Molecular Diversity in Lactuca Species Using Isozymes and RAPD Markers

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Research Article

Keywords: Lactuca species, isozymes, RAPD markers, corresponding analysis, Cluster analysis

Posted Date: February 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-221342/v1

Abstract

Background: understanding molecular diversity in *Lactuca* species is substantial for the management, improvement and efficient uses of their accessions. Therefore, this work aimed to evaluate molecular diversity among and within *Lactuca* species.

Methods and Results: the molecular diversity was assessed using isozymes and RAPD analyses that gave 87.09% and 100% polymorphic percentages respectively, indicating a high genetic variation within and among *Lactuca* species. The number of alleles were higher in the wild species compared to the cultivated species, reflecting a reduction in the richness of alleles in the cultivated species due to domestication that caused a reduction in genetic diversity to meet the demand for high crop productivity. Isozymes and RAPD clustering dendrogrames: (1) separated, *L. sativa* accessions in more than one cluster confirming their polyphyletic origin; (2) collected the accessions of *L. viminea* in one cluster revealed its homogeneity; and (3) divided the accessions of *L. saligna* in two clusters varied in the number of alleles, particularly “A” form. The corresponding analysis associated the accessions of the wild species based on “B” form of the alleles of the tested isozymes, and the cultivated species on the forms “A” and “C”, suggesting that: (1) allele “B” might be the primitive form of these loci that can tolerate the environmental stresses which prevails in the habitats of the wild species, and (2) forms “A” and “C” could be the derived forms.

Conclusions: These results are of great interest for the management of *Lactuca* germplasm and for future breeding programs of lettuce.

Introduction

Genus *Lactuca* L. is a member of subtribe Lactucinae, tribe Lactuceae, family Lactuceae (Asteraceae) (Güzel et al. 2018). It includes annual, biennial or perennial herbs, rarely shrubs with abundant latex. The species of *Lactuca* distributed mainly in warm and temperate geographical regions of the world (van Herwijnen and Manning 2017). However, the continents Asia and Africa are the center of the diversity of the genus. (Doležalová et al., 2002).

The primary gene pool of *L. sativa* L. is represented by a worldwide spread *L. serriola*, further *L. aculeata*, *L. scarioloides*, *L. altaica, L. azerbaijancia*, *L. georgica* originating in Asia and *L. dregeana* native to South Africa (Zohary 1991; van Herwijnen and Manning 2017). The secondary gene pool includes *L. saligna*, whereas the tertiary gene pool includes the species that can be crossed with difficulties with *L. sativa*, e.g. *L. virosa* (Doležalová et al. 2002; Jemelková et al. 2018).

Genetic diversity is the raw material permitting species to adapt to environmental changes. The genetic structure of accessions varies from niche to niche along the distribution range of a species (Mondini et al. 2009). So, estimating of genetic diversity is crucial for providing information for domestication, propagation, breeding programs, conservation and use of the germplasm of the plant species (Yu et al. 2001). Biochemical and Molecular markers have been proved as valuable tools to assess and evaluate genetic diversity between and within species, populations and accessions (Yang et al. 2013; Khan et al. 2019). Each marker reveals a specific class of variation, which is dependent on: (1) the fraction of the genome surveyed by that marker, (2) marker distribution throughout the genome and (3) the extent of the DNA target which is analyzed by that marker (Govindaraj et al. 2015; Bhandari et al. 2017).

Isozymes have been used as a reproducible marker in assessing the amount and distribution of genetic variability and systematic relationships within and between *Lactuca* spp. (Kesseli and Michelmore 1986; Dziechciarková et al. 2004). They exhibited that the genetic variability was lower in intra-species compared with inter-species and suggested that the origin of *L. sativa* was polyphyletic. The systematic relationship in *Lactuca* spp. was investigated using isozymes: (1) confirming the genetic closeness between *L. aculeata* and *L. sativa* as they are members of *L. serriola* complex, (2) revealing a wide genetic distance between *L. saligna* and *L. virosa*, and (3) identifying *L. serriola*, *L. saligna*, *L. virosa*, and landrace *L. sativa* as distinct entities (Kesseli and Michelmore 1986).

The use of molecular markers is also important in assessing the level of genetic diversity and in defining the genetic relationship between and within species, populations and accessions. Randomly Amplified Polymorphic DNA marker (RAPD) is one of the class of DNA markers that has received maximum attention in investigating the genetic variability because it is less expensive, less technical, fast and involves no radioactivity and hybridization. It was used to investigate the genetic variability and interspecific relationship among accessions, cultivars and genotypes of *L. sativa* indicating wide genetic base (Waycott and Fort 1994; Yoo and Jang 2003; Sharma et al. 2017).

