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Resistance gene resources of the genus *Lactuca* against downy mildew (*Bremia lactucae*) and their genetic variability

Ph.D. Thesis

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Declaration

I hereby declare that this Ph.D. thesis has been worked out by myself together with listed coauthors. All literary sources cited in this thesis are listed in the References section.

Olomouc ..... Michaela Jemelková

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Abstract:

This Ph.D. thesis is focused on five various topics related to the *Lactuca* spp. germplasm, their genotypic and phenotypic variation, ecogeography, resistance to pathogens and pests, as well as utilization in lettuce breeding. The first on is the development of genetic variability of 53 Swedish and Slovenian populations of *L. serriola*, two marginal areas of natural distribution of this species. The genetic variability between populations was evaluated with microsatellite (EST-SSR) and amplified fragment length polymorphism (AFLP) markers. The mutual relationships among the analyzed samples were visualized using two approaches, Neighbor-Network diagram and Bayesian clustering method. In the next part, the results of genetic variability (EST-SSR and AFLP marker analysis) of 69 samples of *L. aculeata*, 3 samples of *L. serriola* and 3 putative hybrids *L. aculeata* × *L. serriola,* coming from Turkey, Jordan and Israel are summarized and include data from screening (41 samples of *L. aculeata*) for responses to lettuce downy mildew (*Bremia lactucae*). Our results confirmed a previous determination of three putative hybrids *L. aculeata* × *L. serriola*. Moreover, at least 6 additional hybrid samples showing certain proportion of *L. serriola*-like genotype were detected. The results from the studies of the resistance to demostrated race-specific reaction patterns, which indicate the possible presence of some race-specific resistance factors/genes in the studied samples of *L. aculeata*. The third part describes the results obtained from the study of the population structure, including morphological/genetic variability in Israeli samples of *L. serriola*, *L. aculeata* and *L. saligna* (two populations per each *Lactuca* species). The genetic structure and diversity were analyzed using EST-SSR and AFLP markers. The results showed that although these species have the predominantly self-pollinating character, the populations

were not morphologically and genetically uniform. The genetic variability in a population increases at its periphery, due to the presence of plants with „non-indigenous“ alleles, which most likely come from migration and subsequent interpopulation or interspecific hybridization. The other two parts are complementary and interconnected. The fourth part is focused on reaction of wild *Lactuca* genetic resources to diseases (including viral pathogens, oomycete and fungal pathogens) and pests (incl. nematodes, insects and mites). The last part summarizes the current knowledge of the intensive research of the wild *Lactuca* species in taxonomy, ecogeography, conservation strategy, karyology, molecular biology and the approaches using the wild *Lactuca* species in lettuce breeding programme especially in breeding lines resistant to biotic factors.

Keywords: race-specific resistance, ecogeography, Israel, *Lactuca serriola*, *Lactuca aculeata*, *Lactuca saligna*, genetic variability, lettuce downy mildew, Scandinavia, biodiversity
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Abstrakt:

Předkládaná disertační práce je zaměřena na pět různých témat týkajících se genových zdrojů rodu *Lactuca* L., jejich genotypové a fenotypové variability, ekogeografie, rezistence vůči patogenům a škůdcům a praktického využití ve šlechtění kulturního salátu (*L. sativa*).

První část pojednává o genotypové variabilitě 53 populací *L. serriola* pocházejících ze Švédska a Slovinska, okrajových areálů výskytu tohoto druhu. Variabilita mezi jednotlivými populacemi byla hodnocena pomocí EST-SSR a AFLP markerů. Vzájemné vztahy mezi analyzovanými vzorky byly vizualizovány pomocí dvou přístupů, Neighbor-Network diagramu a Bayesovské shlukovací analýzy. V další části předkládané disertační práce jsou shrnuty výsledky studia genetické variability (EST-SSR a AFLP markerů) u 69 vzorků *L. aculeata*, 3 vzorků *L. serriola* a 3 pravděpodobných hybridních položek *L. aculeata* × *L. serriola*, pocházejících z Turecka, Jordánska a Izraele včetně výsledků studia rezistence vůči plísni salátové (*Bremia lactucae*) u 41 vzorků *L. aculeata*. Podle námi dosažených výsledků se potvrdila dřívější determinace hybridních vzorků *L. aculeata* × *L. serriola* včetně detekce nejméně 6 dalších vzorků, které vykazovaly taktéž podobnost s genotypovými profily *L. serriola*. Dosažené výsledky studia rezistence vykazovaly rasově-specifické reakce indikující pravděpodobnou přítomnost rasově-specifických faktorů/genů ve vzorcích *L. aculeata*. Ve třetí části jsou uvedeny dosažené poznatky hodnocení populační struktury včetně morfologické/genetické variability u izraelských vzorků *L. serriola*, *L. aculeata* a *L. saligna*, z nichž každý druh byl zastoupen dvěma populacemi. Pomocí EST-SSR a AFLP markerů byla analyzována genetická struktura a diverzita. Výsledky ukázaly, že i když jsou tyto tři druhy převážně

samosprašné, populace nebyly morfologicky a geneticky uniformní. Genetická variabilita populací vzrůstala v okrajových částech areálu, a to díky přítomnosti rostlin s „nepůvodními“ alelami, které s největší pravděpodobností získaly migrací či vnitropopulační/vnitrodruhovou hybridizací. Čtvrtá část je zaměřena na reakce planých druhů rodu *Lactuca* k patogenním organismům (zahrnující virové patogeny, oomycety a houbové patogeny) a škůdcům (hlístice, hmyz a roztoči). Pátá část shrnuje dosavadní poznatky intenzivního výzkumu planých druhů rodu *Lactuca* L. v oblasti taxonomie, ekogeografie, konzervační strategie, karyologie, molekulární biologie včetně přístupů využívajících plané druhy rodu *Lactuca* ve šlechtění na odolnost kulturního salátu (*L. sativa*) vůči biotickým faktorům.

Klíčová slova: rasově-specifická rezistence, ekogeografie, Izrael, *Lactuca serriola*, *Lactuca aculeata*, *Lactuca saligna*, genetická variabilita, plíseň salátová, Skandinávie, biodiverzita
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CONTENTS

1. INTRODUCTION

Lactuca L. genus involves more than 97 wild species. Several of them have been cultivated for their economic and medicinal importance since ancient times (Lebeda et al., 2004b; Doležalová et al., 2002). Those species include *Lactuca serriola* L., *L. virosa* L., *L. saligna*., *L. indica*., *L. undulata* Ledeb., *L. viminea* L., *L. dregeana* DC., *L. perennis* L., *L. orientalis* Boiss., *L. altaica* Fisch. & C.A. Mey. and the cultivated lettuce (*L. sativa L.*) (Doležalová et al., 2002).

Cultivated lettuce (*L. sativa* L.) is the most important leafy salad vegetable in the world (McGuire et al., 1993). It is rich in vitamins B and C. The oil of the Oilseed group (e.g., *L. serriola*, *L. sativa*, a hybrid between these two taxa), with a high vitamin E content, is used for human consumption. Several species of the genus *Lactuca* L. are rich in a milky sap that flows freely from any wounds in the plant. This sap contains lactucarium which is used in medicine for its anodyne, atispasmodic, digestive, diuretic, narcotis, aphrodisiac, soporific and sedative properties (Bown, 1995).

Several viral, bacterial and fungal pathogens, such as yellows virus, turnip mosaic potyvirus, *Microdochium*, *Rhizomonas* (corky root disease), *Bremia* (downy mildew), *Golovinomyces* (powdery mildew) (Lebeda et al., 2014) are one of the most important problems affecting lettuce. Therefore, the attention has been paid to genetic resources to find the genes conferring resistance and good agronomic traits. Modern breeding methods of cultivated lettuce are based on utilization of wild related species and progenitors. The study of genetic variability within a wild *Lactuca* species is vital to plant breeders because of its importance for selecting germplasm included in a breeding program. The study of genetic diversity is essential to receive information about propagation, domestication, which can be used in breeding programs and for conservation of genetic resources of *Lactuca* spp.

The aim of the thesis was to broaden the knowledge of relationships among *Lactuca* species and the resistance to diseases (*Bremia lactucae*). Besides, the work focused on the genetic structure and variability of wild *Lactuca* species. The most important results are summarized in chapter Conclusions. The results of the thesis are summarized in four chapters, which involve five papers. The papers are already published or in press.

2. LITERATURE OVERVIEW

2.1. General characterization and ecogeographical distribution of the genus *Lactuca* L.

The genus *Lactuca* L. belongs to tribe Lactuceae, subfamily Cichorioideae, family Asteraceae. It includes 97 wild species of annual, biennial or perennial herbs with erect or ascending habit, which are predominantly self-pollinating (Thompson et al., 1958). The genus *Lactuca* is distributed throughout the temperate and warm regions with 16 species in Europe, 12 species in America, 43 species in Africa and 51 species in Asia (Lebeda et al., 2001a).

Currently, the classification proposed by Lebeda (2004a) is accepted. Based on the recent phylogenetic studies, the genus *Lactuca* is divided into 7 sections (Phoenixopus, Mulgedium, Lactucopsis, Tuberosae, Micranthae, Sororiae and Lactuca) and comprises two geographic groups-the African and the North American ones. The species of the section Lactuca are classified into two subsections Lactuca and Cyanicae based on the different life cycle and the number of chromosomes (Doležalová et al., 2002). The subsection Lactuca include the most common and the most broadly spread species *L. serriola*, *L. aculeata*; *L. saligna* and *L. virosa* which represent a primary, secondary and terciary genepool of a popular leafy vegetable-cultivated lettuce (*L. sativa*). These species are annuals and biennial herbs, with characteristic capitula composed from 10-30 (50) yellow florets and obovate achenes with many ribs. In comparison with subsection Cyanicae, which includes perennial herbs (*L. perennis* and *L. tenerrima*). The subsection Cyanicae is characterized by capitula composed of not more than 22 blue or lilac florets and 1-3 ribbed achenes (Doležalová et al., 2002).

It is likely that, the classification of species will be changed based on data from molecular studies. Wei et al. (2017) describe phylogenetic relationships within *Lactuca* L. based on chloroplast DNA sequence comparisons, in which the genus *Lactuca* is divided into distinct phylogenetic clades – the crop clade (which includes *L. sativa*, *L. serriola*, *L. saligna*, *L. virosa*…) and the *Pterocypsela* clade (*L. indica*, *L. raddeana*, *L. formosana*…), the North American, Asian, and widespread species form smaller clades and African endemic species probably should be treated as a new genus.

The species of the genus *Lactuca* inhabit a wide range of habitat. The most common species such as *L. serriola*, *L. saligna* and *L. virosa* are frequent ruderal species. They prefer disturbed soil e.g. in waste places, embankments, field margins, roadsides and ditches (Feráková, 1977; Lebeda et al., 2001a; 2004a; 2007). Some Mediterranean species (e.g. *L. perennis*, *L. viminea*, *L. graeca*, *L. tenerrima*) are calciphilous occurring on limestone and on rocky slopes (Feráková, 1977). Species like *L. canadensis* and *L. biennis* occur in woods, shrubs and clearings (Lebeda and Astley, 1999). The optimal altitude for the majority of *Lactuca* spp. are between 200 and 600 m but representatives of this genus can be found at higher altitudes (above 2000 m) (Feráková, 1977; Lebeda et al., 2001a).

The most serious disease of *Lactuca* spp. is downy mildew caused by *Bremia lactucae* Regel (Lebeda et al., 2002). *B. lactucae* is a highly variable obligate biotrophic oomycete (Peronosporaceae) pathogen. It attacks not only cultivated lettuce, but also many other Asteraceae species that have worldwide distribution (Lebeda et al., 2002). Fungicide protection often becomes difficult and ineffective (Barrière et al., 2014; Brown et al., 2004; Michelmore and Wong, 2008), and it has strong hygienic limits. Breeding for the resistance is a major activity of most lettuce improvement programmes. There is an increasing need for information and methods to accelerate the development of new disease-resistant cultivars (Lebeda et al., 2007; Michelmore and Wong, 2008). The resistance breeding exploits genotypes with dominant race-specific resistance *Dm* genes (or R-factors) (Lebeda et al., 2014). However, breeding for race-specific resistance is problematic due to extremely high variability (Lebeda et al., 2002, 2008; Lebeda and Zinkernagel, 2003; Sharaf et al., 2007) and adaptability of the pathogen populations (Lebeda and Zinkernagel, 2003). Therefore, the wild *Lactuca* species are intensely studied since the 1970s. Nowadays, some of them (e.g. *L. serriola*, *L. saligna* and *L. virosa*) are almost routinely used in resistance breeding and by crop evolutionists (Zohary, 1991; Lebeda et al., 2007, 2014). However, much less attention has been given to *L. aculeata*, the species, which is fully interfertile with *L. sativa* and with *L. serriola* (Globerson, 1980, Lebeda et al., 2007).

2.2. The species *L. aculeata*, *L. serriola* and *L. saligna*

***Lactuca aculeata* Boiss et Kotschy** is a robust and very prickly annual or biennial plant (*2n =2x* = 18) reaching up to 100 cm. The erect stem is very prickly with rigid pointed emergence. The leaves have a suculent character, are sessile, entire to pinnatisect with densely covered emergence on adaxial and abaxial side. The shape of rosette leaves is spathulate, rosette leaves (cauline) are subacute. The composed inflorescence is formed with many flower heads (capitula). The achenes are brown with a lot of ribs with striking white trichomes (Doležalová et al., 2002).

L. aculeata is restricted to the Near East and the Anatolian plateau (Israel, Lebanon, Turkey, Syria and Jordan) (Zohary, 1991; Danin, 2004; Lebeda et al., 2004b). In Israel, which is considered a centre of its origin (Zohary, 1991), *L. aculeata* grows in a broad span of altitudes (222-968 m) and in various habitats such as roadsides, field margins, dumps, anthropogenic and ruderal places (Beharav et al., 2010a). The lithology on the survey sites is mostly basalt bed rocks. *L. aculeata* is primarily self-pollinating (Zohary, 1991) and fully cross-compatible with *L. sativa* and *L. serriola*. Thus, it is a component of the primary gene pool of *L. sativa* (Globerson et al., 1980; Lebeda et al., 2007; de Vries, 1990). In the North Israel, *L. aculeata* grows, frequently together with *L. serriola* and *L. saligna*, and sporadic intermediate and recombinant individuals have been detected repeatedly there (Zohary, 1991; Beharav et al., 2008, 2010a; Lebeda et al., 2012).

Together with *L. serriola*, *L. dregeana* and some other *Lactuca* species belongs to the primary genepool of cultivated lettuce (*Lactuca sativa* L.) (Koopman et al., 2001; Lebeda et al., 2007, 2009).

***Lactuca serriola* L.** (prickly lettuce, *2n =2x* = 18) is annual or biennial therophyte reaching up to (30)50-200 cm. Stiff and erect stem growing from the basal rosette is prickly on the base. Rosette leaves are oval-rounded to elongated, widest near the apex. On the underside of the leaf is a row of spines along the midrib and the leaf margin is weakly spiny. Basal leaves oblong-ovate in outline, pinnate-lobed to pinnatisect with backwards orientated lateral lobes. Cauline green waxy leaves are alternate, sessile and clasp the stem with small pointed lobes. The cauline leaves are oriented vertically in full sun, in a north-south plane. Pyramidal panicle inflorescence is composed of many small

flower heads (capitula). A head can contain usually yellow, ligulate ray flowers. Achenes are oblong-ovate, olive green to greyish with longitudinal ribs (Doležalová et al., 2002; Dostál, 1989; Feráková, 1977; Grulich, 2004; Weaver and Downs, 2003).

Two primary morphological forms are recognized within *L. serriola* L. based on cauline leaf-shape variability; the pinnatifid-leaved form *L. serriola* L. f. *serriola*, and the unlobed-leaved form *L. serriola* L. f. *integrifolia* (S.F. Gray) S.D. Prince et R. N. Carter. The *serriola* form is recorded as the most frequent species, occurring at a very high density in Europe. The form *integrifolia* is not so common, and has been recorded in e.g., Switzerland, Italy, France, western Germany, the Netherlands, and is prevalent in the UK (Lebeda et al., 2001a, 2004a, 2007). *L. serriola* is native to the Mediterranean Basin and to the Near East (de Vries, 1997) and currently is widely distributed around the world. Its occurrence was recorded from Australia, Tasmania and New Zealand (Lebeda et al., 2004a). In Europe, North America, southern Africa and Argentina, *L. serri*ola is considered as an invasive weed reducing the crop yield or quality (Lebeda et al., 2004a; Weaver and Downs, 2003; Weaver et al., 2006). *L. serriola* is distributed from lowland to montane regions. Its occurrence is limited by warm summers (Lebeda et al., 2004a, 2007). According to Feráková (1977), the northern boundary of the distribution in Europe runs near the latitude 65°N in Finland and 55°N in Great Britain. In Norway and Sweden the northmost localities are at 60°N up to 65°N (Feráková, 1977). The western limit of *L. serriola* distribution in Europe is 5°W (Lebeda et al, 2004a, 2007). The invasive character is caused mainly by climate changes, extensive development in transportation and increasing number of man-made habitats (Carter and Prince, 1985; D'Andrea et al., 2009; Hooftman et al., 2006).

***Lactuca saligna* L.** (least lettuce, willow-leaf lettuce; $2n = 2x = 18$) is annual or biennial, rarely perennial herb. The erect stem is reaching up to 30-100 cm, usually glabrous. The rosette leaves are pinnatilobed, glabrous or prickly-bristly on midvein, the cauline leaves leaves are very long (up to 20 cm), linear to lanceolate. The inflorescence is narrow, spike-like panicle with yellow flower heads. The achenes are dark brown, rough, glabrous, several-veined on each face (Grulich, 2004). Feráková, 1977 describe, that the *L. saligna* is extremely polymorphic, and two different variety are distinguished

according to the cauline leaves in the middle part of main stem: var. *saligna* – with entire margin of leaves, and var. *runcinata* with pinnatipart up to pinnatisect leaves.

L. saligna is a Eurasian species (Feráková, 1977), widely distributed throughout the Mediterranean Basin (Beharav et al., 2008), extending to the Caucasus and parts of temperate Europe (Lebeda et al., 2004b). In Europe its distribution area reaches 52°N (Feráková, 1977). It was likely introduced to North America from Europe (Lebeda et al., 2016). *L. saligna* prefers warm, fertile, semi-arid and slightly salty soils. Its most common habitats are waste and disturbed places, railways, roadsides, borders of wooded areas, arable fields and river banks (Feráková, 1977; Lebeda et al., 2001a, 2004a,b; Beharav et al., 2008). *L. saligna* is a characteristic weedy species of both lowland and hilly areas (Europe to 1000 m in Italy, Cyprus up to 1680 m, Turkey up to 2400 m) (Hegi, 1987; Meusel and Jäger, 1992). Nevertheless, the most frequent occurence of this species in Europe is at altitude between 0 and 300 m (Lebeda et al., 2001a).

2.3. Plant-parasite interactions in wild plant pathosystems

Plants are exposed to a wide range of potential pathogens and pests, during their life and they have developed a various resistance mechanism to protect themselves and survive. Plant pathogens play a substantial role in the structure, dynamics, and evolution of natural plant communities. They might cause increased mortality, reduced fitness of individual plants. On the other hand, pathogens can help maintain plant species diversity, facilitate successional processes, and enhance the genetic diversity and structure of host populations (Gilbert, 2002; Lebeda et al., 2002). The wild plant pathosystem is a self-organising, complex, adaptive system in which people have not interfered (Robinson, 1996). The pathosystem is very specific, and it is regulated by three main components – the host plants, the pathogen and the environmental conditions. In the wild plant pathosystems both the host and the parasite populations exhibit great genetic diversity, while in crop pathosystems the host population normally exhibits high genetic uniformity and in the parasite population the low genetic diversity is commonly assumed. These differences are due to the people activities (Robinson, 1996). Therefore, the wild

pathosystems may be much more flexible in responses to environmental changes than crop pathosystems.

Examples of plant pathogens, diseases and pests, which attack the *Lactuca* spp. are summarized in Lebeda et al. (2014, 2015).

Bremia lactucae Regel is one of the highly variable oomycete pathogen (from the order Peronosporales) with global distribution. It causes an exponentially spreading disease downy mildew of cultivated lettuce (*Lactuca sativa* L.) and many other species from the family Asteraceae (*Sonchus*, *Cirsium*, *Arctium*…) (Crute and Dixon, 1981; Lebeda et al., 2002; Thines et al., 2010; Choi et al., 2011).

The asexual spore germinates directly rather than via zoospores that are used by most other members of *Peronosporaceae* and the interaction between plant and *B. lactucae* begins with penetration through the plant cuticle and epidermal cells. Its biotrophic mode of nutrition involves a close interaction with its host, in which the plant plasmalemma is invaginated around simply lobed haustoria (Michelmore and Wong, 2008). *Lactuca sativa* (lettuce) can be infected by this patogen at any developmental stage, from young seedlings to mature plants. Infected plants develop yellow to pale green lesions that eventually become necrotic due to secondary pathogens following the breakdown of the biotrophic interaction (Simko et al., 2013). *B. lactucae* is predominantly heterothalic, and the sexual reproduction has an important role in genetic recombination (Michelmore, 1981) and is considered to be a major source of virulence variation (Crute, 1992b, Lebeda and Schwinn, 1994).

The control of lettuce downy mildew is possible by either chemical protection or by genetic mechanism of resistance. The fungicide protection often becomes ineffective (Brown et al., 2004; Michelmore and Wong, 2008) and breeding race-specific resistance is problematic due to extremely high variability (Lebeda et al., 2002, 2008; Lebeda and Zinkernagel, 2003; Sharaf et al., 2007) as well as adaptability of the pathogen populations to resistance newly introduced to crop plants (Lebeda and Zinkernagel, 2003).

In the most interactions, the resistance of *Lactuca* sp. to *B. lactucae* is considered as a host-resistance (basin compatibility), according to the phenotypic, tissue and cellular expression (Lebeda et al., 2007). Host resistance (basic compatibility) is a better known phenomenon in this pathosystem because it has been studied from many perspectives since 20 century. The most common three categories of host resistance are reviewed

below, i.e. race-specific resistance, race non-specific resistance and field resistance (Lebeda et al., 2001b, 2002).

Race-specific (differential/vertical) resistance is currently the most intensively studied type of resistance. The interaction between *Lactuca* sp. and *B. lactucae* generally conforms to a gene-for-gene (GFG) relationship (Crute, 1992a,b), in which the resistance is determinated by dominant *Dm* resistance genes (or R-factors) in the hosts, matched by dominant avirulence factors (Avr genes) in the pathogens (Hammond-Kosack and Jones, 1997). The infected plant can exhibit susceptibility or resistance to the pathogen, the results of interaction are dependent on genotypes of both participants (Lebeda and Zinkernagel, 2003). The plant fitted with race-specific resistance show, incompatibility reaction in contact with microorganism. The resistance is limited only to the specific phenotypes of pathogen (race) (Lebeda, 1989; Burdon et al., 1996). The Dm resistance genes provide a high levels of resistance, however, they are effective only temporarily until new virulence genes (v-factors) occur within the pathogen population. The permanent control of lettuce downy mildew therefore requires a continuous supply of new resistance genes (Lebeda et al., 2007) and a great breeding effort is currently focused on introgressing new genes from wild species in response to pathogen changes. This type of resistance is well documented also in wild *Lactuca* spp. (*L. serriola*, *L. saligna*, *L. virosa*) and a few closely related genera (Lebeda et al., 2002). Over fifty resistance genes and factors (*Dm* or *R* factors) have been identified in lettuce (Michelmore et al., 2009; Michelmore and Wong, 2008; Parra et al., 2016b). Wild *Lactuca* species are considered as important and promising sources of these resistance genes (Lebeda et al., 2002, 2007, 2009; Lebeda and Zinkernagel, 2003; Jeuken and Lindhout, 2004; Beharav et al., 2014; Jemelková et al., 2015). A current lettuce breeding program must be focussed on searching for and utilising novel and more durable sources of resistance to *B. lactucae*.

Race-non-specific (non-differential/horizontal) resistance is controlled by many genes (minor gene resistance, polygenic resistance) and characterized by effectiveness against a spectrum of *B. lactucae* races. *Lactuca* spp. genotypes with this type of resistance posses a certain level of non-specific resistance according to phenotypic expression (Lebeda et al., 2002). The presence of race non-specific resistance is not well-

documented for *L. sativa* (Lebeda et al., 2001b). It has been reported in some accessions of *L. serriola* (PI 281876 and PI 281877) for which the genetic background is not well known, and the presence of some major genes and modifiers is predicted (Lebeda et al., 2002). And also in accession of *L. saligna* (CGN05271), where is tested the complete non-host resistance due to the cumulative and additive effects between several quantitative resistance genes (QTLs) (den Boer et al., 2014, Lebeda et al., 2016).

Field resistance is a complex epidemiological phenomenon (Lebeda et al., 2002), expressed by reduced susceptibility of mature plants grown in the field with natural infections of *B. lactucae*. Recent studies suggested simple inheritance of this trait, but the single gene models did not fit the data obtained (Grube and Ochoa, 2005). The field resistance exhibit some lettuce varieties such as Iceberg and Grand Rapids. This resistance trait, which is manifested in adult stages of development, has shown to be quantitatively inherited and using the marker-assisted gene pyramiding of multiple Dm genes in combination with QTLs for field resistance provides the opportunity for more durable resistance to *B. lactucae* (Parra et al., 2016a). Field resistance is also expected in wild *Lactuca* spp., with direct evidence existing for some *L. serriola* accessions (e.g. PI 281876; Lebeda et al., 2002).

2.4. Genetic diversity in plants

Genetic diversity is usually defined as the amount of genetic variability among individuals of a variety, or population of a species (Brown, 1983). It results from the many genetic differences between individuals and may be manifest in differences in DNA sequence, in biochemical characteristics (e.g. in protein structure or isoenzyme properties), in physiological properties (e.g. abiotic stress resistance or growth rate) or in morphological characters such as flower colour or plant form. Four components of genetic diversity can be distinguished; the number of different forms (alleles) ultimately found in different populations, their distribution, the effect they have on performance and the overall distinctness among different populations. The variation, that supports genetic diversity arises from mutation and recombination. Selection, genetic drift and gene flow

act on the alleles present in different populations to cause variation in the diversity in them. The selection can be natural or artificial. Much of the variation occurs in crop species (Frankel, 1977; Nevo et al., 1984; Brown, 1988; Hamrick et al., 1992). It is generally accepted that the genetic variation in plant populations is structured in space and time (Loveless and Hamrick, 1984). The major factors, which probably affect the genetic structure of plant populations include climatic, edaphic and biotic factors as well as those specific to the populations (e.g. population size, selection), or to the species (e.g. ploidy, breeding system, linkage) (Rao and Hodgkin, 2002).

Genetic diversity is the basis for survival and adaptation and makes it possible to continue and advance the adaptive processes on which evolutionary success and, to some extent human survival, depends. The process of extinction can be due to biotic or abiotic stresses, caused by factors such as competition, predation, parasitism and disease, or to isolation and habitat alteration due to slow geological and climatic change, natural catastrophes or human activities. Given these threats, it is essential to understand properly the genetic diversity in plant genetic resources and to conserve and use it efficiently (Rao and Hodgkin, 2002).

Molecular methods have become an essential part of most studies on genetic diversity extend and distribution and in the analyses of breeding system, bottlenecks and other key features affecting genetic diversity patterns. However, it is important to understand that different markers have different properties and will reflect different aspects of genetic diversity (Karp and Edwards, 1995). Comparative studies of various marker systems are needed to determine the relative merits of the different approaches for diverse crops, wild species or situations, in order to permit researchers to make appropriate choice of methodology. In general, there remains a need to develop improved methodologies (assessment of genetic diversity on morphological, biochemical and molecular level) for studying and sampling genetic diversity in populations (Hodgkin et al., 2001, Mondini et al., 2009).

Thus, it is likely that molecular methods (e.g. AFLPs, SSRs, ...) are most useful for evaluation of genetic diversity, for estimating a gene flow, genetic drift and degree of outbreeding. Thus, information generated using different PCR-based molecular markers can provide valuable information on a number of practical issues of germplasm management, including the classification of accessions by known allelic constitution,

detection of redundancy in collections or the detection of genes influencing economically important traits.

2.5. Molecular markers used in study of diversity wild *Lactuca* spp. population

Since the 1980s, molecular markers became frequently used in plant genetic studies and breeding programs, thus shifting the orientation from phenotype-based genetics to genotype-based approaches. Also, the molecular markers have been utilized for a variety of applications including examination of the genetic relationships between individuals, mapping of useful genes, construction of linkage maps, marker assisted selections and backcrosses, population genetics and phylogenetic studies (Simko, 2009; Kalia et al., 2011)

Several types of markers are regularly used for cultivar fingerprinting, linkage map construction, mapping alleles for desirable traits, marker-assisted selection, and assessment of population structure (Simko, 2009). The molecular markers are useful tools for assaying genetic variation and provide an efficient means to link phenotypic and genotypic variation (Varshney et al., 2005). In the recent years, the progress made in the development of DNA based marker systems has advanced our understanding of genetic resources. These molecular markers are classified as: (a) non PCR-based i.e. restriction fragment length polymorphism (RFLP), (b) PCR-based i.e. random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter simple sequence repeats (ISSR) and microsatellites or simple sequence repeats (SSR), sequence specific PCR-based markers i.e. expressed sequence tag based SSR (EST-SSR), (c) single nucleotide polymorphism (SNP), (d) markers based on other DNA than genomic DNA i.e. chloroplast (cpDNA) and mitochondrial (mtDNA) microsatellites, PCR sequencing approaches. (Varshney et al., 2007; Sehgal and Raina, 2008; Mondini et al., 2009; Rauscher and Simko, 2013).

Microsatellites, also called Simple Sequence Repeats (SSRs), are short, tandemly repeated motifs of one to six nucleotide long DNA motifs, generally distributed in all prokaryotic and eucaryotic genomes (Zane et al., 2002). The existence of microsatellites was demostrated by Hamada et al. (1982) in various eukaryotes ranging from yeasts to vertebrates. Subsequent studies by Delseny et al. (1983) and Tautz and Renz (1984) confirmed the abundance of microsatellites in plants and in many eukaryotes. Plants are rich in AT repeats, whereas in animals AC repeats is the most common. This appears to be the general feature distinguishing plant and animal genomes (Powell et al., 1996). SSRs are present in both coding and noncoding regions and are distributed throughout the nuclear genome. These can also found in the chloroplastic (Provan et al., 2001; Chung et al., 2006) and mitochondrial (Soranzo et al., 1999; Rajendrakumar et al., 2007) genomes.

SSR-based molecular markers are frequently used in plant genetics due to their high reproducibility, codominant inheritance, and high information content (Simko, 2009). The high polymorphism is due to different number of repeats in the microsatellite regions, therefore they can be easily and reproducibly detected by polymerase chain reaction (PCR) (Kalia et al., 2011). These markers are amenable to high throughput genotyping and have proven to be an extremely valuable tool for paternity analysis, construction of high density genome maps, mapping of useful genes, marker assisted selection, and for establishing genetic and evolutionary relationships (Parida et al., 2009).

Amplified fragment length polymorphism (AFLP) is a DNA fingerprinting technique developed by Zabeau and Vos (1993). AFLPs are polymerase chain reaction (PCR) based markers for the rapid screening of genetic diversity. This technique is based on the detection of genomic restriction fragments by PCR amplification, and can be used for DNAs of any origin or complexity. Fingerprints are produced without prior sequence knowledge using a limited set of generic primers. The number of fragments detected in a single reaction can be 'tuned' by selection of specific primer sets (Vos et al., 1995). AFLP involves the restriction of genomic DNA, followed by ligation of adaptors complementary to the restriction sites and selective PCR amplification of a subset of the adapted restriction fragments. These fragments are viewed on denaturing polyacrylamide gels either through autoradiographic or fluorescence methodologies (Vos et al., 1995;

Jones et al., 1997). AFLP technique has become the ideal means in situations where there is neither an a priori-sequence information nor suitable established markers (such as microsatellites) (Meud and Clarke, 2007). The main advantage of AFLP is that large numbers of genetic markers can be typed relatively quickly and effectively at low cost (Evanno et al., 2005, Nicolè et al., 2007). The disadvantages of AFLP are its dominant (heterozygotes cannot be distinguished from dominant homozygotes) and biallelic character (for a given size, the fragment is either present or absent) and homoplasy of bands (Vekemans et al., 2002; Meud and Clarke, 2007; Paris et al., 2010).

