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

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ABSTRACT

Isozymes were used to investigate the genetic variability, population structure, and relationships of *Lactuca* germplasm. The isozyme systems revealed 16 putative loci of a total of 31 alleles. Out of these 16 loci, 11 were polymorphic. The average values of expected heterozygosity (H_e), observed heterozygosity (H_o), mean number of alleles per locus (A) and effective number of alleles per locus (A_e) were 0.2227, 0.266, 1.3005 and 1.369, respectively. The average fixation indices were lower than zero for most of the accessions studied, indicating an excess of heterozygotes. Genetic differentiation among accessions (F_{ST}) exhibited that 51.3% of the isozyme variation was recorded among accessions, and 48.7% of the genetic variation resided within accessions. The average values of total heterozygosity (H_T) and intra-accessional genetic diversity (H_S) were 0.352 and 0.171, respectively. Moreover, the inter-accessional genetic diversity (D_{ST}) ranged from 0 to 0.424 with an average of 0.18. Cluster analysis revealed that *L. sativa* cultivars were distributed throughout different *Lactuca* species. Thereby, isozymes results confirms the hypothesis of the polyphyletic origin of *L. sativa*. This high level of genetic variation proved that isozymes are efficient for polymorphism analysis of *Lactuca* germplasm.

1. Introduction

Lactuca L. genus is a member of the tribe Lactuceae of subfamily Cichorioideae, family Asteraceae. It involves more than 97 species of a highly economic importance (Lebeda et al., 2001; Doležalová et al., 2002). Those species include *Lactuca serriola* L., *L. virosa* L., *L. saligna* L., *L. indica* L., *L. undulata* Ledeb., *L. viminea* L., *L. dregeana* DC., *L. perennis* L., *L. orientalis* Boiss., *L. altaica* Fisch. & C.A. Mey. and cultivated lettuce (*L. sativa* L.) (Doležalová et al., 2002). Cultivated species including *L. sativa* and *L. serriola* are widely grown as food crops. Additionally, *L. serriola* seeds are rich in edible oils used for human consumption. Currently, *Lactuca* productivity is limited due to the significant effects of biotic and abiotic factors (Doležalová et al., 2002). To overcome this issue, *Lactuca* genetic resources should be well-characterized, managed and conserved for future use in agriculture and human food security. Furthermore, evaluation of genetic variability and population structure of *Lactuca* germplasm would enhance breeding and management practices as well as developing improved and more effective varieties.

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Various genetic tools have been successfully used to study the biology and genetic variability of different plant species (El-Esawi et al., 2015, 2016a,b). Isozymes are among these tools. Isozymes are biochemical protein markers encoding gene products, and arise as a result of changes in amino acids which cause changes in the structure, charge or electrophoresis mobility of enzyme molecules (Doležalová et al., 2002; El-Esawi, 2016a). Isozymes exhibit significant advantages over cytological and morphological traits that are affected by both genetic and environmental factors (Basu et al., 2004; El-Esawi and Sammour, 2014). The significant advantages of isozymes also include their codominance nature and ability to reveal considerable level of genetic variability between and within populations. Codominant isozyme data could also be used to estimate the genetic variability of plant populations in terms of effective number of alleles per locus and average levels of heterozygosity and polymorphism (Hedrick, 1984).

Isozyme markers have been used to evaluate the genetic variability and relationships, study population genetic structure, and to enhance breeding practices of *Lactuca* germplasm (Roux et al., 1985; Vries, 1996; Lebeda et al., 2001). The results of these studies revealed a lower level of intra-specific variability, and suggested a polyphyletic origin of *Lactuca sativa* (Vries, 1996). *L. aculeata* Boiss. & Kotschy has been a part of *L. serriola* complex, indicating their relatedness with *L. sativa* (Roux et al., 1985). However, most of these studies focused on the genetic characterization of *Lactuca* germplasm collected from few countries, and little information is therefore available on the intra- and inter-specific genetic diversity of *Lactuca* genetic resources. The main objective of the current study was to assess the relationships, genetic structure and genetic variability of a wide range of *Lactuca* species collected from different geographical origins worldwide based on isozyme markers.

2. Material and methods

2.1. Plant material

Twenty-six accessions of nine different *Lactuca* species and a hybrid between *Lactuca serriola* x *Lactuca sativa* obtained from the Centre for Genetic Resources (CGN) in the Netherlands, were examined in this study (Table 1). The accessions were selected to represent and cover diverse geographic locations and origins worldwide.

