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Genetic variability in accession of *Lathyrus inconspicuous* based on protein analysis on SDS-PAGE

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ABSTRACT

Eighteen *L. inconspicuous* accessions were evaluated for variations in 100 seeds weight, seed proteins content, and electrophoretic patterns of the seed proteins. The 100 seeds weight and seed proteins content ranged between 0.885 g to 1.995 g and 147 mg/g seed meal to 109 mg/g seed meal respectively, exhibiting a reasonable genetic diversity for these traits. The variation between the seed size of these accessions was attributed to the development process and the environmental condition to which the mother plant is exposed, whereas the variation in total seed proteins content may be due to genotype and/or seasonal influences. Interestingly, there is no correlation between seed proteins content and 100 seeds weight indicating that the two traits are genetically independent. The variation in the electrophoretic pattern located in the regions of the gel contain the bands with molecular weight more than 98 KD, heavy subunits of alpha-lathyrin subunits and bands with molecular weight around 70KD. Multivariate analysis of SDS-PAGE data showed reasonable genetic variability among the accessions and a low variability among the accessions of the same region. It also showed that the accessions collected from Turkey were distributed between more than on cluster, indicating relatively high variation in the genetic diversity of these accessions. Moreover, it revealed there is no relation between genetic diversity and geographic distribution. The separation of all accessions on the first principal coordinate analysis indicated a good association between accessions which was probably attributed to parallel evolution. Based on genetic diversity between these accessions, improvement through simple selection for these traits is possible.

KEYWORDS

100 Seeds weight;
Protein content;
SDS/PAGE;
Cluster analysis;
Principal coordinate analysis.

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INTRODUCTION

L. inconspicuous belongs to genus *Lathyrus*, a member of the tribe Vicieae (Fabaceae, Papilionoideae). It is annual autogamous herbaceous plants. It has abroad distribution all over the world in Europe, North America, Asia, tropical East Africa and temperate South America[1]. The main center of diversity is the eastern Mediterranean region, with smaller centers in North and South America[2]. *L. inconspicuous* has agronomic importance as forage plant.

To establishment core collections, facilitate efficient sampling and utilize germplasm and select desirable genotypes to be used in breeding programs, knowledge of genetic variation is a useful tool to achieve a good breeding program for useful agronomic traits. There are a good collections of L. inconspicuous in numbers of gene banks; for example USDA Germplasm and ICARDA Germplasm. However, there were sporadic studies on the characterization of *L. 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[3]. "Despite it's tolerance to drought it is not affected by excessive rainfall and can be grown on land subject to flooding[4-6].

Characterization of germplasm using morphological, biochemical and molecular markers is very important to plant breeders to make use the collected germplasm of any plant species. Therefore, the genetic markers including biochemical markers received a great attention in the last decades[7-11]. This attention was attributed to the increased recognition of germplasm resources in the improvement of the croplands. Storage proteins as biochemical marker is useful for screening germplasm with the minimum cost in time and labor[10-12]. The qualitative traits of the seed proteins obtained by electrophoresis have been successfully used to evaluate the genetic variation among the accessions of the wild and cultivated species[13,14]. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS/PAGE) is among the biochemical techniques that are used on wide scale due to its simplicity and effectiveness for describing the genetic structure of the accessions of wild and cultivated plant species. Seed storage proteins have been used as genetic markers in identifying variation among the taxa of each species; screening the purity of the ever expanding number of cultivars; establishing genome relationships, exploiting the important traits of landraces and wild relatives to provide increasing crop production and stabilizing yield[15-18], and 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[19-23].

As far is known, there is no study on the characterization of *L. inconspicuous* accessions. Such study is considered as the first practical effort toward building an applicable breeding program to improve the agronomic traits of this interested crop, especially in lands subject to drought and flooding. In this stressful environment, this plant prevails and can be used as forage or a fodder for the animal stocks of the people living in these lands. Therefore, 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 and SDS-PAGE markers.

MATERIAL AND METHODS

Plant material

The designated germplasm of *Lathyrus inconspicuous* that used in this study included 18 different accessions distributed world wide. They were obtained from the International Center for Agricultural Research in The Dry Areas ICARDA, Aleppo, Syria.

Methods

Seed protein extraction

The seed meal 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 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[24]. 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 ^{0}C for 4 minutes. One dimentional SDS-PAGE was performed according to the method of Lammeli[25] 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 elecrtophoretic 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 110 to15 KD). The genetic diversity among the accessions was evaluated by Jaccard similarity index, cluster analysis and factor analysis. 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 0 for WINDOWS VERSION 7.0 COPYRIGHT © 1997, SPSS INC.