The studies related to use of molecular markers (AFLP, SSR) in the *Lactuca* spp. germplasm collections have been reviewed by Dziechciarková et al. (2004) and Lebeda et al. (2014). During the last three years, the AFLPs and SSRs methods have been used in study of genetic diversity of *L. aculeata* populations from the Near East (Jemelková et al., 2015) and in study of population structure of three predominantly self-pollinating wild *Lactuca* species (*L. serriola*, *L. saligna* and *L. aculeata*) collected from Israel (Kitner et al., 2015). These publications are part of this Ph.D. thesis. The microsatellites were also used in D'Andrea et al. (2017), where, together with chloroplast RFLP-markers, evaluated interpopulation diversity and the recent range expansion process of *L. serriola* in Europe. Beside these methods, Sevindik et al. (2016), in phylogenetic study of Turkish *Lactuca* species, used sequence analysis of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA), or chloroplast *trn*L-F (cpDNA) region. One of the most recent title, which deals with assessment of genetic variability, population structure and relationships of *Lactuca* L. species using the isozyme analysis is El-Esawi (2017).

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3. AIMS OF THE PH.D. THESIS

The main aims of the Ph.D. study are summarized in the following points:

1. Process available literature relating to the topic;

2. Testing resistance with inoculation tests under laboratory conditions and evaluation a variability of the resistance of the wild *Lactuca* genetic resources to lettuce downy mildew (*Bremia lactucae*);

3. The analysis of a genetic variability of the *Lactuca* species using microsatellite and AFLP markers;

4. Genetic resources of wild *Lactuca* L. species and their exploitation in lettuce breeding – critical analysis.

4. RESULTS

4.1. Genetic and morphological variability of *Lactuca serriola* populations

4.2. Biodiversity of *Lactuca aculeata* germplasm including the resistance variation to *Bremia lactucae*

4.3. Genetic and morphological variability of the wild *Lactuca* species in natural populations in Israel

4.4. Wild *Lactuca* genetic resources – summary of the intensive research

4.1. Genetic and morphological variability of *Lactuca serriola* populations

4.1.1. Genetic variability and distance between *Lactuca serriola* L. populations from Sweden and Slovenia assessed by SSR and AFLP markers

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Genetic variability and distance between *Lactuca serriola* L. populations from Sweden and Slovenia assessed by SSR and AFLP markers

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Running title: Genetic variability of *L. serriola* populations

Abstract

The study involved 121 samples of the common weed, *Lactuca serriola* L. (prickly lettuce), representing 53 populations from Sweden and Slovenia. The seed materials, originating from different habitats, were regenerated and taxonomically validated at the Department of Botany, Palacký University in Olomouc, Czech Republic. The morphological characterizations of the collected plant materials classified all 121 samples as *L. serriola* f. *serriola*; one sample was heterogeneous, and also present was *L. serriola* f. *integrifolia*. Differences in the amount and distribution of the genetic variations between the two regions were analyzed using 257 amplified fragment length polymorphism (AFLP) and 7 microsatellite (SSRs) markers. Bayesian clustering and Neighbor-Network were used for visualization of the differences among the samples by country. Under the Bayesian approach, the best partitioning (according to the most frequent signals) was resolved into three groups. While the absence of an admixture or low admixture was detected in the Slovenian samples, and the majority of the Swedish samples, a significant admixture was detected in the profiles of five Swedish samples collected near Malmö, which bore unique morphological features of their rosette leaves. The Neighbor-Network divided samples into 6 groups, each consisting of samples coming from a particular country. Reflection of morphology and eco-geographical conditions in genetic variation is also discussed.

Keywords: Biogeography, Dinaric Alps and the Pannonian Plain, DNA polymorphism, Ecology, Habitats, Morphological variation, Prickly lettuce, Scandinavia

Introduction

Prickly lettuce (*Lactuca serriola* L., Asteraceae) is the most common species in the genus *Lactuca* L. (Feráková 1977), and has a circumglobal distribution (Lebeda et al. 2004). It is an annual or winter-annual therophyte (Feráková 1977), and an ‘r’strategist (Tilman 1988). Its evolution has trended towards a short life cycle, strong self-fertilization

ability, good adaptation for wind dispersal, and quick germination (Frietema de Vries 1992; Lebeda et al. 2001). *L. serriola* is a drought-tolerant species (Werk and Ehleringer 1986), mainly growing in sunny microhabitats within anthropogenic habitats such as roadsides, railways, dumps, and urban areas (Feráková 1977; Lebeda et al. 2001, 2004); it is considered a good colonist of a wide spectrum of different habitats with different degrees of invasivity. Prickly lettuce is of Euro-Asian origin, also being native in North Africa (Feráková 1977). It has primarily spread in the Mediterranean and the Near East (de Vries 1997; Lebeda et al. 2007a), and is considered an archaeophyte dependent on a culture from the northern part of central Europe (Meusel and Jäger 1992). The species belongs to a group of Mediterranean ruderal plants that have enlarged their distribution area during the last few centuries (Landolt 2001).

The northern boundary of the European distribution area runs near latitude 65 °N through Finland, and 55 °N through Great Britain (Feráková 1977). The expanding distribution of this species is accomplished with the transport of reproductive propagules, the achenes. The ripened achenes with attached pappus are primarily dispersed by the wind, probably also by water (Weaver and Downs 2003). The spread of this species is also closely related with human activities, which primarily produce an increase in their transport (Lebeda et al. 2001). Prickly lettuce has drastically increased its geographical range, invading many European, (North-) American, and Australian regions during the last 50-60 years (de Vries 1996; Lebeda et al. 2001, 2004); recently *L. serriola* has spread as an invasive weed throughout Europe (Lebeda et al. 2004, 2007b; D'Andrea et al. 2009), including Scandinavia (Rydberg 2013). Its synanthropic distribution has also been recorded from Australia, including Tasmania and New Zealand (Burbinge and Gray 1970; Webb et al. 1988), as well as Taiwan (Wang and Chen 2010), North America, southern Africa, and Argentina (Strausbaugh and Core 1978; Zohary 1991; Zuloaga and Morrone 1999). The study by Alexander (2010) supported a genetic basis for the differences in the elevation limits of *L. serriola* populations between two parts of its native and introduced ranges.

Two primary morphological forms are recognized within *L. serriola* L. based on cauline leaf-shape variability; the pinnatifid-leaved form *L. serriola* L. f. *serriola*, and the unlobed-leaved form *L. serriola* L. f. *integrifolia* (S.F. Gray) S.D. Prince et R. N. Carter. The *serriola* form is recorded as the most frequent species, occurring at a very high density in Europe; the form *integrifolia* is not so common, and has been recorded in e.g., Switzerland, Italy, France, western Germany, the Netherlands, and is prevalent in the UK (Lebeda et al. 2001, 2004, 2007a,b).

Lactuca serriola is the best known wild species of the genus *Lactuca*, the geographic distribution, morphological, and phenological variations of which have been intensively studied (Lebeda et al. 2004, 2007a; Alexander 2010). *L. serriola* is also an important genetic resource for new resistance to diseases and pests (Lebeda et al. 2014), abiotic factors, as well as for genes responsible for physiological and quality characters (Lebeda et al. 2007a). Prickly lettuce has been used in commercial lettuce breeding for more than 80 years (Lebeda et al. 2007a), especially as a source of race-specific resistance genes against lettuce downy mildew (*Bremia lactucae* Regel) (Parra et al. 2016). It has also been used over the last decade in various molecular studies to characterize genetic variation and diversity in both germplasm collections and natural populations (e.g. Koopman et al. 2001; Kitner et al. 2008, 2015).

The most commonly used methods for the analysis of DNA polymorphism include amplified fragment length polymorphism (AFLP; Vos et al. 1995), and microsatellites (simple sequence repeats, SSRs); Simko (2009) contributed significantly to the development of these for the genus *Lactuca*, and in particular for *L. serriola* Riar et al. (2011). These markers have been successfully applied in *Lactuca* research, addressing e.g., the distribution of genetic variation of prickly lettuce across Europe (Lebeda et al. 2009a), distribution of genetic variation in natural populations of *L. serriola*, *L. saligna*, and *L. aculeata* in Israel (Kitner et al. 2015), or analyses of gene flow from crops to their wild relatives (Uwimana et al. 2012).

Southern and central Sweden is the northern limit of *L. serriola* distribution in Europe; Slovenia represents an area between the Central European and Mediterranean / Balkan distributions (Feráková 1977). Both areas differ in their climatic, ecogeographic, and ecologic conditions. In Slovenia prickly lettuce is distributed throughout the entire territory, from the lowlands to the mountain regions (Martinčič and Sušnik 1984), and it most often grows in association with *Stellarietea mediae* - annual weed communities species (Šilc and Košir 2006). In Sweden, *L. serriola* populations are found in southeastern, and mostly grow on surfaces and among stones in dry and sunny exposures (Doležalová et al. 2001).

The genetic structure of populations represented by prickly lettuce plants growing at a specific time in a particular site could emerge in at least four different ways: i) achenes can survive in a soil seed bank for 1 to 3 years (Marks and Prince 1982); at the moment of soil disturbation, the seeds can germinate, and these plants bear/represent "old" genotypes for a given population; ii) plants can grow from achenes newly transported to a particular locality by wind, humans, or other transport mechanisms, with such plants bearing "new" genotypes; iii) plants can grow on permanently disturbed soil from generation to generation, and such plants represent a "modified" genotype resulting from continuous evolution under local conditions; iv) "hybrid" plants may appear after natural hybridization between different plant species within the genus *Lactuca*.

The main purpose of this research was to describe the differences in genetic variability and population genetic structures between populations of prickly lettuce (*Lactuca serriola*) coming from two different and distant biogeographic areas of the species distribution in Europe.

Materials and methods

Plant materials

A set of 121 samples of *L. serriola* L. plants, representing 53 populations, was collected by the authors in Sweden (47 samples) and Slovenia (74 samples) during 2000 (Doležalová et al. 2001). The collected seed samples were regenerated in a greenhouse at the Department of Botany (Palacký University in Olomouc, Czech Republic). During regeneration, the plants were described morphologically according to Doležalová et al. (2002), and the taxonomic status of each sample was verified (Feráková 1977; Doležalová et al. 2002). From each plant two mature leaves were used for DNA extraction (i.e., 121 samples). Data from the individual samples are provided in On-line Suppl. Tab. 1., with the geographic positions of the collection sites given in Fig. 1.

DNA extraction, SSR, and AFLP analyses

Total genomic DNA was extracted from 100 mg of fresh leaf tissue using the CTAB method (Kump and Javornik 1996), with minor modifications. After DNA extraction, the quality of the DNA was inspected by 1.5% agarose gel electrophoresis, and the concentration measured on a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Delaware, USA).

For microsatellite genotyping, seven EST-SSR loci were used: SML-002, SML-019, SML-045, SML-055 (Simko 2009), as well as WSULs-18, WSULs-75, and WSULs-163 (Riar et al. 2011). The primer pairs were selected according to their high diversity indices in previously published papers (Simko 2009; Riar et al. 2011); however, randomly without any previous knowledge of their chromosome positions. Amplification of the SSRs was performed according to Jemelková et al. (2015). The length of the SSR allele was scored based on their migration relative to the molecular weight size markers 30-330bp AFLP® DNA ladder (Invitrogen, Carlsbad, California, USA). The AFLP analyses were carried out according to the protocol of Vos et al. (1995), with modifications, and the AFLP fragment detection according to Kitner et al. (2008, 2012). Five selective primer combinations, with two to three selective nucleotides, were chosen to generate the AFLP profiles (Table 2).

The PCR products were separating on a 6%, 0.4 mm thick denaturating polyacrylamide gel using a T-REX sequencing gel electrophoresis apparatus (Thermo Scientific Owl Separation Systems, Rochester, NY, USA).

Data scoring

Microsatellite profiles were scored based on the length of the PCR product. The allele frequencies, proportions of polymorphic loci (*P%*), number of private alleles (*PA*), observed and expected heterozygosity (H_O and H_E) were all performed using GENALEX 6 software (Peakall and Smouse 2012). The mean number of alleles per locus (*A*) was calculated manually. The relative discriminatory value of each microsatellite locus was estimated by the polymorphic information content (*PIC*), which measures the information content as a function of a marker system´s ability to distinguish between genotypes (Powell et al. 1996). The number of different genotypes (N_G), number of samples with a heterozygous constitution (N_{HET}), and maximal number of heterozygous loci (N_{HETmax}) were calculated manually.

AFLP profiles were checked visually, and only clear and unambiguous bands were scored for their presence (1) or absence (0) across all samples. For AFLP data, the number of private bands (*PA*), the proportion of polymorphic loci (*P%*) and gene diversity (H_E) were calculated using GENALEX 6 software (Peakall and Smouse 2012).

To evaluate the population genetic structure, a Bayesian clustering approach was used as implemented in STRUCTURE 2.3.4 (Falush et al. 2007). STRUCTURE attempts to assign individuals to clusters/groups/populations on the basis of their genotypes, while simultaneously estimating population allele frequencies. This allows one to compute the likelihood of a given genotype having originated in a predefined number (*K*) of clusters. In the simplest, 'no-admixture' model, it assumes that each individual belongs to a single cluster. In the more general 'admixture model' it estimates admixture proportions for each individual, allowing one to identify admixed individuals represented by a proportional mixture of two or more signals characteristic for the various clusters. In our analyses, SSR co-dominant data were transferred into binary data based on the presence/absence

of a particular allele, and merged with the AFLP binary data; the samples were then ordered according to the increasing latitude of the sampling site within a particular country. An admixture model was used, with correlated allele frequencies. *K* was set at 1-10, and the highest *K* value was identified as the run with the highest likelihood value, as recommended by Pritchard et al. (2000). In addition, *K* values were averaged across 10 replicate runs for each *K* (100.000 burn-in iteration followed by 1.000.000 MCMC iterations). For the graphical interpretation of clustering for the appropriate *K*, STRUCTURE HARVESTER (Earl and von Holdt 2012), CLUMPP (Jacobsson and Rosenberg 2007), and DISTRUCT (Rosenberg 2004) software were used. The optimal *K* value was selected according to Evanno et al. (2005), who suggested the use of the ΔK value for identifying the correct number of clusters.

To visualize the genetic relationships within and among the analyzed samples, a Neighbor-Network based on Dice´s similarity coefficient (*D*) was constructed in SPLITSTREE 4 (Huson and Bryant 2006). The Nexus input file for SPLITSTREE 4 was exported from GENALEX. Also, for this purpose, the SSR data were transformed into a binary matrix and merged with the AFLP binary data. The reliability and robustness of the network were tested by bootstrap analysis with 1.000 bootstrap replicates.

Results

Taxonomic verification of *L. serriola*

For all 121 plants, the taxonomic status of *Lactuca serriola* f. *serriola* according to Feráková (1977) was confirmed. Moreover, in one sample (no. 205_00, Bostahusen, Sweden) the plants were morphologically heterogeneous; with divided stem leaves belonging to *L. serriola* f. *serriola*, plants with entire stem leaves that ranged toward *L. serriola* f. *integrifolia*. In our analyses, this sample was split into two subsamples 205_00A (f. *serriola*) and 205_00B (f. *integrifolia*) and treated (analyzed) separately.

Genetic polymorphism

The seven polymorphic SSR loci produced a total of 32 alleles across the 121 individual *L. serriola* plants. The number of alleles per locus ranged from 2 to 7, with an average of 4.57 alleles per locus (Table 1). The allele sizes varied from 161 to 240bp. The mean *PIC* per SSR polymorphic allele was 0.762, within a range of 0.494 to 1.072. Null alleles only appeared in two accessions from Slovenia (13_00 and 22_00) at the locus SML-055.

Private alleles (*PA*) were present within both sampled regions (Table 3). The *L. serriola* samples from Sweden possessed 5 unique alleles: 193bp, 204bp, and 207bp for locus SML-002, 221bp for locus SML-055 (i.e., $221bp^{SML\text{-}055}$), and $188bp^{WSULs\text{-}163}$. The samples from Slovenia possessed eight unique alleles: 172bp, 198bp for locus SML-002, $238bp^{SML\text{-}045}$, $228bp^{SML\text{-}055}$; 217bp and 235bp for locus WSULs-18, and lastly 183bp and 195bp for locus WSULs-163.

The observed and expected heterozygosity (H_O and H_E) ranged from 0.036 to 0.054 (mean 0.045), and from 0.341 to 0.432 (mean 0.387), respectively. The proportion of polymorphic loci (*P%*) was higher in Slovenian samples (84.4%) compared with the Swedish ones (75%). Based on SSR data, a total of 51 different genotypes (N_G) were recognized (Sweden = 17; Slovenia = 34) (On-line Suppl. Tab. 2,3). Genotype G3 was the most common in the samples from Sweden (36.2%), while genotype G29 represented

32.4% of the Slovenian samples (On-line Suppl. Tab. 2,3). We recorded 17 Slovenian samples having at least one heterozygous locus (N_{HET} = 17), in contrast to eight samples from Sweden (On-line Suppl. Tab. 2,3). Three samples from Slovenia and one sample from Sweden bore the maximum number of heterozygous loci (N_{HETmax} = 3) observed from among all analyzed samples.

In total, five primer combinations, with two to three selective bases, were applied for AFLP genotyping (Table 2), resulting in 257 unambiguously scored fragments. Detailed overall statistics calculated for each primer combination used are presented in Table 2. The number of private bands (*PA*) ranged from 19 (Slovenian samples) to 20 (Swedish samples). The expected heterozygosity (H_E) ranged from 0.130 to 0.149 (mean H_E = 0.140) (Table 3), and the proportion of polymorphic loci (*P%*) in the *L. serriola* samples ranged from 44.8% (Swedish population) to 52.9% (Slovenian population). The genetic variability indices for all populations are summarized in Table 3.

Cluster analysis of molecular data

Based on seven microsatellite and 257 AFLP markers, Bayesian clustering and construction of a Neighbor-Network were used for visualization of the putative relationships among the analyzed individuals. Under the Bayesian approach implemented in STRUCTURE, the best partition into three clusters (K = 3, Fig. 2) was resolved (ΔK = 214.73; St. dev. LnP(K) = 6.07), which are represented by the green (G-cluster), red (R-cluster), and blue (B-cluster) color signals in Figure 2. In general, a relatively low admixture was detected in the Slovenian samples, being clearly identified as genotypes from the G- or B-cluster. While the B-cluster can be considered as characteristic for *L. serriola* genotypes from the southern part of Central Europe and the northern Balkans (representing ca. 1/3 of the Slovenian samples), the G-cluster represents the genotype largely dispersed across Europe, contributing significantly to the genotypic composition of the Swedish populations. The signal characteristic for genotypes from the R-cluster was nearly absent in the Slovenian samples, but was recorded in each sample from Sweden; and 48.9% of the Swedish samples fell into the R-cluster with no admixture signal (Fig. 2). For 19 samples, the signal from the R-cluster contributes up to 30% of a particular genotype, and is accompanied with an admixture of the G signal, which prevails in the Slovenian samples (Fig. 2). Further, we observed a nearly equal admixture of signals from all three clusters in five samples collected in southern Sweden near Malmö.

The Neighbor-Network divided the analyzed samples into 6 groups (A-F; Fig. 3), each consisting of samples coming from a separate country. The results fit to the results of the Bayesian clustering in terms of assigning individuals from a separate country to the revealed clusters (R-, G-, B-). The samples from Sweden were placed into the A, C, and D groups. While individuals placed in Group C represent the genotype from the R-cluster, Group D is formed by samples with the G-cluster prevailing. Finally, Group A is formed by five samples 215_00, 217_00, 218_00, 219_00, and 220_00, having a strong admixture signal from all three STRUCTURE clusters. These samples represent populations no. 16 and 17 from collecting sites close to Malmö (On-line Suppl. Tab. 1). The samples from Slovenia were split into three groups: a majority of the samples fell in groups B and E, both representing the G-cluster in Fig. 2. Samples originating from Slovenian localities below 46°14′34′′ lat. fell into a separate Group F, which represents genotypes from a unique B-cluster (Fig. 2). It is interesting, that all three "G-cluster" groups from both countries are in the center of the Neighbor-Network, which resemble their characteristics

closely. On the other hand, Group C (SWE, R-cluster) and Group F (SLO, B-cluster) are placed on opposite sides of the network.

Discussion

Verification of the taxonomic status of the plants showed that *Lactuca serriola* f. *serriola* is predominant in both countries. In the entire territory of Slovenia only *L. serriola* f. *serriola* was recorded, which is in agreement with previous observations in Central Europe (Lebeda et al. 2001, 2004, 2007b). Within one sample from southern Sweden (Bostahusen, sample 205_00), apart from *L. serriola* f. *serriola* plants, there were plants identified as *L. serriola* f. *integrifolia*. All remaining samples from Sweden were represented only by *L. serriola* f. *serriola*. It is evident that both populations are very taxonomically homogeneous on the subspecific level. The very rare occurrence of *L. serriola* f. *integrifolia* in southern Sweden could be caused by the repeated introduction (e.g., through truck or ship transportation) of this form from the Netherlands or UK, where it is prevalent (Lebeda et al. 2007a,b). However, from our previous results (Doležalová et al. 2001) it is evident, that this variety is not spreading into northern Scandinavia, where the northern limit of the European distribution for this species is (Feráková 1977). These conclusions are supported by recent observations in Sweden made by Rydberg (2013). Also, in Norway only *L. serriola* f. *serriola* has been recorded (Lebeda 2013, unpubl. results).

The leaf shape (i.e., the division of the leaf blade), can be interpreted as an ecological adaptation of the plant to different factors, including a means of leaf thermoregulation in arid or hot environments, or in reaction to hydraulic constraints (Nicotra et al. 2011). Doležalová et al. (2009) also confirmed the differences in the morphology of rosette and cauline leaves of Swedish and Slovenian *L. serriola* samples. The cauline leaves of Swedish *L. serriola* plants were longer and wider; plants from Slovenia had longer and narrower rosette leaves (divided) (Doležalová et al. 2009). The width and length of cauline leaves (divided) correlate with the latitude, which could be explained as adaptations of the plants to drought. Drier areas of lower latitudes are increasingly represented by plants with smaller leaves. Regarding altitude, a negative correlation with the length and width of the leaves was found (Doležalová et al. 2009), which could mean they are adapting to ecologically worse conditions at higher elevations. The occurrence of *L. serriola* f. *integrifolia* in temperate areas without a dry season (but with a warm summer) in the UK, western part of Germany, Benelux, and France (Peel et al. 2007) supports the theory on the ecological adaptation of leaves presented by Nicotra et al. (2011). Areas in Sweden, where lettuce samples were collected, belong to the cold climate type, without a dry season or warm summer (Peel et al. 2007).

The higher phenotypic and genetic variability of the Slovenian samples can be explained by the more favorable climatic and ecological conditions in the country (see Peel et al. 2007). *L. serriola* is distributed throughout the entire country, and movement of diaspores among the surrounding countries is feasible (Lebeda et al. 2004). This is in opposition to Sweden, where the distribution is limited to the southern part alone (Doležalová et al. 2001), with very limited migration from the surrounding countries. In general, plant species occurring almost in and/or near the center of their diversity, with suitable environmental and ecological conditions, display more genetic/phenotypic variability. Conversely, at the edge of the distribution area, where less favorable

conditions exist, the selection prioritizes stable and well-adapted genotypes. Our results on genetic variability are in relationship to the general principles of diversity and allele distribution formulated by Vavilov (1950). Kuang et al. (2008) suggested that eastern Turkey and Armenia, along with the surrounding regions, might be the center of diversity of *L. serriola* (and possibly its center of origin). *L. serriola* might have spread from its center of origin first to the Mediterranean basin and then to Central and Western Europe after the glaciers retreated in the Upper-Pleistocene Holocene period (Kuang et al. 2008). Recent climatic changes and anthropogenic disturbances contributed substantially to the rapid spread of *L. serriola* into new areas (D´Andrea et al. 2009; Rydberg 2013), as well as increasing the genetic diversity of their populations in the central parts of their natural distribution areas (Lebeda et al. 2009a; van de Wiel et al. 2010; Kitner et al. 2015). This phenomenon was also clearly demonstrated in the genetic diversity of the Central European population of *L. serriola* (van de Wiel et al. 2010), as well as the resistance of the same population to *Bremia lactucae.* Whereas, the Czech Republic has the greatest diversity of resistance phenotypes, the lowest was recorded in the UK (Lebeda et al. 2008; Petrželová and Lebeda 2011).

The results of our study on genetic variability are in good agreement with the different climatic conditions in Sweden and Slovenia. From the viewpoint of genetic variation, the results have proven the existence of *L. serriola* genotypes characteristic for each country. These clearly differ from one another, as is evident from Bayesian clustering and Neighbor-Network, where the R-cluster characteristic for the Swedish samples (Group C), and the B-cluster (Group F) unique for Slovenian samples were distinguished (Figs. 2, 3). A number of samples from both countries were characterized by genotypes characteristic for the G-cluster, which might represent a common genotype resulting from the rapid spread of *L. serriola* in Central Europe (Lebeda et al. 2001, 2007b; D´Andrea et al. 2009). We have not recorded a prevailing microsatellite genotype for the samples representing this G-cluster, also no linkage to the latitude nor altitude of the sampled sites. The same phenomenon was described by Lebeda et al. (2009a), demonstrating that some *L. serriola* populations (e.g., Scandinavian, British, some Mediterranean) are quite isolated genetically from the heterogeneous Central and West European populations. Genetic analysis (PCR-RFLP and SSR markers) on 101 populations of *L. serriola* from seventeen countries of Western and Central Europe made by
D´Andrea et al. (2006) revealed a strong genetic differentiation between populations, and high inbreeding coefficients within populations. A clear geographical pattern of isolation by increasing distance was found; however, only a weak pattern of correlation between genetic diversity and geographical distance was found on the continental scale. The greatest amount of genetic diversity was characterized in Central Europe, while populations from the western Mediterranean (Spain and Portugal), southern Italy, Great Britain, the Alps, and southern Scandinavia generally possessed lower gene diversities (D´Andrea et al. 2006). Discrepancies were present in Scandinavia with some polymorphic populations, and a monomorphic one. Further, in a recent study, higher genetic variability in the Slovenian samples was observed in terms of the recorded genetic variability indices (Table 3) and the higher number of SSR genotypes ($^{SWE}N_G = 17/^{SLO}N_G = 34$) (On-line Suppl. Tab. 2,3). The level of genetic variation within and between populations can also result from intraspecific crossing. Although autogamy is the predominant breeding system within the genus *Lactuca* L., especially in the marginal

parts of the distribution area (Feráková 1977); in the center of the distribution, a higher occurrence of allogamy was estimated (Stebbins 1957). Lindquist (1960) proved experimentally that all species belonging to the "serriola" group were self-fertile. *L. serriola* is primarily a self-pollinated species; however, not only intermediate plants between the two *L. serriola* forms, but also interspecific hybrids of *L. serriola* can be detected in natural populations (Zohary 1990; Křístková et al. 2012). The main differences between the samples from Sweden and Slovenia can be characterized by the presence of genotypes characteristic for the R- or B-cluster, determined by Bayesian clustering (Fig. 2), with each unique (with a few exceptions) for a given country. The signal from the R-cluster was present in all Swedish samples and prevails in 48.9% of them. These samples formed Group C on the Neighbor-Network (Fig. 3), 65.2% of them represent the SSR genotype G3, with a completely homozygous character at all loci, and originating from localities at a higher latitude (On-line Suppl. Tab. 2).

A rather interesting characteristic of five *L. serriola* samples was found in a group of plants collected near Malmö. These samples forming Group A on the Neighbor-Network, are represented by a significant admixture signal on the Bayesian diagram, and also bore unique morphological features of their rosette leaves. The apical parts of the rosette leaves in samples 215_00, 217_00, 218_00, 219_00, and 220_00 were not divided, forming a long apex; the remaining two-thirds of the leaves were slightly divided (pinnately lobed). Surprisingly, specific DNA patterns fit better to specific phenotypes of the rosette leaves than to phenotypes of the cauline leaves. This is in contrast to the generally accepted view that morphological traits of the cauline leaves have a more significant taxonomic value than do the rosette leaves. The city of Malmö is an international harbor in the region, and it is possible to explain the exceptional phenotypic characteristics of these samples by the human-moderated introduction of non-indigenous genotypes into the southern parts of northern Europe, with subsequent natural hybridization with autochtonous *L. serriola* genotypes. The B-cluster in Slovenian samples showed, with a few exceptions, a continuity with samples from a lower latitude; 96% of these samples are represented by the completely homozygous microsatellite genotype G29 (On-line Suppl. Tab. 3).

This study provides interesting insights into the genetic variability of *L. serriola* populations originating from completely different eco-geographical areas. Specifically those from Slovenia, near the Mediterranean, one of the world diversity hotspots (Myers et al. 2000), the center of the greatest diversity of the genus *Lactuca* (Lebeda et al. 2009b); additionally, those from Sweden, a region at the northern border of *L. serriola* European distribution (Feráková 1977; Lebeda et al. 2004). This study showed, that *L. serriola* populations originating from various eco-geographical conditions differ significantly in their genetic background, which is also reflected in the geographic patterns of their phenotypic features. To obtain more comprehensive information on the genetic structure and variations of this species, it would be interesting to: i) analyze more populations with more individuals from Sweden, and for a comparative study ii) additional samples originating from areas with greater contrasting ecological conditions.

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Tab. 1. SSR loci used to assess genetic variability in *Lactuca sativa* L. and *L. serriola* L.

Marker	Reference	N_A	Allele size (bp)	PIC (%)
SML-002	Simko (2009)	6	168-207	0.594
SML-019	Simko (2009)	2	163-164	0.599
SML-045	Simko (2009)	4	229-238	0.838
SML-055	Simko (2009)	5	221-240	1.072
WSULs-18	Riar et al. (2011)	4	208-235	0.494
WSULs-75	Riar et al. (2011)	4	161-206	0.684
WSULs-163	Riar et al. (2011)	7	183-197	1.052

(N_A - number of alleles; PIC - allelic polymorphic information content)

Tab. 2. AFLP primer sets for amplification reactions with the total number of scored and polymorphic fragments.

Primer combination	N_F	N_{POL}	*PLP* (%)
E - AGC, M - CTG	45	37	82.2
E - AGC, M - CAAC	49	36	73.5
E - AGC, M - CAAT	72	54	75.0
E - ACC, M - CAAC	43	35	81.4
E - ACC, M - CAAT	48	30	62.5
Total	257	192	74.9
Mean	51.4	38.4	74.7

(N_F, total number of fragments; N_{POL}, number of polymorphic fragments; *PLP*, percentage of polymorphic fragments)

Tab. 3. Summary data based on 7 EST-SSR and 257 loci of 121 *L. serriola* samples from Sweden and Slovenia in recent study.