Genetic resources collections of *Lactuca* species are poorly characterized because they are made up of large groups of numbered accessions of *Lactuca* that lack descriptive and (or) pedigree (Lebeda et al. 2019). So, little information is available on the genetic variation among different accessions of *Lactuca* representing different regions in the world. Therefore, the aim of this research was to use isozymes and DNA markers to evaluate the genetic variability and genetic relationships within and between *Lactuca* species.

Material And Methods

Plant materials

The experimental material was obtained from the CGN (Centre for Genetic Resources, Wageningen, The Netherlands). The material was: (A) *Lactuca sativa* group Butterhead lettuce (CGN04706) from Netherlands, (B) *Lactuca sativa* group Butterhead lettuce (CGN04888) from USA, (C) *Lactuca sativa* group Crisp lettuce (CGN05048) from China, (D) *Lactuca sativa* group Latin lettuce (CG N04566) from France, (E) *Lactuca sativa* group Latin lettuce (CGN05835) from Spain, (F) *Lactuca sativa* group Latin lettuce (CGN04557) from Argentina, (G) *Lactuca sativa* group Cos lettuce (CGN04744) from Turkey, (H) *Lactuca sativa* group Cutting lettuce (CGN10956) from Italy, (I) *Lactuca sativa* group Stalk lettuce (CGN11387) from China, (J) *Lactuca sativa* group Stalk lettuce(CGN04546) from USA, (K) *Lactuca serriola* group Oilseed lettuce (CGN04770) from Egypt, (L) *Lactuca serriola* (CGN16210) from Germany, (M) *Lactuca*

*sativa*_x_*Lactuca serriola* group Oilseed (CGN05115) from Egypt, (N) *Lactuca saligna* (CGN13330) from Turkey, (O) *Lactuca saligna* (CGN13327) from Greece, (P) Lactuca saligna (CGN10883) from Portugal, (Q) *Lactuca virosa* (CGN05332) from Italy, (R) *Lactuca virosa* (CGN05145) from France, (S) *Lactuca indica* (CGN14312) from Indonesia, (T) *Lactuca indica* (CGN13392) from China, (U) *Lactuca viminea* (CGN16202) from USSR, (V) *Lactuca viminea* (CGN14301) from France, (W) *Lactuca dregeana* (CGN04790) from Italy, (X) *Lactuca dregeana* (CGN05805) from France, (Y) *Lactuca perennis* (CGN09321) from Switzerland, (Z) *Lactuca perennis* (CGN13299) from France.

Protein Extraction, Electrophoresis And Activity Staining

Protein extraction, electrophoresis and activity staining

Isozymes crude extracts were prepared by macerating 20 mg young leaves of fifteen days old seedlings with 1 mL of extraction buffer consisted of 0.05M sodium phosphate buffer (pH 7.2), 20% v/v glycerol, 14 mM 2-mercaptoethanol and 0.05% v/v triton X-100 (Manchenko 1994). The clear supernatant was applied directly on 7% PAGE at 4°C in a Mini Protean III unit (BioRad, California, USA), under a constant current of 100 mA for 5 to 6hr, until the tracking dye had moved 5 to 7cm from the cathodal end. The gels were subjected to staining for Phosphorylase, Catalase, α-Esterase and β-Esterase isozymes following the protocols of Pasteur et al. (1988). Phosphorylase gels were stained in solution formed of 10 mM I_2 mixed with 14 mM KI after incubation in 100 ml solution of 0.1M sodium phosphate buffer (pH 5.1) at 37 ^{0}C for 3 to 5 h, developing white bands on a dark blue background. The chromatic or light brown bands appeared at the bottom of the gels were amylase bands. The gels of catalase were stained by immersing in 1:1 mixture of solutions 2% potassium ferricyanide and 2% ferric chloride after incubation in a solution of 3% H_2O_2 for about 15 min. The gels were then washed and gently agitated for a few minutes in water. Yellow bands of Catalase activity appeared on a blue-green background. The gels of α and β-esterases were incubated at 37°C for 15 min 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 until brown colored bands appeared. The stained gels were photographed as quickly as possible and stored in 3% acetic acid. At least 5 and generally 10 plants per accession were examined for isozyme patterns.

Dna Extraction For Rapd Analysis

0.5 g young leaves of fifteen days old seedlings were ground with a pestle in liquid nitrogen. The ground sample was suspended in 1 ml preheated CTAB buffer (1.4 M NaCl, 0.2 % 2-mercaptoethanol, 100 mM Tris-Cl and 20 mM EDTA) at 65ºC for one hour. The suspended solution was centrifuge at 1000 rpm and the supernatant was mixed with 0.5 ml of 24:1 chloroform: isomyl, then centrifuge at 14000 rpm (Doyle and Doyle 1990). The aqueous layer was mixed with ice cold isopropanol, then incubated overnight at -20 ºC and centrifuged at 14000 rpm. The supernatant was discarded and the pellet which contained the nucleic acid was carefully washed twice with cold 70% ethanol, dried at room temperature and re-suspended in 100 µl of sterile de-ionized distilled water.

