrophoretic mobility and by numerous side by side comparisons of proteins extracts. The estimation of genetic diversity within and among the samples was based on 38 reproducibly scored bands identified in the zones of highest variation of protein profile (ranging from 15 to 110 kDa). The genetic diversity among the accessions was evaluated by Jaccard similarity index (Table 2), Dendrogram was made by Euclidian distance (Figure 3). The analysis was performed using the frequencies of scored bands calculated for the accessions. A dendrogram was constructed through the Average linkage-joining rule, using the soft-ware package SYSTAT.

Table 2: Jaccard similarity coefficients between accessions of *L. inconspicuous*.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
A	1.000																	
B	0.933	1.000																
C	0.967	0.966	1.000															
D	0.967	0.966	1.000	1.000														
E	0.900	0.897	0.931	0.931	1.000													
F	0.833	0.893	0.862	0.862	0.926	1.000												
G	0.833	0.893	0.862	0.862	0.857	0.923	1.000											
H	0.806	0.862	0.833	0.833	0.767	0.821	0.889	1.000										
I	0.806	0.862	0.833	0.833	0.767	0.821	0.889	0.889	1.000									
J	0.697	0.742	0.719	0.719	0.656	0.700	0.759	0.793	0.793	1.000								
K	0.806	0.862	0.833	0.833	0.767	0.821	0.889	0.926	0.926	0.793	1.000							
L	0.806	0.862	0.833	0.833	0.828	0.889	0.821	0.857	0.857	0.793	0.857	1.000						
M	0.933	0.931	0.966	0.966	0.897	0.893	0.893	0.862	0.862	0.742	0.862	0.862	1.000					
N	0.900	0.964	0.931	0.931	0.862	0.926	0.926	0.893	0.893	0.767	0.893	0.893	0.964	1.000				
O	0.900	0.964	0.931	0.931	0.862	0.926	0.926	0.893	0.893	0.767	0.893	0.893	0.964	1.000	1.000			
P	0.758	0.806	0.781	0.781	0.719	0.767	0.767	0.742	0.742	0.636	0.742	0.742	0.806	0.833	0.833	1.000		
Q	0.867	0.929	0.897	0.897	0.828	0.889	0.889	0.857	0.857	0.733	0.857	0.857	0.929	0.963	0.963	0.862	1.000	
R	0.839	0.774	0.806	0.806	0.742	0.733	0.733	0.710	0.710	0.656	0.710	0.710	0.833	0.800	0.800	0.719	0.828	1.000

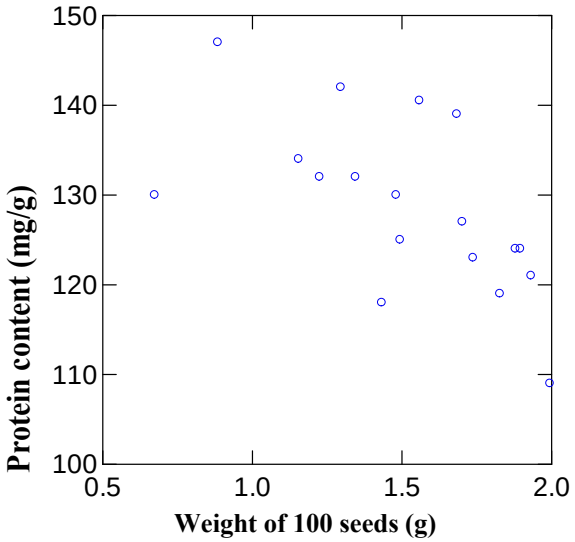

Fig.1: Scatter plot of protein contents and 100 seeds weight of 18 *L. inconspicuous* accessions.

3 RESULTS

Generally, the accessions of *L. inconspicuous* exhibited wide genetic diversity for 100 seeds weight. Moreover, the variation in 100 seeds weight was very evident for the seeds collected from the same country (Table 1). For example in Turkey, it was varied between 1.995 g in Antakya to 0.674 g in Urfa. In Syria, the variation was not such wide as in Turkey; it was ranged between 1.828 g in Tartous and 0.885 g in Damascus.

