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Isozymes variation among and within accessions of *Lathyrus sativus*

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ABSTRACT

This study aims at exploraing genetic diversity between and within sixteen accessions of *L. sativus* using four isozyme systems; *Amylase, Phosorelase,* â- *esterase and* á-*esterase.* The four isozyme systems were examined for from five to ten seedlings of each accessions. All isozymes were polymeric in at least one accession. They are encoded with 12 loci migrated anodally. The total alleles of isozymes were 34. The total number of alleles in each accession for the 12 loci ranged from 24 to 32 with a mean of 27.5 (total = 34). The heterogenesty of all accessions were studied using Hardery Weinbring expectations test, and estimated the genetic structure of accession by using Nei genetic diversity statistics. F statistics were also calculated for all accessions. They indicated that the mean breeding index was significantly higher than zero (0.399). The relationship between the accession size and the mean number of alleles per locus was significant. On the contrary the proportion of polymorphic loci and the heterozygosity observed with population size were non significant. The presented data indicated that about 50% of the total genetic diversity detected was intera-accessional while the 50% was inter-accessional. It was also indicated that the high level of gene flow revealed could be due to the high percent of out-crossing. Significant correlation was also found between the size of the accessions and their level of genetic variations.

KEYWORDS

Polymorphic loci;
Nei genetic diversity;
F statistics;
Total heterozygosity (H_T);
Intra accessional genetic diversity (H_S);
Inter-accessional genetic diversity (D_{ST});
Fixation index.

INTRODUCTION

Lathyrus sativus (grass pea) belongs to genus *Lathyrus*, a member of the tribe *Vicieae,* Family Fabaceae. It is widely cultivated as a food crop[1-5]. Despite it's tolerance to drought, it is not affected by excessive rainfall and can be grown on land subject to flooding[6-12]. This hardiness, together with its ability to fix atmospheric nitrogen, makes the crop one that seems designed to grow under adverse conditions[5,13-16]. Compared with other legumes, the grass pea is resistant to many pests including storage insects[17-21].

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

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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 intraspecific and specific levels. In the meantime, characterization of genetic diversity is necessary to improve strategies for conservation and collection of germplasm and to increase utilization of plant genetic resources in varietals improvement which should have the top priority[22-27]. The advent of the electrophoresis method as an analytical tool provides an indirect methods for genome probing by exposing structural variations in enzymes and other proteins[28-33]. Electrophoretic markers are the encoding products of neutral genes, not linked to any loci that affect the species and value[34,35]. They are also 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.

The electrophoretic markers of the seed storage proteins were used to: (1) differentiate between species, (2) check species identification, (3) assist biosystematics analysis, (4) study phylogenetic relationships of the species, and (5) generate pertinent information to complement evaluation and passport data and thereby increase the knowledge of the genetic diversity of the materials in the germplasm collections[36-39].

Isozymes are useful biochemical markers for assessing genetic variability. They have been used in taxonomic, genetic, evolutionary and ecological studies. Isozymes have also been a good estimators of genetic variability in plant populations[40-43]. They also used to measure intra-population variation in terms of the percent of polymorphic loci, the effective number of alleles per locus and the mean proportion of loci heterozygous per individual.

Isozyme variation has been studied in grass pea without the allelic interpretation of zymogram considering the isozyme banding pattern as a phenotype[44-51].

Little information is available on the genetic variability among accessions of *Lathyrus spp*. Most of the 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.*

The objectives of this study were to evaluate the genetic variablity in accessions of *L. sativus* cover wide range geographical regions using isozymes.

MATERIAL AND METHODS

Material

Seeds of sixteen accessions of *Lathyrus sativus* L., cover a wide range of its distribution, were obtained from International Center for Agricultural Research in the Dry Areas (ICARDA) Aleppo, Syria and germplasm collection of the USDA, ARS, WRPIS Washington State University, Regional Plant Introduction Station, 59 Johnson Hall, P.O. 646402 Pullman, Washington, United States, 99164-6402 (TABLE 1).

