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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.

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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	<i>Lactuca sativa</i> group butterhead lettuce	CGN04706	Netherlands
2	<i>Lactuca sativa</i> group butterhead lettuce	CGN04888	USA
3	<i>Lactuca sativa</i> group crisp lettuce	CGN05048	China
4	<i>Lactuca sativa</i> group latin lettuce	CGN04566	France
5	<i>Lactuca sativa</i> group latin lettuce	CGN05835	Spain
6	<i>Lactuca sativa</i> group latin lettuce	CGN04557	Argentina
7	<i>Lactuca sativa</i> group cos lettuce	CGN04744	Turkey
8	<i>Lactuca sativa</i> group cutting lettuce	CGN10956	Italy
9	<i>Lactuca sativa</i> group stalk lettuce	CGN11387	China
10	<i>Lactuca sativa</i> group Stalk lettuce	CGN04546	USA
11	<i>Lactuca serriola</i> group oilseed lettuce	CGN04770	Egypt
12	<i>Lactuca serriola</i>	CGN16210	Germany
13	<i>L. sativa</i> x <i>L. serriola</i> group oilseed	CGN05115	Egypt
14	<i>Lactuca saligna</i>	CGN13330	Turkey
15	<i>Lactuca saligna</i>	CGN13327	Greece
16	<i>Lactuca saligna</i>	CGN10883	Portugal
17	<i>Lactuca virosa</i>	CGN05332	Italy
18	<i>Lactuca virosa</i>	CGN05145	France
19	<i>Lactuca indica</i>	CGN14312	Indonesia
20	<i>Lactuca indica</i>	CGN13392	China
21	<i>Lactuca viminea</i>	CGN16202	Union of Soviet
22	<i>Lactuca viminea</i>	CGN14301	France
23	<i>Lactuca dregeana</i>	CGN04790	Italy
24	<i>Lactuca dregeana</i>	CGN05805	France
25	<i>Lactuca perennis</i>	CGN09321	Switzerland
26	<i>Lactuca perennis</i>	CGN13299	France

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