		Microsatellite data					**AFLP data**		
	N	PA_{SSR}	*A*	*P(%)*	*Ho*	*He (SE)*	PA_{AFLP}	*P(%)*	*He (SE)*
Sweden	47	5	3.42	75.0	0.036	0.341 (0.065)	20	44.8	0.130 (0.011)
Slovenia	74	8	3.86	84.4	0.054	0.432 (0.049)	19	52.9	0.149 (0.011)

(*N*, sample size; PA_{SSR}, private microsatellite alleles; PA_{AFLP}, private AFLP bands; *A*, mean number of alleles per locus; *P*, polymorphism; observed *Ho* and expected *He* heterozygosity; (SE) standard error

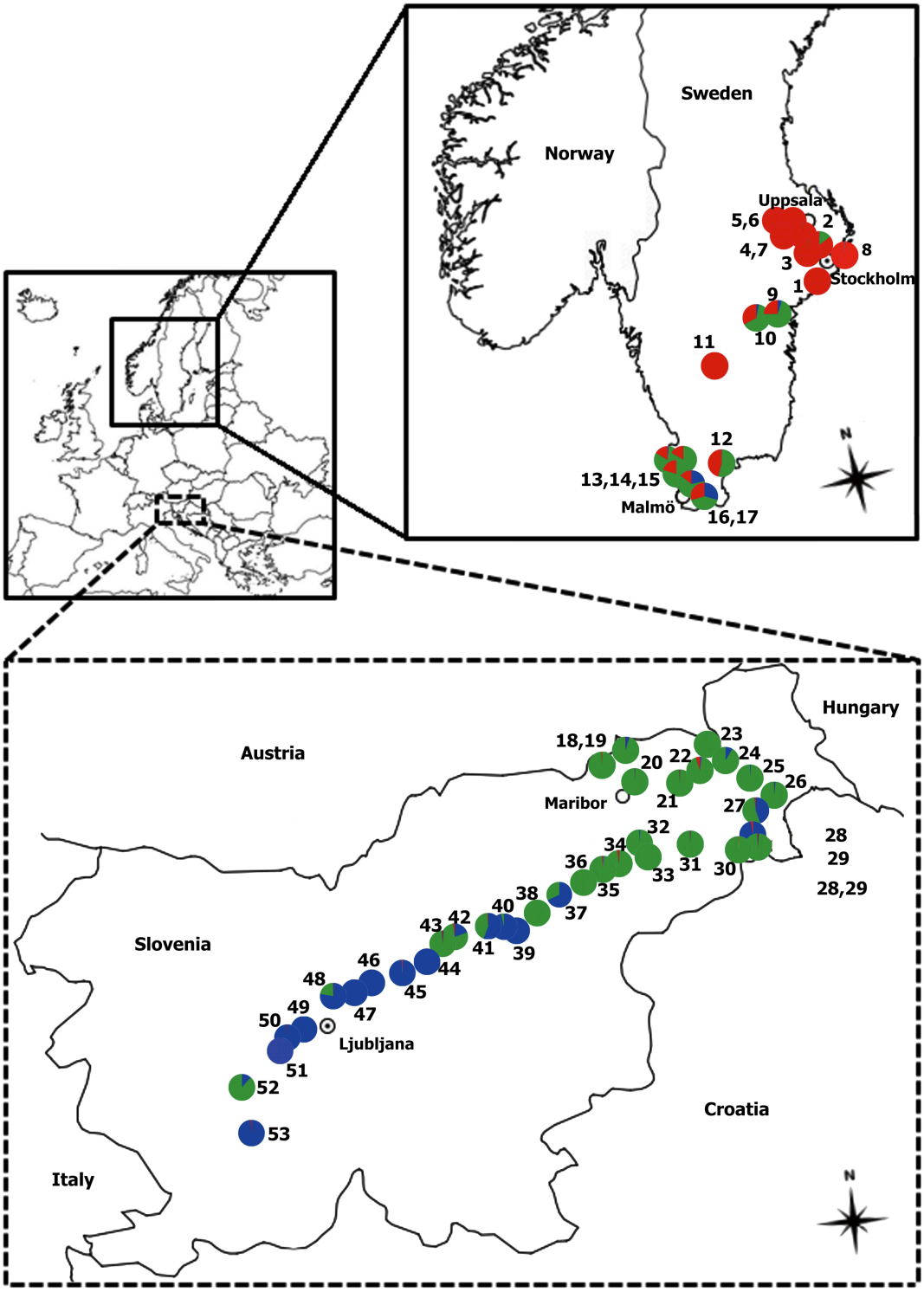

Fig. 1. Collecting sites of the 121 samples *Lactuca serriola* in Sweden and Slovenia. Colours of spots correspond to the results of Bayesian clustering (Fig. 2).

Fig. 2. Results of Bayesian clustering based on the SSR and AFLP data of 121 *L. serriola* samples from Sweden (SWE) and Slovenia (SLO), ordered according to the increasing latitude of the sampling site within a specific country.

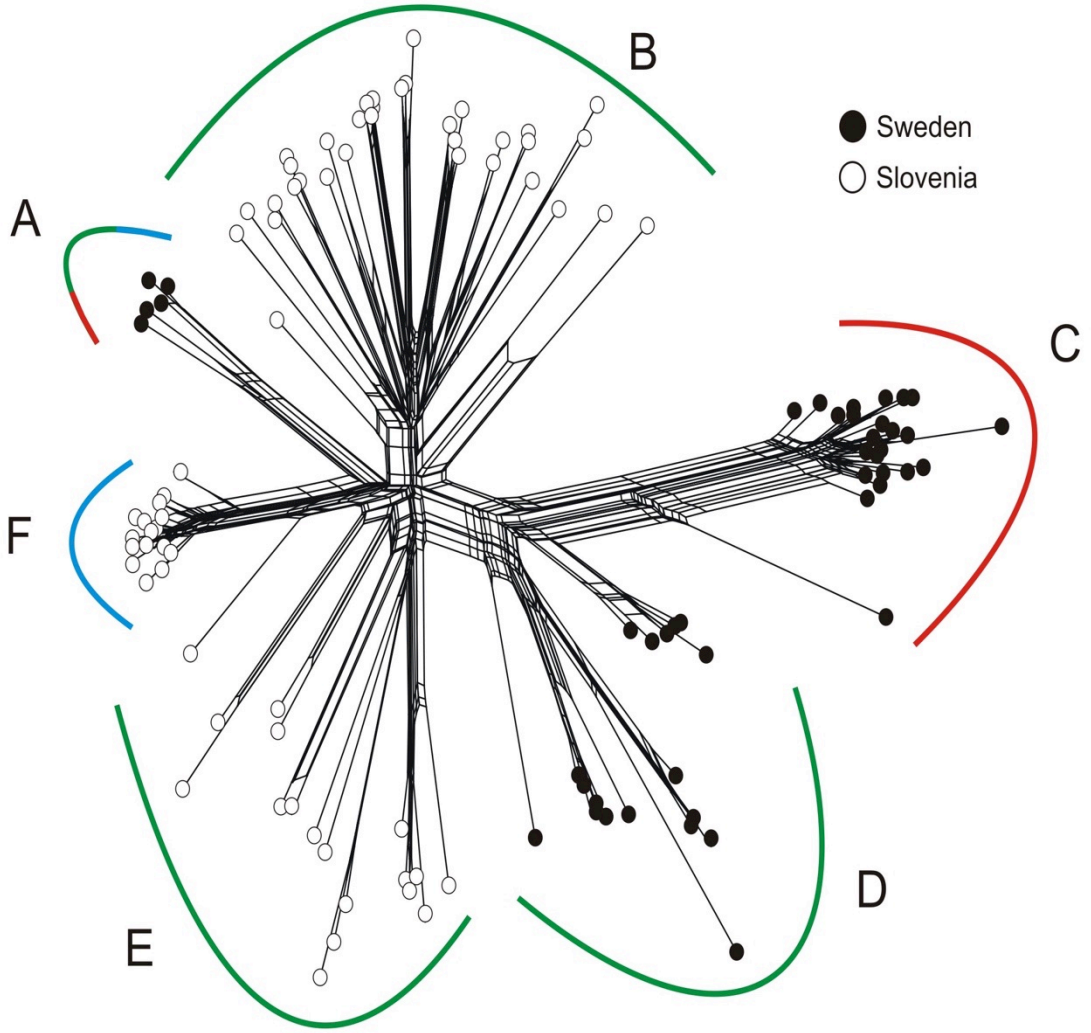

Fig. 3. Neighbor-Network cluster analysis of 121 samples *L. serriola* from Sweden and Slovenia, based on SSR and AFLP analysis. Resulting groups are highlighted by colouring which corresponds to the results of Bayesian clustering (Fig. 2).

Supporting Information File 1 Sampling details of 121 *L. serriola* samples.

Lactuca species	Country	Pop. No.	Sample No.	Locality	Latitude	Longitude	Altitude (m.a.sl.)
L. serriola	Sweden	1	171/00;172/00;173/00	Farstanäs	59°05'49''	17°38'59''	21
	Sweden	2	174/00;175/00;176/00	Stockholm	59°33'03''	17°54'05''	15
	Sweden	3	177/00;178/00	Stockholm	59°34'03''	17°53'57''	10
	Sweden	4	179/00	Uppsala	59°51'29''	17°38'40''	35
	Sweden	5	180/00;181/00	Högsta	59°58'16''	17°34'12''	27
	Sweden	6	182/00	Högsta	59°57'44''	17°34'59''	31
	Sweden	7	183/00;184/00;185/00	Uppsala	59°53'07''	17°39'59''	27
	Sweden	8	186/00;187/00;188/00;189/00	Stockholm	59°29'46''	17°56'25''	21
	Sweden	9	190/00;191/00;192/00	Norrköping	58°35'40''	16°11'01''	62
	Sweden	10	194/00;195/00;196/00;197/00	Linköping	58°26'06''	15°44'11''	118
	Sweden	11	198/00;199/00;200/00	Jönköping	57°46'54''	14°09'30''	158
	Sweden	12	202/00;203/00;204/00	Kristianstad	56°01'52''	14°09'17''	7
	Sweden	13	205/00A;205/00B	Bostahusen	55°52'46''	12°52'43''	10
	Sweden	14	206/00;207/00;208/00;209/00	Landskrona	55°52'13''	12°49'48''	3
	Sweden	15	210/00;211/00;212/00	Malmö	55°42'51''	13°05'35''	8
	Sweden	16	213/00;215/00;217/00	Malmö	55°36'11''	13°00'04''	8
	Sweden	17	218/00;219/00;220/00	Malmö	55°33'35''	13°00'02''	25
	Slovenia	18	1/00;2/00	Šentilj	46°40'50''	15°39'11''	299
	Slovenia	19	3/00;4/00	Šentilj	46°40'39''	15°39'12''	310
	Slovenia	20	5/00;6/00	Pesnica	46°35'46''	15°40'22''	258
	Slovenia	21	9/00	Lenart	46°35'00''	15°51'0''	251
	Slovenia	22	10/00;11/00;12/00	Spodnja Ščavnica	46°37'44''	15°56'22''	221
	Slovenia	23	13/00;14/00;15/00	Gornja Radgona	46°40'41''	15°59'25''	218
	Slovenia	24	16/00	Radenci	46°38'18''	16°03'01''	201
	Slovenia	25	21/00	Iljaševci	46°34'28''	16°07'50''	187
	Slovenia	26	22/00;23/00	Ljutomer	46°31'38''	16°11'35''	183
	Slovenia	27	24/00;25/00	Žerovinci	46°29'15''	16°08'30''	286
	Slovenia	28	26/00;27/00	Pavlovci	46°26'07''	16°08'03''	212
	Slovenia	29	28/00;29/00	Dobrava	46°24'52''	16°08'53''	233
	Slovenia	30	33/00	Ormož	46°24'29''	16°07'53''	196
	Slovenia	31	35/00	Ptuj	46°25'07''	15°52'18''	225
	Slovenia	32	36/00	Gaj	46°27'05''	15°41'00''	259
	Slovenia	33	41/00;42/00;43/00	Šikole	46°24'18''	15°42'11''	243
	Slovenia	34	44/00;45/00;46/00;47/00;48/00	Maribor Stari Log	46°23'55''	15°36'23''	265
	Slovenia	35	51/00	Slovenska Bistrica	46°23'34''	15°34'24''	273
	Slovenia	36	52/00;53/00;54/00	Preloge	46°21'45''	15°30'01''	346
	Slovenia	37	55/00;56/00;57/00	Slovenske Konjice	46°20'13''	15°25'10''	348
	Slovenia	38	58/00;59/00;60/00	Vojnik	46°17'29''	15°17'55''	270
	Slovenia	39	61/00;62/00	Levec	46°14'34''	15°13'10''	242
	Slovenia	40	63/00	Žalec	46°15'03''	15°10'13''	256
	Slovenia	41	64/00;65/00;66/00;67/00	Šempeter	46°15'36''	15°07'12''	271
	Slovenia	42	68/00;69/00	Prekopa	46°15'0''	14°59'0''	319
	Slovenia	43	70/00;72/00;73/00	Vransko	46°14'56''	14°57'45''	346
	Slovenia	44	74/00;75/00;76/00;77/00;78/00	Zavrh	46°11'03''	14°52'20''	573
	Slovenia	45	79/00	Žirovše	46°10'14''	14°47'46''	392
	Slovenia	46	80/00;81/00	Lukovica	46°10'30''	14°41'00''	337
	Slovenia	47	82/00;83/00	Dob	46°09'03''	14°37'45''	305
	Slovenia	48	84/00;85/00	Trzin	46°08'57''	14°33'57''	298
	Slovenia	49	86/00	Brezovica	46°02'02''	14°23'59''	307
	Slovenia	50	87/00	Dragomer	46°01'12''	14°22'48''	301
	Slovenia	51	89/00	Log	46°00'27''	14°21'56''	299
	Slovenia	52	90/00;91/00	Kalce	45°53'42''	14°11'23''	494
	Slovenia	53	96/00	Postojna	45°46'31''	14°12'51''	549

Supporting Information File 2 Microsatellite genotypes of *L. serriola* samples. (*N*, number of analyzed plants; N_G, number of genotypes; N_{HET}, number of samples with heterozygous constitution; $N_{HET\ max}$, maximal number of heterozygous loci; * The inclusion of sample to Red/Green/Blue cluster according the Bayesian clustering; ** The position of sample in group A-F on Neighbor-Network.

		N	N_G	N_{HET}	N_{HETmax}														
L. serriola	Sweden	47	17	8	1														
	Slovenia	74	34	17	3														
Sample	**Country**	**Locus**														**Genotype**	**R/G/B cluster***	**Group A-F****
		SML-002		**SML-019**		**SML-045**		**SML-055**		**WSULs-18**		**WSULs-75**		**WSULs-163**				
171_00	SW	168	168	164	164	233	233	240	240	208	208	206	206	193	188	G 1	R	C
172_00	SW	168	168	164	164	233	233	240	240	208	208	206	206	188	188	G 2	R	C
173_00	SW	168	168	164	164	233	233	240	240	208	208	206	206	188	188	G 2	R	C
174_00	SW	168	168	164	164	233	233	240	240	208	208	203	203	188	188	G 3	R	C
175_00	SW	204	168	163	163	233	233	233	233	208	208	203	188	193	185	G 4	R	C
176_00	SW	168	168	164	164	233	233	240	240	208	208	203	203	188	188	G 3	R	C
177_00	SW	168	168	164	164	233	233	240	240	208	208	203	203	188	188	G 3	R	C
178_00	SW	168	168	164	164	233	233	240	240	208	208	203	203	193	188	G 5	R	C
179_00	SW	168	168	164	164	233	233	240	240	208	208	203	203	188	188	G 3	R	C
180_00	SW	168	168	164	164	233	233	240	240	208	208	203	203	188	188	G 3	R	C
181_00	SW	168	168	164	164	233	233	240	240	208	208	203	161	188	188	G 6	R	C
182_00	SW	168	168	164	164	233	233	240	240	208	208	203	203	188	188	G 3	R	C
183_00	SW	168	168	164	164	233	233	240	240	208	208	203	203	188	188	G 3	R	C
184_00	SW	168	168	164	164	233	233	240	240	208	208	203	203	188	188	G 3	R	C
185_00	SW	168	168	164	164	233	233	240	240	208	208	203	203	188	188	G 3	R	C
186_00	SW	168	168	164	164	233	233	240	240	208	208	203	203	188	188	G 3	R	C
187_00	SW	168	168	164	164	233	233	240	240	208	208	203	161	197	188	G 7	R	C
188_00	SW	168	168	164	164	233	233	240	240	208	208	203	203	188	188	G 3	R	C
189_00	SW	168	168	164	164	233	233	240	240	208	208	203	203	188	188	G 3	R	C
190_00	SW	168	168	164	164	233	233	233	233	208	208	203	203	193	193	G 8	G	D
191_00	SW	193	193	164	164	235	235	235	235	208	208	188	188	191	191	G 9	G	D
192_00	SW	168	168	164	164	233	233	233	233	208	208	203	203	193	193	G 8	G	D
194_00	SW	168	168	164	164	233	233	233	233	208	208	203	203	193	193	G 8	G	D
195_00	SW	168	168	164	164	233	233	233	233	208	208	203	203	193	193	G 8	G	D
196_00	SW	168	168	164	164	233	233	233	233	208	208	203	203	193	193	G 8	G	D
197_00	SW	168	168	164	164	233	233	233	233	208	208	203	203	193	193	G 8	G	D
198_00	SW	168	168	164	164	233	233	240	240	208	208	203	203	188	188	G 3	R	C
199_00	SW	168	168	164	164	233	233	240	240	208	208	203	203	188	188	G 3	R	C
200_00	SW	168	168	164	164	233	233	240	240	208	208	203	203	188	188	G 3	R	C
202_00	SW	168	168	164	164	233	233	240	240	208	208	206	206	188	188	G 2	R	C
203_00	SW	204	204	163	163	233	233	233	233	208	208	203	203	188	188	G 10	G	D
204_00	SW	168	168	163	163	233	233	233	233	208	208	203	203	188	188	G 11	G	D
205_00A	SW	204	204	163	163	229	229	233	233	212	212	188	188	193	193	G 12	G	D
205_00B	SW	168	168	164	164	233	233	233	233	208	208	203	203	193	193	G 8	G	D
206_00	SW	168	168	163	163	235	235	233	233	208	208	188	188	193	193	G 13	G	D
207_00	SW	168	168	163	163	235	235	233	233	208	208	188	188	193	193	G 13	G	D
208_00	SW	168	168	163	163	235	235	233	233	208	208	188	188	193	193	G 13	G	D
209_00	SW	168	168	163	163	235	235	233	233	208	208	188	188	193	193	G 13	G	D
210_00	SW	204	204	163	163	233	233	233	233	208	208	203	203	188	188	G 10	G	D
211_00	SW	204	204	163	163	233	233	233	233	208	208	203	203	188	188	G 10	G	D
212_00	SW	207	207	163	163	233	233	233	233	208	208	203	203	188	188	G 14	G	D
213_00	SW	168	168	163	163	233	233	233	233	208	208	203	203	188	188	G 11	G	D
215_00	SW	168	168	164	164	233	233	240	235	208	208	203	203	193	188	G 15	R/G/B	A
217_00	SW	204	204	164	164	233	233	235	235	208	208	203	188	193	193	G 16	R/G/B	A
218_00	SW	168	168	164	164	233	233	240	240	208	208	203	203	188	188	G 3	R/G/B	A
219_00	SW	168	168	164	164	233	233	240	221	208	208	203	203	188	188	G 17	R/G/B	A
220_00	SW	168	168	164	164	233	233	240	240	208	208	203	203	188	188	G 3	R/G/B	A

Supporting Information File 3 Microsatellite genotypes of *L. serriola* samples. (*N*, number of analyzed plants; N_G, number of genotypes; N_{HET}, number of samples with heterozygous constitution; $N_{HET\ max}$, maximal number of heterozygous loci; * The inclusion of sample to Red/Green/Blue cluster according the Bayesian clustering; ** The position of sample in group A-F on Neighbor-Network.

Sample	Country	Locus														Genotype	R/G/B cluster*	Group A-F**
		SML-002		SML-019		SML-045		SML-055		WSULs-18		WSULs-75		WSULs-163				
001_00	SLO	168	168	163	163	233	233	240	240	208	208	203	203	193	193	G 1	G	B
002_00	SLO	168	168	163	163	233	233	240	240	208	208	203	203	193	193	G 1	G	B
003_00	SLO	172	172	164	164	233	233	240	240	235	235	203	203	193	193	G 2	G	B
004_00	SLO	172	172	164	164	235	235	240	240	208	208	206	206	193	193	G 3	G	B
005_00	SLO	172	172	163	163	235	235	240	240	208	208	203	203	193	193	G 4	G	B
006_00	SLO	172	172	163	163	235	235	240	240	208	208	203	203	193	193	G 4	G	B
009_00	SLO	198	198	164	164	233	233	233	233	208	208	188	188	197	195	G 5	G	E
010_00	SLO	168	168	163	163	235	235	240	240	208	208	206	206	193	193	G 6	G	B
011_00	SLO	168	168	163	163	235	235	240	240	208	208	206	206	193	193	G 6	G	B
012_00	SLO	168	168	163	163	235	235	240	240	208	208	206	206	193	193	G 6	G	B
013_00	SLO	172	168	163	163	235	229	0	0	208	208	203	203	193	185	G 7	G	B
014_00	SLO	172	172	163	163	235	235	240	240	208	208	203	203	193	193	G 4	G	B
015_00	SLO	172	172	163	163	235	235	240	240	208	208	203	203	193	193	G 4	G	B
016_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	195	195	G 8	G	E
021_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	195	195	G 8	G	E
022_00	SLO	168	168	163	163	235	235	0	0	208	208	203	203	197	197	G 9	G	B
023_00	SLO	168	168	163	163	235	235	240	240	208	208	206	203	195	193	G 10	G	B
024_00	SLO	168	168	163	163	233	233	240	240	208	208	203	203	195	193	G 11	G	B
025_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
026_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
027_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
028_00	SLO	168	168	164	164	238	238	240	240	208	208	203	203	193	193	G 13	G	B
029_00	SLO	168	168	164	164	238	238	240	240	208	208	203	203	193	193	G 13	G	B
033_00	SLO	168	168	163	163	233	233	240	240	212	212	203	203	193	193	G 14	G	B
035_00	SLO	172	172	163	163	235	235	228	228	208	208	203	203	193	193	G 15	G	E
036_00	SLO	168	168	163	163	235	235	240	240	208	208	206	206	193	193	G 6	G	B
041_00	SLO	168	168	163	163	229	229	240	240	212	212	203	188	195	195	G 16	G	E
042_00	SLO	168	168	163	163	229	229	240	240	212	212	203	188	195	195	G 16	G	E
043_00	SLO	168	168	163	163	229	229	240	240	212	212	203	188	195	195	G 16	G	E
044_00	SLO	198	198	164	164	233	233	233	233	208	208	188	188	197	197	G 17	G	E
045_00	SLO	198	168	164	164	233	233	233	233	208	208	203	188	197	183	G 18	G	E
046_00	SLO	198	198	164	164	233	233	233	233	208	208	188	188	197	197	G 17	G	E
047_00	SLO	198	168	164	164	233	233	233	233	208	208	188	161	197	197	G 19	G	E
048_00	SLO	198	168	164	164	233	233	233	233	217	208	203	188	197	197	G 20	G	E
051_00	SLO	168	168	163	163	233	233	240	240	217	217	203	203	193	193	G 21	G	B
052_00	SLO	168	168	163	163	233	233	240	240	208	208	203	203	195	195	G 22	G	B
053_00	SLO	168	168	163	163	233	233	240	240	208	208	203	203	195	193	G 11	G	B
054_00	SLO	168	168	163	163	238	233	240	240	217	208	203	203	195	195	G 23	G	B
055_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
056_00	SLO	172	172	164	164	238	235	233	233	212	212	203	203	191	191	G 24	G	E
057_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
058_00	SLO	172	168	164	164	229	229	240	240	208	208	203	203	193	185	G 25	G	B
059_00	SLO	168	168	164	164	229	229	240	240	208	208	203	203	185	185	G 26	G	B
060_00	SLO	168	168	164	164	229	229	240	240	208	208	203	203	185	185	G 26	G	B
061_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
062_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
063_00	SLO	168	168	164	164	229	229	235	235	208	208	203	203	193	193	G 27	B	F
064_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
065_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
066_00	SLO	168	168	164	164	233	233	233	233	212	212	203	203	193	193	G 28	G	E
067_00	SLO	168	168	164	164	238	233	233	233	212	212	203	203	193	193	G 29	G	E
068_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	185	185	G 30	G	E
069_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	185	185	G 30	G	E
070_00	SLO	168	168	163	163	235	235	235	235	217	217	203	203	193	193	G 31	G	B
072_00	SLO	168	168	163	163	235	235	235	235	217	217	203	203	193	193	G 31	G	B
073_00	SLO	168	168	163	163	235	235	235	235	217	217	203	203	193	193	G 31	G	B
074_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
075_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
076_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
077_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
078_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
079_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
080_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
081_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
082_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
083_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
084_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
085_00	SLO	168	168	164	164	235	235	240	240	208	208	203	203	193	193	G 32	G	E
086_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
087_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
089_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F
090_00	SLO	168	168	164	164	233	233	233	233	212	208	203	203	193	193	G 33	G	B
091_00	SLO	168	168	164	164	233	229	240	240	212	208	203	203	193	193	G 34	G	B
096_00	SLO	168	168	164	164	233	233	235	235	208	208	203	203	193	193	G 12	B	F

4.2. Biodiversity of *Lactuca aculeata* germplasm including the resistance variation to *Bremia lactucae*

4.2.1. Biodiversity of *Lactuca aculeata* germplasm assessed by SSR and AFLP markers, and resistance variation to *Bremia lactucae*

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Biodiversity of *Lactuca aculeata* germplasm assessed by SSR and AFLP markers, and resistance variation to *Bremia lactucae*

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ABSTRACT

In total, seventy two *Lactuca aculeata* and three *Lactuca serriola* samples originating from natural populations of these species in Turkey, Jordan, and Israel were analysed by eight microsatellite and 287 amplified fragment length polymorphism (AFLP) markers. Neighbor–Network and Bayesian clustering were used for visualisation of the differences among the analysed *L. aculeata* and *L. serriola* samples, and to confirm hybrid origin (*L. aculeata* × *L. serriola*) of three samples (343-8A, 343-8B, 54/07) previously indicated by their morphological traits. Molecular data reflect the geographical origin, i.e., the clustering of samples according to their country of origin. Samples from neighbouring parts of Jordan and Israel expressed similar genetic characteristics, indicating the possibility of migration or artificial introduction of plant material. Forty-one *L. aculeata* samples were screened for their response to five *Bremia lactucae* races (Bl: 17, Bl: 18, Bl: 24, Bl: 27, and Bl: 28). Susceptible reactions of *L. aculeata* prevailed. *L. aculeata* samples were most frequently susceptible to races Bl: 18, Bl: 24, Bl: 27, Bl: 28; and least susceptible to Bl: 17. No highly efficient source of resistance was detected; however, race-specific reaction patterns were frequently recorded, indicating the possible presence of some race-specific resistance factors/genes in the studied samples of *L. aculeata*. Conservation and exploitation of this material in lettuce breeding is discussed.

1. Introduction

The genus *Lactuca* L. is a member of the family Compositae (Asteraceae), subfamily Cichorioideae, tribe Cichorieae, subclade Lactucinae (Kilian et al., 2009). The genus is distributed in temperate and warm regions of the northern hemisphere (Europe, Asia, Indonesia, North and Central America, Africa) (Lebeda et al., 2004a, 2004b, 2007). The Mediterranean region and the Middle East are considered the centers of *Lactuca* biodiversity, and thought to be the probable center of domestication of cultivated lettuce (*Lactuca sativa*) (Lebeda et al., 2004b, 2007), a very important leafy vegetable worldwide.

The most serious disease of *Lactuca* spp. is downy mildew caused by *Bremia lactucae* Regel (Lebeda et al., 2002). *B. lactucae*, is a highly variable obligate biotrophic oomycete (Peronosporaceae) pathogen. It can not only attack cultivated lettuce, but may also occur on many other Asteraceae species that have worldwide distributions (Lebeda et al., 2002). Fungicide

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protection often becomes difficult and ineffective (Brown et al., 2004; Michelmore and Wong, 2008; Barrière et al., 2014), and has strong hygienic limits. Breeding for resistance is a major activity of most lettuce improvement programmes, and there is an increasing need for information and methods to accelerate the development of new disease-resistant cultivars (Lebeda et al., 2007; Michelmore and Wong, 2008). The resistance breeding exploits genotypes with dominant race-specific resistance *Dm* genes (or R-factors) (Lebeda et al., 2014). However, breeding for race-specific resistance is problematic due to extremely high variability (Lebeda et al., 2002, 2008a; Lebeda and Zinkernagel, 2003; Sharaf et al., 2007) and the adaptability of the pathogen populations (Lebeda and Zinkernagel, 2003). Therefore, wild *Lactuca* species have been intensely studied since the seventies, and some of them (e.g., *Lactuca serriola* and *Lactuca saligna*) are presently almost routinely used in resistance breeding (Lebeda et al., 2007, 2014). However, the response of *Lactuca aculeata* to *B. lactucae* has not yet been adequately studied (Lebeda et al., 2009, 2014; Beharav et al., 2014).

L. aculeata Boiss et Kotschy is a diploid, autogamous species with $2n = 18$ chromosomes. Together with *L. serriola*, *Lactuca dregeana* and some other *Lactuca* species, *L. aculeata* belongs to the primary genepool of cultivated lettuce (*L. sativa* L.) (Koopman et al., 2001; Lebeda et al., 2007, 2009). The close relationship of *L. aculeata* to the primary gene pool of cultivated lettuce (Lebeda et al., 2007) was also supported by analysis of sesquiterpene lactones (Michalska et al., 2009; Michalska and Kisiel, 2010; Beharav et al., 2010b). The *L. aculeata* distribution area covers the Near East and the Anatolian plateau (Zohary, 1991; Danin, 2004). According to a recent biogeographical analysis, *L. aculeata* occurs in five neighbouring countries: Israel, Lebanon, Turkey, Syria, and Jordan (Lebeda et al., 2004b). In Israel, which is considered a centre of its origin (Zohary, 1991), *L. aculeata* grows in a broad span of elevations (222–968 m a.s.l.) and in various habitats such as roadsides, field margins, dumps, as well as anthropogenic and ruderal localities (Beharav et al., 2010a). *L. aculeata* is primarily self-pollinating (Zohary, 1991) and fully interfertile with *L. sativa* and *L. serriola* (Globerson et al., 1980; de Vries, 1990; Lebeda et al., 2007), as evidenced by the spontaneous natural interspecific hybrids between *L. aculeata* × *L. serriola* (Lebeda et al., 2012). In northern Israel, *L. aculeata* frequently grows together with *L. serriola* and *L. saligna*, and sporadic intermediate and recombinant individuals have been repeatedly detected there (Zohary, 1991; Beharav et al., 2008, 2010a; Lebeda et al., 2012). Hybridization between *L. aculeata* and *L. serriola* is more probable, compared to *L. saligna*.

Information concerning the morphology and population variability of *L. aculeata* is very limited. Thus far, *L. aculeata* germplasm collections have been characterized using molecular biological approaches: ITS-1 DNA sequences (Koopman et al., 1998), isozyme analysis (Lebeda et al., 2012), AFLP fingerprints (Koopman et al., 2001), and relative DNA contents (Doležalová et al., 2002b). The existence of race-specific resistance to *B. lactucae* had been postulated for *L. aculeata* (Lebeda et al., 2002), and later preliminary studies (Beharav et al., 2006) confirmed this expectation. The most recent resistance studies were focused on the screening of *L. aculeata* for response to some Californian isolates of *B. lactucae* (Beharav et al., 2014); however, detailed information about race-specificity is still absent.

In this study, we would like to answer the following questions: What is the level of genetic variability within the entire collection of *L. aculeata* samples? Are there genetic differences among the samples of different geographic origins? Is there any variation in the *L. aculeata* samples' resistance to lettuce downy mildew (*B. lactucae*)? Could this species be a potential donor of resistance, useful in lettuce breeding programs?

2. Materials and methods

2.1. Plant materials

In 1996, 1998, 2005, 2007, and 2009, original seed materials were collected in 19 natural populations of *L. aculeata* occurring at 19 locations in Turkey, Jordan, and Israel (Beharav et al., 2010a); additionally, three *L. serriola* samples were collected in Jordan. Data on individual populations are given in Table 1, with the geographic distribution of collection sites in Israel shown in Fig. 1. Each population was represented by one to thirteen seed samples according to the population size, and each seed sample was collected from an individual plant. The distance between sampled plants within a population was at least 2 m (Lebeda et al., 2001, 2007; Beharav et al., 2010a). The set of 72 *L. aculeata* and 3 *L. serriola* seed samples has been deposited in the seed storage facilities of the Institute of Evolution Gene Bank (IoEGB, University of Haifa, Israel), and in parallel at the *Lactuca* Working Collection of the Department of Botany (Palacký University in Olomouc, Czech Republic). Seed samples were regenerated in a greenhouse of the Department of Botany (Palacký University in Olomouc, Czech Republic), and the taxonomic status of each accession was verified according to its morphology.

The set of 75 samples (including 69 samples of *L. aculeata*, 3 samples of *L. serriola*, and 3 putative hybrids *L. aculeata* × *L. serriola*) was used for the study of molecular polymorphism. Out of these, 41 samples were included in the resistance screening (Table 1). The three *L. serriola* samples served as controls, and data for these species were excluded from the subsequent data analyses (except for microsatellite genotyping).

2.2. Microsatellite and AFLP genotyping

2.2.1. DNA extraction

Total genomic DNA for both SSR and AFLP analyses was extracted from fresh young leaf tissue of three week old plants grown in a glasshouse, using an InnuPREP Plant DNA Kit (Analytik Jena AG, Germany) following the manufacturer's protocol.

Table 1
List of 75 samples representing 19 populations (14 in Israel, 4 in Jordan, 1 in Turkey) of *Lactuca*.