Seed germination

Seeds were germinated on filtr paper in previously sterilized Petri dishes. Young leaves were collected from fifteen days old seedlings for isozymes extraction. Crude extracts were prepared by macerating the collected leaves in 30-40 µl of extraction buffer consisted of 0.05M sodium phosphate, pH 7.0, plus 14 mM mercaptoethanol and 0.1% triton X-100[52]. To prevent denaturation of the enzymes, the extraction trays were kept on crushed ice during maceration. The macerates were then centrifuged at 12,000 rpm for 10 min. The residue was discard and the supernatant was used for isozymes analysis. At least 5, and generally 10 plants per accession were examined for isozyme patterns

Isozyme analysis

Preliminary tests were made with eight enzymatic systems 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). α-Amy, α-Est, β-Est and Phos, were selected for further analysis due to good resolution, polymorphism and repeatability of results. The abbreviation and IUBMB of selected isozymes are *α-Amy*, E.C.3.2.1.1; *α-Est*, E.C.3.1.1; *β- Est*, E.C.3.1.1; and *Phos*, E.C.2.4.1.1 respectively. Preliminary tests were performed to identify the best gel and buffer systems, buffer concentrations and tissue amount in the samples.

Aliquots 20 µl of the crude extracts were loaded onto the gels. Ten samples were run in each gel. Elec-

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TABLE 1 : List of accessions of *L. sativus* L.

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

trodes were filled with 1M tris/glycine pH 8.3. The front line was monitored with bromophenol blue (0.1% in ethanol). Migration was performed at approx. 45^{0}C and was stopped when the bromophenol blue line was one cm away from the bottom of the gel. During migration, electric voltage was checked every 25-35 min and adjusted if needed.

Following the removal of the electrrophoretic assembly, gels were stained for the desired isozymes[53]. For α-Amylase (*Amy*), electrophorized PAG (Polyacrylamid gel) containing 0.5% soluble starch was incubate in100ml solution of 50 mM sodium acetate buffer, pH 5.6, at 37 to 50 ^{0}C for 1 h. After the solution was discard, 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 solution was discarded and stained gel was rinsed with water. Then the zymogram was photographed as quickly as possible.

The gels of α and □-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 □-esterases respectively and 50 mg Fast Blue RR. The incubation was carried out in the dark at 37^{0}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 ^{0}C for 3 to 5 h. After the solution was discarded, the gel washed with distilled water and stained in solution formed of 10mM I_2 mixed with 14mM KI. Zones of *Phos* activity appeared as white bands bands on a dark blue background. A chromatic or light brown bands appered at the bottom of the gels. These bands were cromatic bands of amylase. The stained gels was photographed as quickly as possible.

Data analysis

The data of isozymes were analyzed using POPGENE version 1.31 Microsoft Window-based Freeware for Population Genetic Analysis. Spearman rank non-parametric correlation was used to test relationship between populations size and measures of genetic variation (number of alleles per locus, A; proportion of polymorphic loci; P_p; and mean heterozygosity, H_O). This test was excuted using the soft ware package (SYSTAT 0 for WINDOWS VERSION 7.0 COPY-

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RESULTS

Isozyme phenotypes and their variation

α-Amylase (α-Amy, E.C.3.2.1.1)

Two zones of activity were detected, one with faint activity and one with strong activity (Figure 1A). The two zones of activity showed variation between accessions and interpreted as products of two loci (*Amy-1* and *Amy-2*), each locus with two codominant alleles encoding allozymes phenotypes *Amy*-a and *Amy*-b for *Amy*-1[54], and *Amy*-a and *Amy*-b for *Amy*-2. The bands of *Amy*-1 showed three electrophoretic variants, at the anodal staining zone. These variants segregated independently relative to those of the cathodal staining zone, suggesting that two loci control *Amy* isozymes. The alleles that expressed the three allozymes phenotype were detected. At both loci, double banded isozyme phenotypes representing heterozygous individuals were observed (Figure 1A). These results indicated that *Lathyrus Amy* isozymes have a monomeric quaternary structure.