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.

The relationships between total proteins content and 100-seeds weight of *L. inconspicuous* germplasm is presented in 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 total proteins content of the seed meals tends to be less variable for median values of 100-seeds weight.

The total seed proteins of the accessions of *L. inconspicuous* were separated by SDS/PAGE under reducing conditions (Figure 2). The patterns of the bands

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

NO.	species name	Accession	Origin	Wt of	Concentration	No of
A	L.inconspicuus	65037	TUR, Diyarbakir	1.481	130	30
B	L.inconspicuus	65038	TUR, Siirt	1.738	123	28
C	L.inconspicuus	65048	IRN, Lorestan	1.932	121	29
D	L.inconspicuus	65054	IRN, East Azerbaijan	1.433	118	29
E	L.inconspicuus	65077	AUS	1.494	125	27
F	L.inconspicuus	65282	SYR, Homs	1.702	127	25
G	L.inconspicuus	65436	SYR, Alepppo	1.684	139	25
H	L.inconspicuus	65508	SYR, Idlib	1.559	140.5	26
I	L.inconspicuus	65579	SYR, Sweida	1.345	132	26
J	L.inconspicuus	65627	SYR, Damascus	0.885	147	26
K	L.inconspicuus	65638	SYR, Tartous	1.828	119	26
L	L.inconspicuus	65679	TUR, Ankara	1.296	142	26
M	L.inconspicuus	65739	TUR, Antakya	1.995	109	28
N	L.inconspicuus	65847	TUR, Izmir	1.896	124	27
O	L.inconspicuus	65866	TUR, Gaziantep	1.155	134	27
P	L.inconspicuus	65913	TUR, Urfa	0.674	130	28
Q	L.inconspicuus	65935	TUR, K.Maras	1.225	132	26
R	L.inconspicuus	65951	TUR, Adiyaman	1.880	124	27

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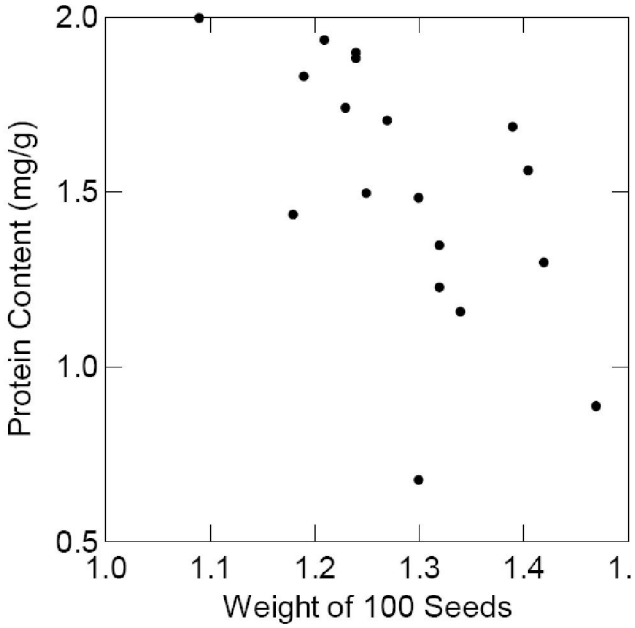

Figure 1: Regression line showing the relationships among protein content and 100-seeds weight of 18 of *L. inconspiuus* L. accessions

weight albumin) and 43 KDa. The variation located in the bands with molecular weight more than 98 KD, the heavy subunits of alpha-lathyrin subunits and the area with molecular weight around 70KD. 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 1), with a total of thirty six bands from eighteen accessions and molecular weights ranged from 110 to 10 KDa (Figure 2).

Jaccard's similarity coefficients were 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 correlation between accessions was close to 0.7.