Lactuca species	Collection year	Pop. No.[a]	Sample No.[a]	Country	Locality	Longitude	Latitude
L. aculeata	1998	131	10[b],11[b],13[b],15[b],24[b]	Turkey	Ankara	32°51′14.81″	39°55′14.75″
	1996	224	22[b]	Jordan	10 km south to Amman		
	1996	232	12[b],22[b],23	Jordan	10 km south to Amman		
	1996	233	1[b],16[b],17[b],19[b],20[b],20A, 20S, 24[b],25[b],26[b],28[b],29[b]	Jordan	Near Mafraq Junction, 35 km north to Zarka		
	1996	241	20	Jordan	Haraj, 3 km west to Irbid	35°51′25.17″	32°32′41.45″
	2005	343	1[b],3[b],5[b],7[b]	Israel	Giv'at Yo'av	35°41′22.00″	32°48′04.00″
	2005	344	1[b],4[b],5[b],6[b],8[b]	Israel	Nov, Haspin	35°47′19.00″	32°49′40.00″
	2007	366	1,9[b]	Israel	Gamla	35°46′15.00″	32°54′40.00″
	2007	367	1,4,6,7,9,10,11,13,17	Israel	Zomet Hamappalin	35°45′01.00″	32°59′14.00″
	2007	368	1,12[b]	Israel	'En Ziwan	35°49′17.00″	33° 06′21.00″
	2007	371	1[b],13[b]	Israel	Kela' Alone	35°41′04.00″	33° 07′58.00″
	2007	372	14[b]	Israel	Zomet Hashiryon	35°44′39.00″	33° 03′44.00″
	2007	373	1[b],22[b]	Israel	Elifelet	35°32′32.00″	32° 57′05.00″
	2007	374	1[b],16[b]	Israel	Mishmar-Hayarden	35°36′10.00″	33° 00′11.00″
	2007	375	1[b],10[b]	Israel	Qidmat-Zevi-1	35°41′46.00″	33° 01′21.00″
	2007	376	1[b],15[b]	Israel	Qidmat-Zevi-2	35°43′13.00″	33° 02′18.00″
	2009	380	1,2,3,4,5,6,7,9,10,11,12,13,14	Israel	Nov, Haspin	35°47′16.70″	32° 49′35.00″
	2007	56/07[b]		Israel	Eli Ad	35°43′39.00″	32° 47′45.00″
L. serriola	1996	224	5, 5A, 5B	Jordan	10 km south to Amman		
L. aculeata × *L. serriola*	2005	343	8A, 8B	Israel	Giv'at Yo'av	35°41′22.00″	32°48′08.00″
L. aculeata × *L. serriola*	2007	54/07		Israel	Giv'at Yo'av	35°41′33.00″	32° 48′00.00″

[a] Population (Pop. No.) and Sample (Sample No.) number corresponds to IoELDB (Institute of Evolution *Lactuca* database), and/or to *Lactuca* database in the Department of Botany, Palacký University in Olomouc, Czech Republic.
[b] Samples, used for screening, resistance against *Bremia lactucae*.

DNA quality and quantity were determinated by agarose gel electrophoresis and by use of a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Delaware, USA).

2.2.2. Molecular marker assays

For microsatellite genotyping, a total of eight microsatellite primers pairs (EST-SSR) were used in this study (for details see Table 2). The primers pairs were selected according to their high diversity indices in previously published papers (Simko, 2009; Riar et al., 2011), but randomly, without any previous knowledge of their chromosome position. Amplification of SSRs was performed according to a modified protocol: 15 μl PCR reactions containing 7 ng/μl of DNA template, 5 U/μl of Taq polymerase, 10 μM of both primers, reaction Buffer, and 10 mM dNTPs. The following PCR program was used: 1 min at 95 °C, 30 to 34 cycles of 1 min for annealing temperature (57.5–64.5 °C), 30 s for extension at 72 °C, and a final step of 5 min at 72 °C. The lengths of the microsatellite products were scored based on their migration relative to molecular weight size markers 30–330 bp AFLP® DNA ladder (Invitrogen). The AFLP analysis was performed as described by Vos et al. (1995), with modifications, and the AFLP fragment detection according to Kitner et al. (2008, 2012). The PCR products were separated by denaturing polyacrylamide gel electrophoresis (T-REX, Thermo Scientific Owl Separation Systems, Rochester, NY, USA).

2.2.3. Data analyses

Allele frequency and polymorphism were evaluated in each SSR locus. AFLP fragments were checked visually, and only clear and unambiguous bands were scored for their presence (1) or absence (0) across all samples.

For SSR data, the proportion of polymorphic loci (*P%*), number of private alleles (*PA*), observed and expected heterozygosity (H_O and H_E) were performed using GenAlEx 6 software (Peakall and Smouse, 2006). The mean number of alleles per locus (*A*) was calculated manually. The relative discriminatory value of each microsatellite locus was estimated by polymorphic information content (PIC), which measures the information content as a function of a marker system's ability to distinguish between genotypes (Powell et al., 1996). The number of different genotypes (N_G), the number of samples with a heterozygous constitution (N_{HET}), and the maximal number of heterozygous loci (N_{HETmax}) were calculated manually.

For AFLP data, the polymorphic rate (*PLP%*) was calculated as follows: $PLP = (loc\ P/loc) \times 100\%$, where *loc P* is the number of polymorphic bands, and *loc* is the total number of amplified bands. The number of private bands (*PA*), the proportion of polymorphic loci (*P%*), and gene diversity (H_E) were calculated using GenAlEx 6 software (Peakall and Smouse, 2006).

To visualize genetic relationships within and among the analysed samples, a Neighbor–Network based on Dice's similarity coefficient (*D*) was constructed in SplitsTree 4 (Huson and Bryant, 2006). The Nexus input file for SplitsTree was exported from GenAlEx. For this purpose, SSR data were transformed into a binary matrix (where the presence of a fragment of a particular length was coded as 1, and its absence as 0), followed by merging with the AFLP binary data. The reliability and robustness of the network were tested by bootstrap analysis with 1,000 bootstrap replicates.

For analysis of the population structure, a Bayesian clustering approach was used as implemented in STRUCTURE 2.2 (Falush et al., 2007) for combined SSR and AFLP binary data (*K* in range 1–10 with ten replicate runs for each *K*, 100,000 burn-

Fig. 1. Geographical distribution of analysed *Lactuca aculeata* in populations Israel.

in iterations followed by 1,000,000 MCMC iterations). For the graphical interpretation of clustering for the appropriate *K*, both CLUMPP (Jacobson and Rosenberg, 2007) and DISTRUCT (Rosenberg, 2004) software were used.

2.3. Screening for resistance to B. lactucae

2.3.1. Isolates of B. lactucae

Five isolates of *B. lactucae* (Bl: 17; Bl: 18; Bl: 24; Bl: 27; Bl: 28), representing the officially denominated races of *B. lactucae*, with known virulence patterns (Van Ettekoven and Van der Arend, 1999; Van der Arend et al., 2006), were used for *L. aculeata* resistance screening. These isolates originated from cultivated lettuce (*L. sativa*), and they are maintained by the Department of Botany (Palacký University in Olomouc, Czech Republic) in the collection of microorganisms (http://botany.upol.cz).

2.3.2. Plant inoculation and incubation

The tests were carried out according to the methods described by Lebeda and Petrželová (2010), with minor modifications. Pathogen races were maintained and multiplied on seedlings of *L. sativa* cvs. 'Cobham Green' and 'Hilde', which also served as susceptible controls in the resistance tests. Thirty seeds in three replications per individual sample were sown on moistened filter paper in plastic boxes. Plants were inoculated at the stage of fully expanded cotyledon leaves, and incubated in a growth chamber as described by Lebeda and Petrželová (2010).

2.3.3. Evaluation of sporulation intensity and interpretation of obtained data

Data on sporulation intensity were recorded in two-day intervals, 6–14 days after inoculation, using a 0–3 scale (Dickinson and Crute, 1974). Intensity of sporulation was expressed as a percentage of the maximum possible scores according to Townsend and Heuberger (1943). The reaction of a particular *L. aculeata* sample was considered as susceptible, if the sporulation intensity was more than 30%, and at least half of the tested seedlings showed a degree of infection of 2 or 3 (Lebeda and Petrželová, 2010). Differentiation of resistance phenotypes (R-phenotypes) of individual *L. aculeata* samples was

Table 2
Microsatellite markers used in this study.

Marker	Reference	N_A	Allele size (bp)	PIC (%)
SML-002	Simko (2009)	9	167–207	0.227
SML-039	Simko (2009)	2	232–237	0.099
SML-045	Simko (2009)	6	214–258	0.163
SML-055	Simko (2009)	6	212–240	0.326
WSULs-18	Riar et al. (2011)	8	209–243	0.512
WSULs-45	Riar et al. (2011)	3	184–189	0.285
WSULs-75	Riar et al. (2011)	4	173–188	0.171
WSULs-163	Riar et al. (2011)	6	178–196	0.242

N_A, number of alleles; PIC (%), allelic polymorphic information content.

Table 3
Total number of AFLP fragments.

Primer combination	N_F	N_{POL}	*PLP* (%)
E – AGC, M– CTG	58	43	74.1
E – AGC, M– CAAC	47	31	65.9
E – AGC, M– CGAT	46	33	71.7
E – ACC, M– CAAC	16	10	62.5
E – ACC, M– CAAT	56	47	83.9
E – ACC, M– CGAT	34	24	70.6
E – ACC, M– CGATC	30	21	70.0
Total	287	209	72.8
Mean	41	29.9	71.2

N_F, total number of fragments; N_{POL}, number of polymorphic fragments; *PLP*, percentage of polymorphic fragments.

used for examination of variation in resistance patterns to *B. lactucae* within and between populations of *L. aculeata*. The aim was to assess whether there are any geographic differences in the resistance of the *L. aculeata* samples, to determine the spatial pattern of resistance variation, and to evaluate at which spatial scale the variation is most pronounced.

3. Results

3.1. Genetic polymorphism

The eight polymorphic SSR loci produced a total of 44 alleles across the 72 individuals of *L. aculeata*. The number of alleles per locus ranged from 2 to 9, with an average of 5.5 alleles per locus (Table 2). The allele sizes varied from to 167 and 258 bp. The mean PIC per SSR polymorphic allele was 0.253, within the range of 0.099–0.512.

Private alleles (*PA*) were present within each sampled region (Table 4). The *L. aculeata* samples from Turkey possessed 5 unique alleles: 177 bp (at SML-002), 237 bp (at SML-039), 214 bp (at SML-045), 218 bp (at WSULs-18), and 180 bp (at WSULs-163). The samples from Jordan possessed only two unique alleles: 207 bp (at SML-002) and 231 bp (at SML-045); and samples from Israel possessed 17 unique alleles: 170 bp, 174 bp, 180 bp, 195 bp, 203 bp (at SML-002); 228 bp, 233 bp (at SML-045); 212 bp, 225 bp, 235 bp (at SML-055); 237 bp, 243 bp (at WSULs-18); 187 bp, 188 bp (at WSULs-75); and alleles 193 bp, 195 bp, 196 bp (at WSULs-163). The highest numbers of loci with private alleles (*PA*) were found for the Turkish sample 131-24 (4 loci), and Israeli samples 343-8A (5), 380-2 (5), and 380-11 (5) (Supplementary file S1). *L. serriola* plants from Jordan possessed several unique alleles (236 bp at SML-055, 247 bp at WSULs-18, and 193 bp at WSULs-75), absent in *L. aculeata* samples. In addition to the unique alleles, they contained at least 7 alleles shared with samples of *L. aculeata* (from a transition zone), indicating their hybrid origin (Supplementary file S1). The observed and expected heterozygosity (H_O and H_E) ranged from 0.085 to 0.141 (mean 0.109), and from 0.164 to 0.305 (mean 0.233), respectively. The proportion of polymorphic loci (*P%*) was of the same value (87.5%) across all samples from Turkey, Jordan, and Israel.

Table 4
Population data – totals for SSR and AFLP band patterns of 72 *L. aculeata* samples.

	N	Microsatellite data					AFLP data		
		PA_{SSR}	*A*	*P(%)*	H_O	H_E *(SE)*	PA_{AFLP}	*P(%)*	H_E *(SE)*
Turkey	5	5	2.13	87.5	0.100	0.305 (0.072)	6	23.00	0.077 (0.009)
Jordan	17	2	2.50	87.5	0.141	0.164 (0.038)	2	37.28	0.074 (0.008)
Israel	50	17	4.38	87.5	0.085	0.229 (0.069)	24	59.93	0.125 (0.010)

N, sample size; PA_{SSR}, private microsatellite alleles; PA_{AFLP}, private AFLP bands; *A*, mean no. of alleles per locus; *P*, polymorphism; H_O, observed heterozygosity; H_E, expected heterozygosity; *SE*, standard error.

Table 5
Survey of *Lactuca aculeata* ($N = 41$) reaction patterns to *Bremia lactucae* in individual countries.

	Response to *Bremia lactucae* race					No. of samples (populations)		
Pattern	Bl: 17	Bl: 18	Bl: 24	Bl: 27	Bl: 28	TUR	JOR	ISR
1	+	+	+	+	+	5 (1)	2 (2)	6 (4)
2	-	+	+	+	+		10 (3)	9 (5)
3	-	-	+	+	+			1 (1)
4	-	+	+	+	-		1(1)	1 (1)
5	-	-	-	+	-			1 (1)
6	-	-	-	-	-			5 (4)
						5 (1)	13(6)	23(16)

Based on SSR data, a total of 37/3 different genotypes were recognized for *L. aculeata*/*L. serriola*. The highest number of different SSR genotypes (N_G) was observed for Israeli samples of *L. aculeata* ($N_G = 21$), followed by Jordanian ($N_G = 11$), Turkish ($N_G = 5$), and Jordanian samples of *L. serriola* ($N_G = 3$) (Supplementary file S1). The highest number of samples, with at least one heterozygous locus, was recorded for samples from Israel ($N_{HET} = 9$), followed by samples from Jordan ($N_{HET} = 8$), and Turkey ($N_{HET} = 4$) (Supplementary file S1). In the case of *L. serriola*, all three samples had a heterozygous constitution. The highest values of maximal number of heterozygous loci (N_{HETmax}) were observed in Israeli *L. aculeata* samples ($N_{HETmax} = 7$) and Jordanian *L. aculeata* samples ($N_{HETmax} = 6$), followed by Jordanian *L. serriola* samples ($N_{HETmax} = 5$) and Turkish *L. aculeata* samples ($N_{HETmax} = 4$) (Supplementary file S1).

In total, seven primer combinations with three to five selective bases were applied for AFLP genotyping (Table 3), resulting in 287 unambiguously scored fragments. The number of loci scored per single primer combination varied from 16 (E-ACC/M-CAAC) to 58 (E-AGC/M-CTG), with an average of 41 loci per single primer combination (Table 3). The range of polymorphism (*PLP%*) found for different primer combinations ranged from 62.5% (E-ACC/M-CAAC) to 83.9% (E-ACC/M-CAAT). The number of private bands (*PA*) ranged from 2 (samples from Jordan) to 24 (samples from Israel). The expected heterozygosity (H_E) ranged from 0.074 to 0.125 (mean 0.092) (Table 4), and the proportion of polymorphic loci (*P%*) in *L. aculeata* samples varied from 23% (Turkish population) to 59.9% (Israeli populations). The genetic variability indices for all populations are summarized in Table 4.

Based on eight microsatellite and 287 AFLP markers, three control samples of *L. serriola* (224-5; 224-5A; 224-5B) were clearly separated apart from *L. aculeata* samples, and helped us to confirm the putative hybrid origin (*L. aculeata* × *L. serriola*) of three samples (348/8A, 343/8B, 54/07), as was proposed by Lebeda et al. (2012). Moreover, our data (Figs. 2 and 3a,b) indicate a hybrid character of at least six (232-23, 343-3, 343-7, 380-2, 380-11, and 56/07) additional samples.

3.1.1. Cluster analysis of molecular data

Based on the SSR and AFLP binary data, the Neighbor–Network was constructed. The individuals fell into 3 groups (Fig. 2). The first group is composed of *L. serriola* samples from Jordan, which were used as control samples in our study. The second group is represented by samples from Turkey. The third group comprises samples of *L. aculeata* originating from Jordan and Israel. Finally, a proportion of *L. aculeata* samples fell into a transition zone between *L. aculeata* and *L. serriola*, indicating their putative hybrid origin. The pattern of genetic structure revealed by STRUCTURE (Fig. 3) showed similar results to the grouping of samples by the Neighbor–Network. *L. serriola* samples showed a different genetic background compared to *L. aculeata* samples, with a further differentiation of *L. aculeata* samples originating in Turkey and those samples from Jordan and Israel. The hybrid character of some *L. aculeata* samples is indicated by the admixture signal in their profiles (i.e., corresponding bars contain a mixture of colours from various groups, indicating that a given individual inherited some fraction of its genome from ancestors in other groups) (Figs. 2 and 3).

3.2. Host-pathogen interaction data

3.2.1. Screening of L. aculeata set for resistance to B. lactucae

The studied set represented by 41 *L. aculeata* samples from Turkey ($N = 5$), Jordan ($N = 13$), and Israel ($N = 23$) generally expressed a high level of susceptibility to five races of *B. lactucae* from *L. sativa* (Bl: 17, Bl: 18, Bl: 24, Bl: 27, Bl: 28) used in this study (Table 5; Figs. 4 and 5). However, sporulation intensity of Bl races on individual samples varied greatly from 30% to 93.7% (Fig. 4). *L. aculeata* samples were most frequently susceptible to the races Bl: 27, Bl: 18, Bl: 24, and Bl: 28; and were least susceptible to race Bl: 17 (only 31.7% of responses to this race were compatible) (Fig. 5). The proportion (%) of susceptible vs. resistant responses was 73.7 vs. 26.3. This is evidence that in *L. aculeata* some new and interesting sources of race-specific resistance, useful for *L. sativa* resistance breeding, might be located.

3.2.2. Determination of L. aculeata reaction patterns to the used races of B. lactucae

Resistance phenotypes (R-phenotypes) were described on the basis of reaction patterns of *L. aculeata* seedlings, derived from seed collections, to the set of *B. lactucae* races. On the whole, six different reaction patterns (R-phenotypes) were

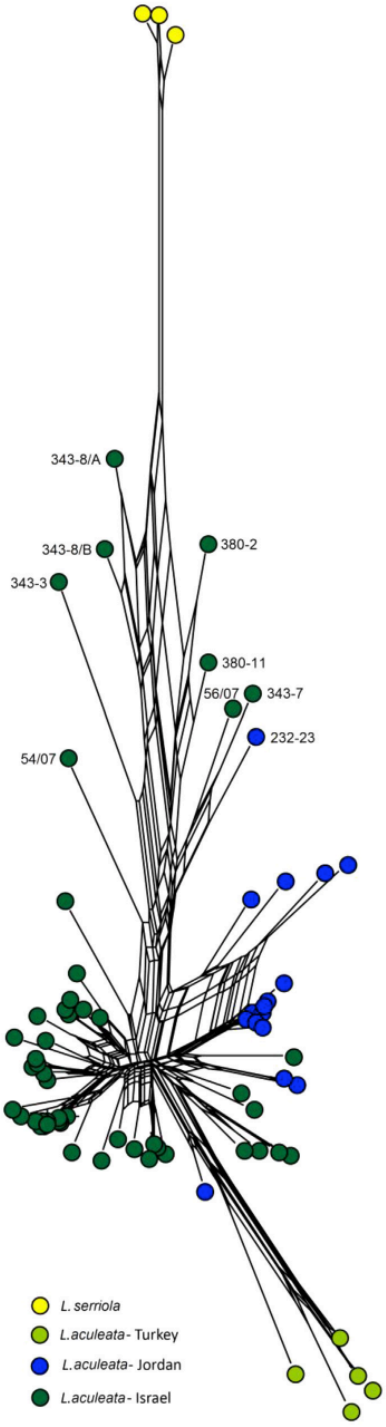

Fig. 2. Neighbor–Network based on joined AFLP and SSR analysis of *L. aculeata* samples from three different countries (Turkey, Jordan and Israel) and three samples of *L. serriola*.

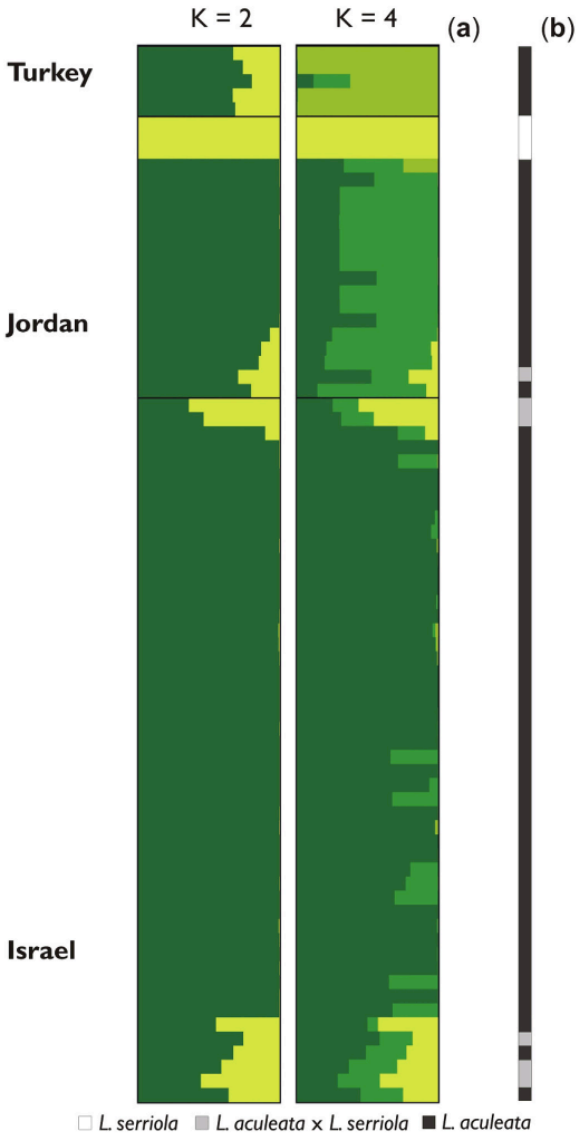

Fig. 3. Results of Bayesian clustering based on AFLP and SSR data (a) and morphological determination (b) of 75 *Lactuca* spp. samples. Each individual is represented by a thin horizontal line, which is partitioned into K colored segments that represent the individual's estimated membership fractions. The hybrid character of some *L. aculeata* samples is indicated by admixture signal in their profiles (i.e., corresponding bars contain mixture of colours from various groups indicating that given individual inherited some fraction of its genome from ancestors in other groups).

recorded among 41 *L. aculeata* samples screened (Table 5). In total, 56.1% of samples expressed a heterogeneous response to the Bl: races used. Complete susceptibility to the Bl: races used (pattern No. 1) was recorded for two samples (232-12, 233-16) from two Jordanian populations and six samples (344-4, 344-5, 344-6, 373-22, 374-1, 376-1) from four Israeli populations. The most common reaction pattern was pattern No. 2 (19 [46.3%] of 41 tested *L. aculeata* samples), which was characterized by susceptibility to 4 Bl: races, and resistance to Bl: 17 (Table 5). In comparison, patterns Nos. 3, 4, and 5 with resistance to 2, 2, and 4 Bl: races, respectively, were recorded only sporadically. Pattern No. 6 was characterized by complete resistance to all five Bl: races, and was represented by 12.2% of samples (Israeli samples 366-9, 371-1, 371-13, 373-1, and 56/07; Fig. 4, Table 5).

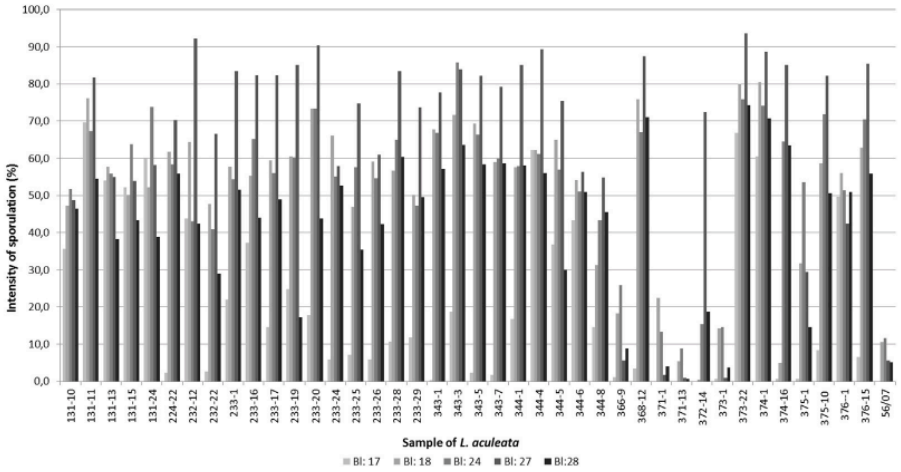

Fig. 4. Intensity of sporulation (%) of *Bremia lactucae* races on 41 individual samples of *L. aculeata*.

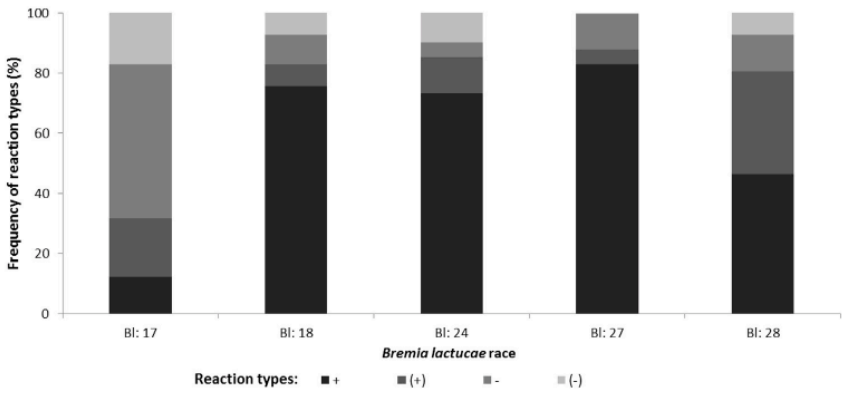

Fig. 5. Proportion (%) of *L. aculeata* reaction types to individual *Bremia lactucae* races (+ Susceptibility; (+) Heterogeneous response; – Resistance; (−) Incompletely compatible response).

All reaction patterns (Nos. 1 to 6) were present in Israel, while 3 patterns (Nos. 1, 2, 4) were present in Jordanian populations. In Turkish population (No. 131) only one pattern (No. 1) with susceptibility to all *B. lactucae* races was recorded (Table 5).

The three samples with a putative hybrid character (343-3, 343-7, and 56/07) were included in a set screened for resistance to *B. lactucae*. In comparison with non-hybrid samples, no differences in resistance phenotypes were observed. Samples 343-3 and 343-7 belong to reaction pattern No. 2, and sample 56/07 to pattern No. 6 (Table 5).

3.2.3. Inter- and intrapopulation variation of L. aculeata in response to B. lactucae

Low variation in reaction patterns (R-phenotypes) was recorded in some populations. In addition to the Turkish population 131, only one pattern (No. 2 or No. 6) was also identified in the Israeli populations 343 (4 samples) and 371 (2 samples), respectively. In population 233, in total, 8 out of 10 samples belonged to the same reaction pattern (No. 2), and the remaining 2 samples belonged to No. 1 and 4. On the contrary, clear intrapopulation variability in responses to Bl: races were recorded within some other populations with more than one *L. aculeata* sample tested: 232 (2 samples, 2 different R-phenotypes) from Jordan; and 344 (5 samples, 2 R-phenotypes), 373 (2 samples, 2 R-phenotypes), 374 (2 samples, 2 R-phenotypes), and 376 (2 samples, 2 R-phenotypes) from Israel (Fig. 4).

4. Discussion

Recent increasing interest in the wild *Lactuca* spp. has brought new valuable information about their diversity, with practical applications (Lebeda et al., 2009, 2014). However, research interest has been focused on a limited number of species.

Except for *L. serriola* and *L. saligna* (e.g., Lebeda et al., 2001, 2008a; Beharav et al., 2008; Petrželová and Lebeda, 2011), our knowledge of the ecology, biology, biodiversity, and interactions with pathogens of wild *Lactuca* species, closely related to cultivated lettuce, is poor (Lebeda et al., 2004b, 2009, 2014).

In our recent study, we focused on molecular polymorphism and the variation in resistance to *B. lactucae*, two not very well explored features of *L. aculeata* variation. Previous studies were based on a limited number of *L. aculeata* genebank accessions (Koopman et al., 1998, 2001; Doležalová et al., 2002a,b) originating from germplasm collections, or on a limited number of samples collected in natural populations (Lebeda et al., 2012). The current study is probably the first detailed research focused on genetic variability in a large set of *L. aculeata* (72 samples from 19 populations), based exclusively on newly collected plant materials from a wide area of distribution (Turkey, Jordan, and Israel), with precise eco-geographical data available for the collection sites.

4.1. Genetic variability

Using two different but widely used molecular techniques (microsatellites and AFLP), the genetic variability of the *L. aculeata* germplasm collection was studied. In our study, AFLP appeared to be more informative than microsatellites, due to its ability to detect fine differences among samples. Nevertheless, the current study of 72 *L. aculeata* samples, originating from 19 populations, showed a relatively low level of genetic variation. The majority (more than 80%) of Jordanian and Israeli samples were grouped in one compact cluster on the Neighbor–Network (Fig. 2). A subset of 30 samples was screened for morphological and isozyme variations in our previous study (Lebeda et al., 2012), and nearly 75% of these samples also formed one very compact cluster, and showed a similar genetic background. The main reasons for the limited genetic variation of *L. aculeata* populations can be seen in the prevailing autogamy of this species, strict habitat requirements (Beharav et al., 2010a), and limited area of its distribution (Zohary, 1991).

Using morphological traits and isozyme analysis, Lebeda et al. (2012) described the occurrence of three putative interspecific hybrids *L. aculeata* × *L. serriola* (343-8A, 343-8B, and 54/07) in *L. aculeata* natural populations. These samples were also used in the present study, and their hybrid character is evident from their microsatellite profiles (Figs. 2 and 3; Supplementary file S1).

L. aculeata together with *L. serriola* and with other species (*Lactuca azerbaijanica*, *L. dregeana*, *Lactuca georgica*, and *Lactuca scarioloides*) belong to the primary genepool of the popular leafy vegetable – cultivated lettuce (*L. sativa*). Additionally, crosses between these species (e.g., *L. sativa* × *L. serriola*) are easily made either way, and the F_1'S are fully fertile. Since the 1930s, many crossing experiments between *Lactuca* and closely related species have been conducted. The crossing experiments and practical utilization of wild *Lactuca* germplasm were mostly carried out with *L. serriola* (Uwimana et al., 2012a). Spontaneous interspecific hybridization between *L. sativa* and *L. serriola* was probably reported for the first time by Bohn and Whitaker (1951), and Lindqvist (1960). Recently, numerous theoretical and experimental studies focused on natural hybrids between *L. sativa* and *L. serriola* have been published (D'Andrea et al., 2008; Hooftman et al., 2009a,b,c; 2011, Uwimana et al., 2012b).

According to Lebeda et al., 2012 (description occurrence of three putative interspecific hybrids *L. aculeata* × *L. serriola* in natural populations) and the recent study, it is also evident that in the wild population *L. aculeata* and *L. serriola* spontaneous interspecific hybridization may occur. This phenomenon was also confirmed with detection of the hybrid origin of several additional samples (232-23, 343-3, 343-7, 380-2, 380-11, 56/07). Morphologically, these six samples were determined as *L. aculeata*, although three samples (232-23, 380-2, 380-11) expressed some morphological traits characteristic for *L. serriola* during their regeneration in a greenhouse (unpubl. data). Furthermore, they shared some alleles (174 and 195 bp at SML-002; 228 bp at SML-045; 209 and 213 bp at WSULs-18; 189 bp at WSULs-45; 195 bp at WSULs-163) with Jordanian samples of *L. serriola*. Eight samples (232-23; 343-3; 343-7; 343/8A; 343-8B; 380-2; 380-11; 54/07) showed the presence of at least one allele (mostly 2 or 3 alleles) shared with *L. serriola* (Supplementary file S1). Their heterozygotic constitution indicates that these samples are offspring arising from crossing *L. aculeata* × *L. serriola*. As for sample 56/07, in addition to the alleles characteristic for *L. aculeata*, the microsatellite profile revealed two unique alleles (233 bp at SML-045, 237 bp at WSULs-18), which were not shared with any other sample (both *L. serriola* and *L. aculeata*) used in this study. Based on our recent results, we are therefore unable to explain the origin of this sample. A future study is needed to determine which *Lactuca* spp. occurring in the collection area of sample 56/07 could contribute to its genome constitution. It could either be another genotype of *L. serriola* or some other species from the primary or secondary gene pool (*Lactuca altaica*, *L. azerbaijanica*, *L. scarioloides*, *L. saligna*).