α-Esterase (*α-Est*, E.C.3.1.1)

Four zones of activity were observed for α-*Esterase* (Figures 1B). Absence of band for some individuals could explain by a locus possessing a null allele. The four zones of activity showed variance and were interpreted as product of four loci (*Est*-1, *Est*-2, *Est*-3, *Est*-4), each with two codominant alleles encoding allozymes phenotypes. Heterozygous individuals with a double-banded isozyme phenotype at locus demonstrates the monomeric quaternary structure of *α-Est*. Nevertheless, it has been widely demonstrated that *α-Est* isozymes are dimeric. Similar results were reported for several *Leguminosae*,[55], *C. arietinum*[56] and *Phaseolus lunatus L.*[54].

Polymorphisms were observed at loci. The most cathodal zone was monomorphic. It was assured that this zone is specified by one gene *Est*-4. This showed two allelic variants one/second null. Three isozymes phenotypes were observed for the loci *Est*-1, *Est*-2, *Est-3* (two alternative homozygous phenotypes and one heterozygous phenotype). The observed banding patterns were similar to those found in *Phaseolus lunatus*[54]. These results suggested that four loci were coding for isozymes expressed in *Lathyrus sativus*. Three of these loci showed two codominant alleles. Each of these genes coded for functionally monomeric protein.

□-Esterase (□-*Est*, E.C.3.1.1)

Gels stained for this enzyme displayed four zones of activity (Figures 1C). The four zones of activity showed variance and were interpreted as product of four loci (*Est*-1, *Est*-2, *Est*-3, *Est*-4), each with two codominant alleles encoding allozymes phenotypes, except *Est*-4. Heterozygous individuals with a double-banded isozyme phenotype at locus demonstrate the monomeric quaternary structure of *α-Est* in loci. Polymorphisms were observed at loci. The most cathodal zone was monomorphic. It was assured that this zone is specified by one gene *Est*-4. This showed one allelic variant. Three isozymes phenotypes were observed for

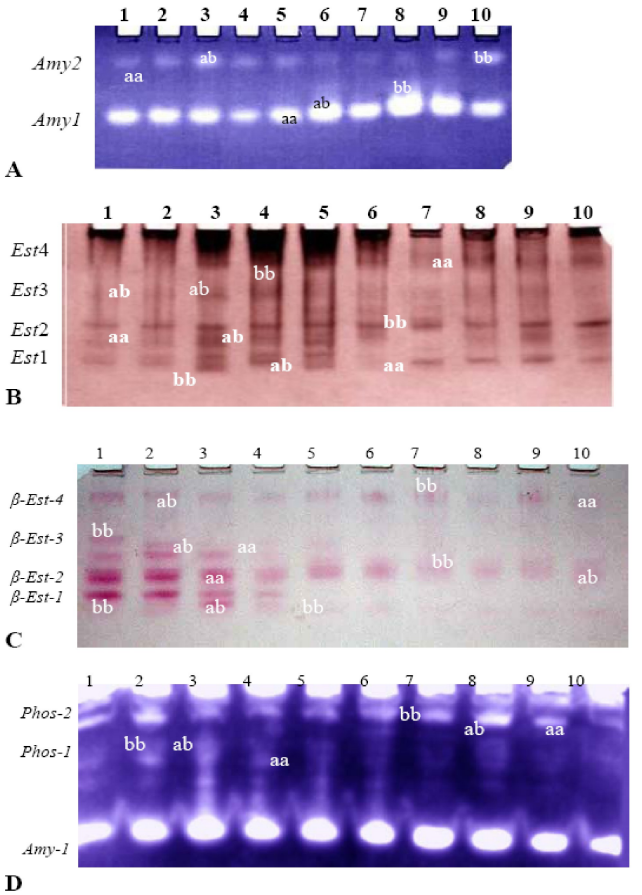

Figure 1 : Isozyme zymogrammes from *Lathyrus sativus* accessions: A, amylase; B, α-estrase; C, □-estrase; D, phosporylase

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the loci *Est*-1, *Est*-2, *Est-3* (two alternative homozygous phenotypes and one heterozygous phenotype). The observed banding patterns were similar to those found in *Phaseolus lunatus*[54,57-60]. These results suggested that four loci were coding for isozymes expressed in *Lathyrus sativus*. Three of these loci showed two codominant alleles. Each of these genes coded for functionally monomeric protein.

