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Isozyme analysis of genetic variability and population structure of *Lathyrus sativus* L. germplasm

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ABSTRACT

This work aimed at exploring the genetic variability, population structure and relationships of *Lathyrus sativus* L. germplasm using isozyme analysis. The data of isozyme analysis revealed 12 putative polymorphic loci of a total 33 alleles, indicating that studied accessions express good allelic richness and had an apparent rate of allogamy. The mean average of the expected heterozygosity (0.483) was more than the mean average of the observed heterozygosity (0.449) suggesting an apparent rate of allogamy taking place in *Lathyrus sativus* L. The average of total heterozygosity (H_T) and intra-accessional genetic diversity (H_S) were 0.559 and 0.428 respectively indicating that majority of genetic diversity was intra-accessional. The low levels and non-significant of genetic diversity among accessions (D_{ST} = 0.190, X^2 = 62.59, p = 0.029) were probably indicative of occurrence of several gene flows. The coefficient of gene differentiation (G_{ST}) showed significant variation, suggesting the heterogonous distribution of *L. sativus* accessions among different geographic regions. The mean average of F_{ST} was 0.327, suggesting the occurrence of random mating system for the studied accessions and reflecting adaptation to strong environmental dissimilarities. Cluster analysis based on isozyme data suggested that the environment had no influence on the genetic diversity and confirmed that *Lathyrus sativus* L. had a polyphyletic origin.

Key words: Genetic variability, Isozyme, Population structure.

INTRODUCTION

Lathyrus sativus L. (grass pea) belongs to genus *Lathyrus*, a member of the tribe *Vicieae*, Family Fabaceae. It is widely cultivated as a food crop (Yunus *et al.*, 1984). Despite its tolerance to drought, it is also not affected by excessive rainfall and can be grown on land subject to flooding (Kaul *et al.*, 2010). This hardiness, together with its ability to fix atmospheric nitrogen, makes the crop one that seems designed to grow under adverse conditions. Compared with other legumes, the grass pea is resistant to many pests including storage insects (Campbell, 1997).

In spite of the importance of *Lathyrus* for human and animal, it has a limited uses due to the presence of a neurotoxic compound, which causes an irreversible paralysis of the lower limbs in human and the four limbs in animal and is known as Lathyrism. To overcome this constrain, scientists pay attention to genetic resources to find the genes conferring high and good agronomic traits, including protein content and less amount of β-N-Oxalyl-l-α,β-diamino-propionic Acid (β-ODAP). The first step in making use of genetic resources, for human interest, is the collection of genetic resources and assessment of genetic diversity at both intra and inter accessional/population levels. The assessment of genetic diversity is necessary to increase utilization of plant genetic resources in varietals improvement (Sammour *et al.*, 2007; Bakshi and Konnert, 2011; Ambade *et al.*, 2015; Indira *et al.*, 2015; El-Esawi *et al.*, 2017). The advent of the electrophoresis method provides an indirect method for genome probing by exposing structural variations in enzymes and other proteins (Sammour, 1999; Thakur *et al.*, 2018; El-Esawi *et al.*, 2017). Electrophoretic markers are independent of species morphology and physiology and offer significant advantages over morphological methods of variety and/or species identification, in that: they are rapid, relatively cheap, eliminate the need to grow plants to maturity and are largely unaffected by the growth environment.

Isozymes are useful biochemical markers for assessing genetic variability in plant populations/accessions (Karam *et al.*, 1999; Sammour and Mustafa, 2013; Pegu *et al.*, 2018). They also used to measure intra-population/ accession variation in terms of the per cent of polymorphic loci, the effective number of alleles per locus and the mean proportion of loci heterozygous per individual (El-Esawi *et al.*, 2017).