4.2. Variation in resistance to B. lactucae

Knowledge about the occurrence of diseases and pests in naturally growing *Lactuca* populations is very limited (Lebeda et al., 2001, 2008a, 2009, 2014). During the field studies in Jordan and Israel, no plants of *L. aculeata* infected by diseases or pests were recorded (Beharav et al., 2010a; Lebeda, unpubl. data). Furthermore, from the previous critical review, it appears that *L. aculeata* is not a natural host of *B. lactucae* (Lebeda et al., 2002). However, this phenomenon does not imply that *L. aculeata* is a host plant variable in resistance to this pathogen. To verify and analyse the status of *L. aculeata* as a host plant with a race-specific reaction pattern to this lettuce downy mildew (*B. lactucae*), we screened 41 *L. aculeata* samples from 15 mostly Israeli natural populations of this species (Table 1) for their resistance to 5 official races of *B. lactucae* from *L. sativa*.

Several samples expressed a differential reaction pattern, i.e., serious infection by one or a few *B. lactucae* races, along with resistance to the other races used; altogether, six different race-specific reaction patterns were recognized (Table 5). The highest variability of race-specific reactions was recorded in *L. aculeata* populations from Israel, compared to those from Jordan and Turkey. However, lower numbers of populations and samples have been studied from the latter two countries.

Data from recent experiments confirm the race-specific character of the interaction between *L. aculeata* and *B. lactucae*, previously conjectured by Lebeda et al. (2002), and Jemelková et al. (2013); on the other hand showing the relatively high susceptibility of *L. aculeata*. Beharav et al. (2006) received similar results for materials from Jordan, Turkey, and Armenia screened for resistance to race Bl: 21. On the contrary, in a set of *L. aculeata* samples from Israel, tested at seedling and true leaf stages against five highly virulent Californian *B. lactucae* isolates (C00O879, C11O1358, C98O648, C11O1342, and C10O1273), resistance was detected in 70% of samples (Beharav et al., 2014).

Two samples (373-1 and 374-16) from the study of Beharav et al. (2014) were included to our recent resistance screening to *B. lactucae*. Sample 373-1, which was resistant to five Californian isolates (both at the seedling and true leaf stages) (Beharav et al., 2014) was also resistant to five Bl: races (Fig. 4). Sample 374-16 was resistant to all Californian isolates (Beharav et al., 2014); however, according to our data, it was susceptible to races Bl: 24, Bl: 27, Bl: 28 (Fig. 4). These results clearly demonstrate the differences in race-specific virulence between European and Californian isolates of *B. lactucae*.

The recent study has also shown that *L. aculeata* could provide additional and interesting race-specific resistance sources against *B. lactucae*. However, we do not know anything about the mechanism of resistance of *L. aculeata* to *B. lactucae* (Lebeda et al., 2008b). The preliminary genetic studies have shown that one dominant R-factor could be expected in *L. aculeata* (Lebeda et al., 2002). According to the recent data, it could be hypothetized that more dominant race-specific resistance genes are localized in *L. aculeata*. This makes the broadly diversified germplasm of this species, growing in its center of origin around the Mediterranean Basin (Lebeda et al., 2004b), interesting for plant breeders as a potential source of new resistance for the breeding of cultivated lettuce (*L. sativa*) (Beharav et al., 2010a; Lebeda et al., 2002, 2009, 2014). From a previous study (Lebeda et al., 2012) and our recent results, it seems highly probable that interspecific hybrids of *L. aculeata*, with at least *L. serriola*, can arise in the center of origin of this species. Unfortunately, the spiny indumentum of *L. aculeata* × *L. sativa* hybrids (Lebeda et al., 2007, 2012) could be considered as a "breeder non-friendly" feature. Therefore, breeding strategies based on unconventional approaches *via* incorporation of desired genes should be used.

An open question remains as to why *L. aculeata* was rather highly variable in the resistance tests, while under natural conditions its interaction with *B. lactucae* is not known (Lebeda et al., 2002). As the race-specific character of the *L. aculeata* – *B. lactucae* interaction has recently been proven, a gene-for-gene nature of the relationship between these two species might be considered. Furthermore, due to the absence of natural infections by *B. lactucae* in the center of its genetic diversity, selection pressure by this pathogen on *L. aculeata* is probably minimum; therefore, the broad diversity of resistance genes may be maintained in these populations.

To conclude, there is a great challenge for further study of *L. aculeata* as a potential source of resistance genes against *B. lactucae* as well as some other diseases and pests (Lebeda et al., 2014). Moreover, this species could also be used as a model system for population and evolutionary studies, and for research of the evolutionary forces acting between *L. aculeata* and pathogens, which are not known and/or common in natural habitats of this species.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bse.2015.07.003.

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Supplementary table 1a: Microsatellite genotypes and genotype diversity of *L. serriola* and *L. aculeata* samples.

Locus		SML-002		SML-039		SML-045		SML-055		WSULs-18		WSULs-45		WSULs-75		WSULs-163	
L. aculeata																	
343-1	ISR	182	182	232	232	226	226	219	219	215	215	184	184	173	173	182	182
343-3	ISR	182	182	232	232	228	228	219	219	215	215	184	184	173	173	182	182
343-5	ISR	182	182	232	232	226	226	219	219	215	215	184	184	173	173	182	182
343-7	ISR	167	182	232	232	226	258	219	230	213	234	184	189	173	188	182	193
343-8/A	ISR	195	195	232	232	226	228	219	235	243	243	184	184	173	173	182	195
343-8/B	ISR	195	195	232	232	228	228	219	219	243	243	184	184	173	173	182	182
344-1	ISR	182	182	232	232	226	226	219	219	220	220	184	184	173	173	182	182
344-4	ISR	182	182	232	232	226	226	219	219	220	220	184	184	173	173	182	182
344-5	ISR	182	182	232	232	226	226	219	219	220	220	184	184	173	173	182	182
344-6	ISR	182	182	232	232	226	226	219	219	215	215	184	184	173	173	182	182
344-8	ISR	182	182	232	232	226	226	219	219	215	215	184	184	173	173	182	182
366-1	ISR	182	182	232	232	226	226	225	225	215	215	187	187	173	173	182	182
366-9	ISR	182	182	232	232	226	226	219	219	215	215	0	0	173	173	182	182
367-1	ISR	182	182	232	232	226	226	225	225	220	220	184	184	173	173	182	182
367-4	ISR	182	182	232	232	226	226	225	225	220	220	184	184	173	173	182	182
367-6	ISR	182	182	232	232	226	226	225	225	220	220	187	187	173	173	182	182
367-7	ISR	182	182	232	232	226	226	219	225	220	220	184	184	173	173	182	182
367-9	ISR	182	182	232	232	226	226	219	219	220	220	184	184	173	173	182	182
367-10	ISR	182	182	232	232	226	226	225	225	220	220	184	184	173	173	182	182
367-11	ISR	182	182	232	232	226	226	219	219	220	220	184	184	173	173	182	182
367-13	ISR	182	182	232	232	226	226	219	219	220	220	184	184	173	173	182	182
367-17	ISR	182	182	232	232	226	226	219	219	220	220	184	184	173	173	182	182
368-1	ISR	182	182	232	232	226	226	219	219	220	220	184	184	173	173	182	182
368-12	ISR	182	182	232	232	226	226	212	212	220	220	184	184	173	173	182	182
371-1	ISR	182	182	232	232	226	226	219	219	215	215	187	187	173	173	182	182
371-13	ISR	182	182	232	232	226	226	219	219	215	215	187	187	173	173	182	182

Supplementary table 1b: Microsatellite genotypes and genotype diversity of *L. serriola* and *L. aculeata* samples.

Locus		SML-002		SML-039		SML-045		SML-055		WSULs-18		WSULs-45		WSULs-75		WSULs-163	
L. aculeata																	
373-1	ISR	182	182	232	232	226	226	219	219	215	215	184	184	173	173	182	182
373-22	ISR	182	182	232	232	226	226	219	219	215	215	0	0	173	173	182	182
374-1	ISR	182	182	232	232	226	226	219	219	220	220	184	184	173	173	182	182
374-16	ISR	182	182	232	232	226	226	219	219	215	215	187	187	173	173	182	182
375-1	ISR	182	182	232	232	226	226	219	219	215	215	184	184	173	173	182	182
375-10	ISR	182	182	232	232	226	226	219	219	220	220	184	184	173	173	182	182
376-1	ISR	180	180	232	232	226	226	219	219	215	215	184	184	173	173	182	182
376-15	ISR	182	182	232	232	226	226	219	219	215	215	187	187	173	173	182	182
380-1	ISR	182	182	232	232	226	226	225	225	215	215	184	184	173	173	182	182
380-2	ISR	182	203	232	232	226	228	225	225	215	234	184	189	173	187	182	196
380-3	ISR	182	182	232	232	226	226	219	219	220	220	184	184	173	173	182	182
380-4	ISR	182	182	232	232	226	226	212	212	215	220	184	184	173	173	182	182
380-5	ISR	182	182	232	232	226	226	219	219	215	220	184	184	173	173	182	182
380-6	ISR	182	182	232	232	226	226	219	219	215	215	184	184	173	173	182	182
380-7	ISR	182	182	232	232	226	226	219	219	215	215	184	184	173	173	182	182
380-9	ISR	182	182	232	232	226	226	219	219	220	220	184	184	173	173	182	182
380-10	ISR	182	182	232	232	226	226	219	219	215	215	184	184	173	173	182	182
380-11	ISR	170	182	232	232	226	228	212	219	215	234	184	189	173	187	182	196
380-12	ISR	182	182	232	232	226	226	219	219	215	215	184	184	173	173	182	182
380-13	ISR	182	182	232	232	226	226	219	219	215	215	184	184	173	173	182	182
380-14	ISR	182	182	232	232	226	226	219	219	215	215	184	184	173	173	182	182
54/07	ISR	174	182	232	232	226	228	219	219	209	215	184	184	173	187	182	182
56/07	ISR	167	182	232	232	226	233	219	240	220	237	184	184	173	173	182	182

Supplementary table 1c: Microsatellite genotypes and genotype diversity of *L. serriola* and *L. aculeata* samples.

Locus		SML-002		SML-039		SML-045		SML-055		WSULs-18		WSULs-45		WSULs-75		WSULs-163	
L. aculeata																	
373-1	ISR	182	182	232	232	226	226	219	219	215	215	184	184	173	173	182	182
373-22	ISR	182	182	232	232	226	226	219	219	215	215	0	0	173	173	182	182
374-1	ISR	182	182	232	232	226	226	219	219	220	220	184	184	173	173	182	182
374-16	ISR	182	182	232	232	226	226	219	219	215	215	187	187	173	173	182	182
375-1	ISR	182	182	232	232	226	226	219	219	215	215	184	184	173	173	182	182
375-10	ISR	182	182	232	232	226	226	219	219	220	220	184	184	173	173	182	182
376-1	ISR	180	180	232	232	226	226	219	219	215	215	184	184	173	173	182	182
376-15	ISR	182	182	232	232	226	226	219	219	215	215	187	187	173	173	182	182
380-1	ISR	182	182	232	232	226	226	225	225	215	215	184	184	173	173	182	182
380-2	ISR	182	203	232	232	226	228	225	225	215	234	184	189	173	187	182	196
380-3	ISR	182	182	232	232	226	226	219	219	220	220	184	184	173	173	182	182
380-4	ISR	182	182	232	232	226	226	212	212	215	220	184	184	173	173	182	182
380-5	ISR	182	182	232	232	226	226	219	219	215	220	184	184	173	173	182	182
380-6	ISR	182	182	232	232	226	226	219	219	215	215	184	184	173	173	182	182
380-7	ISR	182	182	232	232	226	226	219	219	215	215	184	184	173	173	182	182
380-9	ISR	182	182	232	232	226	226	219	219	220	220	184	184	173	173	182	182
380-10	ISR	182	182	232	232	226	226	219	219	215	215	184	184	173	173	182	182
380-11	ISR	170	182	232	232	226	228	212	219	215	234	184	189	173	187	182	196
380-12	ISR	182	182	232	232	226	226	219	219	215	215	184	184	173	173	182	182
380-13	ISR	182	182	232	232	226	226	219	219	215	215	184	184	173	173	182	182
380-14	ISR	182	182	232	232	226	226	219	219	215	215	184	184	173	173	182	182
54/07	ISR	174	182	232	232	226	228	219	219	209	215	184	184	173	187	182	182
56/07	ISR	167	182	232	232	226	233	219	240	220	237	184	184	173	173	182	182

4.3. Genetic and morphological variability of wild *Lactuca* species in natural populations in Israel

4.3.1. Genetic structure and diversity in natural populations of three predominantly self-pollinating wild *Lactuca* species in Israel.

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RESEARCH ARTICLE

Genetic structure and diversity in natural populations of three predominantly self-pollinating wild *Lactuca* species in Israel

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Abstract It is important to optimize strategies for collecting wild germplasm from their natural habitats for the successful conservation of plant genetic resources. We studied the population structure of three predominantly self-pollinating wild *Lactuca* species (*Lactuca serriola*, *L. saligna*, and *L. aculeata*). Seeds for this study were collected from individual plants in northern Israel, along a line transect, and two populations per each *Lactuca* species. The distance between neighboring plants sampled for seeds varied from 1.5 to 37 m. The transect length at single sites ranged from 47.2 to 151.8 m. The taxonomic status of 67 individual plants was morphologically validated during greenhouse multiplication. Both genetic structure and diversity were analyzed by using 11 EST–SSR loci and 230 AFLP markers. Relatively low genetic diversity values were observed, increasing in the following order: *L. aculeata* < *L. serriola* < *L. saligna*. Network analysis clearly separated samples according to their taxonomic determination; also reflecting the gene diversity as well as the genetic distance values among the three species. Nevertheless, given the predominantly selfing character of these species, populations were not uniform (genetically and morphologically). It seems that overall genetic variation in a population increases at its periphery, due to the presence of plants with "non-indigenous" alleles, which are most likely coming from migration and subsequent interpopulation or interspecific hybridization. Mantel tests generally indicated a positive association between genetic distance and micro-geographical distance of a particular population, primarily due to the "outlier" samples collected at a population's periphery.

Keywords Collecting strategy · Genetic variability · Germplasm · *Lactuca serriola* · *Lactuca saligna* · *Lactuca aculeata* · Population genetic structure · Wild lettuce

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Introduction

Conservation of wild genetic resources for improvement of important food crops is an extremely challenging task for the new millennium. Efforts to explore and exploit the genetic variability present in the wild relatives of the world's major crops are especially important (Zamir 2008). Although crop wild relatives, which include crop progenitors and other closely related species, have for centuries been used for crop improvement, they still possess many novel traits that have not been fully exploited (McCouch 2013).

Development of efficient conservation strategies for the maintenance of the highest possible genetic variability of crop progenitors is a key step in plant genetic resource management. To this end, it is necessary to understand the genetic structure of a particular progenitor species at both the population and species levels. Information about the distribution of genetic diversity within populations/species, as well as among populations/species, should serves as driving forces for the collecting strategy. Brown and Marshall (1995) postulated crucial principles for a sampling strategy for wild relatives of domesticated crops, taking into consideration important factors influencing the population structure (e.g., measurement of genetic variation, number of alleles in the sample, etc.). They suggested a practical approach for sampling (e.g., the number and location of sampling sites, number of individual plants sampled at a given site, choice of individuals, etc.).

Thus, knowledge about the genetic variation within a species is important to set the optimal collecting strategy. And, conversely, a proper collecting strategy can lead to the description and explanation of molecular variation present within a population/species. Inappropriate collecting strategies may not do so. For example, the collecting strategy proposed by Brown and Marshall (1995), based on the "benchmark criterion" in which seeds from fifty randomly sampled individual plants are bulked and represent a sample for a particular population, may not be appropriate for many species. It does not allow for detailed analyses of population structure, which can trace the associations of morphology, phenology, disease and pest resistances, and molecular profiles of individual plants in relationship to their original positions within the population. For predominantly autogamous species (such as many species of *Lactuca*), a more suitable collecting strategy was introduced by Lebeda et al. (2001, 2007a), in which one seed sample is represented by achenes collected from one plant. The number of samples per collecting site/population depends upon the population size and habitat.

Once a proper collection strategy has been established, the next important step is the proper maintenance of seeds in gene banks. The regeneration and storage of germplasm seed materials need to be done efficiently in order to avoid the accidental loss of diversity. This raises many questions as to which samples to include in a collection and how to determine redundancies, as well as how to collect and multiply the samples without loss of diversity due to contamination, inadvertent selection, or genetic drift (van Hintum 2003). Common molecular markers (e.g., AFLPs, SSRs, SNPs) may serve as adequate tools for the rapid screening of germplasm collections. Marker data can help curators characterize existing collections, as well as minimize duplication and prioritize the accessions to be maintained (Lebeda et al. 2007a, b). And, recently, Lebeda et al. (2007b, 2009a, 2014) reviewed the various molecular methods and approaches, as applied to the germplasm characterization of wild *Lactuca* species. However, the power of such molecular studies is often limited by the obscure origins of accessions present in collections and specific information about collected populations and their regeneration history.

In the present study, we focused on three wild *Lactuca* species: *L. serriola* L., *L. aculeata* Boiss., and *L. saligna* L. All three are annual, predominantly self-pollinating species (Lindqvist 1960; Zohary 1991; Lebeda et al. 2007b), with their main distribution within the Temperate Zone of the Northern Hemisphere (Kilian et al. 2009). The high level of diversity of *Lactuca* species within both the Mediterranean region and southwest Asia indicates that these areas may be the center of diversity for the genus *Lactuca* (Zohary 1991; Lebeda et al. 2001, 2004a), as well as the original center for the domestication of cultivated lettuce—*L. sativa* L. (Lindqvist 1960; de Vries 1997).

Lactuca serriola (prickly lettuce) is considered to be the direct progenitor of cultivated lettuce and is a component of its primary gene pool (Zohary 1991; Lebeda et al. 2007b). *Lactuca serriola* is widely distributed around the world, especially in the non-tropical parts of Eurasia, North Africa, and North America (Lebeda et al. 2004b, 2012a). *Lactuca aculeata* is also fully cross-compatible with *L. sativa*, and, thus, is another component of the primary gene pool for *L. sativa* (Lebeda et al. 2007b). Its distribution is restricted to the Near East arc and the Anatolian plateau (Zohary 1991; Beharav et al. 2010a). *Lactuca saligna* (least lettuce, willow-leaf lettuce) is not fully cross-compatible with *L. sativa*, and represents the secondary gene pool for cultivated lettuce (Lebeda et al. 2007b). Willow-leaf lettuce is primarily a Mediterranean species—occurring in Europe, northern Africa, and the Middle East (Lebeda et al. 2004b; Beharav et al. 2008). However, it is also naturalized in

North America (Lebeda et al. 2012a). Although all three species are mostly selfers (Lindqvist 1960; Zohary 1991; Lebeda et al. 2012b), some level of outcrossing should be expected. Detection of the interspecific hybrids in nature (Křístková et al. 2012) has highlighted the close phylogenetic relationships of these three lettuce species.

Recently, we initiated studies on the characterization of the population structure of wild *Lactuca* species collected from Israel, Armenia, Jordan, Turkey (Beharav et al. 2008, 2010a; Jemelková et al. 2013), well as other countries (Lebeda et al. 2009a). These studies included: the eco-geographical distribution (Beharav et al. 2008, 2010a; Lebeda et al. 2009a, 2012a), morphological variation (Lebeda et al. 2007b, 2011; Novotná et al. 2011; Křístková et al. 2014), genetic polymorphism (Kitner et al. 2008; Lebeda et al. 2009a, 2014), downy mildew resistance (Petrželová et al. 2011; Beharav et al. 2014; Lebeda et al. 2014), powdery mildew resistance (Lebeda et al. 2012c, 2013), and variation in the content of biologically active secondary lactones (Beharav et al. 2010b; Stojakowska et al. 2013). Recently obtained results strongly support that wild *Lactuca* species, closely related to *L. sativa*, could be the source of large genetic variation for breeding programs (Lebeda et al. 2009a, b, 2014; Petrželová and Lebeda 2011).

The aim of the present study was to obtain detailed information about the genetic structure of natural populations of three wild lettuce species in Israel (*L. serriola*, *L. aculeata*, *L. saligna*). We focused on the genetic variability present both within populations and within species, outcrossing rates of the species investigated, and associations between genetic-distance assessment and actual physical distances between individuals in the population. For these purposes, two common molecular marker classes (AFLPs and SSRs) were used. Such information gathered at both the intra- and inter-population levels can help refine seed collecting strategies, and widen the diversity maintained in germplasm collections without redundancy.

Materials and methods

Seed materials

Two populations of each of three wild *Lactuca* species (*L. serriola*, *L. aculeata*, and *L. saligna*) collected in Northern Israel were included in the present study (Table 1; Fig. 1). Seed collection followed the approach proposed by Lebeda et al. (2001, 2007a). Seeds were collected from individual plants (each referred to as a sample) along a linear transect at each sampled site (Table 1). Distances between neighboring sampled plants within the population ranged from 1.8 to 37 m, according to the density of plants at each site. The transect length ranged from 47.2 m (population 367 *L. aculeata*) to 151.8 m (population 380 *L. aculeata*). At each site, achenes from 8 to 13 individual plants per population were collected, resulting in a total of 67 samples (Table 1; Fig. 1).

Phenotype characterization and taxonomical validation

The regeneration of plants from the collected seeds followed standard multiplication protocols for wild *Lactuca* species (Lebeda et al. 2007b). The plants were cultivated in the greenhouse of the Department of Botany, Palacký University in Olomouc, Czech Republic. Each sample was represented by two to eight plants, according to the germination of the original seeds. Morphological and phenological evaluations of all 428 individual plants, as well as their taxonomic validation, followed pertinent botanical keys (Feráková 1977; Grulich 2004) and descriptor lists for wild *Lactuca* species (Doležalová et al. 2002, 2003).

In the case of morphological heterogeneity of plants within one sample, the "prevailing" and "minor" phenotypes were noted in our molecular analyses, to see if the genetic fingerprints of these heterogenic samples differed. For the species/population analyses and Mantel tests (see below), we used the "prevailing" phenotype for each of the 67 samples; but for construction of a Neighbor-Network (as described below), the "minor" phenotypes were also included (making a total of 75 individual entries). Minor phenotypes have been highlighted by use of the suffix "m" in the text, tables and figures.

Microsatellite and AFLP genotyping

The genomic DNA of 75 individual plants was extracted from fresh leaf tissues, by using an InnuPREP Plant DNA Kit (Analytik Jena AG, Jena, Germany), following the manufacturer's protocol.

Table 1 Eco-geographical data for six populations of wild *Lactuca* species, collected throughout North Israel

Species	Pop. No.[a]	Locality	N	T_L	Lt	Ln	Alt	Rain	MDT1	MDT8	Soil type
L. serriola	365	Avi'el	13	55.0	32°32′22.6″N	34°58′21.5″E	32	572	12.1	25.5	Brown rendzina
L. serriola	377	Kaukab Abu El Hija	12	100.0	32°50′12.1″N	35°14′56.6″E	375	605	10.3	24.4	Terra Rossa
L. aculeata	367	Zomet Hamappalim	8	47.2	32°59′10.7″N	35°45′00.8″E	518	659	6.9	25.7	Alluvial soils
L. aculeata	380	Nov	13	151.8	32°49′35.0″N	35°47′16.7″E	406	616	7.8	26.8	Alluvial soils
L. saligna	369	Metulla	11	124.3	33°16′37.4″N	35°34′33.7″E	485	775	8.4	24.5	Terra Rossa
L. saligna	379	Binyamina	10	105.6	32°31′41.9″N	34°56′49.3″E	11	562	11.6	25.5	Alluvial soils

Population number corresponds to IoELDB (Institute of Evolution *Lactuca* database). T_L, Transect length (m) of analyzed samples; N, Number of analyzed samples; Lt, latitude; Ln, longitude; Alt, altitude (m a. s. l.); Rain, Mean annual rainfall (mm); MDT1, January mean daily temperature (°C); MDT8, August mean daily temperature (°C)

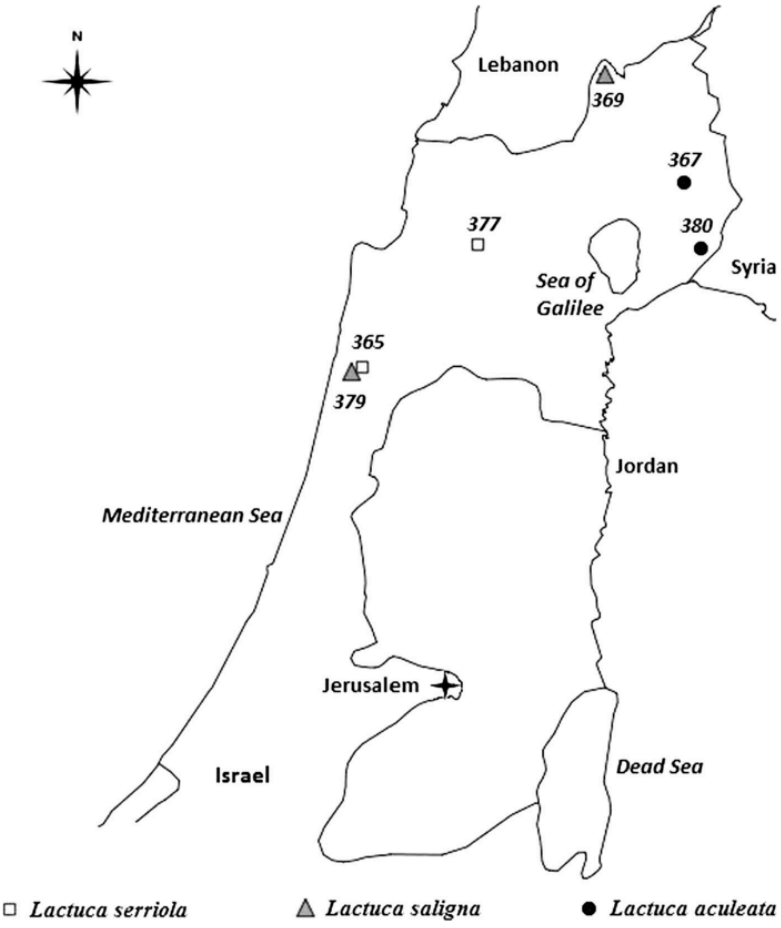

Fig. 1 Map of the study area showing analysed populations of the three *Lactuca* species. Population codes follows their designation in Table 1

For microsatellite genotyping, 11 EST–SSR loci were used: *SML-001*, *SML-019*, *SML-039*, *SML-045*, *SML-055* (Simko 2009); and *WSULs-18*, *WSULs-25*, *WSULs-45*, *WSULs-75*, *WSULs-153*, *WSULs-163* (Riar et al. 2011). These EST–SSR loci were chosen for their high diversity values. Preparation of the PCR mix was performed according to Majeský et al. (2012). The number of cycles and the annealing temperatures were adjusted for each locus, in order to obtain unambiguously scorable products. A 30–330 bp AFLP® DNA ladder (Invitrogen, Carlsbad, California, USA) was used to determine SSR allele length.

AFLP analyses were carried out according to the protocol of Vos et al. (1995) following the protocol described in Kitner et al. (2008). The products of amplification were separated on a 6 %, 0.4-mm-thick denaturing polyacrylamide gel (0.5× TBE buffer) using the T-REX (Thermo Scientific Owl Separation Systems, Rochester, NY, USA) sequencing gel electrophoresis apparatus. Consequent silver staining was used for the visualization of AFLP fingerprints. Four selective primer combinations, with two to four selective nucleotides (Table 2), were chosen to generate the AFLP profiles.

Data analysis

Genetic structure was statistically analyzed for SSR and AFLP data at the species level and also at the population level within a given species.

Microsatellites were scored based on the length of the PCR product. For SSRs data, allele frequencies (including homogeneity G^2 test for the polymorphic loci), the proportion of polymorphic loci (*P*), number of private alleles (*PA*), observed heterozygosity (*Ho*), and gene diversity index (*He*) were all computed in GenAlEx 6.5 (Peakall and Smouse 2012). The mean number of alleles per population (*A*) and per species were calculated in Arlequin 3.5 (Excoffier and Lischer 2010). For each polymorphic locus at each population, the random mating or out-crossing rate (*t*) was calculated from the formula: $t = (1 - Fe)/(1 + Fe)$ (Crow and Kimura 1970), where *Fe* equals: $1 - Ho/He$ (i.e., *Fe* represents the equilibrium inbreeding coefficient under partial selfing). The number of different genotypes (N_G), number of samples with a heterozygous constitution (N_{HET}), and maximal number of heterozygous loci (N_{HETmax}) were calculated manually. Both locus and multilocus *R*- and *F*-statistics were computed with SPAGeDi (Hardy and Vekemans 2002). Analyses of molecular variance (AMOVA) and population pairwise comparisons were computed in Arlequin 3.5. The significance of the covariance components and population pairwise comparisons were tested by 10,000 permutations (Felsenstein 1985).

AFLP profiles were checked visually, and only clear and unambiguous bands were scored for their presence (1) or absence (0). Scored markers ranged from 50 to 500 bp. Allele frequencies (including homogeneity G^2 test for polymorphic loci), percentage of polymorphic loci (*P*), gene diversity (*He*), and unbiased genetic distance coefficients (*D*) were calculated according to Nei (1978) with GenAlEx 6.5. The number of private alleles (PA_B) was calculated in FAMD (Schlüter and Harris 2006), while the number of null alleles (PA_N) was calculated manually.

The Mantel test (Mantel 1967) was used to determine the strength of association between matrices of genetic distances for a given marker (AFLPs and EST–SSRs), and the geographic distance for each population. The Mantel test was also used to check the congruence of the two molecular markers, based on the Cavalli-Sforza and Edwards (1967) chord distance (*Dc*) for SSR data,—calculated in Populations 1.2.32 (Langella 2002), and on Dice's similarity coefficient (Dice 1945, *D*, equivalent to the Nei-Li coefficient) for AFLP data, calculated in FreeTree (Pavlíček et al. 1999).

To elucidate relationships within and among the analyzed samples, a Neighbor-Network based on Dice's similarity coefficient (*D*) was constructed in SplitsTree 4 (Huson and Bryant 2006). Neighbor-Network (Bryant and Moulton 2004) is a variant of

Table 2 Primer sequences used for the AFLP preamplification reaction and primer combinations (PC) used for amplification reactions

Preamplification primer sequences		
EcoRI	5′-G ACT GCG TAC CAA TTC A-3′	
MseI	5′-G ATG AGT CCT GAG TAA C-3′	
Amplification primer combinations		
PC	*EcoRI primer*	*MseI primer*
A	EcoRI-CC	MseI-AAT
B	EcoRI-CC	MseI-GATC
C	EcoRI-CC	MseI-GATG
D	EcoRI-AC	MseI-GATG

Amplification sequence includes preamplification sequence and additional nucleotides

Neighbor-Joining (NJ), which constructs phylogenetic networks instead of phylogenetic trees. Phylogenetic networks generalize phylogenetic trees because they permit to display also conflicting signal or alternative phylogenetic histories (Fitch 1997), especially when reticulate events such as hybridization, horizontal gene transfer, recombination, or gene duplication and loss are expected to play a role in a species history (Huson and Bryant 2006). The Nexus input file for SplitsTree was exported from GenAlEx after transformation of SSR genotypes into a binary matrix (according to the presence or absence of alleles of a specific size at a given locus), which was merged with the AFLP binary matrix. Robustness of the network was tested by 1,000 bootstrap replicates.

Results

Taxonomic validation and phenotypic variation

Morphological observations supported the species identity of all three investigated wild lettuce species following Feráková (1977) and Feinbrun-Dothan (1978). The most frequent phenotypes within the samples were identified as *L. serriola* f. *serriola*, *L. aculeata* and *L. saligna* L. var. *runcinata* Gren. et Godr. Highly uniform traits were observed in samples from populations 365 (*L. serriola*; 365^{SER}), 367 (*L. aculeata*; 367^{ACU}), and 379 (*L. saligna*; 379^{SAL}). Morphological heterogeneity was observed within populations 377 (*L. serriola*; 377^{SER}), 380 (*L. aculeata*; 380^{ACU}), and 369 (*L. saligna*; 369^{SAL}). Heterogeneity of morphotypes was observed among samples within populations, and also among seedlings grown from individual samples.