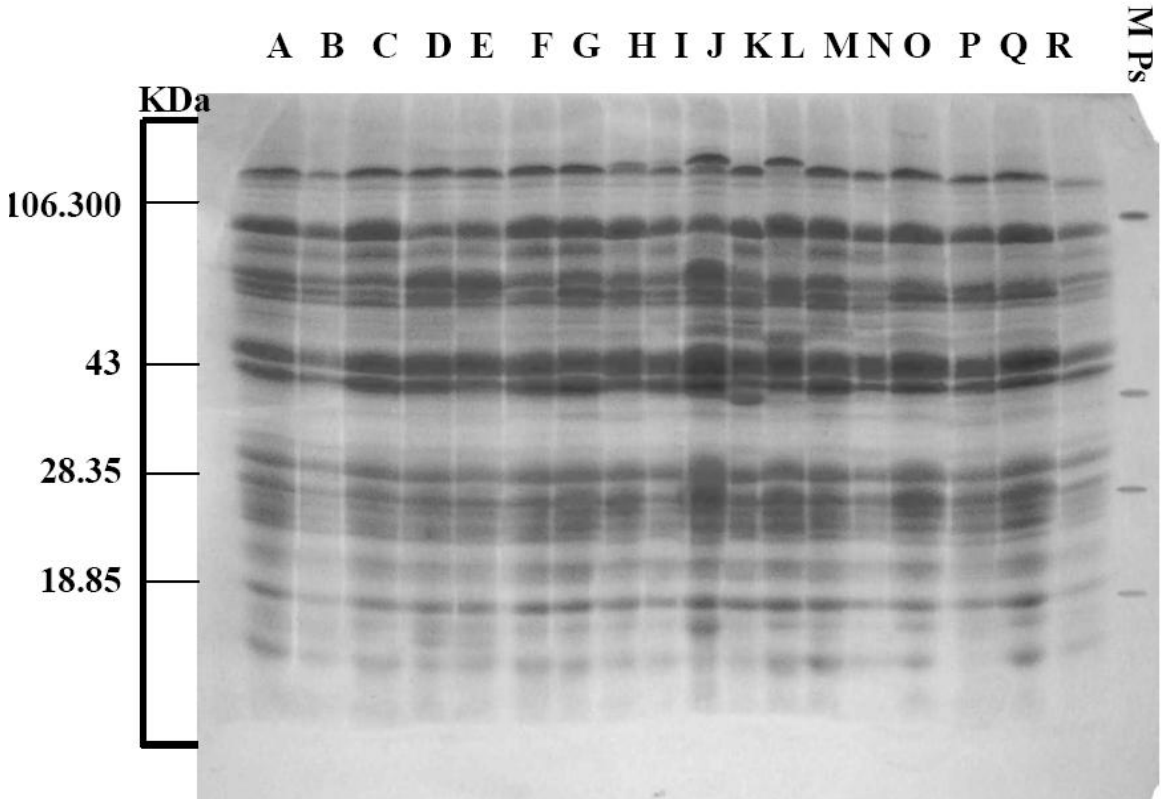

Figure 2 : Electrophorogram produced by SDS/PAGE analysis of seed proteins of accessions of *L. inconspiuus* L.

obtained were varied for the all the examined the accessions. These differences were most marked amongst the proteins with molecular weights ranged between 110 KDa (the weight of the high molecular

This indicated the close relationships between the evaluated accessions, though they are collected from different country.

The dendrogram produced from electrophoretic

TABLE 2 : Jaccard binary similarity coefficients between accessions of *L. inconspiuus*.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
A	1.00																	
B	0.82	1.00																
C	0.93	0.88	1.00															
D	0.93	0.88	1.00	1.00														
E	0.82	0.76	0.88	0.88	1.00													
F	0.69	0.84	0.74	0.74	0.84	1.00												
G	0.69	0.84	0.74	0.74	0.62	0.78	1.00											
H	0.61	0.76	0.66	0.66	0.66	0.84	0.84	1.00										
I	0.57	0.73	0.62	0.62	0.62	0.78	0.78	0.95	1.00									
J	0.52	0.71	0.58	0.58	0.59	0.78	0.78	0.83	0.78	1.00								
K	0.48	0.66	0.54	0.54	0.54	0.73	0.73	0.78	0.84	0.72	1.00							
L	0.39	0.59	0.46	0.46	0.59	0.78	0.60	0.72	0.78	0.77	0.61	1.00						
M	0.69	0.76	0.75	0.75	0.64	0.73	0.73	0.66	0.73	0.71	0.66	710.	1.00					
N	0.60	0.82	0.68	0.68	0.57	0.79	0.79	0.72	0.68	0.77	0.72	0.65	0.82	1.00				
O	0.69	0.88	0.75	0.75	0.64	0.84	0.84	0.78	0.73	0.71	0.78	0.59	0.76	0.94	1.00			
P	0.33	0.57	0.42	0.42	0.32	0.56	0.60	0.48	0.44	0.40	0.48	0.28	0.45	0.62	0.69	1.00		
Q	0.65	0.83	0.71	0.71	0.59	0.78	0.78	0.72	0.67	0.65	0.72	0.54	0.71	0.89	0.94	0.77	1.00	
R	0.56	0.52	0.50	0.50	0.40	0.50	0.50	0.43	0.50	0.35	0.54	0.35	0.64	0.57	0.64	0.45	0.72	1.00

data of the total seed protein extracts of the evaluated accessions, using Euclidean distance matrix on average linkage shows 8 clusters by drawing a horizontal lines at 0.08 distances (Figure 3). Clusters 1, 2, 3 and 8 contain one accession each. The accessions collected from Turkey were included in the clusters 1, 4 and 5. The accessions collected from the other countries were quit homogenous and the accessions of these countries were come together in a cluster.