Isozyme variation has been studied in *Lathyrus sativus* L. without the allelic interpretation of zymogram considering the isozyme banding pattern as a phenotype (Yamamoto *et al.*, 1986; Yunus and Jackson, 1991). In the meantime, little information is available on the genetic variability among accessions of *Lathyrus spp*. Most of the

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study in this area is restricted to the countries of the authors. A general study is sporadic. This is an indication of the poor characterization of *Lathyrus spp.*

MATERIALS AND METHODS

Plant materials: Seeds of sixteen accessions of *Lathyrus sativus* L. cover a diverse geographic locations (Table 1), were obtained from International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria and germplasm collection of the USDA, Regional Plant Introduction Station, 59 Johnson Hall, P.O. 646402 Pullman, Washington, United States.

Seed growth and isozyme extraction: Seeds were germinated on filter paper in sterilized Petri dishes. Isozymes crude extracts were prepared by macerating young leaves of fifteen days old seedlings in 30-40 µl of extraction buffer consisted of 0.05M sodium phosphate, pH 7.0, plus 14 mM mercapto-ethanol and 0.1% triton X-100 (Manchenko, 1994). The macerates were then centrifuged at 12,000 rpm for 10 min. The residue was discarded and the supernatant was used for isozymes analysis. At least 5 and generally 10 plants per accession were examined for isozyme patterns.

Isozyme analysis: α- Amylase (α- Amy), alcohol dehydrogenase (ADH), α-Esterase (α-EST); β-Esterase (β-Est), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), malic enzyme (ME), and phosphorelase (Phos) were preliminary screened for high resolution and polymorphism. Out of these isozymes α-Amy, α-Est, β-Est and Phos, were selected for further analysis due to good resolution, polymorphism and repeatability of results (Fig 1) . 20 µl of the crude extracts of the isozymes were loaded onto the gels. Electrodes were filled with 1M tris/glycine pH 8.3. Migration was performed at approx. 45°C and was stopped when the bromophenol blue line was one cm away from the bottom of the gel. Following the removal of the electrophoretic assembly, gels were stained for the desired isozymes (Manchenko, 1994).

For α-Amylase (*Amy*), electrophorized PAG (Polyacrylamid gel) containing 0.5% soluble starch was incubate in100 ml solution of 50 mM sodium acetate buffer, pH 5.6, at 37 to 50°C for 1 h. After the solution was discarding, the gel was washed with distilled water, and stained in an appropriate volume of solution formed of 10 mM I_2 mixed with 14 mM KI. The areas of enzyme activity developed on a dark blue back ground of the gel as light blue or translucent bands, depending on incubation time and the enzyme activity. The stained gel was rinsed with water and photographed as quickly as possible.

The gels of α and β-esterases were incubated in 100 ml staining solution consisted of 0.05 M phosphate buffer, pH 7.2, containing 1% α or β naphthyl acetate for α and β-esterases respectively and 50 mg Fast Blue RR. The incubation was carried out in the dark at 37°C. When light to dark reddish color bands appeared, the stained gels were washed with water and fix in 3% acetic acid.

For phosphorelase, electrophorized PAG containing 0.1 to 0.5% soluble starch was incubated in 100 ml solution of 0.1 M sodium phosphate buffer, pH 5.1, at 37°C for 3 to 5 h. After the solution was discarded, the gel washed with distilled water and stained in solution formed of 10 mM I_2 mixed with 14 mM KI. Zones of *Phos* activity appeared as white bands on a dark blue background. Chromatic or light brown bands appeared at the bottom of the gels. These bands were chromatic bands of amylase. The stained gels were photographed as quickly as possible.

Data analysis: The data of isozymes were analyzed using POPGENE version 1.31 software package. Dendrogram was constructed based on Nei,s genetic distance using UPGMA (Nei, 1973).

Table 1: Number, origin and sub-geographical region of the studied accessions of *Lathyrus sativus* L.