2.2. Seed growth and isozyme extraction

Seeds were sown in sterilized Petri dishes containing moistened cotton mats. For isozymes extraction, young leaves were collected from seven-day-old seedlings. Crude extracts were then prepared, according to Manganaris and Alston (1987), by macerating leaves in 1 ml of extraction buffer kept on ice and comprised 0.05 M Na phosphate pH 7.2, 20% v/v glycerol, 0.05% v/v triton X-100 and 14 mM mercaptoethanol. The extracts were centrifuged at 15000 rpm for 10 min, and the supernatant was then used for isozyme analysis. At least 3–5 plants per accession were analyzed for isozyme patterns.

Table 1
Number and origin of *Lactuca* accessions studied.

No.	Species	Accession number	Origin
1	*Lactuca sativa* group butterhead lettuce	CGN04706	Netherlands
2	*Lactuca sativa* group butterhead lettuce	CGN04888	USA
3	*Lactuca sativa* group crisp lettuce	CGN05048	China
4	*Lactuca sativa* group latin lettuce	CGN04566	France
5	*Lactuca sativa* group latin lettuce	CGN05835	Spain
6	*Lactuca sativa* group latin lettuce	CGN04557	Argentina
7	*Lactuca sativa* group cos lettuce	CGN04744	Turkey
8	*Lactuca sativa* group cutting lettuce	CGN10956	Italy
9	*Lactuca sativa* group stalk lettuce	CGN11387	China
10	*Lactuca sativa* group Stalk lettuce	CGN04546	USA
11	*Lactuca serriola* group oilseed lettuce	CGN04770	Egypt
12	*Lactuca serriola*	CGN16210	Germany
13	*L. sativa* x *L. serriola* group oilseed	CGN05115	Egypt
14	*Lactuca saligna*	CGN13330	Turkey
15	*Lactuca saligna*	CGN13327	Greece
16	*Lactuca saligna*	CGN10883	Portugal
17	*Lactuca virosa*	CGN05332	Italy
18	*Lactuca virosa*	CGN05145	France
19	*Lactuca indica*	CGN14312	Indonesia
20	*Lactuca indica*	CGN13392	China
21	*Lactuca viminea*	CGN16202	Union of Soviet
22	*Lactuca viminea*	CGN14301	France
23	*Lactuca dregeana*	CGN04790	Italy
24	*Lactuca dregeana*	CGN05805	France
25	*Lactuca perennis*	CGN09321	Switzerland
26	*Lactuca perennis*	CGN13299	France

2.3. Isozyme analysis

Seven isozyme systems were selected from literature and screened for high resolution and polymorphisms. Following the screening, 4 isozymes exhibited high resolution and polymorphism and were used to examine all the 26 accessions. These isozymes comprised acid phosphatase (ACP), catalase (CAT) and two esterases (EST; α- and β-naphthyl acetate). Aliquots (20 μl) of the extracts were loaded onto 10% polyacrylamide gels, and bromophenol blue was added as an indicating dye. Electrodes were filled with 0.025 M tris/glycine buffer pH 8.8. Electrophoresis was performed at 25 mA/gel for 3 h. Gels were then stained for the four isozymes selected (Manchenko, 1994). For acid phosphatase, a solution of 0.05% phenolphthalein monophosphate dissolved in 0.1 M citrate buffer of pH 5.5 was poured on filter papers, and the gels were overlayed and incubated at 37 °C for 2 h. Gels were then immersed in NH_4OH solution. Red gel bands showing ACP activity zones appeared and zymograms were photographed.

For catalase, gels were incubated in 3% H_2O_2 for 15 min. Gels were then washed in water and immersed in 1:1 mixture of 2% ferric chloride and 2% potassium ferricyanide with a gentle agitation for a few minutes. Yellow gel bands revealing CAT activity appeared. Gels were then washed in water and zymograms were photographed. For the two esterase isozymes, gels were stained in 100 ml 0.05 M phosphate buffer pH 7.2 containing 50 mg Fast Red TR and 10 mg α- or β-naphthyl acetate dissolved in 1 ml of acetone, in the dark at 37 °C till bands appeared. Gels were then washed in water and fixed in 3% acetic acid, and zymograms were photographed.