Rapd Amplification

Genomic DNAs of the studied accessions were amplified in 25µl reaction mixture, containing 20 ng DNA, 0.5 unit Taq polymerase (Sigma-Aldrich, St. Louis, MO, USA), 0.2 mM PCR Nucleotide Mix (Boehringer Mannheim, Tubingen, Germany), 0.5 µM RAPD primers, 5µl amplification buffer, 1.5µl of $MgCl_2$ and 9.75µl of distilled H_2O (Williams et al. 1990). Amplification was performed for 45 cycles using a Biometera Uno thermal cycler (SPW Industrial, Laguna Hills, CA, USA): One cycle at 95 ^{0}C for 3 minutes, then 44 cycles at 92 ^{0}C for 2 minutes, 37 ^{0}C for 1 minute and 72^{0}C for 2 minutes. The reactions were finally run at 72 ^{0}C for 10 min and further incubated on ice, at 4 ^{0}C. The primers were chosen based on their ability to produce reproducible amplification patterns. The primers used were: OPA08 (5-$GTGACGTAGG$-3), OPA09 (5-$GGGT\forall CG$C-3), OPA14 (5-$TCTGTGCTGG$-3), OPA20 (5-$G\top GCGAT$C-3) and OPZ12 (5-$TC\forall CGGGAC$-3). The amplification DNAs were separated by electrophoresis on 2% agarose in 50X Tris-Acetate EDTA buffer consisted of 242g Tris-base, 57.1 ml Glacial acetic acid and 100 ml EDTA (0.5 M pH 8.0). The electrophoresed gels were stained in 0.2 µg/ml ethidium bromide, then photographed under UV light. DNA ladder (Axygen, Union City, CA, USA) was run side by side with the samples, consisted of the following DNA fragments: 1031, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp

Data analysis

The isozymes and RAPD bands were scored as “0” for presence and “1’ for absence for a band at a particular locus in each accessions, which were transformed into a binary character matrix. The binary character matrix of each marker was subjected to correspondence and cluster analyses using the software package “PAST”, Version 4.02, Natural History Museum, University of Oslo, 1999–2020. The cluster analyses were performed using the unweighted pair-group method with arithmetic mean (UPGMA).

Results

Isozymes analysis

The electrophoretic patterns of the four isozyme systems viz. phosphorylase, catalase, α- Esterase and β-Esterase in 26 accessions of *Lactuca* spp. exhibited 31 alleles (Table 1). 27 alleles were polymorphic and 4 were monomorphic (*ACP*-1A, *ACP*-4A, *CAT*-3A and α*EST*-4) with 87.09% polymorphism (Table 1). Furthermore, the polymorphism in the accessions of *L. sativa* was 74.2%.

Table 1
The accession number and the origin of the studied accessions of *Lactuca* species

Code	Species	Accession no.	Origin	Code	Species	Accession no.	Origin
A	*Lactuca sativa* group Butterhead lettuce	CGN04706	Netherlands	N	*Lactuca saligna*	CGN13330	Turkey
B	*Lactuca sativa* group Butterhead lettuce	CGN04888	USA	O	*Lactuca saligna*	CGN13327	Greece
C	*Lactuca sativa* group Crisp lettuce	CGN05048	China	P	*Lactuca saligna*	CGN10883	Portugal
D	*Lactuca sativa* group Latin lettuce	CGN04566	France	Q	*Lactuca virosa*	CGN05332	Italy
E	*Lactuca sativa* group Latin lettuce	CGN05835	Spain	R	*Lactuca virosa*	CGN05145	France
F	*Lactuca sativa* group Latin lettuce	CGN04557	Argentina	S	*Lactuca indica*	CGN14312	Indonesia
G	*Lactuca sativa* group Cos lettuce	CGN04744	Turkey	T	*Lactuca indica*	CGN13392	China
H	*Lactuca sativa* group Cutting lettuce	CGN10956	Italy	U	*Lactuca viminea*	CGN16202	Union of Soviet
I	*Lactuca sativa* group Stalk lettuce	CGN11387	China	V	*Lactuca viminea*	CGN14301	France
J	*Lactuca sativa* group Stalk lettuce	CGN04546	USA	W	*Lactuca dregeana*	CGN04790	Italy
K	*Lactuca serriola* group Oilseed lettuce	CGN04770	Egypt	X	*Lactuca dregeana*	CGN05805	France
L	*Lactuca serriola*	CGN16210	Germany	Y	*Lactuca perennis*	CGN09321	Switzerland
M	*Lactuca sativa*_x_ *Lactuca serriola* group Oilseed	CGN05115	Egypt	Z	*Lactuca perennis*	CGN13299	France