No	Accession Number	Accession origin (Abbreviation)	Sub-geographical region
1	PI 283546	Egypt (Egy)	Northern Africa
2	PI 283569	Libya (Lib)	Northern Africa
3	PI 283564	Sudan (Sud)	Sudano-Sahelian
4	PI 358601	Ethiopia (Eth)	Eastern Africa
5	PI 210342	Soviet Union (USSR)	Eastern Europe
6	PI 422533	Iran (Iran)	Near East (Middle East)
7	PI 317438	Afghanistan (Afghan)	Central Asia
8	W6 12869	Bangladesh (Ban)	Southern and Eastern Asia Indian Subcontinent)
9	PI 577136	Turkey (Tur)	Near East (Middle East)
10	PI 283563	Spain (Spain)	Mediterranean Europe
11	PI 422540	Italy (Italy)	Mediterranean Europe
12	PI370600	Yugoslavia (Yogh)	Central Europe
13	PI 283558	Canada (Can)	Northern America
14	PI 422543	Hungaria (Hun)	Central Europe
15	PI 391431	India (India)	Southern and Eastern Asia (Indian Subcontinent)
16	PI 426886	Pakistan (Pak)	Southern and Eastern Asia (Indian Subcontinent)

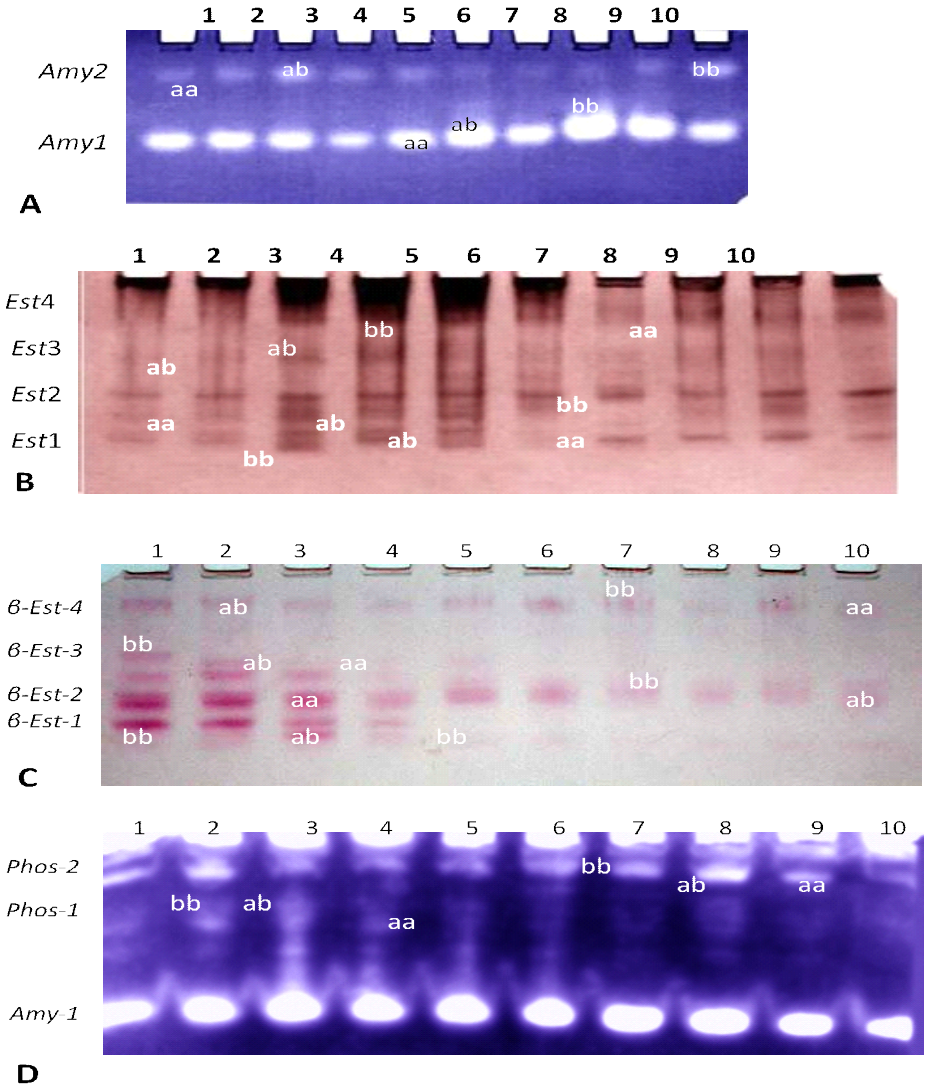

Fig 1: Isozyme zymogrammes from *Lathyrus sativus* accessions: A, amylase; B, α-estrase; C, β-estrase; D, phosporylase.

RESULTS AND DISCUSSION

Loci and alleles scored: Enzyme electrophoresis resulted in detecting 12 putative polymorphic loci, with mean allele frequency ranged between 0.78 in αEst-4-1 to 0.05 in βEst-3-3 (Table 2). Although the breeding system of *Lathyrus sativus* L is predominantly autogamous, the frequency of the 12 putative loci is greater than it is expected for this type of breeding system in *Lathyrus sativus* L. and other grain legumes (Yunus and Jackson, 1991). However, the high allelic frequencies can be interpreted on the basis of the wide range of morphological diversity in accessions of *Lathyrus sativus* L. (Yunus *et al*., 1984; Yunus and Jackson, 1991) and on the wide geographical distance between the locations of the collected accessions (Yunus and Jackson, 1991).

Although, the twelve loci were polymorphic, the per cent of polymorphism varied from 72.7 in accessions from Bangladesh and Pakistan to 93.9 in accession from Egypt. Our data did not agree with data of Chowdhury and Slinkard (2000), who reported that 68.42 % of the detected loci were polymorphic. This might be attributed to the similarity between the accessions they analyzed in plant type, flower colour, seed traits and maturity and share the Mediterranean climate, where they were collected from South Asia and Sudan–Ethiopia.

The mean frequency of βEst-3.3 was 0.05% (Table 2). The allele had this percent of frequency known as rare alleles. The presence of this allele could be due to deleterious mutations or may be due to evolutionary relics (Bakshi and Konnert, 2011). The detection of rare allele in combination with high allelic frequency of other loci leads to the conclusion that the studied accessions had obvious genetic differentiation.

Genetic diversity and accession-level homozygosity: The average of observed heterozygosity (H_o) was 0.449, ranging

Table 2: Allele frequencies at the 12 analyzed enzyme loci for 16 accessions of *L. sativus* L.