2.4. Data analysis

Isozymes data were analyzed using POPGENE version 1.31 software package in order to assess the genetic variability within and among *Lactuca* accessions through analysis of the allele frequencies, percentage of polymorphic loci (%P), observed and expected heterozygosity (H_o and H_e), mean number of alleles per locus (A), effective number of alleles per locus (A_e), fixation index (F), F-statistic values, χ^2 test, and gene flow estimate according to Wright's (1951) equation. Dendrogram was constructed based on Nei's genetic distance using UPGMA (Nei, 1978).

3. Results

3.1. The genetic interpretation of the studied isozymes

Both of acid phosphatase and catalase isozymes had the same genetic interpretation. Each of these 2 isozyme systems revealed 4 loci. Two of these loci were monomorphic with one allele each, while the other 2 loci were polymorphic. One of the 2 polymorphic loci was triallelic and had 6 isozyme phenotypes (3 homozygous and 3 heterozygous phenotypes), while the second one was diallelic and had 3 isozyme phenotypes (one heterozygous and 2 homozygous phenotypes).

α-Esterase isozyme exhibited 4 loci. One of them was monomorphic. The other 3 loci were polymorphic with different numbers of alleles. Two of these polymorphic loci were diallelic with 3 isozyme phenotypes each (2 homozygous phenotypes and one heterozygous phenotype). The third polymorphic locus was triallelic and had 6 isozyme phenotypes (3 homozygous and 3 heterozygous phenotypes).

β-Esterase isozyme revealed 4 polymorphic loci with different numbers of alleles. Three of these polymorphic loci were diallelic with 3 isozyme phenotypes each (2 homozygous phenotypes and one heterozygous phenotype). The fourth polymorphic locus was triallelic and had 6 isozyme phenotypes (3 homozygous and 3 heterozygous phenotypes). One of the 4 polymorphic loci of β-Esterase was characteristic for all wild *Lactuca* species studied except *L. serriola* and *L. dregeana*.

3.2. Isozyme loci and alleles displayed

The 4 isozyme systems studied revealed 16 putative loci. Of these 16 loci, 5 (*ACP-1*, *ACP-4*, *CAT-1*, *CAT-3* and *αEST-4*) were monomorphic for all accessions, while the other 11 loci were polymorphic (Table 3). A total of 31 alleles were detected across all loci. Only allelic frequencies of 7 loci (*ACP-2*, *ACP-3*, *CAT-2*, *αEST-3*, *βEST-1*, *βEST-2* and *βEST-4*) were able to distinguish among the 26 accessions analyzed ($p < 0.05$) (Table 3), while the allelic frequencies of the other loci exhibited inter- and inter-accessional genetic variability, but were not statistically significant.

3.3. Genetic variation and heterozygosity

The total number of alleles in each accession for the 16 loci varied from 17 to 27 with an average of 20.8. Table 2 shows the estimates of genetic diversity of *Lactuca* accessions analyzed. The observed heterozygosity (H_o) varied from 0.0667 in *L. virosa* CGN05332 from Italy to 0.5476 in *L. serriola* CGN16210 from Germany with an average of 0.266. The expected heterozygosity (H_e) ranged from 0.0711 in *L. virosa* CGN05145 from France to 0.3905 in *L. serriola* CGN04770 from Egypt with an average of 0.2227. The proportion of polymorphic loci (P) varied from 12.5% in *L. virosa* CGN05145 from France to 56.3% in the accessions of *L. sativa* group Crisp CGN05048 from China, *L. serriola* CGN04770 from Egypt, *L. serriola* CGN16210 from Germany and *L. sativa* x *L. serriola* CGN05115 from Egypt with a mean of 36.3%. The mean number of alleles per locus (A) ranged from 1.0625 in *L. virosa* CGN05145 from France to 1.6875 in *L. sativa* x *L. serriola* CGN05115 from Egypt with an average of 1.3005. The effective

Table 2
Sample size, genetic diversity and average fixation index in the 26 accessions of *Lactuca* species analyzed.