The number of alleles ranged from 17 in *L. virosa* CGN05145 from France to 27 in *L. sativa* x *L. serriola* group Oilseed CGN05115 from Egypt with an average 20.8 and the mean number of alleles per locus ranged from 0.55 to 0.87 with an average 0.67 (Table 2). The mean frequency of alleles of the 26 accessions was 0.64. It was higher in cultivated species (0.52) compared to the wild ones (0.48). The lowest allele frequency (0.07) was observed for the allele *CAT*-1A in the accessions of *L. perennis.*

Table 2
Allelic frequencies for 16 isozyme loci in 26 accessions of Lactuca species.

Code	Allele Frequency		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	ACP-1	A	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
2	ACP-2	A	0.25	0.33	0.5	0.5	0	0	0.5	0	0.5	0.67	0	0.33	0.4	0.4	0	0	0	0
3		B	0.75	0.5	0.33	0.5	0.37	0.67	0.5	0.67	0.5	0.33	0.67	0.67	0.2	0.6	0.37	0.5	1	1
4		C	0	0.17	0.17	0	0.63	0.33	0	0	0	0	0.33	0	0.4	0	0.63	0.5	0	0
5	ACP-3	A	1	0.33	0.67	0.2	1	0.33	1	1	0	0.33	0.67	0.67	0.5	0.2	1	1	0	1
6		B	0	0.67	0.33	0.8	0	0.67	0	0	1	0.67	0.33	0.33	0.5	0.8	0	0	1	0
7	ACP-4	A	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
8	CAT-1	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	CAT-2	A	0	0.67	0.67	1	0	1	0.33	0	1	0.67	0.67	0.5	0.6	1	0	0	0	0
10		B	1	0.33	0.33	0	1	0	0.67	1	0	0.33	0.33	0.5	0.4	0	1	1	1	1
11	CAT-3	A	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
12	CAT-4	A	0.37	0.5	0.5	0.5	0	0	0.5	0	0.5	0.33	0.17	0.5	0.4	0.3	0	0	0	0
13		B	0.63	0.17	0.17	0.5	0.63	0.67	0.5	0.5	0.5	0.67	0.5	0.5	0.2	0.7	0.63	0.67	1	1
14		C	0	0.33	0.33	0	0.37	0.33	0	0.5	0	0	0.33	0	0.4	0	0.37	0.33	0	0
15	αEST-1	A	1	1	1	1	1	1	1	1	1	1	1	1	0	0.6	0.37	0.5	0.33	0
16		B	0	0	0	0	0	0	0	0	0	0	0	0	1	0.4	0.63	0.5	0.67	0
17	αEST-2	A	0.5	0.5	0.5	0.5	0	0	0.5	0	0.5	0.5	0.17	0.5	0.5	0.5	0	0	0	0
18		B	0.5	0	0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0	0.5	0.5	0.5	1	1
19		C	0	0.5	0.5	0	0.5	0.5	0	0.5	0	0	0.33	0	0.5	0	0.5	0.5	0	0
20	αEST-3	A	1	0	0.33	0	1	0	1	1	0	0	0.5	0.5	0.5	0	1	1	1	1
21		B	0	1	0.67	1	0	1	0	0	1	1	0.5	0.5	0.5	1	0	0	0	0
22	αEST-4	A	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**
23	βEST-1	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24		B	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
25	βEST-2	A	0.63	0.5	0.67	0.5	0.33	0.33	0.5	0.33	0.67	0.33	0.67	0.5	0.25	0.5	0.63	0.5	0.83	0
26		B	0.37	0.5	0.33	0.5	0.67	0.67	0.5	0.67	0.33	0.67	0.33	0.5	0.75	0.5	0.37	0.5	0.17	0
27	βEST-3	A	0.5	0.5	0.5	0.4	0	0.33	0.67	0.17	0.5	0.33	0.17	0.33	0.5	0.5	0	0	0	0
28		B	0.5	0	0	0.6	0.5	0.5	0.33	0.33	0.5	0.67	0.5	0.67	0.2	0.5	0.5	0.5	0.67	1
29		C	0	0.5	0.5	0	0.5	0.17	0	0.5	0	0	0.33	0	0.3	0	0.5	0.5	0.33	0
30	βEST-4	A	0.25	0.67	0.67	0.8	0.25	1	0.33	0	0.67	1	0.67	0.67	0.6	1	0	0	0	0
31		B	0.75	0.33	0.33	0.2	0.75	0	0.67	1	0.33	0	0.33	0.33	0.4	0	1	1	1	1
	Total number of alleles		20	24	25	21	20	21	21	19	20	21	26	23	27	22	22	21	18	1
	Mean Number of alleles per locus		0.65	0.77	0.80	0.68	0.65	0.68	0.68	0.61	0.65	0.68	0.84	0.74	0.87	0.71	0.71	0.68	0.58	0