Allele frequency		EGY	LIB	SUD	ETH	USS	IRA	AFG	BAN	TUR	SPA	ITA	YUG	CAN	HUN	IND	PAK	Mean Frequency of allele
	A	0.00	0.20	0.60	0.30	0.37	0.40	0.70	1.00	0.40	0.60	0.00	0.20	0.10	0.25	0.00	0.40	0.19
αEst-1	**b**	0.87	0.30	0.30	0.50	0.25	0.30	0.30	0.00	0.40	0.10	0.60	0.70	0.70	0.50	0.60	0.60	0.62
	C	0.13	0.50	0.10	0.20	0.37	0.30	0.00	0.00	0.20	0.30	0.40	0.10	0.20	0.25	0.40	0.00	0.19
	A	0.30	0.50	0.10	0.30	0.25	0.30	0.40	0.00	0.50	0.10	0.50	0.10	0.40	0.40	0.10	0.50	0.3
αEst-2	**b**	0.20	0.30	0.40	0.30	0.50	0.50	0.20	0.30	0.30	0.40	0.40	0.50	0.40	0.50	0.30	0.50	0.44
	C	0.50	0.20	0.50	0.40	0.25	0.20	0.40	0.70	0.20	0.50	0.10	0.40	0.20	0.10	0.60	0.00	0.26
	A	0.20	0.10	0.30	0.50	0.00	0.25	0.00	0.00	0.87	0.00	0.83	0.50	0.50	0.30	0.20	0.10	0.32
αEst-3	**b**	0.80	0.90	0.10	0.50	0.00	0.75	0.25	0.50	0.00	0.75	0.00	0.17	0.00	0.00	0.60	0.80	0.31
	C	0.00	0.00	0.60	0.00	1.00	0.00	0.75	0.50	0.13	0.25	0.16	0.33	0.50	0.70	0.20	0.10	0.37
αEst-4	**A**	0.60	0.60	1.00	0.75	1.00	0.20	1.00	0.00	0.37	0.00	0.00	1.00	1.00	0.75	1.00	0.13	**0.78**
	B	0.40	0.40	0.00	0.25	0.00	0.80	0.00	1.00	0.62	1.00	1.00	0.00	0.00	0.25	0.00	0.87	0.22
	A	0.17	0.20	0.10	0.75	0.67	0.00	0.00	0.00	0.00	0.25	0.00	0.16	0.50	0.00	0.37	0.00	0.21
βEst-1	**b**	0.50	0.40	0.60	0.25	0.17	0.50	0.38	0.33	0.25	0.37	0.80	0.16	0.50	0.66	0.25	0.00	0.31
	C	0.33	0.40	0.30	0.00	0.17	0.50	0.63	0.66	0.75	0.37	0.20	0.67	0.00	0.33	0.37	1.00	0.47
	A	0.10	0.40	0.40	0.20	0.13	0.40	0.40	0.00	0.10	0.40	0.50	0.12	0.40	0.40	0.10	0.37	0.28
βEst-2	**b**	0.70	0.40	0.40	0.40	0.37	0.40	0.50	0.50	0.50	0.50	0.40	0.50	0.50	0.10	0.30	0.00	0.28
	C	0.20	0.20	0.20	0.40	0.50	0.20	0.10	0.50	0.40	0.10	0.10	0.37	0.10	0.50	0.60	0.62	0.44