Sample	Sample size	Polymorphic loci (%P)	H_o	H_e	A	A_e	F
L. sat. 04706	8	37.5	0.2857	0.2194	1.2500	1.3546	−0.302
L. sat. 04888	6	50.0	0.3571	0.3476	1.5000	1.6102	−0.027
L. sat. 05048	6	56.3	0.3333	0.3810	1.5625	1.6531	0.125
L. sat. 04566	9	43.8	0.2857	0.2476	1.3125	1.4189	−0.154
L. sat. 05835	8	37.5	0.2976	0.2269	1.2500	1.3689	−0.312
L. sat. 04557	6	37.5	0.2857	0.2476	1.3125	1.4122	−0.154
L. sat. 04744	6	43.8	0.2857	0.2857	1.3125	1.4571	0.000
L. sat. 10956	6	31.3	0.2857	0.2143	1.2500	1.3694	−0.333
L. sat. 11387	6	37.5	0.3810	0.2476	1.2500	1.4000	−0.539
L. sat. 04546	6	43.8	0.2619	0.2714	1.3125	1.4143	0.035
L. ser. 04770	6	56.3	0.5238	0.3905	1.6250	1.6939	−0.341
L. ser. 16210	6	56.3	0.5476	0.3667	1.4375	1.5857	−0.493
L. sat. x *ser.* 05115	9	56.3	0.5133	0.3515	1.6875	1.7089	−0.460
L. sal. 13330	9	43.3	0.3200	0.2370	1.3750	1.4027	−0.350
L. sal. 13327	7	37.5	0.3333	0.2190	1.3125	1.3686	−0.522
L. sal. 10883	5	37.5	0.3778	0.2356	1.3125	1.3867	−0.604
L. vir. 05332	6	18.8	0.0667	0.0933	1.1250	1.1323	0.285
L. vir. 05145	6	12.5	0.0889	0.0711	1.0625	1.1067	−0.250
L. ind. 14312	5	18.8	0.0889	0.1067	1.1250	1.1600	0.167
L. ind. 13392	5	18.8	0.0889	0.1067	1.1250	1.1600	0.167
L. vim. 16202	6	18.8	0.1333	0.1156	1.1875	1.1867	−0.153
L. vim. 14301	6	25.0	0.1111	0.1467	1.4375	1.2267	0.243
L. dre. 04790	6	25.0	0.2143	0.1667	1.1250	1.2714	−0.286
L. dre. 05805	6	25.0	0.1667	0.1571	1.1250	1.2429	−0.061
L. per. 09321	6	31.3	0.1458	0.1458	1.1250	1.2106	0.000
L. per. 13299	11	43.8	0.1354	0.1898	1.3125	1.3049	0.287
Mean	6.6	36.3	0.2660	0.2227	1.3005	1.3695	−0.155

H_o: observed heterozygosity; **H_e**: expected heterozygosity; **A:** mean number of alleles per locus; **A_e**: effective number of alleles per locus; **F:** fixation index.

Table 3
F-statistics, Nei's (1973) genetic diversity indices, and gene flow estimate among *Lactuca* accessions.

Locus	*F*-statistics				Nei's genetic diversity				X^2	*p*-value
	F_{IS}	F_{IT}	F_{ST}	Nm_w	H_T	H_S	D_{ST}	G_{ST}		
ACP-1	–	–	0.0000	–	0.000	0.000	0.000	–	–	–
ACP-2	−0.453	0.0965	0.3780	0.4113	0.550	0.342	0.208	0.378	8.50	0.0367*
ACP-3	0.6432	0.8508	0.5819	0.1796	0.499	0.209	0.290	0.581	63.08	0.0000*
ACP-4	–	–	0.0000	–	0.000	0.000	0.000	–	–	–
CAT-1	–	1.0000	1.0000	0.0000	0.000	0.000	0.000	–	–	–
CAT-2	0.3971	0.8075	0.6807	0.1173	0.498	0.159	0.339	0.681	53.47	0.0000*
CAT-3	–	–	0.0000	–	0.000	0.000	0.000	–	–	–
CAT-4	−0.639	0.0635	0.4284	0.3336	0.583	0.333	0.250	0.429	3.37	0.3388
αEST-1	−0.702	0.0984	0.4702	0.2817	0.447	0.237	0.210	0.470	0.90	0.3421
αEST-2	−0.954	−0.126	0.4238	0.3399	0.648	0.373	0.275	0.424	4.00	0.2611
αEST-3	−0.543	0.7683	0.8498	0.0442	0.499	0.075	0.424	0.850	51.18	0.0000*
αEST-4	–	–	0.0000	–	0.000	0.000	0.000	–	–	–
βEST-1	−0.527	0.9466	0.9650	0.0091	0.289	0.010	0.279	0.965	10.12	0.0015*
βEST-2	−0.536	−0.389	0.0958	2.3607	0.499	0.451	0.048	0.096	11.32	0.0008*
βEST-3	−0.507	0.0731	0.3849	0.3995	0.612	0.376	0.236	0.386	4.33	0.2276
βEST-4	0.3770	0.7842	0.6535	0.1325	0.499	0.173	0.326	0.653	51.41	0.0000*
Mean	−0.404	0.3163	0.5129	0.2374	0.352	0.171	0.180	0.538	23.79	0.1100