* Average of mean frequency of allele

The clustering dendrogram of the isozymes data gave two main groups (G1 and G2) at genetic distances 2.25 (Fig. 1). The accessions of *L. sativa* were distributed in the two groups (Fig. 1). G1 included *L. serriola* group Oilseed lettuce, *L. serriola*, *L. sativa* x *L. serriola* group Oilseed and six accessions of *L.* [illegible] *dregeana* with the rest of the accessions of *L. sativa*. At genetic distances 1.9, *Lactuca* accessions were

separated into five clusters and two singletons (*L. sativa* x *L. serriola* group Oilseed (CGN05115) and *L. perennis* CGN13299). Cluster one (C1) contained the accessions of *L. indica* and *L. perennis* CGN09321, whereas C2 included *L sativa* (group Butterhead lettuce CGN04888, group Crisp lettuce CGN05048, group Latin lettuce CGN04566, group Latin lettuce CGN04557, group Cos lettuce CGN04744 and group Stalk lettuce CGN11387), *L. serriola group Oilseed lettuce* CGN04770, *L. serriola* CGN16210 and *L. saligna* CGN13330. *L. saligna* CGN13327, *L. saligna* CGN10883 and the accessions of *L. viminea* were collected in C3. The accessions of *L. virosa* and *L. dregeana* were separated in C4. *L. sativa* group Butterhead lettuce CGN04706, *L. sativa* group Latin lettuce CGN05835, *L. sativa* group Cos lettuce CGN04744 and *L. sativa* group Cutting lettuce CGN10956 were grouped in C5.

The accessions in the top right quadrant of the corresponding analysis of the isozyme data included *L. sativa* group Butterhead lettuce CGN04888, *L. sativa* group Crisp lettuce CGN05048, *L. sativa* group Latin lettuce CGN04566, *L. sativa* group Latin lettuce CGN04557, *L. sativa* group Cos lettuce CGN04744, *L. sativa* group Stalk lettuce CGN04546, *L. serriola* group Oilseed lettuce CGN04770, *L. serriola* CGN16210, *L. sativa* x *L. serriola* group Oilseed CGN05115, *L. perennis* CGN13299 (Fig. 2). These accessions were closely associated by the alleles, namely *CAT*-1A, *CAT*-2A, *CAT*-4A, *αEST*-1A, *αEST*-2A, *αEST*-3B and *βEST*-3A (Fig. 2). The left top quadrant consisted of the accessions (*L. sativa* group Butterhead lettuce CGN04706, *L. sativa* group Latin lettuce CGN05835, *L. sativa* group Cutting lettuce CGN10956 and the accessions of *L. viminea*) with the alleles *ACP*-2C, *ACP*-3A, *CAT*-4C, *αEST*-2C, *βEST*-1A, *βEST*-2A and *βEST*-3C. The right bottom quadrant comprised the accessions *L. saligna* (CGN13330, *L. indica* CGN14312, *L. indica* CGN13392, *L. perennis* CGN09321) that were closely associated with *ACP*-2B, *ACP*-3B, *CAT*-4B, *αEST*-2B, *βEST*-2B, *βEST*-3B. The accessions (*L. saligna* CGN13327, *L. saligna* CGN10883, *L. virosa* CGN05332, *L. virosa* CGN05145, *L. dregeana* GN04790, *L. dregeana* CGN05805) in the bottom left were also closely related in terms of *CAT*-2B, *αEST*-1B, *αEST*-3A, *βEST*-1B, *βEST*- 4B.

Rapd Analysis

Among 18 random primers tested in this study, 5 primers generated reproducible bands. A total of 186 polymorphic bands were identified using the five primers. Maximum and minimum percentages of polymorphic bands were observed by primers OPA3 (46 fragments) and OPA1 (31 fragments) respectively. The average percentage of polymorphic bands was 100 %. The average size of DNA fragments ranged between 33bp and 600bp (Fig. 3). The fragment with DNA size of: 33 bp is unique marker for *L. sativa* x *L. serriola* group Oilseed CGN05115; 500 bp DNA fragment for *L. indica* CGN13392 (Primer 1 OPA09); 200 bp DNA fragment for *L. serriola* group Oilseed lettuce CGN04770; 500 bp DNA fragment for *L. serriola* group Oilseed lettuce CGN04770 (Primer 2 OPA14); 200 bp DNA DNA fragment for *L. serriola* group Oilseed lettuce CGN04770; 350 bp DNA fragment for *L. saligna* CGN13330, 47 bp DNA fragments for *L. saligna* CGN10883 (Primer 3 OPZ12).