Two plants in population 380^{ACU} expressed specific traits that pointed to possible interspecific hybridization. Divided stem leaves, a yellow base to its stem trichomes, and a pyramidal-paniculate form to its inflorescence clearly distinguished plant 380-2m *L. aculeata* from the rest of the plants in this population with entire stem leaves, white trichomes, and spike-like panicles. Plant 380-11m only differed morphologically from the remaining plants by displaying a yellow base to its stem trichomes.

Two samples in the 377^{SER} population (377-1 and 377-14) uniformly differed from the other samples from this population by displaying well-developed, persistent rosette leaves, the grey-green color of those rosette leaves, and by narrow lateral lobes on the stem leaves. An interesting example, tending to affirm the hybrid origins of these two plants, is the number of florets; the 16–18 florets of sample 377-1 could indicate hybridization with *L. saligna*, while the 18–22 florets of sample 377-14 corresponds to the description of *L. serriola*.

Within the otherwise morphologically uniform population 365^{SER} there were two individual plants (365-4m and 365-17m), with dentate lobes on their stem leaves, distinguishing them from the remaining plants from this population. Approximately 100 days after sowing, at the developmental stage of bolting but before flower set, all plants of population 365 (except for plants from 365-2 and, partly, from 365-20) collapsed for unknown reasons. All of the plants from population 377^{SER}, as well as those from both populations of *L. aculeata* and *L. saligna,* cultivated in the same greenhouse, remained vital. A detailed assessment of the fine morphological traits on the stem leaves showed that the lobe margins on the stem leaves of the plants in sample 365-2 were dentate, in contrast to the entire margin of the leaf lobes on all other samples within this population.

Within the morphologically highly heterogeneous population 369^{SAL}, interesting "minor" phenotypes were observed: e.g., the lack of anthocyanin in the florets and anther tubes in 369-2m; lengths of the terminal lobes on stem leaves being >1/3 of the whole lamina in 369-4m and 369-14m, in contrast to other individuals within samples 369-4 and 369-14; and lengths of terminal lobes on stem leaves were <1/3 of the whole lamina in 369-3m, in contrast to other individuals within sample 369-3.

Microsatellite loci

The 75 individual seedlings that were sampled, representing three related *Lactuca* species, were polymorphic for 11 EST–SSR loci, resulting in 67 distinct alleles. Private alleles were present within each population, and more than 10 private alleles were also observed for all three of the species (Table 3). Although most genotyped individuals were homozygous, we also observed some heterozygotes in each population. The highest number of individuals with at least one heterozygous locus was observed for *L. saligna* population 369^{SAL} ($N_{HET} = 8$ out of 15 analyzed plants) (Table 6). The lowest values were observed for

L. aculeata population 367 ($N_{HET} = 1$) and *L. serriola* population 377^{SER} ($N_{HET} = 1$).

The highest number of different SSR genotypes (N_G) was observed for *L. saligna* ($N_G = 22$), followed by *L. aculeata* ($N_G = 16$) and *L. serriola* ($N_G = 11$). At the population level, N_G ranged from 5 (populations 367^{ACU}, and 377^{SER}) to 13 (population 369^{SAL}) (Tables 4, 5, 6). Comparisons from the overall data (Table 3) of the three species, showed that for most diversity parameters the lowest values observed were for *L. aculeata* ($He^{ACU} = 0.206$), increasing to *L. serriola* and *L. saligna* ($He^{SER} = 0.307$; $He^{SAL} = 0.374$). As expected, the highest portion of observed genetic variability was partitioned among the three species (50.35 %). Within the three species, 26.81 % of observed variation was present among particular populations, as we observed population specific genotypes at several loci (see Tables 4, 5, 6). While populations were more-or-less highly homogeneous (only 18.42 % of total variability was detected among individuals within populations), within individuals, only 4.42 % of the overall variability could be explained. Particular covariance components of AMOVA were highly significant ($p < 0.001$), except for the covariance component for the variance among the species ($p = 0.065$). Multilocus estimates of the inbreeding coefficient were high $F_{IS}/R_{IS} = 0.823/0.855$, pointing to the predominant autogamy of the species studied.

When comparing SSR fingerprints of samples exhibiting the "prevailing" and "minor" phenotypes consisting of specific traits in the margins on the stem leaves, the *L. serriola* plants 365-17 and 365-17m were genetically identical, and individuals 365-4 and 365-4m differed only at one locus (WSULs_18) with a heterozygous constitution (Table 4). Two pairs of samples (380-2, 380-11) with a "minor" phenotype in the *L. aculeata* population 380^{ACU} differed at 9 loci (Table 5). For the *L. saligna* population 369^{SAL}, we observed a difference at 3–5 loci between the "minor" and "prevailing" phenotypes (Table 6).

The multilocus values of F_{ST}/R_{ST} were high (0.736/0.917), which only stressed the high level of differentiation between the investigated species and the sampled populations. Similarly, the values of F_{ST}/R_{ST} for specific loci were also high (data not shown). Pairwise F_{ST} (based on the number of different alleles) in population comparisons showed large differences among the three wild lettuce species investigated. F_{ST}-pairwise values for specific species pairs are as follows: $ACU/SAL = 0.671$; $ACU/SER = 0.641$; $SAL/SER = 0.634$ ($p < 0.001$ in all cases). Lower, but also highly significant ($p < 0.001$) pairwise F_{ST} values were obtained for population pairs (data not shown), again pointing to clear genetic differences among populations within these species.

AFLP loci

Combinations of four primer pairs yielded 230 unambiguously scorable markers. A summary of genetic

Table 3 Summary of genetic diversity, based on 11 EST–SSR loci and on 230 AFLP loci, of six populations belonging to the three wild *Lactuca* species

		Microsatellite data						AFLP data				
	N	PA_{SSR}	*A*	*P*	*Ho*	*He* (SE)	*t*	PA_B	PA_N	PA_{SUM}	*P*	*He* (SE)
L. serriola	25	12	2.36	81.82	0.036	0.307 (0.071)	n.a.	46	8	54	26.09	0.088 (0.011)
365	13	2	1.64	54.55	0.063	0.115 (0.038)	0.377	14	3	17	8.70	0.023 (0.006)
377	12	4	2.00	81.82	0.008	0.217 (0.034)	0.018	7	0	7	19.13	0.063 (0.010)
L. aculeata	21	12	2.00	45.45	0.013	0.206 (0.085)	n.a.	35	8	43	7.83	0.029 (0.007)
367	8	3	1.36	27.27	0.011	0.085 (0.049)	0.065	0	0	0	0.87	0.004 (0.003)
380	13	3	1.64	36.36	0.014	0.143 (0.061)	0.051	5	0	5	7.39	0.029 (0.007)
L. saligna	21	22	3.64	63.64	0.061	0.374 (0.102)	n.a.	76	28	104	33.04	0.099 (0.011)
369	11	8	2.64	63.64	0.066	0.361 (0.091)	0.100	13	1	14	22.61	0.077 (0.010)
379	10	5	1.91	36.36	0.055	0.207 (0.091)	0.153	11	3	14	16.96	0.050 (0.009)

A, mean no. of alleles per locus; observed (*Ho*) and expected (*He*) heterozygosity; N, sample size; n.a., not analysed; *P*, polymorphism; PA_{SSR}, private microsatellite alleles; PA_B, private alleles (AFLP bands), PA_N, private null alleles; $PA_{SUM} = PA_B + PA_N$; *t*, outcrossing rate

Table 4 Microsatellite genotypes and results of Mantel test of *L. serriola* populations (abbreviations are explained below Table 5)

Population	T_L (m)	N_P	N_G	N_{HET}	N_{HETmax}	Mantel test:	SSR	AFLP
365	55.0	15	6	4	5		$r^2 = 0.029$; $p = 0.110$	$r^2 = 0.020$; $p = 0.157$
377	100.0	12	5	1	1		$r^2 = 0.143$; $p = 0.016$	$r^2 = 0.242$; $p = 0.000$

Locus	SML-001		SML-019		SML-039		SML-045		SML-055		WSULs_18		WSULs_25		WSULs_45		WSULs_75		WSULs_153		WSULs_163	
365-1	195	195	163	163	232	232	229	229	230	230	234	234	370	370	184	184	187	187	202	202	196	196
365-2	195	195	163	163	232	232	229	229	230	230	240	240	370	370	184	189	187	187	194	194	194	194
365-3	195	195	163	163	232	232	229	229	230	230	234	234	370	370	184	184	187	187	202	202	196	196
365-4	195	195	163	163	232	232	229	229	230	230	234	240	370	370	184	184	187	187	202	202	196	196
365-4m	195	195	163	163	232	232	229	229	230	230	234	234	370	370	184	184	187	187	202	202	196	196
365-5	195	195	163	163	232	232	229	229	230	230	234	240	370	370	184	184	187	187	202	202	188	196
365-6	195	195	163	163	232	232	229	229	230	230	234	234	370	370	184	184	187	187	202	202	196	196
365-10	195	195	163	163	232	232	229	229	230	230	234	234	370	370	189	189	187	187	202	202	196	196
365-11	195	195	163	163	232	232	229	229	230	230	234	234	370	370	184	184	187	187	202	202	196	196
365-14	195	195	163	163	232	232	229	229	230	230	234	234	370	370	184	184	187	187	202	202	196	196
365-17	195	195	163	163	232	232	229	229	230	230	234	234	370	370	184	184	187	187	202	202	196	196
365-17m	195	195	163	163	232	232	229	229	230	230	234	234	370	370	184	184	187	187	202	202	196	196
365-18	195	195	163	163	232	232	229	229	230	230	234	234	370	370	184	184	187	187	202	202	196	196
365-19	195	195	163	163	232	232	229	229	230	230	234	234	370	370	184	184	187	187	202	202	196	196
365-20	195	195	163	163	232	232	229	229	230	230	234	240	360	370	184	189	177	177	194	202	194	196
377-1	195	195	170	170	238	238	229	229	228	228	244	244	370	370	189	189	184	184	194	194	188	188
377-2	195	195	163	163	232	232	229	229	230	230	240	240	360	360	189	189	187	187	194	194	194	194
377-5	195	195	163	163	232	232	229	229	230	230	240	240	360	360	189	189	187	187	194	194	194	194
377-6	195	195	163	163	232	232	229	229	230	230	240	240	360	360	189	189	187	187	194	194	194	194
377-7	195	195	163	163	232	232	229	229	230	230	240	240	360	360	189	189	187	187	194	194	194	194
377-8	195	195	163	163	232	232	229	229	230	230	240	240	360	360	189	189	187	187	194	194	194	194
377-9	195	195	163	163	232	232	229	229	230	230	240	240	360	360	189	189	187	187	194	194	194	194
377-10	195	195	163	163	232	232	229	229	230	230	240	240	360	360	189	189	187	187	194	194	194	194
377-11	195	195	163	163	232	232	229	229	230	230	240	240	360	360	184	184	187	187	194	194	194	194
377-12	195	195	163	163	232	232	229	229	230	230	240	240	360	360	189	189	187	187	192	196	194	194
377-13	195	195	163	163	232	232	229	229	230	230	240	240	360	360	189	189	187	187	194	194	194	194
377-14	195	195	170	170	238	238	229	229	228	228	244	244	370	370	175	175	184	184	194	194	188	188

Table 5 Microsatellite genotypes, genotype diversity and results of Mantel test of *L. aculeata* populations

Population	T_L (m)	N_P	N_G	N_{HET}	N_{HETmax}	**Mantel test:**	**SSR**	**AFLP**
367	47.2	8	5	1	1		$r^2 = 0.021$; $p = 0.333$	$r^2 = 0.002$; $p = 0.345$
380	151.8	15	11	3	9		$r^2 = 0.113$; $p = 0.012$	$r^2 = 0.288$; $p = 0.001$

Locus	**SML-001**		**SML-019**		**SML-039**		**SML-045**		**SML-055**		**WSULs_18**		**WSULs_25**		**WSULs_45**		**WSULs_75**		**WSULs_153**		**WSULs_163**	
367-1	183	183	170	170	232	232	226	226	225	225	220	220	360	360	184	184	173	173	212	212	182	182
367-4	183	183	170	170	232	232	226	226	225	225	220	220	360	360	184	184	173	173	212	212	182	182
367-6	183	183	170	170	232	232	226	226	225	225	220	220	360	360	187	187	173	173	212	212	182	182
367-7	183	183	170	170	232	232	226	226	219	225	220	220	360	360	184	184	173	173	212	212	182	182
367-9	183	183	170	170	232	232	226	226	219	219	220	220	360	360	184	184	173	173	212	212	182	182
367-10	183	183	170	170	232	232	226	226	225	225	220	220	360	360	184	184	173	173	214	214	182	182
367-11	183	183	170	170	232	232	226	226	219	219	220	220	360	360	184	184	173	173	212	212	182	182
367-13	183	183	170	170	232	232	226	226	219	219	220	220	360	360	184	184	173	173	212	212	182	182
380-1	183	183	170	170	232	232	226	226	225	225	215	215	360	360	184	184	173	173	202	202	182	182
380-2	195	195	170	170	232	232	226	226	225	225	215	215	360	360	184	184	173	173	210	210	182	182
380-2m	195	195	163	170	232	232	226	228	225	225	215	234	358	360	184	189	173	187	184	210	182	196
380-3	183	183	170	170	232	232	226	226	219	219	220	220	360	360	184	184	173	173	210	210	182	182
380-4	195	195	170	170	232	232	226	226	212	212	215	215	360	360	184	184	173	173	217	217	182	182
380-5	183	195	170	170	232	232	226	226	219	219	215	220	360	360	184	184	173	173	210	210	182	182
380-6	195	195	170	170	232	232	226	226	219	219	215	215	360	360	184	184	173	173	210	210	182	182
380-7	195	195	170	170	232	232	226	226	219	219	215	215	360	360	184	184	173	173	210	210	182	182
380-9	195	195	170	170	232	232	226	226	219	219	220	220	360	360	184	184	173	173	210	210	182	182
380-10	195	195	170	170	232	232	226	226	219	219	215	215	360	360	184	184	173	173	210	210	182	182
380-11	195	195	170	170	232	232	226	226	219	219	215	215	360	360	184	184	173	173	210	210	182	182
380-11m	195	195	163	170	232	232	226	228	212	219	215	234	358	360	184	189	173	187	184	214	182	196
380-12	195	195	170	170	232	232	226	226	219	219	215	215	360	360	184	184	173	173	210	210	182	182
380-13	183	183	170	170	232	232	226	226	219	219	215	215	360	360	184	184	173	173	208	208	182	182
380-14	195	195	170	170	232	232	226	226	219	219	215	215	360	360	184	184	173	173	208	208	182	182

Explanation to Tables 3, 4 and 5: m, plant with minor phenotype; N_P, number of analysed plants; N_G, number of genotypes; N_{HET}, number of samples with heterozygous constitution; N_{HETmax}, maximal number of heterozygous loci; T_L, transect lenght, r^2, correlation coefficient; *p*, probability value

Table 6 Microsatellite genotypes and results of Mantel test of *L. saligna* populations (abbreviations are explained below Table 5)

Population	T_L (m)	N_P	N_G	N_{HET}	N_{HETmax}	Mantel test: SSR	AFLP
369	124.3	15	13	8	4	$r^2 = 0.050$; $p = 0.075$	$r^2 = 0.202$; $p = 0.012$
379	105.6	10	9	4	2	$r^2 = 0.025$; $p = 0.142$	$r^2 = 0.551$; $p = 0.000$

	SML-001		**SML-019**		**SML-039**		**SML-045**		**SML-055**		**WSULs_18**		**WSULs_25**		**WSULs_45**		**WSULs_75**		**WSULs_153**		**WSULs_163**	
369-1	183	183	164	164	238	238	214	214	207	207	215	215	324	324	189	189	162	162	190	208	210	210
369-2	189	189	169	169	232	232	214	214	207	207	221	221	324	324	189	189	162	162	198	198	208	208
369-2m	189	189	163	163	238	238	214	214	207	207	221	221	324	324	189	189	162	162	202	202	200	208
369-3	183	183	169	169	232	232	214	214	207	207	221	221	324	324	189	189	162	162	192	196	196	208
369-3m	183	183	169	169	232	232	214	214	207	207	221	221	326	326	189	189	162	162	192	198	196	206
369-4	189	189	164	164	232	232	214	214	207	207	221	221	324	324	189	189	162	162	196	196	206	206
369-4m	189	189	169	169	232	232	214	214	207	207	221	221	324	326	189	189	162	162	192	202	196	206
369-8	183	183	169	169	238	238	214	214	201	201	215	215	324	324	189	189	162	162	190	190	205	205
369-11	183	195	221	221	232	238	214	214	201	201	215	215	324	324	189	189	162	162	190	196	194	206
369-12	183	183	163	163	238	238	214	214	201	201	215	215	324	324	189	189	162	162	190	190	206	206
369-14	189	189	169	169	232	232	214	214	207	207	221	221	324	324	189	189	162	162	196	196	196	200
369-14m	183	189	163	163	232	238	214	214	207	207	221	221	324	324	189	189	162	162	196	202	196	210
369-17	183	183	163	163	238	238	214	214	201	201	215	215	324	324	189	189	162	162	190	190	206	206
369-18	183	183	163	163	238	238	214	214	201	201	215	215	324	324	189	189	162	162	190	190	206	206
369-19	183	183	163	163	238	238	214	214	207	207	215	215	324	324	189	189	162	162	190	190	210	210
379-1	177	177	166	166	238	238	214	214	201	201	215	215	324	324	189	189	162	162	202	202	198	198
379-2	177	177	166	166	238	238	214	214	201	201	215	215	324	324	189	189	162	162	194	200	198	208
379-3	177	177	164	164	238	238	214	214	201	201	215	215	324	324	189	189	162	162	200	200	198	198
379-6	177	177	166	170	238	238	214	214	201	201	215	215	324	324	189	189	162	162	198	198	198	208
379-8	177	177	166	166	238	238	214	214	201	207	215	215	324	324	189	189	162	162	186	186	206	206
379-9	177	177	166	166	238	238	214	214	201	201	215	215	324	324	189	189	162	162	186	198	198	198
379-10	177	177	164	164	238	238	214	214	207	207	215	215	324	324	189	189	162	162	192	192	206	206
379-11	177	177	164	164	238	238	214	214	201	201	215	215	324	324	189	189	162	162	186	186	198	198
379-12	177	177	164	164	238	238	214	214	207	207	215	215	324	324	189	189	162	162	192	192	206	206
379-14	177	177	166	166	238	238	214	214	201	201	215	215	324	324	189	189	162	162	192	192	206	206

diversity for the AFLP data is given in Table 3. The number of private alleles differed among the species investigated ($PA^{SER}_{SUM} = 54$; $PA^{ACU}_{SUM} = 43$; $PA^{SAL}_{SUM} = 104$). In most cases, private alleles represented unique bands for the species; only 8 and 28 null alleles were detected for *L. serriola* and *L. saligna*, respectively.

Polymorphism detected at the species level ranged from $P^{ACU} = 7.83$ % to $P^{SAL} = 33.04$ %; while at the population level, the most polymorphic was population 369^{SAL}, with $P^{369/SAL} = 22.61$ %. The average value of gene diversity (*He*) was 0.041 for populations, and 0.072 for species. Consistent with the microsatellite data, diversity estimates increased in the order: *L. aculeata* ($P = 7.83$ %, $He = 0.029$), *L. serriola* ($P = 26.09$ %, $He = 0.088$), and *L. saligna* ($P = 33.04$ %, $He = 0.099$) (Table 3).

The hierarchical AMOVA analysis revealed that most of the observed molecular variance (75.67 %) is present among the three species; only a minor part (14.77 %) observed among populations within the species; and only 9.57 % observed within populations. Except for the covariance component of the variance among the species ($p = 0.06$), the rest of the covariance components were highly significant ($p < 0.001$). The highest genetic distance was observed for the pair *ACU/SAL* ($D = 0.575$). Distances for the other two pairs were lower: *SAL/SER* ($D = 0.496$), *ACU/SER* ($D = 0.317$).

Mantel test

The Mantel tests indicated a weak, but positive, association between pair-wise genetic distances for the genetic markers (EST–SSR and AFLP) and the physical distances between plants collected at each of the six populations (Tables 4, 5, 6). This association was significant ($p < 0.05$) for four of the six populations. The positive correlation resulted from a few "outlying" genotypes, originating from the peripheral parts of these populations. A non-significant correlation was observed for populations 365^{SER} and 367^{ACU}, both having relatively short transects, as well as less diverse population structure (Tables 4, 5).

Clustering of the investigated populations/species in the Neighbor-Network

Correlation of the genetic distance matrices of $Dc^{EST-SSR}$ and D^{AFLP} for each of the three investigated species in the Mantel test verified a reasonable degrees of congruence between the two classes of molecular markers ($r^2 = 0.618$, 0.324, and 0.441—for *L. serriola*, *L. aculeata*, and *L. saligna*, respectively, $p < 0.001$). An even higher degree of congruence was observed for the overall comparison of both marker types ($r^2 = 0.849$, $p < 0.001$). Based upon this high degree of congruence between the two classes of markers, the SSR-allelic matrix was transformed into a binary matrix, and both binary matrices (AFLP and SSR) were merged. From this merged matrix (302 markers), the Neighbor-Network was constructed (Fig. 2). In the resulting network, individuals divided into three groups according to their taxonomic determinations. Sub-separation, corresponding to the sampled populations, was visible for *L. serriola* and *L. saligna*, but the two *L. aculeata* populations did not form separate groups. Moreover, sub-separation of the *L. saligna* population 369^{SAL} was apparent, where several individuals formed intermediate group to population 379^{SAL}. Samples from this sub-group resembled population 379^{SAL} at several SSR loci (e.g., SML-055, SML-039, and WSULs_18, Table 6). The marked separation of several plants from their maternal populations was observed in our Neighbor-Network: (1) four *L. serriola* samples (365-2, 365-20, 377-1, and 377-14); and (2) two *L. aculeata* individuals (380-2m and 380-11m). These genetically different individuals differed either in their morphological characteristics, or in their growth and phenology (see below).

Discussion

In the present study, our goal was to provide insights into the morphological and genetic structure of three closely-related, primarily autogamous wild lettuce species. We analyzed the morphological and genetic variation in the offspring of the samples, all of which were collected from natural sites in Israel. Knowledge about population structure and reproductive behavior can help optimize collecting strategies, both to broaden the genetic variation maintained in germplasm collections and to foster their use for breeding purposes (Spooner and van der Berg 1992). Herein, we followed both regeneration protocols and morphological characterization methodology commonly used for lettuce germplasm collection (Doležalová et al. 2002,

2003; Lebeda et al. 2007b; Maggioni et al. 2008, 2014). Our sampling strategy followed the recommendations of Lebeda et al. (2001, 2007a); one seed sample represents seeds taken from one plant in the population. Sampling of the plants was done along linear transects throughout the populations. In the analyses, we compared two non-adjacent populations per plant species, with the smallest distance (18.1 km) found between the *L. aculeata* populations (*L. serriola*: 42.0 km; *L. saligna*: 99.3 km). Therefore, we were able to verify patterns of intrapopulation diversity ascertained at two independent sites.

Genetic variation

It is well known that these three *Lactuca* species (*L. serriola, L. aculeata,* and *L. saligna*) differ substantially in their geographic distribution, ecological plasticity, morphology, genetic variation, and reaction to biotic factors (e.g., reaction to pathogens and pests) (Lebeda et al. 2004b, 2007a, b, 2009a, 2014). The three species are also clearly distinguished by both of the molecular-marker classes we employed, with the presence of unique SSR alleles or AFLP bands for each species and population. This resulted in the sub-separation of samples in the Neighbor-Network, according to the determination of species and the population of origin (Fig. 2). The extent of genetic variation in a species, and its distribution among and within populations, can be determined by multiple factors, such as: the breeding behavior, habitat availability, population size, migration between populations, as well as many biotic and abiotic factors (Nybom et al. 2014). The breeding system has a profound effect on gene flow, as well as on the partitioning of genetic variation within and between populations. Species of *Lactuca* are predominantly considered as selfers, and are typically early successional taxa, which generally allocate more variation among populations (Nybom et al. 2014). We substantiated this by the results of hierarchical AMOVA, which ascribed only a minor part of the variation within populations, with more substantial parts of the variance present both among populations and species.

Earlier studies from our laboratory have described differences in the genetic backgrounds of various *Lactuca* species on a large geographical scale, i.e., genetic differentiation of samples originating from different countries (Kitner et al. 2008; Lebeda et al. 2009b, 2011; Jemelková et al. 2013). D'Andrea et al. (2006) also described a high genetic differentiation among individual *L. serriola* populations. This large-scale structuring of genetic variation is ascribed to selection and colonization history (Nybom et al. 2014). However, none of the above-mentioned studies compared actual populations, but assayed pseudopopulations representing individual plants collected from a large geographical area. Our data demonstrate that, on a finer geographical scale, natural populations of wild *Lactuca* species are well differentiated genetically, and that each population can represent a unique combination of genotypes, which differ from other populations of the same species. These populations are not genetically (or morphologically) uniform, and on relatively short transects (<200 m), different genotypes can often be sampled (Tables 4, 5, 6).

The separation of populations within a particular species on the Neighbor-Network can be explained by their geographical distance. The distance of only 18.1 km between the two *L. aculeata* populations that we sampled could help explain their weak separation; i.e., a high number of shared genotypes between the two populations for eight SSR loci. The high similarity of these two *L. aculeata* populations is also reflected in the lowest number of private SSR alleles and AFLP loci (Table 3). On the other hand, the more distant *L. serriola* (42.0 km) or *L. saligna* (99.3 km) populations possessed more private loci, resulting in their more discrete separation in the Neighbor-Network (Fig. 2).

In general, relatively low values of genetic diversity were observed within a particular species, which increased in the order *L. aculeata* < *L. serriola* < *L. saligna* (Table 3). When looking at genotype diversity calculated from merged AFLP and SSR data, each analyzed plant in both *L. saligna* populations represented a unique genotype ($N_G^{SAL} = 22$). This is partly in agreement with data obtained in previous studies (Kitner et al. 2008; Lebeda et al. 2009b, 2012b). This was apparent in the lower genetic diversity values in the *L. aculeata* sample set compared to *L. serriola* (Lebeda et al. 2012b), or less morphological variation in the *L. aculeata* populations in comparison with *L. saligna* (Beharav et al. 2008, 2010a), which is supported by the wide variation of leaf shapes in *L. saligna* (Křístková et al. 2009, 2010; Lebeda et al. 2012b). Nevertheless, a relatively large and unexpected variation of race-specific resistance against *Bremia lactucae* has been detected within

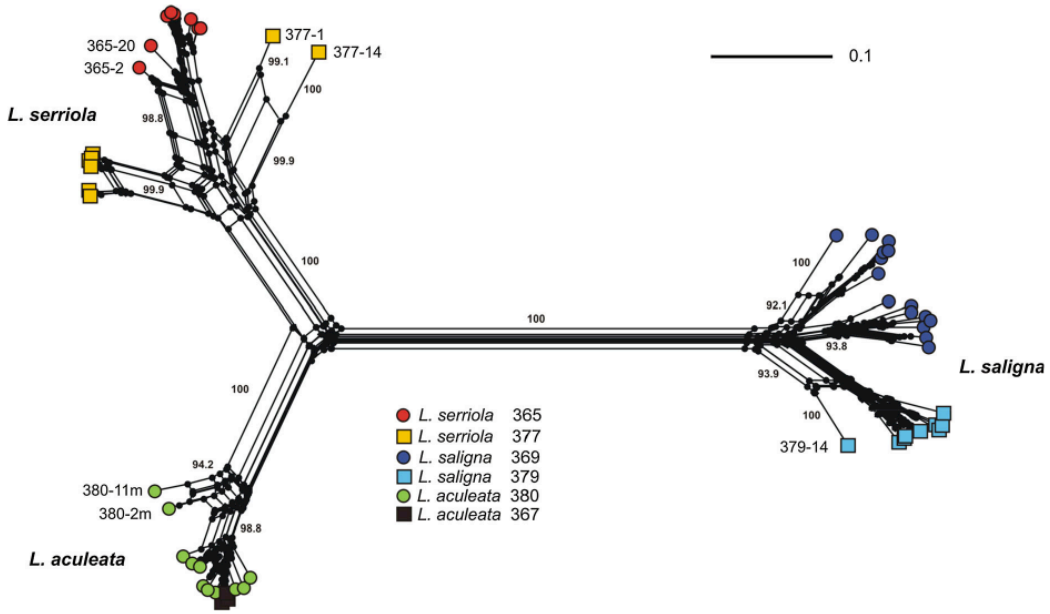

Fig. 2 Neighbor-network cluster analysis of the three wild *Lactuca* species based on EST–SSR and AFLP data (Dice coefficient of similarity, 1,000 bootstraps)

L. aculeata (Beharav et al. 2014; Jemelková et al. 2013).

Comments on variation in *L. serriola*

Population 365^{SER} represents the most uniform population with only 6 genotypes among 15 analyzed plants (calculated from merged AFLP and SSR data). It is also interesting to note that, for both *L. serriola* populations, the largest physical distances between two plants having identical genotypes were observed: 45.5 m between plants 365-1 and 365-18; 46.7 m between plants 377-5 and 377-10. Population 365^{SER} was also morphologically uniform. Morphologic trait on the stem leaves corresponds to the position of sample 365-2 in the Neighbor-Network (Fig. 2), where this sample was placed apart the main 365^{SER} cluster, as well as plant 365-20. Plants 365-2 and 365-20 possessed alleles characteristic of population 377^{SER} at five genotyped loci, which were absent in the other plants from population 365^{SER}. This may point to the presence of gene-flow or immigration between *L. serriola* populations. Another very specific feature that distinguished samples 365-2 and 365-20 from all the remaining samples of population 365^{SER} was connected to their vitality, in contrast to the collapse of the other 365^{SER} plants (approx. 100 days after sowing).

The individual plants from population 377^{SER} were uniform, but some morphological heterogeneity among samples was observed. Two striking exceptions have been observed for samples 377-1 and 377-14, being uniform and resembling each other but distinct from the other samples from this population. Some morphological traits of these plants were characteristic of *L. saligna*, suggesting the involvement of this species in the origin of these two samples. During this experiment, the development and persistence of rosette leaves was observed exclusively on plants of samples 377-1 and 377-14. The formation of leaf rosettes is typically associated with *L. serriola* and *L. saligna* from Europe. In our experience, *L. serriola* and *L. saligna* plants from Israeli climatic conditions do not develop leaf rosettes in either their original habitats, or during their cultivation in temperate parts of Central Europe. Also, the presence of a 238 bp

allele at locus SML-039 may support the morphological data for hybridization with *L. saligna*, while a 170 bp allele at locus SML-019 points to a connection with *L. aculeata* (Fig. 2; Tables 5, 6). Due to the morphological uniformity of plants within samples 377-1 and 377-14 and their homozygous SSR-genotypes, we hypothesize that a hybridization event might have occurred several generations ago.

Comments on variation in *L. aculeata*

While population 367^{ACU} was morphologically uniform, population 380^{ACU} was more heterogeneous for the morphological traits evaluated (e.g., the shapes of the stem leaves varied from spathulate to elliptic). Similarly, the number of observed SSR genotypes was relatively low in the 367^{ACU} population, with only a single sample (367-7) having one heterozygous state at locus SML-055. On the other hand, a higher level of intrapopulation heterogeneity of population 380^{ACU} was confirmed by SSR and AFLP markers (Table 5; Fig. 2), with a noticeable separation of individual plants, 380-2m and 380-11m. These plants were characterized by their distinctive morphology and phenology from the rest of the population. Moreover, these two plants bore the highest number of heterozygous loci observed among all of the plants analyzed; unique alleles, absent in the rest of population sample; and some alleles (e.g., 163 bp at SML-019, 187 bp at WSULs_75, 196 bp at WSULs_163, Table 5) identical to those characteristic of *L. serriola*. A heterozygotic constitution, together with morphological data, provides hints for the likely recent hybrid origins of these two plants, very likely *L. aculeata* × *L. serriola*.