For a better characterization of the *L. inconspicuous* germplasm, relationships among protein content and 100 seeds weight were considered (Figure 1). The distribution of the points indicates clearly a reverse relationship between protein content and 100 seeds weight. Nevertheless, it may be noticed that the protein content tends to be less variable for median values of 100 seeds weight. In other words, a median range of 100 seeds weight variation corresponds to a less wide range of protein content variation.
When the total seed proteins of the studied accessions were separated by SDS/PAGE under reducing conditions, the patterns of the bands obtained were different for all the evaluated accessions, except the two accessions collected from Iran showed identical electrophoretic patterns (Figure 2). These differences were most marked amongst the proteins with molecular weights ranged between 110 kDa (the weight of the high molecular weight albumin) and 43 kDa. The electrophoretic patterns of the total seed proteins of the accessions collected from Damascus and Tartous in Syria and Ankara in Turkey were unique and very characteristic. The number of protein bands in the electrophoregram of the studied accessions ranged between 25 and 30 bands (Table 2), with a total of thirty six bands from eighteen accessions and molecular weights ranged from 10 to 110 kDa (Figure 2).

Jaccard's similarity coefficients was based on the data of SDS/PAGE profiles of the evaluated accessions (Table 2). It ranged from 100.00 (between an accession from Lorestan in Iran and East Azerbaijan in Iran) to 0.697 (between two accessions from Diyarbakir in Turkey and Damascus in Syria). It was noticed that the majority of the similarity coefficients between accessions was close to 0.7. This indicated the close relationships between the evaluated accessions, though they are collected from different country.

The dendrogram produced from electrophoretic data of the total seed protein extracts of the evaluated accessions, using Euclidean distance matrix on average linkage shows three groups, two contain each one accession (G2 and G3) and the rest of accessions in the third group (G1) (Figure 3). G1 is divide into two subgroups (G1A and G1B); G1A includes one accession and G1B divided into three clusters (C1, C2 and C3). C1 includes accessions from Turky and Iran; C2 from Syria and Australia; and C3 from Syria and Turkey.

The matrix of eigenvectors and values of the principal components (PCs) resulting from electrophoretic data of the total seed proteins (Table 3) shows that the protein data influencing 82.88% of the variability accumulated up to the first two components. All the studied accessions were separated on the first principal component.

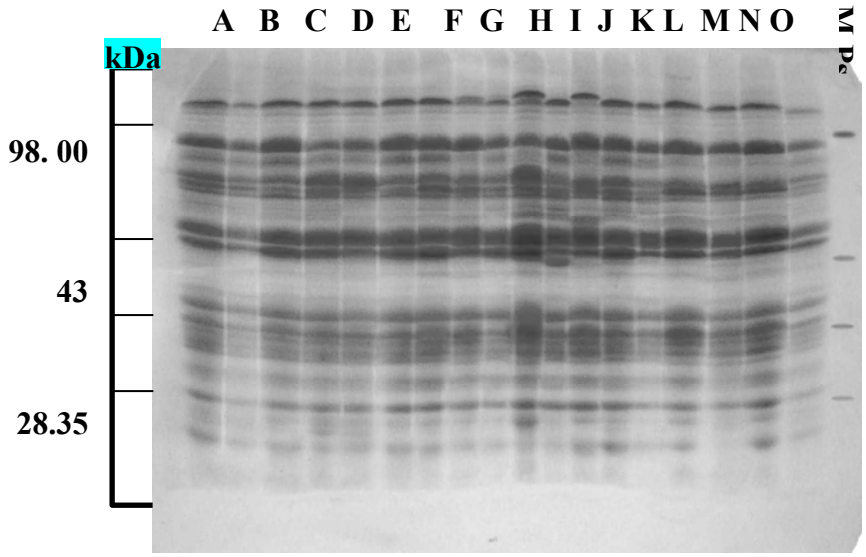

Fig.2: Electrophorogram produced by SDS/PAGE analysis of seed proteins of 18 accessions of *L. inconspicuous* L.