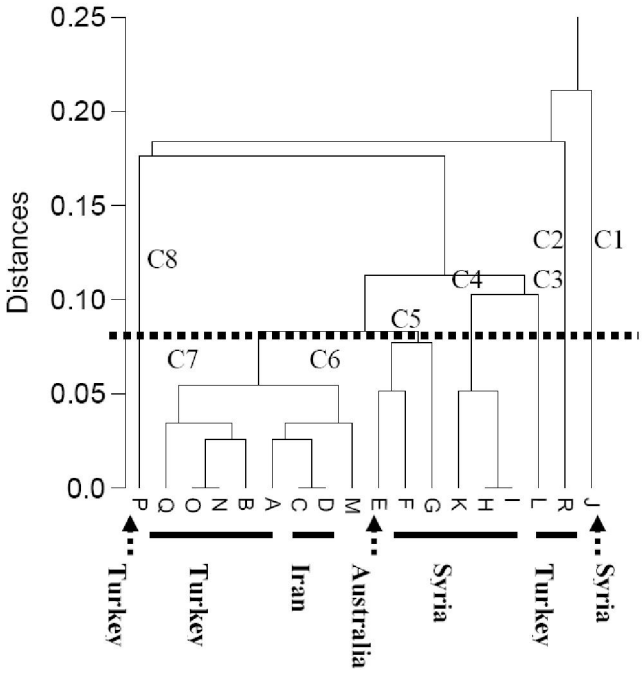

Figure 3 : Dendrogram showing the genetic relationships among of 16 accessions of L. inconspiuus L. based on genetic distance of SDS/PAGE. Horizontal axis indicates genetic

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 82.875% of the variability accumulated up to the first two components. All the studied accessions were separated on the first principal component..

DISCUSSION

Genetic variation in *L. inconspicuous* represents the heritable variation within and between accessions of this species. The pool of genetic diversity 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 L. inconspicuous is essential for its conservation and utilization. It 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, and seed

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TABLE 3 : Origin, matrix of eigenvectors and values of the principal components for protein data of L. inconspiuus L. accessions

	Species	Accession (IG)	Origin	Principal components	
				C1	C2
A	L.inconspicuus	65037	TUR, Diyarbakir	0.802	-0.564
B	L.inconspicuus	65038	TUR, Siirt	0.802	-0.564
C	L.inconspicuus	65048	IRN, Lorestan	0.802	-0.564
D	L.inconspicuus	65054	IRN, East Azerbaijan	0.802	-0.564
E	L.inconspicuus	65077	AUS	0.878	-0.091
F	L.inconspicuus	65282	SYR, Homs	0.901	0.190
G	L.inconspicuus	65436	SYR, Alepppo	0.871	0.146
H	L.inconspicuus	65508	SYR, Idlib	0.855	0.325
I	L.inconspicuus	65579	SYR, Sweida	0.835	0.376
J	L.inconspicuus	65627	SYR, Damascus	0.795	0.400
K	L.inconspicuus	65638	SYR, Tartous	0.782	0.373
L	L.inconspicuus	65679	TUR, Ankara	0.695	0.487
M	L.inconspicuus	65739	TUR, Antakya	0.838	0.119
N	L.inconspicuus	65847	TUR, Izmir	0.867	0.205
O	L.inconspicuus	65866	TUR, Gaziantep	0.913	0.113
P	L.inconspicuus	65913	TUR, Urfa	0.600	-0.174
Q	L.inconspicuus	65935	TUR, K.Maras	0.889	-0.183
R	L.inconspicuus	65951	TUR, Adiyaman	0.781	-0.081
Variance Explained by Components				12.114	2.261
Percent of Total Variance Explained				67.300	12.564
Accumulated Eigenvectors				67.300	79.864

proteins content and electrophoretic patterns (SDS-PAGE) of 18 accessions of L. inconspicuous. The genetic variability of these traits revealed 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)[26,27].