Phosphorylase (*Phos*, E.C.2.4.1.1.)

Starch *phosphorylase* was assayed in the seedling of studied accessions. Two zones of activity were detected on the gel, designated *Phos*-1 and *Phos*-2 (Figures 1D). The other zone at the bottom of the gel was a cromatic bands of amylase, which can develop a chromatic bands by this methods[53]. A control staining for amylase was carried out to differentiate between the bands of starch phosphorylase and amylase.

Polymorphism was observed at both *Phos*-1 and *Phos*-2 loci. Two allelic variants that produced the two allozyme phenotypes were detected at *Phos*-2. Heterozygous individuals with banded isozymes phenotype were detected at both loci, demonstrating their monomeric quaternary structure. For *Phos* no intergenic or intra-allelic products were detected. These observations are in accordance with the monomeric quaternary structure of *Phos* isozyme.

Loci and alleles scored

Enzyme electrophoresis resulted in clear staining for four enzymes encoded by 12 putative loci (TABLE 2). All enzymes migrated anodally. All 12 loci were polymorphic in at least one accession. A locus was considered to be polymorphic if two or more alleles were detected, regardless of their frequencies. A total of 34 alleles were observed a cross *L. sativus* (X = 2.283).

TABLE 2 : Allelic frequencies for 12 isozyme loci in 16 accessions of *Lathyrus sativus*

Allele frequency		EGY	LIB	SUD	ETH	USS	IRA	AFG	BAN	TUR	SPA	ITA	YUG	CAN	HUN	IND	PAK
αES-1	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
	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
	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
αES-2	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
	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
	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
αES-3	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
	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
	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
αES-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
	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
βES-1	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
	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
	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
βES-2	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
	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
	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
βES-3	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
	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
	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
βES-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
	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
PH-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
	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
	c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PH-2	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
	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
	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
AM-1	a	0.20	0.60	1.00	0.00	0.80	0.00	0.60	0.40	0.00	0.40	0.200.	0.80	0.00	0.20	0.60	0.70
	b	0.40	0.40	0.00	0.40	0.20	0.60	0.40	0.60	1.00	0.20	70	0.20	0.80	0.80	0.40	0.10
	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
AM-2	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
	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
	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
Mean number of allele / locus		0.94	0.88	0.82	0.73	0.79	0.88	0.76	0.70	0.76	0.88	0.79	0.82	0.79	0.79	0.87	0.70

Number of allele range from 24 to 32 with mean number 27.4; The total mean number of allele per locus = 0.80

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Most loci have a common allele. One private allele was observed, *Amy*-1 in accession number PI 283546 from Egypt. Only frequencies of loci *α Est-1, α Est-3, α Est-4 β Est-3, Pho*-1, *Amy-1 and Amy-2* were able to discriminate among the 16 accessions studied ($p < 0.001$) (TABLE 1), while the allelic frequencies of the other isozymes showed intra and inter accessional genetic variation but not statistically significant.

Genetic diversity and accession-level homozygosity

The number of polymorphic alleles per locus (A_p = 2.283), percentage of polymorphic loci (P = 89.75%) and expected heterozygosity (H_e = 0.483) showed high mean value compared to the accepted mean values for all plant species (A_p = 1.64, P = 36.8% and H_e = 0.141,[31]. The expected heterozygosity ranged between 0.393 in accession PI 422533 from Iran to 0.557 in the accession PI 283569 from Libya. The range of variation is not too high. However, the accessions with expected heterozygosity more than 0.5 (Egypt, Libya, USSR, Afghanistan, Spain, Hungary, India) are considered rich in alleles frequencies. They represent a valuable repository for genetic variation.