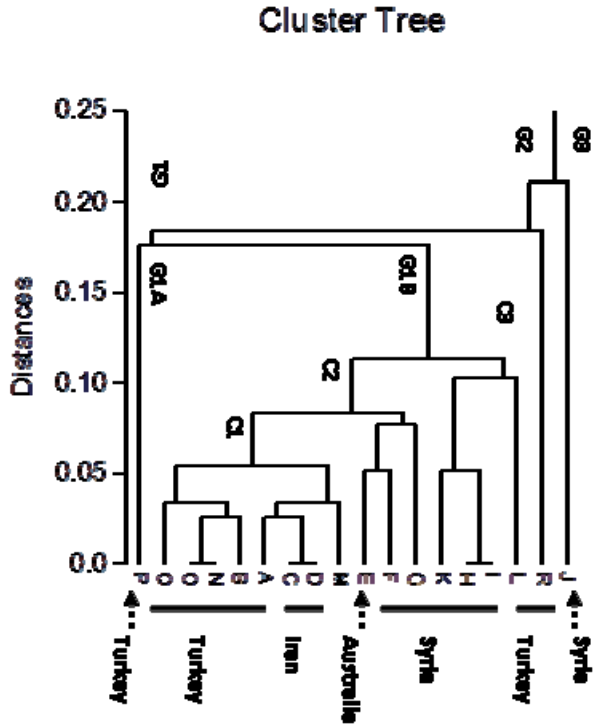

Fig.3: Dendrogram showing the genetic relationships among of 18 accessions of *L. inconspicuous* L. based on Euclidian genetic distance of SDS/PAGE.

Table 3: Origin, matrix of eigenvectors and values of the principal components for protein data of *L. inconspicuous* L. accessions

	Accession number (IG)	Origin	Principal components	
			C1	C2
A	65037	TUR, Diyarbakir	0.869	0.346
B	65038	TUR, Siirt	0.946	0.117
C	65048	IRN, Lorestan	0.924	0.279
D	65054	IRN, East Azerbaijan	0.924	0.279
E	65077	AUS	0.835	0.327
F	65282	SYR, Homs	0.902	0.053
G	65436	SYR, Alepppo	0.915	-0.119
H	65508	SYR, Idlib	0.864	-0.392
I	65579	SYR, Sweida	0.864	-0.392
J	65627	SYR, Damascus	0.618	-0.557
K	65638	SYR, Tartous	0.856	-0.354
L	65679	TUR, Ankara	0.849	-0.216
M	65739	TUR, Antakya	0.953	0.146
N	65847	TUR, Izmir	0.977	-0.008
O	65866	TUR, Gaziantep	0.977	-0.008
P	65913	TUR, Urfa	0.666	0.036
Q	65935	TUR, K.Maras	0.935	0.035
R	65951	TUR, Adiyaman	0.668	0.281
Variance Explained by Components			**13.612**	**1.305**
Percent of Total Variance Explained			**75.624**	**7.251**
Accumulated Eigenvectors			**75.624**	**82.875**

4 DISCUSSION

The pool of genetic variation within accessions of this species is the basis for selection as well as for plant improvement. A better understanding of genetic diversity and its distribution in the accessions of the studied plant is essential for its conservation and use. It will help greatly in determining what to conserve as well as where to conserve, and will enhance our knowledge and understanding of the taxonomy, origin and evolution of *L. inconspicuous.*

In the present investigation, a reasonable genetic variation was observed for 100 seeds weight, total protein content and electrophoretic pattern (SDS-PAGE) of the total protein of the seed meal of accessions of *L. inconspicuous.* The genetic variability of these traits reveals that improvement through simple selection for these traits is possible, particularly if we broaden the genetic base from diverse habitats to include most of the genetic determinants of a trait of interest (i.e. productivity, disease resistance, a biotic stress tolerance, and/or quality) (Ghafoor and Arshad, 2008).