The clustering dendrogram constructed based on RAPD results showed seven clusters (C1-C7) (Fig. 4). The first and second clusters (C1 and C2) included *L. serriola* group Oilseed lettuce CGN04770 and all the accessions of *L. sativa* except *L. sativa* group Butterhead CGN04706 which was separated as singleton at far genetic distance. The wild species *L. serriola* CGN16210 and *L. sativa* x *L. serriola* group Oilseed CGN05115 were clustered in C7 which is connected with other accessions at genetic distance 7.5. The accessions of *L. viminea*, *L. dregeana* and *L. perennis* were included in C5. The accessions of *Lactuca saligna* (C3 and C6), *L. virosa* (C3 and C4) and *L. indica* (C4 and C5) were distributed in two clusters. It can be noticed that two accessions of *Lactuca saligna* (*Lactuca saligna* CGN13327 from Greece and *Lactuca saligna* CGN10883 from Portugal) were collected with *Lactuca serriola* and *Lactuca sativa x Lactuca serriola* group Oilseed and the third accession (*Lactuca saligna* CGN13330 from Turkey) was clustered with *Lactuca virosa* and *Lactuca indicia*.

Discussion

Estimating the genetic diversity between and within accessions of crop species and its wild relatives assist in decision-making to select the best accessions for selection of parents for hybridization and is crucial for providing information for domestication and propagation (Yu et al. 2001; Khan et al. 2019). It is also enhancing the understanding of the plant germplasm for germplasm management and potential users, and helping in producing new varieties best adapted to regional environmental conditions (El-Esawi, et al. 2017; van Herwijnen and Manning 2017).

In the present study, the estimation of genetic diversity with isozyme markers showed high polymorphism between the examined accessions (87.09%) which was attributed to outcrossing of the majority of the species and infrequent interspecific hybridization (Jemelková, et al. 2018). It also showed that the polymorphism between *L. sativa* accessions (74.2%) was not with same magnitude of the polymorphism between the wild species, reflecting the impact of domestication (successive rounds of selection) on reducing the genetic diversity of the domesticated species, leaving them with less allelic richness than their wild progenitors and other crop wild relatives (Abbo et al. 2014; Dempewolf et al. 2017).

The highest number of alleles in the wild species (31) compared with the cultivated species *L. sativa* (27) reflected a reduction in the richness of alleles due to domestication which was reported to modify agronomic phenotypes and genetic signature of the domesticated species, resulted in the reduction in genetic diversity to meet the demand for high crop productivity and crop uniformity in the field and the marketplace (Dempewolf et al. 2017; Zhang et al. 2017). The variation between cultivated and wild species was not only limited to the number of alleles, but also it extended to the mean frequency of alleles which was found to be higher in the cultivated species (0.52) comparing with the wild ones (0.48). This might be attributed to the absence of selection pressure on the wild species, as the existing variations in these species are natural (Das 2011). The allele with low mean allele frequency (0.07) or what was known as rare alleles was observed only for the allele *CAT*-1A in *L. perennis.* The presence of this allele could be due to deleterious mutations or may be due to evolutionary relics (Sammour et al. 2019). The detection of rare allele in combination with high allelic frequency of other loci leads to the conclusion that the studied accessions had wide genetic differentiation.

The separation of *L. dregeana* with *L. virosa* in the same cluster in isozymes and RAPD cluster analyses confirmed their closely relatedness as they belong to the same section *Lactuca* (Lebeda and Astley 1999), having one pair of satellites (El-Esawi and Sammour 2014) and similar morphological characters (De

confirmed the very close relationship between *L. serriola* and *L. dregeana* and considered the two taxa to be

conspecific based on AFLP fingerprints, nuclear and plastid DNA sequence comparison, isozymes and RAPD results of the present research recognized differences between them adequate to maintain them as distinct species, confirming the previous morphological, karyological, historical occurrence, and ecological studies (Zohary 1991; El-Esawi and Sammour 2014; van Herwijnen and Manning 2017).