	A	0.40	0.50	0.20	1.00	1.00	0.60	0.50	0.75	1.00	0.00	1.00	0.50	0.30	0.87	0.63	0.30	0.52
βEst-3	**b**	0.40	0.50	0.20	0.00	0.00	0.20	0.30	0.00	0.00	0.75	0.00	0.50	0.70	0.00	0.37	0.60	0.43
	C	0.20	0.00	0.60	0.00	0.00	0.20	0.20	0.25	0.00	0.25	0.00	0.00	0.00	0.13	0.00	0.10	**0.05**
βEst-4	**A**	0.20	0.80	0.75	0.75	0.88	0.20	0.40	0.40	0.80	0.50	0.80	0.75	0.00	0.00	0.00	0.00	0.15
	B	0.80	0.20	0.25	0.25	0.13	0.80	0.60	0.60	0.20	0.50	0.20	0.25	1.00	0.00	0.00	0.00	0.25
Pho-1	**A**	0.20	0.17	0.20	0.10	0.25	0.90	1.00	0.62	0.70	0.80	0.40	0.70	0.40	0.25	0.60	0.50	0.49
	b	0.80	0.83	0.80	0.90	0.75	0.10	0.00	0.37	0.30	0.20	0.60	0.30	0.60	0.75	0.40	0.50	0.51
	A	0.30	0.00	0.10	0.30	0.25	0.33	0.17	0.50	0.40	0.10	0.10	0.20	0.20	0.60	0.30	0.30	0.32
Pho-2	**b**	0.60	0.40	0.80	0.70	0.75	0.33	0.50	0.33	0.20	0.60	0.20	0.00	0.40	0.20	0.40	0.70	0.34
	C	0.10	0.60	0.10	0.00	0.00	0.33	0.33	0.16	0.40	0.30	0.70	0.80	0.40	0.20	0.30	0.00	0.34
	A	0.20	0.60	1.00	0.00	0.80	0.00	0.60	0.40	0.00	0.40	0.20	0.80	0.00	0.20	0.60	0.70	0.46
Amy-1	**b**	0.40	0.40	0.00	0.40	0.20	0.60	0.40	0.60	1.00	0.20	0.70	0.20	0.80	0.80	0.40	0.10	0.46
	C	0.40	0.00	0.00	0.60	0.00	0.40	0.00	0.00	0.00	0.40	0.10	0.00	0.20	0.00	0.00	0.30	0.10
	A	0.20	0.25	1.00	0.00	0.10	0.10	0.00	0.00	0.00	0.00	0.66	0.50	0.00	0.10	0.60	0.50	0.34
Amy-2	**b**	0.60	0.25	0.00	1.00	0.80	0.70	0.50	0.90	0.50	0.25	0.33	0.50	0.60	0.40	0.20	0.50	0.44
	C	0.20	0.50	0.00	0.00	0.10	0.20	0.50	0.10	0.50	0.75	0.00	0.00	0.40	0.50	0.20	0.00	0.22
Total number of alleles		31	29	28	25	27	30	26	24	26	29	26	28	26	27	27	24	
Percent of polymorphic loci		93.9	87.9	84.4	75.8	81.8	90.9	78.8	72.7	78.8	87.9	78.8	84.4	78.8	81.8	81.8	72.7	