The total number of alleles in each accession for the 12 loci ranged from 24 to 32 with a mean of 27.4 (total = 34). Genetic diversity in accessions was quantified using standard measure of genetic diversity (TABLE 3). These values varied among accessions, with a number of allele / locus ranging from 0.7 in Bangladesh and Pakistan, and 0.94 in Egypt with mean X = 0.80.

From TABLE 3, the proportion of polymorphic loci (P_p) varied from 75 % e.g. Sudan, Iran to 100 % e.g..Egypt, Libya and USSR with a mean of 89.75 %. The mean number of alleles per locus (A) and the effective number of alleles per locus (A_e) varied respectively from 1.9167 (e.g. Bangladesh) to 2.5833 (e.g. Egypt) with a mean of 2.283, and from 1.7031 (e.g. Bangladesh) to 2.1268 (e.g. India) with a mean of 1.905.

From TABLE 3, the average of H_o was 0.449, ranging from 0.256 (e.g. Bangladesh) to 0.563 (e.g. Hungary) and the average of H_e was 0.483, ranging from 0.393 (e.g. Iran) to 0.557 (e.g. Libya). These results indicated that in the accessions of *L. sativus* enzyme loci express a moderate allelic richness (A=2.283) the polymorphic loci represented uneven allele frequencies

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 20 accessions of *L. sativus*

Sample	Sample size	Polymorph loci %	H_o	H_e	A	A_e	F	G_{st}
L. sativus EG	10	100%	0.420	0.535	2.5833	2.0506	0.214	0.539
L. sativus LIB	9	100%	0.544	0.557	2.4167	2.1108	0.023	0.4369
L. sativus SUD	10	75%	0.316	0.411	2.333	1.7949	0.231	0.619
L. sativus ETH	8	89%	0.425	0.446	2.0833	1.8348	0.047	0.599
L. sativus USSR	7	75%	0.3944	0.393	2.1667	1.7086	-0.002	0.659
L. sativus IRA	9	100%	0.541	0.550	2.5000	2.0966	0.016	0.418
L. sativus AFG	8	89%	0.437	0.501	2.1667	1.9512	0.127	0.559
L. sativus BAN	8	89%	0.256	0.428	1.9167	1.7203	0.401	0.739
L. sativus TUR	8	89%	0.433	0.454	2.1667	1.8771	0.046	0.499
L. sativus SPA	8	91%	0.479	0.533	2.4167	2.0056	0.101	0.499
L. sativus ITA	8	89%	0.483	0.413	2.1667	1.7031	-0.169	0.459
L. sativus YUG	8	91%	0.533	0.494	2.3333	1.8776	-0.078	0.459
L. sativus CAN	8	89%	0.416	0.475	2.1667	1.9024	0.124	0.519
L. sativus HUN	9	91%	0.563	0.527	2.4545	1.9596	-0.068	0.406
L. sativus IND	9	89%	0.481	0.551	2.4545	2.1268	0.127	0.448
L. sativus PAK	9	89%	0.463	0.4521	2.1818	1.7591	-0.024	0.512
Mean	8.5	89.75%	0449	0.483	2.2825	1.905	0.070	0.492

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; F: - Wright's *F* [$F = (1 - H_o / H_e)$] the inbreeding coefficient, measures the deviation of population genotypic composition from Hardy-Weinberg (H-W) expectations; G_{st} :- The among-accessions gene differentiation coefficient

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(A_e = 1.905).

In most accessions, observed heterozygosity was less than Weinbering expectations except in four accessions (Iran, Bangladesh, Hungary, and Pakistan). These results may be due to small sample size in these accessions. The average fixation index (F) is significant higher than zero for the analysis accessions, except for Iran, Italy, Yugoslavia, Hungary and Pakistan (TABLE 3), the negative value indicated an excess of heterozygote.