The distribution of the accessions of *L. sativa* in more than one cluster in the isozymes and RAPD dendrograms confirmed the polyphyletic origin of *L. sativa*. Furthermore, the clustering of some of the accessions of *L. sativa* with *L. serriola group Oilseed lettuce* based on isozymes data and the clustering of the rest of the accessions with *L. dregeana* confirmed that *L. serriola* and *L. dregeana* were members of the primary gene pool of *L. sativa* (Zohary 1991) and was consistent with the work of Kesselli and Michelmore (1986) who also suggested that domestication of *L. sativa* may be due to the repeated domestication from wild progenitors or may be due to the use of interspecific hybridizations in breeding programmes to introduce characters of interest into cultivated lettuce. However, the prevailing of the allele “B” in the wild species and alleles “A” and “C” in the cultivated species and its progenitors, as it has been shown in the corresponding analysis, indicating that the cultivated species was firstly domesticated from wild progenitors and later breeders used interspecific hybridizations to introduce characters of interest into cultivated lettuce. The prevailing of the allele “B” in wild species and alleles “A” and “C” in cultivated ones also suggested that: (1) allele “B” might be the primitive form of studied alleles that enable the wild species to resist the environmental stresses which prevails in their habitats, and (2) alleles “A” and “C” could be derived forms that evolved as a result of domestication.

The separation of the accessions of *L. saligna* in two clusters in the cluster analysis of the isozymes data was consistent with the works of Esawi et al. (2017). The heterogeneity of this species attributed to the difference in the number and types of alleles in the studied accessions. The accessions *Lactuca saligna* CGN13327 from Greece and *Lactuca saligna* CGN10883 from Portugal did not have the allele “A” of the loci *ACP*-2, *CAT*-2, *CAT*-4, *αEST*-2, *βEST*-3, *βEST*-4 which characterize the cultivated species, whereas the accession *Lactuca saligna* CGN13330 from Turkey had these alleles. This lead to infer that the accession from Turkey might be subjected to a natural mutation or a sort of unintended domestication. Although Güzel et al. (2018) observed several samples in the field and herbaria belonging to *L. viminea* that easily fell into two distinct subspecies according to their habits and morphological traits, the clustering dendrograms of isozymes and RAPD analyses separated the accessions of *L. viminea* in one cluster which was inconsistent with their observation. The variation observed by Güzel et al. (2018) could be attributed ecogeographical conditions in which the accessions of *L. viminea* were originated; the conditions that cause a significant variation in the phenotypic characters. The clustering dendrogram based on RAPD data indicated a close genetic relationship between *L. sativa, L. serriola*, *L. saligna* and *L. virosa* which was consistent with the phylogenetic trees based on chloroplast DNA sequence comparison (Wei et al. 2017), chromosomal studies (Matoba et al. 2007), nrITS1 and AFLP fingerprints (Koopman et al. 2001). So, *L. serriola*, *L. saligna* and *L. virosa* can be considered important resources for *L. sativa* breeding.

Conclusion

Genetic variability was considerable high within and among *Lactuca* species based on isozymes or RAPD analyses, opened the path for the improvement of the cultivated species. The number of alleles were lower in the cultivated species compared to the wild species, reflecting a reduction in the richness of alleles in the cultivated species due to domestication. The cluster and corresponding analyses revealed: (1) the polyphyletic origin of *L. sativa*, (2) the homogeneity of the accessions of *L. viminea* ruling out the previous study showed that *L. viminea* has two distinct subspecies, (3) the grouping of the wild accessions based on the form “B” of the studied alleles and the cultivated accessions based on the forms “A” and “C”, suggesting that form “B” might be the primitive form of the alleles of the loci of the assessed isozymes, and “A” and “C” could be the derived forms, and (4) the separating of the accessions of *L. saligna* in two clusters was due to the variation in the number of alleles, particularly “A” form, suggesting that some accessions could be wild and the other might be subjected to natural mutation or unintended domestication. The accessions of *L. sativa* x *L. serriola* group Oilseed, L. *indicia*, *L. saligna* were characterized with unique DNA fragments that can be used as markers for identifying these accessions. The association between specific forms of alleles and both cultivated and wild species needs deep insights to be manipulated for lettuce improvement. The considerable genetic variation in the accessions of *Lactuca saligna*, *Lactuca virosa* and *Lactuca indica* open the door for more detailed studies on big number of accessions cover their distribution range using multiple markers.

Declarations

Acknowledgment

This project was supported by King Saud University, Deanship of Scientific Research, College of Science, Research Center, Saudi Arabia.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

Authors’ contributions

This study was conceived and designed by R.H.S and M. E-M. Data were collected by M E-M. and validated by R.H.S and A.M. The initial draft was edited by M E-M and A.M. The revision and the final draft were edited by R.H.S.

Consent to publication

The authors are consent to publish this work in Molecular Biology Reports

Consent to participate

The authors are consent to participate in this manuscript.