It is well established that seed size reflects an underlying trade-off between seed size and seed number. The seedling survival increases constantly with increasing seed size (Turnbull et al., 2006). However, it is useful to consider whether a plant can vary its position in this trade-off in response to environmental conditions or /and if seed size is solely a genetic trait (Sammour et al., 2007a). The suggestion that seed size is solely a genetic trait was based on the study of Lopes et al. (2003) on genetic control of cowpea seed sizes, where they found that the mid-parental value and the additive effect were the most important genetic parameters for the determination of the seed character. However, the size of the seed is the result of three different growth programs: those of the diploid embryo, the triploid endosperm, and the diploid maternal ovule (Sundaresan, 2005). The control and coordination of these growth programs are

under genetic regulation. When the paternal genome is in excess, seed growth is promoted, and conversely, excess of the maternal genome results in smaller seeds. This is true for diploid x tetraploid crosses of plants as described in Sundaresan (2005). This confirmed the finding of Lopes et al. (2003) that the variation of the seed size among different populations of the species was attributed to the development process or the life cycle of the plant. However this variation, which is a development process, may itself enhance fitness. The variation in seed size in an individual plant may make that plant more able to adapt to a changing environment. In other context, Wulff (1986 a, b) stated that seed size as well as seed germination characteristics may vary with the environmental condition to which the mother plant is exposed. In conclusion, seed size is not solely a genetic trait, but it is affected by environment.

The seed protein content in the studied accessions varied between 109 mg/g seed meal in accession number IG65739 from Antakya in Turkey to 147 mg/g seed meal in accession number IG 65627 from Damascus in Syria. It is very interesting to notice that the accession that showed the lowest quantity of the total seed proteins was the accession that exhibited highest weight of 100 seeds and nearly vice versa. This clearly indicated the reverse relationship between protein content and 100 seeds weight. This conclusion was in agreement with that of Saxena et al. (1987) on pigeonpea, Kaushik et al. (2007) on *Jatropha curcas* and Afzal et al. (2003a, b) on mungbean. It was found that investigated accessions had significant variation in protein content. This variation was attributed to environmental factors such as geographical area, season of collecting, elevation, and annual temperature, precipitation, soil fertility and/or genotypes variation (Ries and Everson, 1973; Vollmann et al., 2000).

In general, each accession gave a different electrophoretic pattern except the two accessions collected from Iran, exhibited an identical electrophoretic pattern. The difference in 100 seed weight and total protein content of these accessions indicated that they are not genetically identical (identical duplicate). The suggestion that these two accessions may be derived from the same original population that are mixtures of lines with differing genotype frequencies, or random mating populations with the same alleles but differing allele frequencies, as reported by Van-hintum and Knüpffer (1995). However, this can not stand up, because the two accessions were collected from two different provinces far apart from each other (Lorestan and East Azerbaijan). In this study, the electrophoregam of SDS/PAGE was carried out under reducing conditions, exhibiting that variation between the different accessions located in the bands with molecular weight more than 98 kDa, the bands might include higher molecular weight albumin (Sammour,1987), the heavy subunits of alpha-lathyrin subunits (Rosa and Ferreira, 2000) and the area with molecular weight around 70 kDa. It can be noticed that the two subunits of γ-lathyrin, 24 kDa (major albumin) and 20 kDa (lectin) showed no variation between the different accessions. The genetic variability information can help the plant breeders to select the accessions to be utilized in hybridization programme or to be utilized as parents for the development of future cultivars through hybridization. It can also be used to assess its association with quantitative traits that helps in screening crop germplasm for identified markers.

The results of cluster analysis based of SDS/PAGE under reduction conditions indicated that genetic diversity between Turkish, Syrian, Iranian and Australian accessions is large. Cluster analysis showed that Turkish accessions are closer to both Syrian and Iranian accessions which they are relatively more distant from each other. On the basis of these results, it is clear that crosses between the Iranian and Syrian accessions could create more genetic variability than crosses between Turkish and those gene pools. The distribution of Turkish and Syrian accessions between more than one clusters showed that genetic diversity and geographic distribution were independent of each other and no definite relationship existed between genetic diversity and geographic diversity. SDS-PAGE under reduction conditions results revealed that the total amount of variability accounted for the first two principal components was 82.88%. All accessions were separated on the first principal component, representing 75.62% of the total variability. The variability within the investigated accessions based on SDS/PAGE, 100 seed weight, and quantitative and qualitative traits of the total seed proteins is associated with the expression of the genome.

However, to express all the variability of *L.inconspicuous* gene pools, more studies focused on detailed agronomic, biochemical and molecular traits on a wide range of accessions covering wide geographical regions are recommended and needed.

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