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 0.598 and 0.436 respectively. The inter-accessional genetic diversity (D_{ST}) varied from 0.018 (locus *Phos-1*) to 0.304 (locus *βEst-3*) and the coefficient of genetic differentiation among population (G_{ST}) varied from 0.034 (locus *Phos-1*) to 0.625 (locus *βEst-3*). The result indicated that in *L. sativus* about 27 % of the total genetic diversity is among accessional genetic diversity and 73% representing inter-accessional genetic diversity. The low levels of genetic differentiation among population (G_{ST} = 0.276, x^2 = 62.59, p < 0.001) and the inter accessional genetic diversity (D_{ST} 0.165) were probably indicative of high gene flow which was confirmed by the estimates of the number of migrates per generation based on Wrights equation (Nm_W =0.738) such result corresponded to the occurrence of genetic divergence in *L. sativus* accessions given that genetic drift result in substantial local differentiation if Nm < 1[61,62]. However this drift is going with slow paces.

F statistics for the 16 accessions (TABLE 4), indicated that the mean breeding index was significantly higher than zero (F_{IT} = 0.399). Then the genotypic composition of *L. sativus* showed a deviation from the expected H-W proportions. A high and significant value was obtained for F_{ST} with mean = (0.327) suggesting the occurrence of random mating system for the studied accessions. Although relatively low the estimate of F_{ST} was non-significant (F_{ST} = 0.327, x^2 = 62.59 P > 0.001).

The relationship between population size expressed by the plants number (m) and the proportion of polymorphic loci (P_p), the mean number of allele per locus (A), and the mean observed heterozygosity (H_O) as well as the correlation coefficient (r) describing this relationship are shown in Figure 2. Positive correlation were observed between the three genetic diversity indices (P_p, A, and H_O) and accession size expressed as both number of individuals in the accession and collected seeds numbers (Figure 2). Considering sample size as

TABLE 4 : Nei's (1973) genetic diversity indices, F statics, and estimates of inter-accessions gene flow

Locus	F statistics				Genetic variation				X^2	Probability
	F_{IS}	F_{IT}	F_{ST}	Nm_w	H_T	H_S	D_{ST}	G_{ST}		
αEst-1	-0.114	0.1426	0.2303	0.8355	0.641	0.493	0.148	0.230	63.8	0.0003
αEst-2	-0.489	0.3186	0.1142	1.9397	0.667	0.588	0.079	0.118	36.4	0.1951
αEst-3	-0.228	0.2940	0.4252	0.3380	0.667	0.380	0.287	0.430	97.9	0.000
αEst-4	0.301	0.7550	0.6496	0.1349	0.492	0.470	0.022	0.044	65.6	0.0000
βEst-1	0.1657	0.3812	0.2583	0.7179	0.621	0.574	0.047	0.075	50.8	0.0100
βEst-2	-0.533	0.3390	0.1267	1.7235	0.655	0.361	0.294	0.448	38.9	0.1258
βEst-3	-0.183	0.2225	0.3426	0.4797	0.579	0.275	0.304	0.625	63.8	0.0003
βEst-4	0.6480	0.8545	0.5866	0.1762	0.486	0.349	0.137	0.281	29.1	0.0155
Phos-1	-0.159	0.1890	0.3000	0.5834	0.499	0.517	0.018	0.034	40.1	0.0004
Phos-2	0.0946	0.2795	0.2042	0.9746	0.650	0.397	0.253	0.389	57.3	0.0018
Amy-1	0.6226	0.7548	0.3503	0.4637	0.611	0.412	0.199	0.325	108.5	0.0000
Amy-2	-0.131	0.2521	0.3384	0.4887	0.611	0.416	0.195	0.319	98.9	0.0000
Mean	-0.005	0.399	0.327	0.738	0.598	0.436	0.165	0.276	62.59	0.029

***Notes*:- H_T, the total genetic diversity; H_S, the genetic diversity within accessions; D_{ST}, the genetic diversity among accessions; G_{ST}, the among accessions gene differentiation coefficient, 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[41] equation**