Comments on *L. saligna* variation

Population 379^{SAL} was uniform in its morphological traits, in contrast to the more variable population 369^{SAL}. The presence/absence of trichomes on the stem corresponds (excepting sample 369-1) to the division of population 369 into two clusters. It is interesting that samples 369-8, -11, -12, -17, -18, and -19 with trichomes are close to samples of the "trichomeless" population 379^{SAL}. These samples formed a separate group in the Neighbor-Network (Fig. 2), with intermediate characteristics between populations $369^{trichomes}$ and $379^{trichomless}$. These plants bore identical alleles with population 379^{SAL} at several SSR loci (WSULs_18, WSULs_153 and SML-055; Table 6).

Four individuals from the 369^{SAL} population exhibited the "minor" phenotype. The SSR profiles of samples with the "prevailing" and "minor" phenotypes were considerably different. These samples were germinated from achenes collected from a single maternal plant, and the achenes would be expected to have resulted from self-pollination. When looking at the allelic profiles (WSULs_153 and WSULs_163) (Table 6) of plant pairs analyzed within samples 369-4 and 369-14, the genotypes do not strictly follow the expectation for a segregation of alleles during meiosis. The presence of three different alleles for pairs (e.g., samples: 369-3/m at WSULs_153 and WSULs_163; 369-4/m at WSULs_153; and 369-14/m at WSULs_163; Table 6) indicates that flowers on a single maternal plant can be fertilized by pollen coming from several different plants.

In contrast to *L. serriola* and *L. aculeata*, both of the *L. saligna* populations exhibited higher diversity values, a greater number of SSR genotypes, including more plants with a heterozygous constitution. This might point to the possibility of exchange of individuals between populations (confirmed by the higher out-crossing rate), and/or that selfing is not the strict mode of reproduction in natural population of *L. saligna* in the Near East. However, these statements need to be tested with more-focused research based on comparisons of SSR profiles of maternal plants and their offspring of several populations, sampled in two or more transects from different directions.

Comments on hybridization and the position of outlying plants in populations

There was a noticeable separation of four *L. serriola* samples (365-2, 365-20, 377-1 and 377-14) and two *L. aculeata* plants (380-2m and 380-11m) from other members of their maternal populations on the Neighbor-Network, which was confirmed by their exceptional morphological traits and microsatellite profiles (Tables 4, 5), as described above. The SSR profiles of *L. serriola* 365-2 and 365-20 individuals point to interpopulation gene-flow, due to the absence of alleles present in other species. The pronounced separation of *L. serriola* samples (377-1, 377-14) and *L. aculeata* plants (380-2m, 380-11m) on the Neighbor-Network (Fig. 2), as well as their SSR

genotypes and differences in phenotypes, may point to ongoing interspecific hybridization in natural populations of the Near East.

Although autogamy is considered as the prevailing breeding system within *Lactuca*, especially in the marginal regions of its geographic distribution (e.g. southern Scandinavia) (Feráková 1977), the occurrence of natural interspecific hybrids in the Near East supports the theory that *Lactuca* species of subsection *Lactuca* are more allogamous near their center of origin (Lindqvist 1960) and in their center of distribution (Stebbins 1957; Rick et al. 1977).

The presence of interspecific hybrids can be observed in populations where several *Lactuca* species coexist, as was recently demonstrated in studies by Lebeda et al. (2012b) and Křístková et al. (2012), which described natural interspecific hybridization in wild populations of *L. aculeata* and *L. serriola*. This has also been documented between *L. altaica* and *L. saligna* and *L. serriola*, between *L. aculeata* and *L. sativa* and *L. serriola*, and between *L. serriola* and *L. dregeana* (Zohary 1991; Lebeda et al. 2007b).

The question regarding the source of "non-indigenous" alleles for a given population produced by plants found at the edge of those populations is a rather interesting one. We can consider three mechanisms that can bring new alleles into a population and increase population diversity, cross-pollination due to the transfer of pollen from a distant population by (I) insect or (II) wind, or (III) long-distance seed dispersal. Feráková (1977) described cross-pollination of *Lactuca* facilitated by various Hymenoptera and Diptera. D'Andrea et al. (2008) observed frequent cross-pollination (hybridization rates up to 26 %) over short distances in sympatric populations of cultivated lettuce and its wild relative (*L. serriola*). More than 80 % of the *L. serriola* plants produced at least one hybrid at distance <1 m; and even at 40 m, 4–5 % of the wild plants produced hybrids (D'Andrea et al. 2008).

Lu et al. (2007) reported the movement of *L. serriola* achenes over distances of up to 43 km in South Australia. Our data support the scenario of long-distance seed dispersal and subsequent cross-pollination after successful immigrant seedling emergence. This is mainly visible in individuals collected from the population margins (377^{SER}, 365^{SER}, and 380^{ACU}; Tables 4, 5). Maternal plants 377-1 and 377-14, and 380-2 and 380-11 were located at the opposite ends of our transects. *Lactuca serriola* plants 377-1 and 377-14 were 100 m apart, and the distance between *L. aculeata* plants 380-2 and 380-11 was even higher (135 m). The periphery of wild lettuce populations could act as a transition zone for the transport of pollen and achenes from neighboring populations and may be an important source of novel genetic variation. Especially in relatively open landscapes, the margins of populations can act as a trap for achenes blown by wind from distant populations. Ecological conditions on the periphery of populations can differ slightly from those in the inner parts, or can be more greatly disturbed by human activities. Such environmental shifts may provide opportunities for the successful germination of trapped achenes, and the subsequent crossing with plants from the indigenous population, due to the absence of barriers for interspecific/interpopulation hybridization. This phenomenon could result in introgressive hybridization, where rare hybrids tend to backcross within populations, leading to gene transfer between distinct populations or species (Rieseberg et al. 2003; Baskett and Gomulkiewicz 2011). Empirical examples suggest that introgressive hybridization can play a key role in adaptation to novel environments and environmental changes in a diverse array of taxa (Anderson 1948; Lewontin and Birch 1966; Dowling and Secor 1997; Arnold et al. 2008; Arnold and Martin 2010).

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4.4.1. Resistance of wild *Lactuca* genetic resources to diseases and pests, and their exploitation in lettuce breeding

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Resistance of wild *Lactuca* genetic resources to diseases and pests, and their exploitation in lettuce breeding

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Abstract

The reported variation in the reaction of wild *Lactuca* species to pathogens and pests is summarized, including viral pathogens (e.g. Lettuce mosaic virus – LMV, *Mirafiori lettuce virus*/*Lettuce big vein virus* – LBVV, *Cucumber mosaic virus* – CMV), bacterial pathogens (corky root – *Rhizomonas suberifaciens*, bacterial leaf spot – *Xanthomonas campestris* pv. *vitians*), oomycete and fungal pathogens (downy mildew – *Bremia lactucae*, powdery mildew – *Golovinomyces cichoracearum*, anthracnose – *Microdochium panattoniana*, stemphylium leaf spot – *Stemphylium* spp., sclerotinia drop – *Sclerotinia* spp., verticillium wilt – *Verticillium dahliae*, fusarium wilt – *Fusarium* spp., pythium wilt – *Pythium tracheiphylum*), nematodes (potato cyst nematode – *Globodera rostochiensis*, root-knot nematode – *Meloidogyne* spp., *incognita*, *hapla*, *javanica*, *enterolobii*), insects and mites (the green lettuce aphid – *Nasonovia ribisnigri*, the green peach aphid – *Myzus persicae*, the potato aphid – *Macrosiphum euphorbiae*, leafminer – *Liriomyza* spp.). The approaches used to exploit wild *Lactuca* spp. in lettuce breeding are discussed and known examples of lettuce cultivars with traits derived from wild *Lactuca* spp. are described.

Keywords: biodiversity, germplasm, gene pools, *L. serriola*, *L. saligna*, *L. virosa*, wild lettuce, breeding strategies, transfer of resistance

INTRODUCTION

The value of the use of wild *Lactuca* species in lettuce breeding is being demonstrated by means of classical biology and modern approaches and the study of their diversity has been a subject of theoretical research and practical application during the last decade (Lebeda et al., 2007; Mou, 2008). The currently available knowledge of biodiversity of wild *Lactuca* species and their exploitation as donors (sources) of traits important in lettuce breeding was thoroughly analyzed by Lebeda et al. (2009, 2014). The exploitation of natural sources of resistance corresponds to modern strategies of plant breeding and plant cultivation techniques (Barrière et al., 2014). The main aim of this paper is to summarize the available information about wild *Lactuca* species and their exploitation in lettuce resistance breeding.

VARIATION OF WILD *LACTUCA* SPP. IN REACTION TO PATHOGENS AND PESTS

Viral pathogens

Lettuce mosaic virus, *Mirafiori* (big vein) *lettuce virus*, *Beet western yellows virus*, *Tomato spotted wilt virus*, *Cucumber mosaic virus* and *Lettuce necrotic stunt virus* are the most serious lettuce viruses. Sources of resistance within the wild *Lactuca* species *L. serriola*, *L. saligna*, *L. virosa* and *L. perennis* offer a way of introgressing new resistances. Crossing between *L. virosa* bearing resistance to LBVV and *L. sativa* cultivars was difficult to perform, nevertheless introgression of big vein tolerance from *L. virosa* to cultivars of lettuce has been successful (Hayes and Ryder, 2007).

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Resistance to CMV has been derived from *L. saligna*, introgression of resistance into lettuce was done by backcrossing with *L. sativa*. By the F_7 generation of 'Montello' × ('Vanguard 75' × *L. saligna* PI 261653) (Tamaki et al., 1995), and *Lactuca saligna* × *L. sativa* 'Saladcrisp' (Provvidenti et al., 1980) the introgression was successful.

Bacterial pathogens

Corky root of lettuce (*Sphingomonas suberifacien*, formerly *Rhizomonas suberifaciens*) has been observed in several major lettuce producing areas of the world. The first resistant lettuce cultivars 'Marquette', 'Montello' and 'Green Lake' developed by Sequiera (1978) were released from crosses with a resistant line PI 171669. Brown and Michelmore (1988) identified resistant lines within the wild species *L. serriola*, *L. saligna*, *L. dentata*, *L. virosa* and *Lactuca* spp. Mou and Bull (2004) identified three *L. serriola* and one *L. virosa* accessions consistently resistant to corky root in growth chamber, greenhouse and field experiments. The resistance to corky root is conferred by a recessive allele (*cor*) at a single locus (Brown and Michelmore, 1988), which is present in many modern crisphead lettuce cultivars, e.g. 'Bronco', 'Cannery Row', 'Glacier', 'Premiere', 'Misty Day', 'Sharp Shooter', 'Sniper' (Mou et al., 2007).

Bacterial leaf spot of lettuce caused by *Xanthomonas campestris* pv. *vitians* has been reported from different countries, since the beginning of the 20th century. *X. campestris* pv. *vitians* can infect not only cultivated lettuce but also the wild *Lactuca* species (*L. serriola* and *L. biennis*), and these species may serve as a reservoir for this pathogen (Toussaint et al., 2012). Several commercial cultivars of lettuce have been screened for resistance to this bacterial pathogen (Carisse et al., 2000) and activities aimed at the development of lettuce breeding lines resistant to bacterial leaf spot have been briefly reported (Hayes et al., 2005).

Fungal pathogens

Lettuce downy mildew (*Bremia lactucae*) has a very significant economic impact on lettuce production in many lettuce producing areas, and the study of its biology and epidemiology, sources of resistance, including wild *Lactuca* germplasm evaluations have been a high priority of researchers and breeders (Lebeda et al., 2014).

Currently, the search for new sources of resistance and genes suitable for practical lettuce breeding in wild *Lactuca* germplasm (Lebeda et al., 2014) is considered to be very important. Accessions of *L. serriola* (reported as PI 91532 but subsequently shown to be PI 104584 and PI 167150) originating from Russia and Turkey were used in the 1930s in the USA as sources of race-specific resistance against *B. lactucae* (Lebeda et al., 2002). These sources created the breeding pool for a new generation of lettuce cultivars ('Imperial 410', 'Calmar', 'Valmaine') for outdoor cropping which were introduced in the 1940s and 1950s (Whitaker et al., 1958). In the 1970s and 1980s other sources of resistance to *B. lactucae,* derived from *L. serriola* with race-specific resistance genes (factors) described as *Dm*16 and R18 were used in The Netherlands. All of these genes have been used frequently in breeding programs in Europe during the last twenty years. However, resistance based on these genes is no longer effective against many *B. lactucae* isolates (Lebeda and Zinkernagel, 2003). From the end of the 1980s there was increasing interest (esp. in The Netherlands and UK) for the utilization of resistance located in the hybrid line *L. serriola* (Swedish) × *L. sativa* 'Brunhilde' and the line CS-RL (Lebeda and Blok, 1991) was derived from this material.

L. saligna and *L. virosa* may possess novel and very interesting resistance to *B. lactucae* (Lebeda et al., 2002). As a result of studies in the 1990s a new lettuce cultivar Titan (Sluis & Groot) with the race-specific gene *Dm*6 plus resistance derived from *L. saligna* (K. Reinink, Rijk Zwaan, the Netherlands, pers. commun.) was released in The Netherlands (Lebeda et al., 2002). However, this resistance is no longer effective (Lebeda and Zinkernagel, 2003). Recently, a very intensive program of lettuce breeding aimed at the introduction of newly located sources of resistance from *L. serriola*, *L. saligna* and *L. virosa* was developed in the USA and Europe (Lebeda et al., 2009, 2014), and various sources of resistance are being used. Currently, one of the most challenging resistances for lettuce breeders to exploit is the apparent nonhost resistance located in *L. saligna* (Lebeda et al., 2002, 2014; Petrželová et al.,

2011).

Lettuce powdery mildew is considered as a disease of increasing importance. The Ascomycete *Golovinomyces cichoracearum* V.P. Gelyuta (previously *Erysiphe cichoracearum* DC. s.str.) is the predominant powdery mildew species on lettuce (Lebeda and Mieslerová, 2011). The screening of more than one hundred accessions of wild representatives of the genus *Lactuca* (e.g. *L. aculeata*, *L. saligna*, *L. serriola*, *L. tatarica*, *L. virosa*) under conditions of natural infection by *G. cichoracearum* revealed high variability in resistance (Lebeda, 1985, 1994). The accessions of *L. serriola* were attacked most severely and *L. saligna* showed highly variable levels of resistance, while the lowest levels of infection were found in accessions of *L. virosa*, *L. viminea*, *L. tenerrima* and *L. tatarica*. In some species (e.g. *L. saligna*, *L. serriola*) the interaction with the pathogen is based on race-specific resistance (Lebeda and Mieslerová, 2011).

Anthracnose (shothole disease, ringspot) is caused by *Microdochium panattoniana* (Berl.) Sutton, Galea & Price. Sources of resistance have been identified in wild *Lactuca* species *L. angustana*, *L. livida*, *L. perennis*, *L. serriola*, *L. saligna*, and *L. virosa* (Galea and Price, 1988).

Stemphylium leaf spot (*Stemphylium botryosum* f. *lactucum* Wallr.) has been reported in many parts of the world, but it has relatively small economic impact. The only reported source of resistance to the disease is an unspecified line of *L. saligna* collected in Israel. Resistance is controlled by two genes, with one allele dominant Sm_1 for resistance and the other recessive sm_1 (Netzer et al., 1985).

Two fungal species, *Sclerotinia minor* and *Sclerotinia sclerotiorum*, both surrviving mainly as sclerotia in soil cause sclerotinia drop of lettuce, one of the most widespread and destructive diseases worldwide in lettuce (Subbarao, 1998). *Lactuca* sp. (PI 274376) and *L. serriola* (PI 271938) were shown to be highly resistant to *S. minor* (Abawi et al., 1980), and the latter accession was also resistant to *S. sclerotiorum* (Whipps et al., 2002). In the *S. sclerotiorum*-infested field experiments, three *L. virosa* accessions (SAL 012, IVT 280, IVT 1398) showed high levels of resistance (Hayes et al., 2010).

Verticillium wilt (*Verticillium dahliae* Kleb) is a relatively newly recognised lettuce disease (Bhat and Subbarao, 1999). Partial resistance to race 2, in the form of reduced disease incidence or delayed expression of symptoms, has been found in four PI accessions *L. sativa* (Hayes et al., 2011). The *L. virosa* accession IVT 280 and some other accessions have shown high levels of resistance in field tests (Grube et al., 2005).

Fusarium wilt, a disease of the root vascular system, is caused by *Fusarium oxysporum* f. sp. *lactucae* n.f. (same as f. sp. *lactucum*. Three races of the fungus have been identified (Fujinaga et al., 2003). Resistance sources have been identified for races 1 and/or 2, but not for race 3 (Garibaldi et al., 2004; Tsuchiya et al., 2004). All sources are cultivars of the various lettuce types, so resistance within wild *Lactuca* spp. is not known (Lebeda et al., 2014).

Nematodes

The most important nematodes with documented impact on lettuce growth and yield include the needle nematode (*Longidorus africanus*), root-knot nematode (*Meloidogyne* spp.), root lesion nematode (*Pratylenchus penetrans*), and the spiral nematode (*Rotylenchus robustus*). Reports of wild *Lactuca* species hosting nematodes and on resistant genotypes are summarised by Lebeda et al. (2014).

Insects and mites

Three aphid species, the green lettuce aphid (*Nasonovia ribisnigri*), the potato aphid (*Macrosiphum euphorbiae*) and lettuce root aphid (*Pemphigus bursarius*) and the leafminer (*Liriomyza langei*) are the most important pests both on cultivated and wild lettuces (Lebeda et al., 2014).

Resistance to biotype 0 (Nr: 0) *Nasonovia ribisnigri* was first reported in *L. virosa* accession IVT 280 and it was successfully transferred to lettuce by a bridging cross to *L. serriola* (e.g. Eenink et al., 1982; van der Arend et al., 1999). There are a large number of

modern lettuce cultivars with *N. ribisnigri* resistance, e.g. 'Barcelona', 'Campionas', 'Dynamite', 'Elenas', 'Fortunas', 'Irina', 'Krinas', 'Veronas', '83-67RZ' (Liu and McCreight, 2006). McCreight (2008) identified two new potential sources for resistance to Nr:0 in *L. serriola* acc. PI 491093 (partial resistance) and *L. virosa* PI 274378 (complete resistance). Reports on the genetics of resistance all economically important aphids described in wild *Lactuca* species were critically summarized by Lebeda et al. (2014). Promising sources of resistance to the leafminer (*Liriomyza langei*) within wild *L. saligna*, *L. serriola* and *L. virosa* were identified by Mou and Liu (2004).

EXPLOITATION OF WILD *LACTUCA* SPP. IN LETTUCE RESISTANCE BREEDING

Interspecific hybridization is the basic breeding method used for crossing of cultivated lettuce with wild *Lactuca* species from the primary and secondary gene pools. Interspecific hybrids between the species with a low sexual compatibility with lettuce (e.g. *L. virosa* and *L. saligna*) have been obtained by using a bridging species (e.g. *L. serriola*) i.e. crossing one parent to the bridging species and then crossing the resultant F1 with the other parent (Lebeda et al., 2007).

Techniques and approaches used for in vitro culture of lettuce have been reviewed thoroughly by Lebeda et al. (2014), however there are no recent reports of in vitro culture technologies exploiting wild *Lactuca* species in lettuce improvement. In vitro rescue of immature embryos was used successfully for sexual hybridization between *L. sativa* and *L. virosa* (Maisonneuve, 2003). Protoplast fusion permitted the regeneration of somatic hybrids between *L. sativa* and either *L. tatarica* or *L. perennis* (Maisonneuve et al., 1995). Somatic hybrids between cultivated lettuce and *L. virosa* were produced by protoplast electrofusion (Matsumoto, 1991). Hybrids had normal flower morphology, but all were sterile. *L. indica* (section *Tuberosae*) can be somatically hybridized with *L. sativa* to produce a viable callus (Mizutani et al., 1989). So far, fertile hybrids have only been produced by using somatic hybridization.

During the last ten years, there are numerous reports on the gene introduction into lettuce by transformation (Klocke et al., 2010). Utilization of some other techniques (e.g. plastid transformation, resistance conferred by viral genes, the cloning of resistance genes, induced mutations, etc.) was reviewed elsewhere (Lebeda et al., 2007, 2014).

KNOWN EXAMPLES OF LETTUCE CULTIVARS ISSUED FROM EXPLOITATION OF WILD *LACTUCA* SPP.

Lettuce breeders have increased genetic diversity and achieved disease resistance through crossing cultivated lettuce with non-cultivated or wild lettuce types. Reviews of wild *Lactuca* species used in lettuce breeding and a description of breeding approaches and methods have been published previuosly (Lebeda et al., 2007, 2014; Mou, 2008; Davey and Anthony, 2011). The pedigree analysis of 328 proprietary and publicly developed lettuce cultivars registered in the USA from 1970 through to 2004 showed that 1% of these cultivars were developed from interspecific crosses (Mikel, 2007). Three wild *Lactuca* species, *L. serriola*, *L. saligna* and *L. virosa* were involved in this process, and the cultivars 'Vanguard' and 'Salinas' followed by the cultivar 'Calmar' (which has no *L. virosa* in its ancestry) were determined as elite programme cultivars, frequently used in lettuce breeding (Mikel, 2007).

Lebeda and Blok (1991) reported downy mildew resistance in the hybrid of *L. sativa* × *L. serriola*. More details about this and the historical consequences about the influence of *L. serriola* on lettuce resistance breeding to *B. lactucae* were summarized by Lebeda et al. (2002). *Lactuca saligna* is known to produce, hybrids with *L. sativa* and *L. serriola* when used as the female parent (Pink and Keane, 1993). *L. saligna* was crossed to a cultivated iceberg type by R.W. Robinson (Provvidenti et al., 1980) who developed the cultivar 'Salad Crisp' from that cross. Viable hybrid plants from crosses between *L. sativa* and *L. virosa* were obtained only when *L. serriola* was used as a bridging species (Eenink et al., 1982). In 1958, the cultivar Vanguard was developed from a cross between a *L. sativa* × *L. serriola* which was then crossed to *L. virosa*. However, with some manipulations, crosses have been made with *L virosa* and have led to development of cultivars (Ryder, 1999).

CONCLUSIONS AND CHALLENGES

A detailed survey of recent achievements and future prospects of wild *Lactuca* germplasm exploitation has been published elsewhere (Lebeda et al., 2009, 2014). During the last seventy years of lettuce breeding a strategy of utilizing germplasm of wild relatives has been used with very high practical impact (Lebeda et al., 2007, 2014; Mou, 2008). Unfortunately, the process of resistance breeding is complicated because the variation in host plant resistance is mirrored by the diversity (emergence of new different strains, pathotypes and races) within pathogen/pest populations (Lebeda et al., 2007) and future developments will be focused on detection of new sources of resistance. Multiple disease and pest resistance, methodologies of interspecific hybridization, as well as transfer of resistance genes all play an important role in accessing the genetic variation present in wild *Lactuca* germplasm (Lebeda et al., 2007, 2014; Davey and Anthony, 2011). This will improve our efficiency in exploiting the *Lactuca* genepool for lettuce crop improvement and allow the combination of different resistance mechanisms in a single cultivar in order to provide potentially more durable resistance (Lebeda et al., 2014).

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4.4.2. Wild *Lactuca* species, their genetic diversity, resistance to diseases and pests, and exploitation in lettuce breeding

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Wild *Lactuca* species, their genetic diversity, resistance to diseases and pests, and exploitation in lettuce breeding

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Abstract Current knowledge of wild *Lactuca* L. species, their taxonomy, biogeography, gene-pools, germplasm collection quality and quantity, and accession availability is reviewed in this paper. Genetic diversity of *Lactuca* spp. is characterized at the level of phenotypic and phenological variation, variation in karyology and DNA content, biochemical traits, and protein and molecular polymorphism. The reported variation in reaction to pathogens and pests of wild *Lactuca* spp. is summarized, including the viral pathogens (Lettuce mosaic virus-*LMV*, Mirafiori lettuce virus/Lettuce big vein virus-*LBV*, Beet western yellows virus-*BWYV*, Tomato spotted wilt virus-*TSWV*, Cucumber mosaic virus-*CMV*, Lettuce necrotic stunt virus-*LNSV*), bacterial pathogens (corky root-*Rhizomonas suberifaciens*, bacterial leaf spot-*Xanthomonas campestris* pv. *vitians*), fungal pathogens (downy mildew-*Bremia lactucae*, powdery mildew-*Golovinomyces cichoracearum*, anthracnose-*Microdochium panattoniana*, stemphylium leaf spot-*Stemphylium* spp., sclerotinia drop-*Sclerotinia* spp., verticillium wilt-*Verticillium dahliae*, fusarium wilt-*Fusarium* spp., pythium wilt-*Pythium tracheiphylum*, *P. uncinulatum*), nematodes (potato cyst nematode-*Globodera rostochiensis*, root-knot nematode-*Meloidogyne* spp., *incognita, hapla, javanica, enterolobii*), insects and mites (the green lettuce aphid-*Nasonovia ribisnigri*, the green peach aphid-*Myzus persicae*, the potato aphid-*Macrosiphum euphorbiae*, leafminer-*Liriomyza* spp., *L. langei*). The approaches used to exploit wild *Lactuca* spp. in lettuce breeding (interspecific hybridization, cell and tissue culture, transformation) are dicussed, and known examples of lettuce cultivars with traits derived from wild *Lactuca* spp. are described.

Keywords Taxonomy · Biodiversity · Gene-pools · Gene banks · Disease resistance · Molecular polymorphism · *L. serriola* · *L. saligna* · *L. virosa* · Pest resistance · Breeding strategies · Transfer of resistance · Wild lettuce · Germplasm

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Introduction

The potential of wild *Lactuca* species to be used in lettuce breeding is being demonstrated by means of classical biology and modern approaches and the study of their diversity has been a subject of theoretical research and practical application during the last 25 years (Ryder 1999; Lebeda et al. 2007c; Mou 2008). The currently available knowledge of wild *Lactuca* species as donors (sources) of traits important in lettuce breeding was thoroughly analyzed in our previous paper focused on wild *Lactuca* germplasm (Lebeda et al. 2009a, b). The main aim of this paper is to critically summarize the available information

about interactions between wild *Lactuca* species and the most important lettuce pathogens and pests from the viewpoint of their resistance and their potential exploitation and utilization in lettuce breeding.

Taxonomy of *Lactuca* spp.

The genus *Lactuca* L. (family Compositae/Asteraceae) is composed of one cultivated species-lettuce (*Lactuca sativa* L.), and about 100 wild *Lactuca* spp. of which nearly 95 % are autochtonous in Asia and Africa (Lebeda et al. 2007c). Species are arranged into seven sections and two geographic groups (Table 1). This broader generic concept summarized by Lebeda et al. (2007c) should be critically re-considered with regard to the molecular data on phylogenetic relationships among *Lactuca* species (Koopman et al. 1998, 2001).

Eco-geographic characteristics

The genus *Lactuca* L. comprises annual, biennial or perennial herbs and rarely shrubs with various ecological requirements. The species *L. serriola*, *L. saligna* and *L. virosa* are weedy and occur on waste places and ruderal habitats, along roads, highways and ditches, *L. perennis*, *L. viminea*, *L. graeca*, *L. tenerrima* are calciphilous plants and colonise limestone and dolomite areas, mostly rocky slopes. Endemic lianalike species are found in rain forests of East Africa (Lebeda et al. 2001, 2004b, 2007c).

The greatest diversity of *Lactuca* species is confined to the Mediterranean basin and Southwest Asia (Doležalová et al. 2001; Beharav et al. 2008; Kitner et al. 2008; Lebeda et al. 2001, 2009a, b). The occurrence of valuable germplasm is expected in the Central and South Africa, Southwest and Central Asia, and North America regions (Lebeda et al. 2007c, 2011, 2012a).

Lactuca germplasms and their availability

The concept of conservation and management of wild crop relatives, and conservation priorities were proposed by Maxted et al. (2008) and Ford-Lloyd et al. (2008). The linking of in-situ and ex-situ conservation with the use of wild crop relatives is the leading principle of their conservation and management (Maxted and Kell 2008). Access to wild genetic resources and the possibility to explore and exploit them depend upon the successful and reasonable protection of wild species *in–situ*, i.e. in their natural habitats (Iriondo and De Hond 2008), upon the complex study of wild species in natural habitats and upon the possibility to exchange information and biological material (Azzu and Collette 2008). These research activities are regulated by national policies and the international conventions and protocols, e.g. the recent "Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity" adopted by the Conference of the Parties to the Convention on Biological Diversity at its tenth meeting on

Table 1 Taxonomy of the genus *Lactuca* L. (modified according Lebeda et al. 2007c)

Section	Subsection	Species	Biology
Lactuca L.	*Lactuca* L.	*L. aculeata*, *L. altaica*, *L. dregeana*, *L. livida*, *L. saligna*, *L. sativa*, *L. serriola*, *L. virosa*	annual, overwintering, annual, biennial
	Cyanicae DC.	*L. perennis*, *L. tenerrima*	perennial
Phaenixopus (Cass.) Bentham		*L. viminea*	perennial
Mulgedium (Cass.) C.B. Clarke		*L. tatarica*, *L. sibirica*, *L. taraxacifolia*	perennial
Lactucopsis (Schultz Bip. ex Vis. et Pančić) Rouy		*L. quercina*	perennial
Tuberosae Boiss.		*L. indica*	perennial
Micranthae Boiss.		*L. undulata*	perennial
Sororiae Franchet		*L. sororia*	perennial
Groups (geographical view)			
North American		*L. biennis*, *L. canadensis*, *L. floridana*, *L. graminifolia*	biennial
African		*L. capensis*, *L. dregeana*, *L. homblei*	annual, perennial

29 October 2010 in Nagoya, Japan (http://www.cbd.int/abs/about/ from 16 January 2013).

A recent inventory of The International Lactuca Database (ILDB) with passport data for 11,643 *Lactuca* accessions, and of the Dutch national *Lactuca* germplasm collection (van Treuren and van Hintum 2009; van Treuren et al. 2011) confirmed the conclusions regarding gaps in collection structures reported previously by Lebeda and Boukema (2001) and Lebeda et al. (2004a, 2009a, b). Wild *Lactuca* germplasms are not adequately conserved by official gene banks and the species spectrum and world geographic distribution of the genus are not adequately represented in their germplasm collections (Lebeda et al. 2004a, 2007b, c).

Basic errors in the taxonomic status of wild *Lactuca* accessions as declared by gene banks were found during recent studies (Doležalová et al. 2004, Lebeda et al. 2007b) and duplicates between and within germplasm collections were identified (van Hintum and Boukema 1999, Doležalová et al. 2007; Sretenović-Rajičić et al. 2008). In addition, we are missing basic information on wild *Lactuca* germplasm resistance to the most important diseases and pests of lettuce (Lebeda et al. 2009a, b).

Gene pools of *Lactuca* spp.

The categorization of many *Lactuca* spp. into gene pools based on their crossing ability and fertility of F1 hybrids is still questionable and needs to be clarified. The primary gene pool of cultivated lettuce *L. sativa* comprises its cultivars and landraces, wild *L. serriola*, *L. aculeata*, *L. altaica*, *L. azerbaijanica*, *L. georgica*, *L. scarioloides,* and *L. dregeana* (Lebeda et al. 2007c). The categorization of *L. saligna* and *L. virosa* to secondary and tertiary gene pools is not resolved yet. Koopman et al. (1998) suggested that section Lactuca subsection Lactuca comprises the primary and secondary gene pool, while the sections Phaenixopus, Mulgedium and Lactucopsis include the tertiary gene pool (Table 1). Modern lettuce breeding has been mainly based on the utilization of wild *Lactuca* germplasm from the primary gene pool (*L. serriola*), however more recently it has shifted to the exploitation of secondary and tertiary *Lactuca* germplasm (Maisonneuve et al. 1995; Jeuken and Lindhout 2004). The main reason for this strategy is to broaden the genetic variation of cultivated lettuce by interspecific hybridization (Chupeau et al. 1994; Jeuken et al. 2001), including the introduction of a new and broader spectrum of resistances to diseases and pests (Jeuken and Lindhout 2002; Lebeda et al. 2002, 2007c, 2009a, b)

Genetic diversity of *Lactuca* spp.

Phenotypic variation

Descriptor lists as a tool for the correct taxonomic determination of wild *Lactuca* genetic resource accessions and for a definition of both interspecific and intraspecific variation have been produced at the national (Boukema et al. 1990; McGuire et al. 1993) and international levels (Doležalová et al. 2002, 2003a).