F_{IS}: the fixation index related to non-random mating within accessions; **F_{IT}**: the mean inbreeding coefficient of a set of accessions; **F_{ST}**: the genetic differentiation among accessions due to genetic drift; **Nm_w**: the gene flow estimate according to Wright's (1951) equation; **H_T**: the total genetic diversity; **H_S**: the genetic diversity within accessions; **D_{ST}**: the genetic diversity among accessions; **PIC:** the polymorphic information content of a marker; **X^2**: Chi^2 to test F_{ST} for significant difference from zero; **P:** the probability value (*significant F_{ST} at $P < 0.05$). - Monomorphic loci with no variation.

number of alleles per locus (A_e) varied from 1.1067 in *L. virosa* CGN05145 from France to 1.7089 in *L. sativa* x *L. serriola* CGN05115 from Egypt with a mean of 1.3695.

The average fixation indices values (F) were lower than zero for all the accessions analyzed, except for *L. sativa* group Crisp CGN05048 from China, *L. sativa* group Cos CGN04744 from Turkey, *L. sativa* group Stalk CGN04546 from USA, *L. virosa* CGN05332 from Italy, *L. indica* CGN14312 from Indonesia, *L. indica* CGN13392 from China, *L. viminea* CGN14301 from France, *L. perennis* CGN09321 from Switzerland and *L. perennis* CGN13299 from France (Table 2). The negative value indicated an excess of heterozygotes.

3.4. Genetic structure and gene flow

F-statistics displayed varying fixation indices among loci (Table 3). The estimates of F_{IS} showed 8 loci (*ACP-2*, *CAT-4*, *αEST-1*, *αEST-2*, *αEST-3*, *βEST-1*, *βEST-2*, *βEST-3*) with excess of heterozygotes, as indicated by the negative mean fixation indices values. The remaining 8 loci revealed heterozygote deficiencies. The F_{IS} values varied from −0.954 at αEST-2 to 0.6432 at ACP-3 with an average of −0.404. F_{IT} values varied from −0.389 at βEST-2 to 1 at CAT-1 with a mean of 0.3163. The inter-accession genetic differentiation (F_{ST}) revealed that 51.3% of the total isozyme variation was among accessions, and 48.71% of the genetic variation resided within accessions. The F_{ST} values varied from 0 to 1 with an average of 0.5129.

Nei (1973) genetic diversity indices are shown in Table 3. The averages of total heterozygosity (H_T) and intra-accessional genetic diversity (H_S) were 0.352 and 0.171, respectively. Moreover, the inter-accessional genetic diversity (D_{ST}) ranged from 0 to 0.424 with an average of 0.18, and the coefficient of genetic differentiation among accessions (G_{ST}) ranged from 0.096 (locus βEST-2) to 0.965 (locus βEST-1) with a mean of 0.538. The high genetic differentiation between accessions ($G_{ST} = 0.538$, $p > 0.05$) and the inter-accession genetic diversity ($D_{ST} = 0.18$) may indicate a low gene flow which was confirmed by the estimate of the number of migrates per generation based on Wright's equation ($Nm_w = 0.2374$) (Table 3).

3.5. Genetic distance and dendrogram

Dendrogram constructed based on isozyme data revealed 2 major groups (Fig. 1). The first group splited into 2 subgroups. The first subgroup splited into 2 clusters; one cluster contained *L. sativa* group Butterhead CGN04706, *L. sativa* group Cos CGN04744, *L. sativa* group Latin CGN05835 and *L. sativa* group Cutting CGN10956, and the second one included *L. virosa* CGN05332, *L. virosa* CGN05145, *L. dregeana* CGN04790 and *L. dregeana* CGN05805. The second subgroup involved *L. saligna* (CGN13327 and CGN10883) and *L. viminea* (CGN16202 and CGN14301). The second group splited into 3 subgroups; the first one included *L. indica* (CGN14312 and CGN 13392) and *L. perennis* CGN09321, the second one involved the accession of *L. sativa x L. serriola* CGN05115, while the third one comprised all the other accessions studied.