Number of allele range from 31 to 24 with mean number 27.4

Table 3: Accessions of *Lathyrus*, sample sizes, estimates of genetic diversity, average fixation index (F) and summary of results of tests for deviations of genotypic frequencies from Hardy-Weinberg equilibrium in 16 accessions of *L. sativus.*

Accession origin (Abbreviation)	H_o	H_e	A	A_e
Egypt (Egy)	0.420	0.535	2.583	2.0506
Libya (Lib)	0.544	0.557	2.417	2.1108
Sudan (Sud)	0.316	0.411	2.333	1.7949
Ethiopia (Eth)	0.425	0.446	2.083	1.8348
Soviet Union (USSR)	0.394	0.393	2.167	1.7086
Iran (Iran)	0.541	0.550	2.500	2.0966
Afghanistan (Afgn)	0.437	0.501	2.167	1.9512
Bangladesh (Ban)	0.256	0.428	1.917	1.7203
Turkey (Tur)	0.433	0.454	2.167	1.8771
Spain (Spain)	0.479	0.533	2.417	2.0056
Italy (Italy)	0.483	0.413	2.167	1.7031
Yugoslavia (Yogh)	0.533	0.494	2.333	1.8776
Canada (Can)	0.416	0.475	2.167	1.9024
Hungaria (Hun)	0.563	0.527	2.455	1.9596
India (India)	0.481	0.551	2.455	2.1268
Pakistan (Pak)	0.463	0.4521	2.183	1.7591
Mean	0.449	0.483	2.283	1.905

H_o: The observed heterozygosity.
H_e: The expected heterozygosity.
A: The mean number of alleles per locus.
A_e: The effective number of alleles per locus.

from 0.256 in accession from Bangladesh to 0.563 in accession from Hungary and the average of expected heterozygosity (H_e) was 0.483 ranging between 0.393 in accession from Soviet Union to 0.557 in accession from Libya (Table 3). In general, the expected heterozygosity was more than the observed heterozygosity for all the studied accessions, suggesting an apparent rate of allogamy taking place in *Lathyrus sativus* L. (Lioi *et al*., 2011). The mean average of the observed heterozygosity and expected heterozygosity (0.449 and 0.483) were higher than 0.143 and 0.052 observed by Lioi *et al*. (2011). The difference was attributed to the genetic nature of plant material. Lioi *et al*. (2011) studied with Italian grasspea which had low diversity and the accessions that we studied were collected from different geographical regions showed a wider heterozygosity. The level of apparent heterozygosity in the studied accessions reflects a wider heterozygosity in *Lathyrus sativus* L., which can be exploited in releasing types with desirable agronomic characters, particularly for the trait of low ODAP concentration.