It is well established that seed weight reflects a relation between seed size and seed number. The seedling survival increases constantly with increasing seed size[28]. However, it is useful to consider whether a plant can vary its position in the relation between seed size and seed number in response to environmental conditions or /and if seed size is solely a genetic trait[29]. The suggestion that seed size is solely a genetic trait was reported in the study of Lopes et al.[30] on genetic control of cowpea seed sizes, where they found that the mid-parental value and the additive effect were the more important genetic parameters for the determination of the seed character. However, the size of the seed is the result of the growth of the diploid embryo, the triploid endosperm, and the iploid maternal ovule[31]. The control and coordination of these growths 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 confirmed the finding 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[30]. However this development process variation may itself enhance fitness. The variation in seed size in an individual plant makes the plant more able to adapt to a changing environment. In other context, it was 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 genetically and environmentally controlled[26].

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 the previous works of Saxena et al.[32] and Kaushik et al.[33]. It was found that investigated accessions of L. inconspicuous had significant variation in protein content. This variation was attributed to environmental factors such as geographical area, elevation, season of collecting, and annual precipitation, temperature, soil fertility and/or genotypes variation[29,34,35].

In general, each accession gave a specific 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 Theo et al.[36] can not stand up, because the two accessions were collected from two different provinces far apart from each other (Lorestan and East Azerbaijan). However, their resemblance in the electrophoretic patterns can be interpreted in the light of the fact that the similarity in the molecular weight of two protein bands does not always mean that the two bands are identical because the amino acid sequences of these bands may be different, and in turn their coding genes are different too[9,19,22]. The electrophoregam of SDS/PAGE analyzed under reducing conditions, exhibited that variation between the different accessions located in the bands with molecular weight more than 98 KD, the bands might include higher molecular weight albumin[37], the heavy subunits of alpha-lathyrin subunits[38-41] and the area with molecular weight around 70KD. 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 results of cluster analysis based of SDS/PAGE under reduction conditions indicated that genetic diversity between Turkish, Syrian, Iranian and Australian accessions is quite large. It 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 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 analyzed under reduction conditions revealed that the total amount of variability accounted for the first two principal components was 82.875%. All accessions were separated on the first principal component, representing 75.624 % of the total variability. This percentage indicated that the accessions show a good association, due, probably, to parallel evolution. 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 for more and detailed agronomic, biochemical and molecular traits on a wide range of accessions covering wide geographical regions are recommended.

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REFERENCES

[1] D.J.Goyder; The genus Lathyrus.-In A.Kaul, D.Combes (Eds); Lathyrus and lathyrism Third Whorled Medical Research Foundation.New York, 3-7, **(1986)**.

[2] F.K.Kupicha; Notes from the Royal Botanic Garden Edinburgh, **41**, 209-244 **(1983)**.

[3] V.S.Palmer, A.K.Kaul, P.S.Spencer; In: P.Spencer

(Ed); The Grass Pea: Threat and Promise.Proc. of the International Network for the Improvement of Lathyrus sativus and the Eradication of Lathyrism, Third World Medical Research Foundation, New York, 219-223 **(1989)**.

[4] A.K.Kaul, M.Q.Islam, A.Hamid, In: A.K.Kaul, D.Combes (Eds); Lathyrus and Lathyrism Third World Medical Research Foundation, New York, 130-141 **(1986)**.

[5] K.L.Rathod; In: P.Spencer (Ed); The Grass Pea: Threat and Promise.Proc. of the International Network for the Improvement of Lathyrus sativus and the Eradication of Lathyrism, Third World Medical Research Foundation, New York, 219-223 **(1989)**.

[6] C.Campbell, R.B.Mehra, S.K.Agrawal, Y.Z.Chen, A.M.Abd, El.Moneim, H.I.T.Kawaja, C.R.Yadav, J.U.Tay, W.A.Araya; Euphytica, **73**, 167-175 **(1994)**.

[7] M.A.Karam, R.H.Sammour, M.F.Ahmed, F.M.Ashour, L.M.El.Sadek; Journal of Union Arab Biology, **9**, 269-279 **(1999)**.

[8] M.A.Karam, Y.S.Moris, R.H.Sammour, R.M.Ali; Assessment of genetic relationships within Brassica rapa subspecies based on isozyme Polymorphism. Proc. 6th Edtion Int.Con.Biol.Sci., **6**, 22-28 **(2010)**.