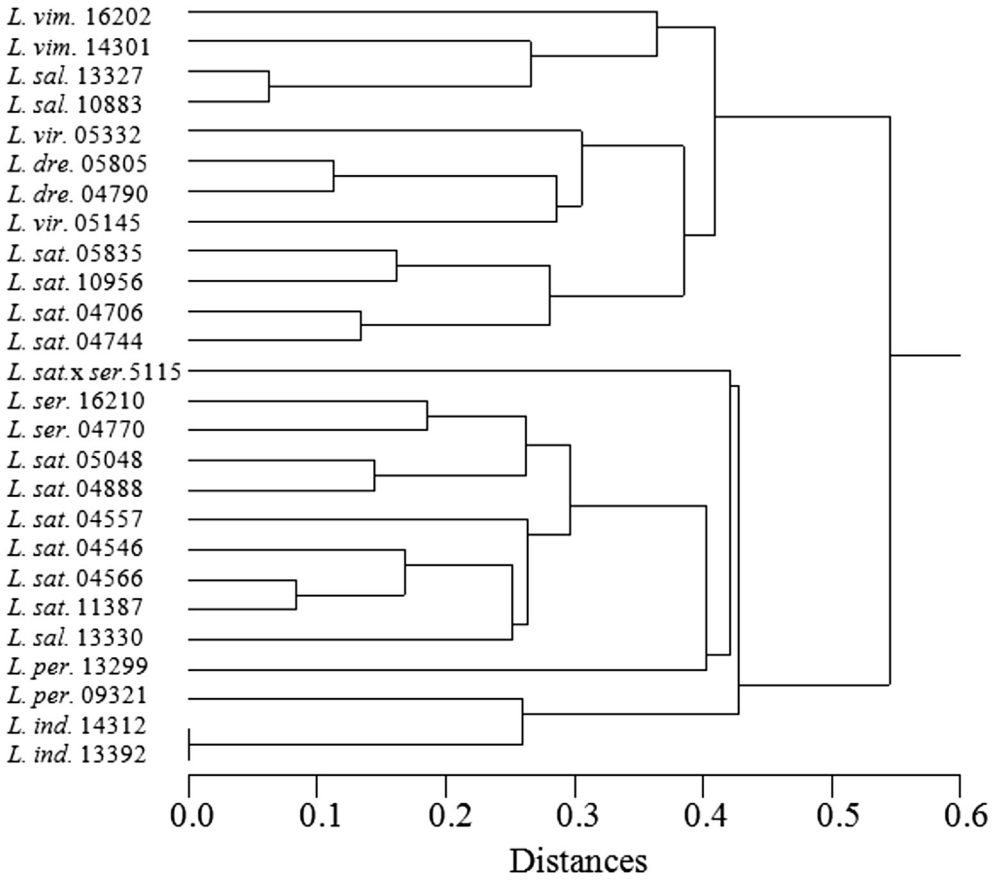

Fig. 1. UPGMA dendrogram showing the relationships among the 26 accessions of *Lactuca* species based on isozyme data.

4. Discussion

The genetic variability, population structure, and relationships of *Lactuca* germplasm were assessed based on isozyme markers. The allelic frequencies of 7 loci (*ACP-2*, *ACP-3*, *CAT-2*, *αEST-3*, *βEST-1*, *βEST-2* and *βEST-4*) were able to distinguish among the 26 accessions analyzed ($p < 0.05$) (Table 3), while the allelic frequencies of the other loci exhibited inter- and inter-accessional genetic variability, but were not statistically significant. The studied accessions have a high genetic diversity, but the distribution of this diversity is not homogenous. The results showed that the observed heterozygosity was higher than the expected one for the accessions analyzed, except for *L. sativa* group Crisp CGN05048 from China, *L. sativa* group Stalk CGN04546 from USA, *L. virosa* CGN05332 from Italy, *L. indica* CGN14312 from Indonesia, *L. indica* CGN13392 from China, *L. viminea* CGN14301 from France and *L. perennis* CGN13299 from France. The high values of the observed heterozygosity might be due to small sample size in these accessions.