The mean number of alleles per locus (A) varied respectively from 1.917 in accession from Bangladesh to 2.583 in accession from Egypt with a mean of 2.283 and the effective number of alleles per locus (A_e) varied from 1.703 from accession of Italy to 2.127 in accession from India with a mean of 1.905 respectively (Table 3). The average number of alleles per locus (2.283) was almost similar to that (2.02) reported by Chowdhury and Slinkard (2000). But it was high, compared to the value (1.26) observed by Lioi *et al*. (2011). This discrepancy could be attributed to the genetic nature of plant material. Lioi *et al*. (2011) studied Italian grasspea which have less diversity and Chowdhury and Slinkard (2000) studied accessions collected from different geographical regions and had a wider heterozygosity. The mean number of alleles per locus indicated that the studied accessions express good allelic richness, which call for the importance of establishing conservation programs to retain the existing diversity which represent a valuable repository for genetic variation.

Genetic structure and gene flow: The estimates of accessions genetic structure using Nei genetic diversity statistics are shown in Table 4. The average of total heterozygosity (H_T) and intra-accessional genetic diversity (H_S) were 0.559 and 0.428 respectively indicating that majority of genetic diversity was intra-accessional. The excess of heterozygosity observed might come from a strong selection in favor of heterozygotes.

The average of inter-accessional genetic diversity (D_{ST}) of isozyme loci was 0.190 (Table 4). The low levels and non-significant of genetic diversity among accessions (D_{ST} = 0.190, X^2 = 62.59, p = 0.029) were probably indicative of occurrence of several gene flows which was confirmed by the estimates of the number of migrates per generation based on Wright's equation (NmW =0.738). The level of gene flow revealed could be due to the outcrossing reported in *L. sativus* (Ben Brahim *et al*., 2001).

As shown in Table 4, the coefficient of gene differentiation (G_{ST}) showed significant variation. It ranged between 0.044 for αEst-4 and 0.625 for βEst- 3 with value

Table 4: Genetic diversity, coefficient of gene differentiation, F statics and estimates of inter-accessions gene flow among accessions for isozyme traits in *Lathyrus sativus* L.

Locus	Genetic diversity									X^2	Probability (*p*-value)
	H_T	H_S	H_S/H_T	D_{ST}	G_{ST}	F_{IT}	F_{ST}	F_{IS}	Nm_w		
αEst-1	0.641	0.493	76.91	0.148	0.230	0.1426	0.2303	-0.114	0.8355	63.80	0.0003
αEst-2	0.667	0.588	88.12	0.079	0.118	0.3186	0.1142	-0.489	1.9397	36.40	0.1951
αEst-3	0.667	0.380	56.97	0.287	0.430	0.2940	0.4252	-0.228	0.3380	97.90	0.0000
αEst-4	0.492	0.470	95.53	0.022	0.044	0.7550	0.6496	0.301	0.1349	65.60	0.0000
βEst-1	0.621	0.574	92.43	0.047	0.075	0.3812	0.2583	0.166	0.7179	50.80	0.0100
βEst-2	0.655	0.361	55.11	0.294	0.448	0.3390	0.1267	-0.533	1.7235	38.90	0.1258
βEst-3	0.579	0.275	47.5	0.304	0.625	0.2225	0.3426	-0.183	0.4797	63.80	0.0003
βEst-4	0.486	0.349	71.81	0.137	0.281	0.8545	0.5866	0.648	0.1762	29.10	0.0155
Phos-1	0.499	0.499	100.0	0.018	0.034	0.1890	0.3000	-0.159	0.5834	40.10	0.0004
Phos-2	0.650	0.397	61.08	0.253	0.389	0.2795	0.2042	0.095	0.9746	57.30	0.0018
Amy-1	0.623	0.623	100.0	0.350	0.464	0.7548	0.3503	0.623	0.4637	108.5	0.0000
Amy-2	0.131	0.131	100.0	0.338	0.489	0.2521	0.3384	-0.131	0.4887	98.90	0.0000
Mean	0.559	0.428	78.79	0.190	0.302	0.399	0.3270	-0.0003	0.7380	62.59	0.0291