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Figures

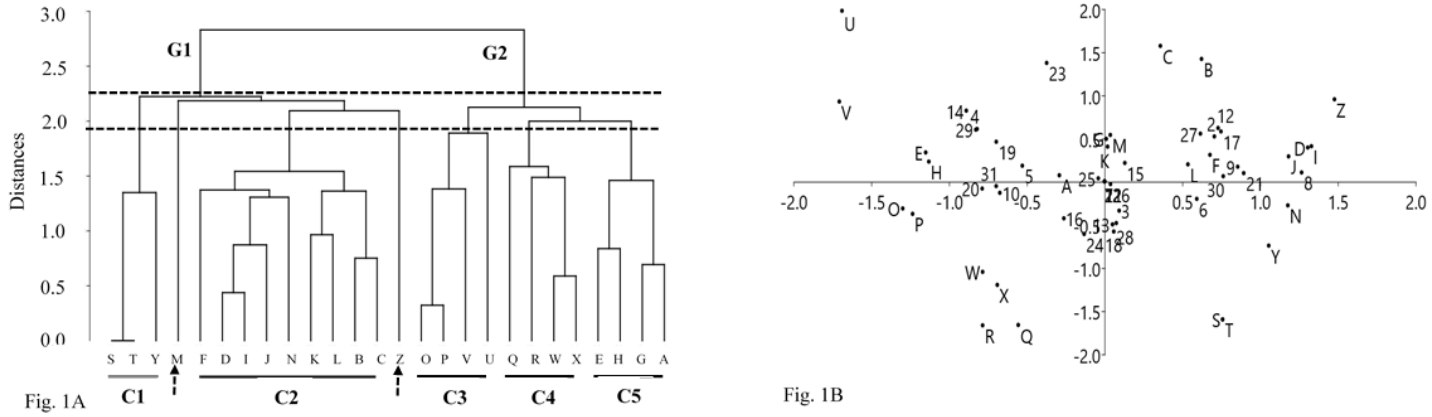

Figure 1

A. UPGMA clustering of 26 accessions of Lactuca species based on isozymes data. B. Corresponding analysis of 26 accessions of Lactuca spp. based on their isozymes characteristics explained by the first and second principal components.

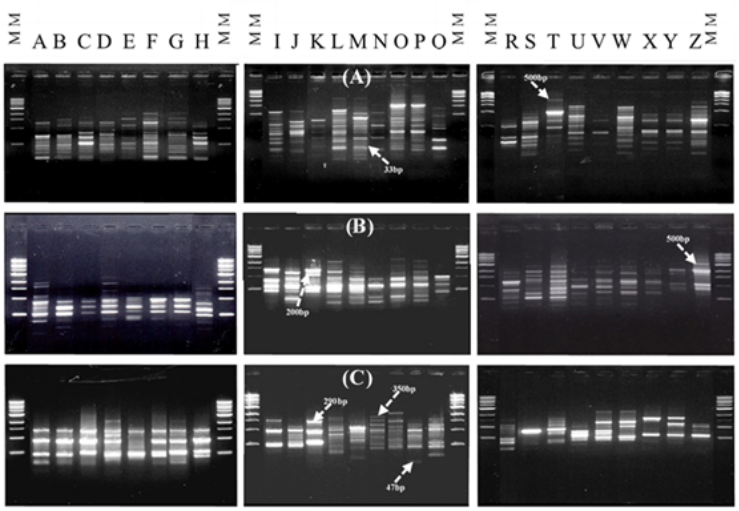

Fig. 2A

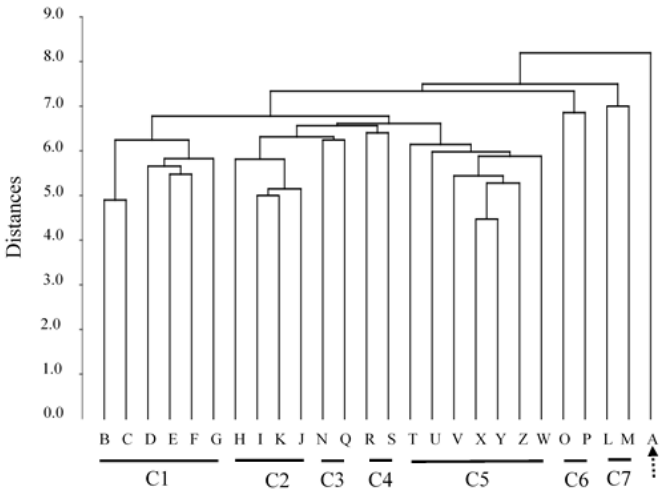

Fig. 2B

Figure 2

A. RAPD profiles of 26 accessions of Lactuca species produced by primer OPA09 (A), primer OPA14 (B), primer OPZ12 (C). The arrows indicates accession specific marker. B. UPGMA dendrogram of 26 accessions of Lactuca species based on RAPD marker data.