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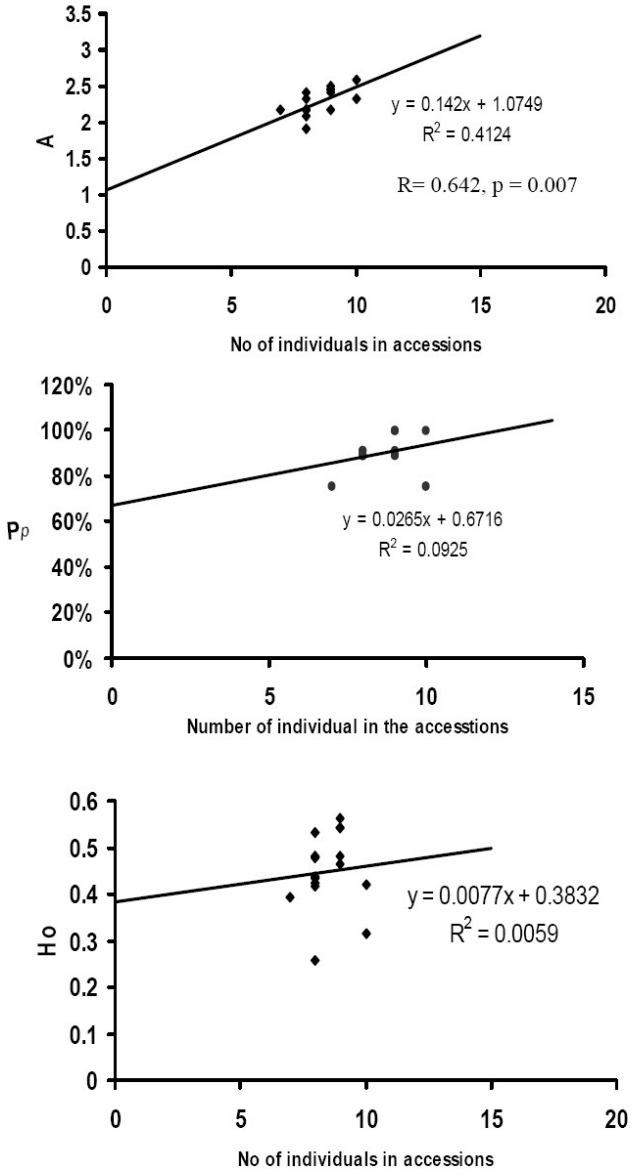

Figure 2 : The relationship between populations size and measures of genetic variation (number of alleles per locus, A; proportion of polymorphic loci; P_p; and mean heterozygosity, H_O). Examined using a spearman rank non-parametric correlation for 16 accessions of *L. sativus*.

the collected seed number, the following results were obtained, r = 0.304 with P = 0.252 for P_p and r = 0.077 with P = 0.778 for H_O and r = 0.642 with P = 0.007 for A. However the correlation between accession size and these indices was non-significant, with the exception of the correlation between accession size and the mean number of alleles per locus.

DISCUSSION

Only frequencies of seven loci (*α-Est-1*, *α-Est-3*, *α-Es-4*, *β-Est-1*, *phos-1*, *Amy*-1 and *Amy*-2) were able to discriminate among the 16 accessions studied ($P < 0.001$). While the allelic frequencies of the other loci showed intra-and interaccessional genetic variations, but were not statistically significant. To infer the significance of this result, it is necessary to known the genetic relatedness of the allelic frequencies for the accessions analyzed. Differences among allelic frequencies for the private loci were found to be statistically significant among the studied accessions.

The number of polymorphic alleles per locus (A_p = 2.283), percent of polymorphic loci (P = 89.75%) and expected heterozygosity (H_e = 0.483) showed high mean values compared to the accepted mean value for all plant species (A_p = 1.69, P = 36.8%, and H_e = 0.141, Soltis and Soltis[63]. The accessions studied contain high genetic diversity, but the distribution of this diversity is not homogenous, as indicated by A_p, P, and H_e values (see TABLE 2). Accession number PI 283546 from Egypt, accession number PI 283569 from Libya and accession number PI 422533 from Iran exhibited the highest P values (2.583). Very high H_e (0.557) was shown by accessions number PI 210342 from USSR. That indicates the importance of conservation programmes to retain the existing diversity. In most accessions, observed heterozygosity was less than Hardy-Weinberg expectations except in five accessions (Iran, Italy, Yugoslavia, Hungary, and Pakistan). These results may be due to small sample size in these accessions.