Notes: H_T, the total genetic diversity; H_S, the genetic diversity within accessions; H_S/H_T, percentage mean genetic diversity within accessions; D_{ST}, the genetic diversity among accessions; G_{ST} (D_{ST}/H_T), coefficient of gene differentiation among accessions, F_{IT}, the mean inbreeding coefficient of a set of accessions; F_{IS}, the fixation index related to non-random mating within accessions; F_{ST}, the inter-accessions genetic differentiation due to genetic draft and Nm_w, the gene flow estimate according to Wright's (1951) equation.

0.302, suggesting the heterogonous distribution of *L. sativus* accessions among different geographic regions.

Table 4 shows the values of Wrights' fixation indices for each locus. The fixation indices were lower for *αEst-1, βEst-2* and *Phos-1* compared with the other loci, indicating that accessions were in panmictic equilibrium for these loci. On the other hand, *αEst-4*, *βEst-4* and *Amy-1* loci showed higher fixation indices F_{ST} and F_{IT} which illustrated their deficits in heterozygotes. In general, the mean breeding index was significantly higher than zero (F_{IT} = 0.399) exhibiting a deviation from the expected H-W proportions. The mean average of F_{ST} was 0.327, suggesting the occurrence of random mating system for the studied accessions and reflecting adaptation to strong environmental dissimilarities or high level of genetic drift maintained by restricted gene flow between populations. Similar observations have been noted in barley (Tadesse and Bekele, 2001).

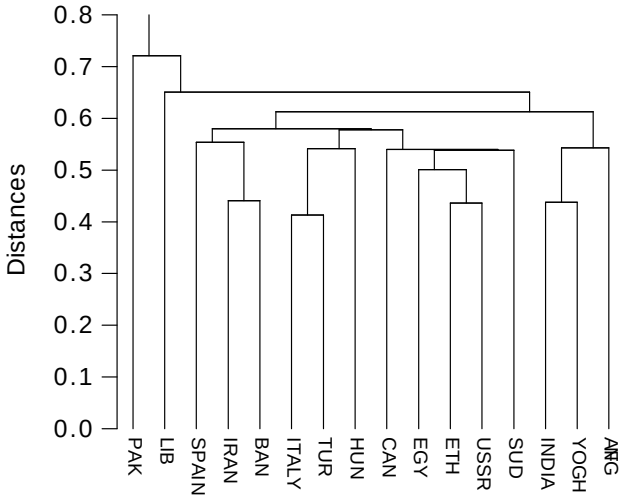

Fig 2: UPGMA dendrogram showing the relationships among the 16 accessions of *Lathyrus sativus* based on isozyme data.

Genetic distance and dendrogram: Dendrogram constructed based on isozyme data showed that accessions from different geographical regions were grouped in one cluster (Fig 2), suggesting that the environment had no influence on the genetic diversity of *Lathyrus sativus* L. and confirmed that *Lathyrus sativus* L. had a polyphyletic origin (Kislev, 1989; Vavilov, 1951).

CONCLUSION

Based on the isozymes electrophoresis, it can be concluded that studied accessions express good allelic richness and had an apparent rate of allogamy, reflecting their adaptation to strong environmental dissimilarities The good allelic richness attracts the attention for the importance of establishing conservation programs to retain the existing diversity. Moreover, the majority of genetic diversity in the studied accessions was intra-accessional and the narrow inter-accessional genetic diversity confirmed that there were several gene flows between accessions. The wider heterozygosity in accessions can be exploited in releasing plant forms with desirable agronomic characters, particularly the trait of low ODAP concentration. It can also be concluded that environment had no influence on the genetic diversity of *Lathyrus sativus* L. and *Lathyrus sativus* L. had a polyphyletic origin.

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