[9] R.HSammour; Journal of Agronomy and Crop Science, **159**, 282-286 **(1987)**.

[10] R.H.Sammour; Botanica.Bulletin of Academica Sinica, **38**, 171-177.

[11] R.H.Sammour, S.A.Radan, M.Mira; Research and Review of Bioscience, **6**, 351-360 **(2012)**.

[12] R.H.Sammour, A.E.Z.Mustafa; Research and Review of Bioscience, **7**, 19-26 **(2013)**.

[13] A.A.Elham, A.El.Hady, A.Atef, A.Haiba, R.Nagwa, A.El.Hamid, A.Aida; Journal of American Science, **6**, 434-441 **(2010)**.

[14] K.Vishwanath, K.P.R.Prasanna, H.M.Pallivi, Rajendra Prasad, S.Ramegowda, P.J.Devaraju, T.V.Anantharararayanan; Research Journal of Agricultural Sciences, **2**, 8-12 **(2011)**.

[15] R.H.Sammour; Plant Breeding, **104**, 196-201 **(1989)**.

[16] R.H.Sammour; Folia Geobotanica et Phytotaxonomica, **26**, 95-100 **(1991)**.

[17] R.H.Sammour, M.A.Hamoud, A.S.Haidar; Cytologia, **56**, 289-291 **(1991)**.

[18] R.H.Sammour; Fedds Repertorium, **103**, 555-557 **(1992)**.

[19] Journal of Agronomy and Crop Science, **160**, 271-276 **(1988)**.

[20] R.H.Sammour; Egyptian Journal of Botany, **33**, 169-174 **(1990)**.

[21] R.H.Sammour; Feddes Repertorium, **105**, 191-196 **(1994)**.

[22] R.H.Sammour, M.A.Hamoud, A.S.Haidar, A.Badr; Feddes Repertorium, **104**, 251-257 **(1993)**.

[23] R.H.Sammour; Acta Agronomica Hungarica, **55**, 131-147 **(2007)**.

[24] M.M.Bradford; Analytical Biochemistry, **72**, 248–254 **(1976)**.

[25] U.K.Lammeli; Nature, **227**, 680–685 **(1970)**.

[26] R.H.Sammour, A.E.Z.Mustafa, S.Badr, W.Tahr; Germplasm.Acta.Agric.Slovenica., **88**, 33-43 **(2007)**.

[27] A.Ghafoor, M.Arshad; Journal of Botany, **40**, 2307-2313 **(2008)**.

[28] L.A.Turnbull, L.Santamaria, T.Martorell, J.Rallo, A.Hector; Biology Letters, **22**, 397-400 **(2006)**.

[29] R.H.Sammour, A.E.Z.Mustafa, S.Badr, W.Tahr; Acta Botanica Croatica, **66**, 1–13 **(2007)**.

[30] F.D.Lopes, R.F.Gomes, F.F.Filho; Scientia Agricola, **60**, 315-318 **(2003)**.

[31] V.Sundaresan; Proceeding of National Academia of Science USA, **102**, 17887–17888 **(2005)**.

[32] K.B.Saxena, D.G.Faris, U.Singh, R.V.Kumar; Plant Foods for Human Nutrition, **36**, 335-340 **(1987)**.

[33] N.Kaushik, K.Kumar, S.Kumar, N.Kaushik, S.Roy; Genetics and Plant Breeding, **31**, 497–502 **(2007)**.

[34] S.K.Ries, E.H.Everson; Agronomy Journal, **65**, 884-886 **(1973)**.

[35] J.Vollmann, C.N.Fritz, T.L.H.Wagentrist, P.Ruckenbauer; Journal of the Science Food and Agriculture, **80**, 1300-1306 **(2000)**.

[36] J.L.Theo, T.J.L.Van Hintum, H.Knuffer; Genetic Resources and Crop Evolution, **42**, 127-133 **(1995)**.

[37] R.H.Sammour; Ph.D. thesis, Tanta University, Tanta, Egypt, **(1985)**.

[38] M.J.Rosa, R.B.Ferreira; Storage proteins from Lathyrus sativus seeds. Journal of Agriculture and Food Chemistry, **48**, 5432–5439 **(2000)**.

[39] R.H.Sammour; Turk J.Bot., **29**, 177-184 **(2005)**.

[40] R.H.Sammour; Plant Var.Seeds, **12**, 11–21 **(1999)**.

[41] R.H.Sammour; M.A.Hamoud, S.A.A.Alla; Botanica.Bulletin of Academica Sinica, **34**, 37-42 **(1993)**.