The noticed allelic richness in the accessions of *Lathyrus sativus* was congruent with the work of Gutiérrez- Marcos *et al*.[64]. They did not found any evidence of high identity between accessions, even when originating from the same geographical location. We reached the same conclusion in the present study. They also found great genetic variability at the intra-region level than at the inter-regional level.

The frequent heterozygote deficiency observed in some of the accessions studied e.g. the accession from USSR could be attributed to a number of causes, founder effects, a high and somewhat steady selfing rate.[54, 65-68], assortative mating (homogamy), selection favoring homozygote individuals, and Wahlund effects. In this study the heterozygote deficiency observed in some accessions was attributed to the high and somewhat steady selfing rate. The out crossing rate of *L.*

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sativus ranged from 5 to 36%[69,70] indicating that the plant has a high level autogamy. However the other effetcing factores on heterozygote deficiency require tobe study in this species. However all pod-bearing plants were sampled in each accession. The sampling procedure, combined with the fact that the mean seed germination rate within a year was more than 90% and most of the seeds reach maturity within the same year allowed us to discard the hypothesis concerning Wahlund effects.

The mean expected heterozygosity (H_e = 0.483) estimated for the studied accessions was higher than the value reported for the study of Tadesse and Bekele[71] H_e = 0.2507. The difference in the two data could be attributed to the difference between sampling schemes adopted for these investigation or may be attributed to differences in the enzymatic systems used.

The extent of genetic heterogeneity within accessions as measured by D_{ST} (0.165) was indicative of occurrence of several genetic gene flow. Generally in annual predominantly autogamous species such as *L. sativus*, gene differentiation among accessions expressed by G_{ST} is high (0.276). *L. sativus* is a mixed mating predominantly autogamous[72] that is expected to express high levels of accession genetic divergence and low levels within accession genetic diversity. In inbreeding accessions, the mean genetic heterogenity could be estimated by G_{St} = 0.276. The G_{ST} obtained for the 16 studied *L. sativus* accessions was 0.492. In outbreeeding populations, the mean genetic heterogeneity could be estimated by G_{ST} = 0.11130[73]. Our data indicated that about 50% of the total genetic diversity detected was inter-accessional while 50% was intra accessional. The loci that detected greatest differentiation among accessions (G_{St} = 0.739) were found in (Bangladesh). Generally the lowest genetic divergence (G_{ST}= 0.40) was found in *L. sativus* from Hungary. The estimates of the accession genetic structures indices analyzed in this study were also in accordance with the designated trend, F_{IT} = 0.339, F_{ST}= 0.327, F_{IS} = -0.005. This mating trait coupled with founder effects associated with recruitment events in accession studied[71], could explain the high level of genetic divergence and lower level of genetic diversity in *L. sativus* accessions. The estimate of gene flow based on Wright[74] equation was high: Nm_W = 0.738. The high level of gene flow revealed could be partly due to the high percent of out crossing reported in *L. sativus*, and partly to the density of the flowering plants reported in[73]. The F_{ST} value reflects 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[72].

We found a significant correlation between the size of the investigated accessions and their level of genetic variations. Thus our data were consistent with the idea that genetic variation within accessions related to population size. Such results have been observed previously in other plant species[73,67]. Various explanations have been formulated for the correlation between accessions size and intra-accessional genetic variability indices. In our case the most likely phenomena to explain this correlation is the breeding system[72-75]. Inbreeding reveals itself through a higher number of homozygotes than would be expected under panmictic mating. In smaller population we mainly observed fewer alleles and simultaneously found lower levels of heterozygosity (computed as H_O). The lower level of heterozygosity was mainly due to fixed alleles. A correlation between heterozygosity and effective accessions size is also expected for loci under weak heterozygote advantage in selection when populations are small in size.

In a big population with a mixing mating system as in *L. sativus*, we mainly observed more alleles and simultaneously found high level of heterozygosity (computed as H_O). The higher heterozygosity was mainly due the rarity of mixed alleles.

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