The mean value of the expected heterozygosity ($H_e = 0.2227$) exhibited a high mean value compared to the accepted values for all plant species ($H_e = 0.141$) (Soltis and Soltis, 1989). However, this value ($H_e = 0.2227$) was lower than that reported by Yu et al. (2001) on short-lived herbaceous forage plants. On the other hand, the mean number of polymorphic alleles per locus ($A = 1.3005$) and percentage of polymorphic loci ($P = 36.3\%$) showed relatively low mean values compared to the accepted values for all plant species ($A = 1.64$ and $P = 36.8\%$) (Soltis and Soltis, 1989) and that reported by Yu et al. (2001) on short-lived herbaceous forage plants ($A = 1.93$ and $P = 53.3\%$). The average fixation indices values (F) were lower than zero for the studied accessions, except for *L. sativa* group Crisp CGN05048 from China, *L. sativa* group Cos CGN04744 from Turkey, *L. sativa* group Stalk CGN04546 from USA, *L. virosa* CGN05332 from Italy, *L. indica* CGN14312 from Indonesia, *L. indica* CGN13392 from China, *L. viminea* CGN14301 from France, *L. perennis* CGN09321 from Switzerland and *L. perennis* CGN13299 from France. The negative value indicated an excess of heterozygotes which might be attributed to the result of selection pressure. However, the heterozygote deficiency detected in some accessions may be attributed to founder effects and the high selfing rate in *Lactuca* species (Zoro Bi et al., 2003). This lack of heterozygosity may also be attributed to the gene flow restriction within populations and of increase of relatedness among individuals (Gregorius and Namkoong, 1983).

The current study revealed that 51.3% of the isozyme variation was among accessions, and 48.7% of the genetic variation resided within accessions. These data may be attributed to short-lived herbaceous plants that gained relatively high genetic variability but most of this genetic variability resided among accessions. The distribution of genetic variability among accessions is a product of interactions among various evolutionary factors, including effective population size, selection and the capability of plants to disperse seeds and pollen. The averages of total heterozygosity and intra-accessional genetic diversity were 0.352 and 0.171, respectively. Furthermore, the inter-accessional genetic diversity ranged from 0 to 0.424, with an average of 0.18. These values were higher than those reported by Kesselli and Michelmore (1986) on isozyme analysis of *Lactuca* species. This difference in data may be attributed to the differences in accessions or the enzymatic systems analyzed. The high genetic differentiation between accessions and the inter-accession genetic diversity were indicative of a low gene flow that was confirmed by the estimates of the number of migrates per generation. These results confirmed the high degree of self-pollination in *Lactuca* species.

Cluster analysis based on our isozyme data confirmed that *L. dregeana* is closely related to *L. virosa*. This result was consistent with that reported by De Candolle (1838) on morphological characters, but conflicted with our previous morphological data (unpublished). The difference in this result may be attributed to the limited number of morphological traits we used in the previous *Lactuca* study. Furthermore, it might be due to differences in the methodologies or accessions analyzed. Our isozyme results indicate the polyphyletic origin of *L. sativa*. This is because the cultivars of *L. sativa* were distributed throughout the accessions of *Lactuca* species. These results were consistent with that reported by Kesselli and Michelmore (1986) and Vries (1996). This may explains the repeating of the domestication process from wild progenitors or the utilization of inter-specific crosses in breeding practices to introduce traits of interest into *Lactuca sativa*. To distinguish between these 2 possibilities, further analysis is still required. Moreover, the cluster analysis showed that the most distantly related genotypes comprised *L. sativa* and *L. perennis*, suggesting their inclusion in future breeding program to broaden the genetic base of *Lactuca* germplasm as well as developing varieties with highly agronomic traits. In conclusion, the considerable level of genetic variation revealed by the used isozymes in this study supports our recommendation to use acid phosphatase, catalase and α- and β-esterase isozymes for the analysis of the genetic diversity, structure and polymorphism of *Lactuca* germplasm. Hence, like other biotechnological tools (El-Esawi, 2016b,c,d) and cell biology technologies (Jourdan et al., 2015; Consentino et al., 2015), isozymes could be efficiently used for improving crops of different genera.

Authors' contribution

M.E. designed the research, performed the experiments, analyzed the data and wrote the manuscript. R.S. designed the research and revised data analysis and manuscript. A.M. and S.B. participated in designing the research and revising the work.

Disclosure of interest

The authors declare that they have no conflict of interest.

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