A large degree of variation in plant phenotypes has been described in greenhouse experiments among samples of two world-wide distributed species, *Lactuca serriola* (Doležalová et al. 2005; Lebeda et al. 2007a, 2011; Novotná et al. 2011), and *Lactuca saligna* (Křístková et al. 2007a; Beharav et al. 2008). This results from the evolutionary adaptation of plants under different climatical and ecological condition in their original habitats in different countries from Europe, Near East and North America. In contrast, a low level of phenotypic variation within *Lactuca aculeata* reflects the relatively limited distribution area of this species (Beharav et al. 2010a).

A high level of intraspecific variation is reported for many other species, e.g. for *L. virosa* (Feráková 1977) and has also been recently observed by the authors of this paper. However, this variation was not described in relation to the ecogeographic conditions and distribution of the accessions. The intraspecific classification of this and many other *Lactuca* species has not as yet been critically described. Recent broad application of wild *Lactuca* species in lettuce breeding and their influence on *L. sativa* phenotypic variation need a new treatment arrising from the previous one (Lebeda et al. 2007c) based on application of various approaches (i.e. phenotyping, digital image analysis, numerical taxonomy, molecular polymorphism etc.).

Variation in phenology features

A high level of variation in phenological characteristics within the genus *Lactuca* was recorded among accessions grown in greenhouse experiments. Substantial differences in the time of flowering were recorded between samples of *L. serriola* originating from various

countries (Doležalová et al. 2005; Lebeda et al. 2007b, c, 2011, unpublished results). A similar phenomenon was recorded for *L. saligna* samples (Křístková et al. 2011). Differences in developmental rates of plants, which are influenced by the original eco-geographic conditions of samples (Lebeda et al. 2001), persist when plants are cultivated in uniform environmental conditions and have a genetic basis (Křístková et al. 2007b).

Karyology and DNA contents variation

Perennial wild *Lactuca* species of Europe and the Himalayas have haploid chromosome number n=8; the haploid chromosome number n=9 characterizes the majority of European and Mediterranean species, and species from the Middle East, Africa and India; species autochtonous from Canada to Florida possess the haploid chromosome number of n=17 (Feráková 1977, Lebeda and Astley 1999). However, the chromosome numbers of numerous *Lactuca* species are not known (Lebeda and Astley 1999) and the actual chromosome numbers of many North American species may differ from the reported data (Doležalová et al. 2003b).

Chromosomal studies (Matoba et al. 2007) and approaches combining analysis of karyotype and variation in relative DNA content serve as tools for distinguishing some *Lactuca* species (Koopman 1999, 2000; Doležalová et al. 2003b), characterization of their evolutionary relationships (Koopman and De Jong 1996), and intraspecific variation (Koopman 2002). The relative DNA content was analysed for large sets of *L. serriola* and *L. saligna* samples originating from different eco-geographical conditions in Europe, Near East and North America (Lebeda et al. 2004c, 2007c, 2011). However, there was little variation and it seems that *Lactuca* species are highly conservative in DNA content at the intraspecific level.

Biochemical trait variation

Nearly 10 % of plant species, including *Lactuca* spp. produce latex which contains complex mixtures of terpenoids, phenolics, proteins, glycosides and alkaloids (Agrawal and Konno 2009). The most important subgroups of sesquiterpenoids within the tribe Cichorieae (Asteraceae) are costus lactone type quaianolides and lactucin derivates, and they make up large numbers of the total of 360 different sesquiterpene lactones and precursorss reported in the tribe (Zidorn 2008). Based on sesquiterpene profiles the 31 genera of the tribe Cichorieae form seven main clusters, and within the second group with eleven genera the genus *Lactuca* is very close to the genera *Notoseris* and *Cichorium*. The integration of chemosystematic data to the botanical systematics is limited by the lack of standard specimens for verifying the identity of plant material (Zidorn 2008). When correctly identified plant material is available, the results of chemical analyses bring new light to evolutionary concepts and taxonomical relationships between different *Lactuca* spp. (Sessa et al. 2000; Kisiel and Michalska 2009; Michalska and Kisiel 2009, 2010; Lebeda et al. 2009a, b; Michalska et al. 2009; Beharav et al. 2010b).

The HPLC profile of sesquiterpene lactones from latex of several *L. sativa* cultivars differed to those of *L. serriola* and *L. virosa* genotypes resistant to important races of lettuce downy mildw (*Bremia lactucae*). Although this resistance was not corrrelated to the "SL" profile, heritability of this profile was demonstrated by analysis of progeny from a cross between *L. sativa* and *L. virosa* (Sessa et al. 2000).

Pharmacological exploitation of some chemical compounds in wild *Lactuca* species (e.g. sesquiterpene lactones, phenolics and glucosides, flavonoids) (Rees and Harborne 1984; Kisiel and Barszcz 1998; Kisiel and Zielinska 2000; Chen et al. 2007; Kim et al. 2007) or plant-produced antigens (Pniewski 2013) is another goal of studies aimed at their detection and characterization. This research is stimulated by the promising results of analgesic and sedative activities of lactucin from *Cichorium intybus*, a species chemotaxonomically closely related to lettuce (Wesolowska et al. 2006).

Most studies have focused on latex as a substance that reduces herbivory or the preference or performance of herbivores (Agrawal and Konno 2009). Latex from the resistant variety of lettuce 'Valmain' inhibited feeeding of *Diabrotica balteata* when painted on leaves of lima bean, conversely the latex from the susceptible variety 'Tall Guzmaine' did not inhibit feeding (Huang et al. 2003). To our knowledge, there is no report of antiherbivoral activities of latex from wild *Lactuca* species.

Protein and molecular polymorphism

Genotyping with molecular markers for genetic diversity detection, assesment of population structure,

selection of desirable genotypes for lettuce breeding, mapping of genes, identification and variation of resistance genes has become common place in modern exploitation of wild lettuce progenitors. The studies related to use of protein and molecular markers in *Lactuca* spp. germlasm collections have been reviewed by Dziechciarková et al. (2004). In the present contribution we want to summarise recent progress on the comprehensive molecular based research on the genus *Lactuca* spp. The survey of studies including wild *Lactuca* species as well as studies using offspring from crosses between cultivated lettuce and wild progenitors and mapping populations derived from these crosses are presented in Table 2.

In general, in the first decade of 21st century there has been a dramatic shift in the number of papers based on isozyme studies (Lebeda et al. 2009a, b, 2012a, b) to more advanced studies utilising microsatellite and AFLP markers (e.g. Kitner et al. 2008; van de Wiel et al. 2010; Lebeda et al. 2009a, b), NBS profiling (e.g. van Treuren and van Hintum 2009) and high-resolution DNA melting analysis (Simko et al. 2009, 2010). However, during the last 2 years there has been an increase in the number of papers using new high throughput marker technologies based on single nucleotide polymorphism (SNP) or single position polymorphism (SPP) arrays on Affymetrix and Illumina genechips (e.g. Kwon et al. 2012; Stoffel et al. 2012; Uwimana et al. 2012b, c).

There are several studies by Koopman et al. (1998, 2001) and Koopman (2002) using molecular markers primarily describing relationships among *Lactuca* species and related genera, which are based on ITS1 sequencing and AFLP's. Several other studies have commented on the phylogenetic relationships between *Lactuca* species as well, but either this has not been a primary aim of the study, or has been restricted to a more narrow frame of *Lactuca* genetic pools (Matoba et al. 2007; Yang et al. 2007; Stoffel et al. 2012) (Table 2).

Several types of molecular markers have been applied in studies of genetic variation in natural populations and for germplasm maintenance and characterisation (Table 2). These studies were frequently carried out with microsatellites (Lu et al. 2007; Riar et al. 2011; Uwimana et al. 2012a) or AFLP markes (Kitner et al. 2008; Kuang et al. 2008; Lebeda et al. 2009a, b) or combination of both methods, sometimes extended with additional markers (van Treuren and van Hintum 2009; van de Wiel et al. 2010; Hooftman et al. 2011). Several studies describing genetic diversity were primarily foccused on marker development, espetially on EST- or genomic-microsatellite design (Simko 2009; Rauscher and Simko 2013). These SSR-based markers with publicly available primer sequences provide an important tool for researchers for future studies of wild lettuce populations. Some of these SSR markers are linked to herbicide resistance genes 2,4-D and ALS resistance (Riar et al. 2011). In fact, the number of population-based studies is limited and was mainly performed with AFLP's or non-publically available SSR's, on lettuce germplasm pseudopopulations originating from a larger geographical scale and/or with a sampling period of several years (van de Wiel et al. 2010; Lebeda et al. 2009a, b, 2012b; Uwimana et al. 2012a) than e.g. comparisons of true populations of local character originating from several regions/countries and sampled within a short period of time. To conclude, we expect that current advances in lettuce genomics (Kwon et al. 2012; Stoffel et al. 2012) will stimulate researchers to use these comercially available genechips based on SNP or SPP features for fast and cost-efficient population-genetic studies in the near future.

Several genetic linkage maps have been published for lettuce. Truco et al. (2007) presented a consensus map of 2,744 markers integrating seven intra- and inter-specific mapping populations and included information from five previously published genetic maps (Kesseli et al. 1994; Witsenboer et al. 1997; Waycott et al. 1999; Johnson et al. 2000; Jeuken et al. 2001). These maps are continuously updated according to marker developments (Schwember and Bradford 2010; Argyris et al. 2011; Aruga et al. 2012; Rauscher and Simko 2013). Such studies are closely associated to QTL studies, design of molecular markers for marker-assisted selection (Simko et al. 2009, 2010, 2011), detection of interspecific hybrids, distribution of crop alleles in natural populations (Uwimana et al. 2012b, c), and finally studies analysing and describing the background of resistance gene clusters, their identification and variability screening (Kuang et al. 2008; McHale et al. 2009).

To conclude, the genome of lettuce has been sequenced using 'next-generation' DNA sequencing, the sequenced genome has been assembled and annotation is underway (Michelmore 2012).

Table 2 Timeline of different types of molecular marker studies of wild *Lactuca* species

Lactuca spp.							Method											References
cross	sat	ser	sal	vir	per	ind	other	AFLP	CHIP	HRM	NBS	RFLP	RAPD	SEQ	SNP	SSR	SSAP	TRAP	other	
	*	*	*	*	*	*						*								Kesseli et al. (1991) [D,T]
	*	*	*	*	*		a)					*								Vermeulen et al. (1994) [T]
	*	*	*	*	*	*		*												Hill et al. (1996) [T]
	*	*	*	*	*	*										*			SAMPL	Witsenboer et al. (1997) [M,D]
	*	*	*	*	*	*	b)							*						Koopman et al. (1998) [T]
	*	*	*	*	*	*	c)									*			SCAR	Sicard et al. (1999) [R]
	*	*	*	*												*				van de Wiel et al. (1998) [M,D]
	*	*	*	*												*				van de Wiel et al. (1999) [M]
	*	*	*	*	*	*	d)	*												Koopman et al. (2001) [T]
x	*		*					*								*				Jeuken et al. (2001) [G,H]
	*	*					e)	*												van Hintum (2003) [D]
x	*		*					*												Jeuken and Lindhout (2004) [G,H]
x	*	*						*								*			EST	Argyris et al. (2005) [Q,H]
	*	*	*															*		Hu et al. (2005) [D,T]
	*	*	*	*	*	*	b)	*												Koopman (2002) [T]
	*	*	*											*						Kuang et al. (2006) [R]
	*	*	*								*						*			Syed et al. (2006) [G,H]
x	*	*						*								*				Lu et al. (2007) [R]
	*	*	*	*	*														FISH	Matoba et al. (2007) [C,T]
x	*	*	*					*				*	*			*				Truco et al. (2007) [G,H]
	*	*	*	*	*		f)	*				*	*			*				Yang et al. (2007) [D,T]
x	*	*											*							D'Andrea et al. (2008) [H]
				*				*												Kitner et al. (2008) [D]
			*					*						*		*				Kuang et al. (2008) [R,T]
	*	*	*															*		Simko and Hu (2008) [D,G]
	*	*						*											ISO	Lebeda et al. (2009b) [D]
x	*		*					*		*						*			EST	Jeuken et al. (2009) [R,H]
x	*	*							*		*								SSCP	McHale et al. (2009) [R,H]
	*	*	*	*												*				Simko (2009) [M,D]
	*	*								*									EST	Simko et al. (2009) [G]

Table 2 (continued)

Lactuca spp.							Method											References
cross	sat	ser	sal	vir	per	ind	other	AFLP	CHIP	HRM	NBS	RFLP	RAPD	SEQ	SNP	SSR	SSAP	TRAP	other	
x	*	*							*											van Leeuwen et al. (2009) [G,H]
	*	*	*				e)	*			*					*	*	*	SRAP	van Treuren and van Hintum (2009) [D]
x	*	*							*											Argyris et al. (2011) [Q,H]
x	*	*						*	*							*				Schwember and Bradford (2010) [Q,H]
x	*	*	*	*						*				*	*	*				Simko et al. (2010) [M,R,H]
		*						*			*					*				van de Wiel et al. (2010) [D]
x	*	*							*										SSCP	Argyris et al. (2011) [Q,H]
x	*	*						*			*						*			Hooftman et al. (2011) [R,H,G]
		*						*												Lebeda et al. (2011) [D]
	*	*	*	*	*											*				Riar et al. (2011) [M,D]
	*	*	*	*	*	*	g)		*											Simko et al. (2011) [G,M]
x	*	*							*										NGS	Michelmore (2012) [G,,H,M,R,Q]
	*	*	*	*	*				*											Stoffel et al. (2012) [M,D,T]
	*	*														*				Uwimana et al. (2012a) [D,H]
x	*	*							*											Uwimana et al. (2012b) [Q,H]
x	*	*							*											Uwimana et al. (2012c) [Q,H]
	*	*	*	*												*				Rauscher and Simko (2013) [M,G]

Explanation to terms and acronyms used in the table: cross, interspecific hybridisation; sat, *L. sativa*; ser, *L. serriola*; sal, *L. saligna*; vir, *L. virosa*; per, *L. perenis*; ind, *L. indica*; AFLP, Amplified Fragment Lenght Polymorphism; CHIP, genechip; EST, markers based on Expressed Sequence Tags; FISH, Fluorescence *In Situ* Hybridization; HRM, High-Resolution DNA Melting analysis; ISO, isozymes; NGS, Next Generation Sequencing; NBS, Nucleotide Bindig Site profiling; RAPD, Randomly Amplified Polymorphis DNA; RFLP, Restriction Fragment Lenght Polymorphism; SAMPL, Selectively Amplified Microsatellite Polymorphic Loci; SCAR, Sequence Characterized Amplified Regions; SEQ, sequencing; SNP, Single Nucleotide Polymorphism; SSAP, Sequence-Specific Amplification Polymorphism (retrotransposomes); SSCP, Single Strand Conformation Polymorphism; SSR, microsatellites; SRAP, Sequence-Related Amplified Polymorphism; TRAP, Target Region Amplification Polymorphism; **a)** *L. alpina*, *L. tatarica*; **b)** *L. aculeata*, *L. tatarica*, *L. dregeana*, *L. altaica*, *L. tenerrima*, *L. sibirica*, *L. quercina*, *L. viminea*, other species; **c)** *L. augustana*, other species; **d)** *L. altaica*, *L. tatarica*, *L. dregeana*, *L. tenerrima*, *L. sibirica*, *L. quercina*, *L. viminea*; **e)** *Lactuca* spp., other species; **f)** *L. georgica*, other species; **g)** *L. aculeata*. Aims of study: C, cytological; D, population/germplasm diversity; G, genetic mapping; H, interspecific hybridisation; M, marker development; Q, quantitative trait loci; R, variation/identification of resistance genes; T, taxonomical/phylogenetical study

Variation of wild *Lactuca* spp. in reaction to pathogens and pests

Viral pathogens

The viral pathogens are after fungi the most economically important pathogens of plants. The impact of virus infection is seen in a reduction of yield, decrease quality of size, shape, taste, structure, composition (sugar content) of plants, decrease of viability (sensitivity to dry, cold), predisposition to infection of other pathogens, short shelf life and decrease of fertility. All viruses are obligate parasites that depend on the cellular machinery of their hosts to reproduce (Gergerich and Dolja 2006). Most plant viruses are transmitted by passive transmission from plant to plant and active transmission from infected to healthy plants by a living organism termed a vector. Plant-feeding arthropods, nematodes and plant-parasitic fungi are the major types of vector organisms for plant viruses (Walkey 1991). All types of plant viruses are important disease causing agents and are responsible for losses in crop yields and quality in all parts of the world. Among the most serious lettuce viruses are: *Lettuce mosaic virus*, *Mirafiori* (big vein) *lettuce virus*, *Beet western yellows virus*, *Tomato spotted wilt virus*, *Cucumber mosaic virus* and *Lettuce necrotic stunt virus*. Lettuce is at risk of infection by these viruses and production of new resistant cultivars is a priority. In breeding programmes crossess between cultivars and wild species such as *Lactuca serriola*, *L. saligna*, *L. virosa* and *L. perennis* offer a way of introgressing new resistances.

Lettuce mosaic virus (LMV)

Lettuce mosaic virus has been a serious worldwide disease problem in lettuce and a few other leafy vegetable species, for a long time (Ryder 2002). It was as first described in Florida (Jagger 1921) and now is distributed worldwide, probably because the virus is seed transmitted and lettuce seeds have been exchanged internationally over many years (Dinant and Lot 1992). The worldwide distribution of LMV includes Europe, North and South America (Mexico, USA, Argentina, Brazil, Uruguay), the West Indies (Bermuda), Africa, the Middle East (Egypt, Israel, Iraq, Iran, Jordan and Turkey), Asia (China and Japan) and Oceania (Australia, Tasmania, New Zealand) (German-Retana et al. 2008).

LMV belongs to the genus *Potyvirus* of the family *Potyviridae*, which is seed-borne in lettuce and disseminated by aphid vectors-*Myzus persicae*, *Macrosiphum euphorbiae, Aphis gossipii*. The characteristic symptoms on susceptible lettuce cultivars are dwarfism, mosaic, distortion and yellowing of the leaves with sometimes a much reduced heart (failure to form heads). The differences in virus strains, cultivars and the physiological stage of the host at the moment of the attack cause different symptom severity; from a very slight discoloration of the veins to severe necrosis leading to death of the plant (German-Retana et al. 2008). The genomic organization of LMV is typical of potyviruses, with a single positive-sense genomic RNA of 10,080 nucleotides encapsidated as flexuous rods (Krause-Sakate et al. 2002). The viral genomic RNA has a viral encoded protein covalently linked at the 5′end, a poly-A tail at the 3′end, and contains a single open reading frame (ORF) which encodes a large polyprotein with 3,255 amino acids (Revers et al. 1997a). This polyprotein undergoes self-cleavage as it is translated, generating 8–10 viral proteins (Shukla et al. 1994; Revers et al. 1997b).

Three phylogenetic groups of LMV isolates were discriminated, correlating with geographical origin of the isolates rather than with their pathogenicity. The largest group includes isolates from western Europe and California. A second group includes three isolates from Greece whereas the third group consists, so far, of a single isolate from the Yemen Arab Republic (Revers et al. 1997b). LMV isolates have been classified into four pathotypes, according to their virulence on lettuce varieties carrying the three resistance or tolerance genes mo ($mo1^1$, $mo1^2$) and Mo2, which were identified in *L. sativa* cultivars (Pink et al. 1992a; Bos et al. 1994) and LMV genes Mo3 and Mo4, which are described in *L. virosa* sources but which are difficult to introgress and not well-characterized. These dominant genes have not currently been used in the field.

In Brazil, and in most European countries, LMV has been controlled through the use of resistant cultivars (Krause-Sakate et al. 2001). Two sources of resistance were identified in the late 1960's – the first recessive gene *$mo1^1$* (formely named g) in Argentina, in a Latin-type cultivar Gallega de Invierno (Bannerot et al. 1969) and European lettuce breeders used the Gallega source of resistance to incorporate the *g* gene in numerous varieties of lettuce, including butterhead, Batavia, cos and crisphead types (Pink et al. 1992b). Later in the

USA the recessive gene *mo1*^{2} (previously *mo*) was identified in three Egyptian wild *Lactuca sativa* lines (Ryder 1970), this recessive gene *mo* has been used by North American breeders, who introduced it into crisphead and cos types of lettuce. Two of these genes *mo1*^{1} and *mo1*^{2}, are recessive and are believed to be either closely linked or allelic (Pink et al. 1992b) and which encode alleles of the cap-binding protein, elF4E. ElF4E-this identification was based on three converging lines of evidence: (1) allelic sequence co-variation between the elF4E gene and *mol*^{1} and *mol*^{2} resistance status of plants; (2) co-segregation of mutations in the elF4E gene and the *mol*^{1} and *mol*^{2} resistance status and finally (3) functional complementation using a viral transient expression vector to vector to restore LMV susceptibility in *mol*^{1}*-or mol*^{2} carrying lettuce plants using the elF4E allele from susceptible plants (Nicaise et al. 2003). The resistant alleles of the eIF4E gene in lettuce, *mo1*^{1} and *mo1*^{2}, are currently the only genetic determinants used to protect lettuce crops from LMV; the third resistance (dominant) gene Mo2, found in the cv. Ithaca (Pink et al. 1992a, b) a is not effective in practice for LMV control, because it is overcome by most LMV isolates.

More of studies were done on cultivars of *L. sativa* (the most frequently tested cultivars were: Trocadéro, Mantilia, Floribibb, Ithaca, Salinas 88, and Vanguard). Among the most frequently tested isolates of lettuce mosaic virus on these cultivars were isolates LMV- 0, LVM-1, LMV-9, LMV-E, LMV-13 and AF-199. Additional sources of resistance to lettuce mosaic virus are known in accessions of *Lactuca virosa* and *Lactuca serriola* (Table 3).

Mirafiori lettuce virus (Lettuce big vein virus, LBV)

Lettuce big vein disease (LBVD) was first described in California (Jagger and Chandler 1934), and it occurs widely in regions of the world with temperate or Mediterranean-type climates (Coutts et al. 2004). LBVD is associated with a complex of two viruses, *Lettuce big-vein associated virus* (LBVV and LBVaV, genus *Varicosavirus*) and *Mirafiori lettuce big vein virus* (MLBVV, *Ophiovirus*) (Rogero et al. 2000). Its natural host range is limited to lettuce (*Lactuca sativa*), endivie (*Cichorium endivia*) and sow thistle (*Sonchus oleraceus*). The vector for both viruses is the root-infecting fungus *Olpidium brassicae* (Coutts et al. 2004).

Both LBVaV and MLBVV have segmented ssRNA genomes, and their virions contain RNA molecules of both polarities. The LBVaV geonome contains two RNA segments-RNA1 is 6797 nucleotide length with a single large open reading frame (ORF), and RNA2 has a slightly smaller size than RNA1 (6081 nt) having coding capacities for five ORFs (Navarro et al. 2005). LBVV particles of virus are fragile, rather rigid rods 320 to 360 nm in length and 18 nm in diameter, with central canal and an obvious helix of pitch=5 nm, MiLV particles, like those of recognized ophioviruses, are highly kinked filaments =3 nm in diameter that form masses of two distinct sizes but of undetermined contour length; they probably form closed circles, because free DNAs are very seldom seen (van Regenmortel et al. 2000)

The mechanism of resistance in cultivated lettuce is not known, and more research is needed to determine the relative role of virus resistance and symptom expression in big vein resistance. Among wild relatives of lettuce, only accesions of *L. virosa* have demonstrated a complete lack of symptom expression in inoculation trials (Bos and Huijberts 1990) (Table 3). *L. virosa* accession IVT280 was identified as 100 % asymptomatic in the greenhouse inoculation trials. Analysis by RT-PCR demonstrated no viral amplification, indicating apparent immunity in this accession (Hayes et al. 2006) Currently, no genotype of *L. sativa* has been identified as immune to big vein (Ryder and Robinson 1995), cultivars Pacific, Thompson, Margarita and Pavane are considered resistant (Ryder and Robinson 1995; Hayes et al. 2006). Crossing between *L. virosa* and *L. sativa* cultivars was dificult to perform (Hayes et al. 2004), nevertheless introgression of big vein tolerance from *L. virosa* to cultivars of lettuce has been successful (Hayes et al. 2004; Hayes and Ryder 2007).

Beet western yellows virus (BWYV), Turnip yellows virus (TuYV)

Beet western yellows virus (BWYV) was originally identified in the USA during the late 1950s as an important virus causing stunting and chlorosis in a wide range of plant species resulting in yield losses in crops such as sugar beet, spinach, lettuce and turnip (Duffus 1961). Beet western yellows virus has been associated with lettuce production since at least the 1950s, when it and the complex of virus diseases affecting spring crops of lettuce were referred to as June Yellows (Davis et al. 1997). BWYV belongs to the genus *Polerovirus* in the family *Luteoviridae* and recently a BWYV isolate,

Table 3 Resistance of wild *Lactuca* species to viruses

Name of pathogen	Source of resistance				
	Lactuca spp.	No. of accession/sample	Type of resistance	Remark	References
LMV 0	*L. virosa*	PIVT 1398 (CGN 9365)	Complete resistance	LMV resistance gene Mo3 effective against all known isolates	Maisonneuve et al. (1999) Maisonneuve (2003) Mazier et al. (2003)
		PIVT 280 (CGN 4683)	Complete resistance		Maisonneuve (2003)
LMV-1	*L. virosa*	PIVT 1398 (CGN 9365)	Resistance	LMV resistance gene Mo3 effective against all known isolates	Maisonneuve et al. (1999) Mazier et al. (2003)
LMV-9	*L. virosa*	PIVT 1398 (CGN 9365)	Complete resistance	*in vitro* experiment, DAS ELISA test	Mazier et al. (2003) Maisonneuve (2003)
		PIVT 280 (CGN 4683)	Complete resistance		Maisonneuve (2003)
LMV-13	*L. virosa*	PIVT 1398 (CGN 9365)	Resistance	LMV resistance gene Mo3 effective against all known isolates	Maisonneuve et al. (1999) Maisonneuve (2003)
		PIVT 280 (CGN 4683)	Complete resistance		Maisonneuve (2003)
LMV-E	*L. virosa*	PIVT 1398 (CGN 9365)	Complete resistance	LMV resistance gene Mo3 effective against all known isolates	Maisonneuve et al. (1999) Maisonneuve (2003)
		PIVT 280 (CGN 4683)	Complete resistance		Maisonneuve (2003)
LMV_Ls-252	*L. perennis*	n.s.	Resistance	Plants symptomless, but positive in ELISA	Bos et al. (1994)
	L. tatarica	n.s.	Resistance?		Bos et al. (1994)
LMV (strain not specif.)	*L. serriola*	PI 251245	Resistance	Original source of resistance used in breeding program	Ryder (2002)
LBVV	*L. virosa*	IVT 280	No symptoms, resistance; high level of resistance		Bos and Huijberts (1990) Ryder et al. (2003)
		IVT 280	Asymptomatic	14/14 plants remained asymptomatic by testing RT-PCR	Hayes et al. (2006)
		n.s.	No symptoms, resistance; high level of resistance		Bos and Huijberts (1990) Ryder et al. (2003)
		n.s.	n.s.	Greenhouse reaction-62 asymptomatic *L. virosa*; 7*L. virosa* with leaf crinkling and 1*L. virosa* with typical vein banding symptoms	Hayes et al. (2008)
LBVV (big-vein)	*L. virosa*	IVT 280, PI 271938, SAL 012	n.s.	Asymptomatic	Hayes et al. (2008)
		CGN 16272	n.s.	34 tested plants-9 % symptomatic	Hayes et al. (2008)
		CGN 16273	n.s.	35 tested plants-17 % symptomatic	Hayes et al. (2008)
		CGN 16274	n.s.	32 tested plants-13 % sympomatic	Hayes et al. (2008)

Table 3 (continued)

Name of pathogen	Source of resistance				
	Lactuca spp.	No. of accession/sample	Type of resistance	Remark	References
		CGN 16275	n.s.	34 tested plants-15 % symptomatic	Hayes et al. (2008)
		CGN 16276	n.s.	34 tested plants-6 % symptomatic	Hayes et al. (2008)
		CGN 16277	n.s.	35 tested plants-3 % symptomatic	Hayes et al. (2008)
		SAL 177	n.s.	34 tested plants-3 % symptomatic	Hayes et al. (2008)
MLBVV	*L. virosa*	CGN 16272	n.s.	9 plants tested-89 % symptomatic	Hayes et al. (2008)
		CGN 16273	n.s.	12 plants tested-92 % symptomatic	Hayes et al. (2008)
		CGN 16274	n.s.	10 plants tested-100 % symptomatic	Hayes et al. (2008)
		SAL 177	positive MLBVV	12 plants tested-33 % symptomatic (by the help NASH test-92 % positive)	Hayes et al. (2008)
		SAL 012	n.s.	12 plants tested-0 % symptomatic (by the help NASH test-17 % positive)	Hayes et al. (2008)
		IVT 280	n.s.	12 plants tested-17 % syptomatic (by the help NASH test-75 % positive)	Hayes et al. (2008)
		IVT 280	Asymptomatic	14/14 plants remained asymptomatic-by testing RT-PCR	Hayes et al. (2006)
BWYV- FL1	*L. virosa*	IVT 280	Resistant	in the field conditions	Maisonneuve et al. (1991)
		IVT 280	Complete resistance	lab conditions; maintained by replication *Myzus persicae* on *Physalis floridana*; (no symptoms, and no virus detectable ELISA) Wageningen NL	Maisonneuve (2003)
		IVT 1145	Partly resistant		Maisonneuve et al. (1991)
		IVT 1398	Complete resistance	lab conditions; maintained by replication *Myzus persicae* on *Physalis floridana*; (no symptoms, and no virus detectable ELISA) Wageningen NL	Maisonneuve (2003)
LNYV	*L. serriola*	273597	Field tolerance	+ 21 samples *L. serriola* susceptible + 61 species *L. serriola* moderately tolerant	Ray et al. (1989)
		491118	Field tolerance	+ 21 samples *L. serriola* susceptible	Ray et al. (1989)
		491133	Field tolerance	+ 61 samples *L. serriola* moderately tolerant	Ray et al. (1989)
	L. saligna	PI 261653	Resistance		Haley and McCreight (1990)
		490999, 491001, 491206, 491208, WP233, WP238, WP239, WP242, WP246, WP246A, WP247, 261653	Field tolerance		Ray et al. (1989)

Table 3 (continued)

Name of pathogen	Source of resistance				
	Lactuca spp.	No. of accession/sample	Type of resistance	Remark	References
TSWV	*L. scariola*=*L. serriola*	n.s.	n.s.	North Carolina, experiment	Groves et al. (2002)
CMV	*L. saligna*×*L. sativa*	PI 261653×Saladcrisp	Viral strain specific		Provvidenti et. al. (1980)
	L. saligna×*L. sativa*	F7 generation of var. Montello× (Vanguard 75×*L. saligna* PI 261653)	Resistance		Tamaki et al. (1995)
	L. serriola	n.s.	Tolerance	Tolerant to three strains of CMV studied, but this tolerance has not been transferred to any current commercial variety	Zitter and Murphy (2009)
CMV-B	*L. saligna*	PI261653	Resistance		Edwards et al. (1983)
CMV-L2 (previously LsS)	*L. serriola*	ACC 500-4	Resistance	No symptoms	Edwards et al. (1983)
CMV-L3	*L. serriola*	n.s.	Necrosis		Edwards et al. (1983)
CMV-LsR	*L. saligna*×*L. sativa*	F1 plants from crosses of PI261653 with varieties of *L. sativa*	Not specified		Provvidenti et. al. (1980)
LNSV	*L. serriola*	UC96US23, PI 491178, PI271940	Resistant		Grube et al. (2005a)
	L. saligna	PI490999, PI509525, PI 491204	Resistant		Grube et al. (2005a)
	L. virosa	PI 273597, IVT 280	Resistant		Grube et al. (2005a)

which does not infect sugar beet was renamed *Turnip yellows virus* (TuYV) (Stevens et al. 2005).

BWYV is transmitted by aphid vectors-especially *Myzus persicae* (Sulz.), *Macrosiphum euphorbiae* (Thos.) and induced symptoms are chlorotic (yellow) symptoms, which are first observable at the tips or margins of leaves and soon spread to cover whole leaves. Interveinal chlorosis first occurs in older leaves and progress acropetally, followed by necrosis (Maisonneuve et al. 1991; Hampton et al. 1998).

The genome of BWYV is composed of a single-stranded plus-sense RNA, approximately 5,6 kb in length. The genome contains six large open reading frames (ORFs, ORF0-ORF5), a short 5′- untranslated region (UTR), a 3′-UTR without tRNA-like or poly (A) structure, and an intergenic non-coding region (NVR) between ORF2 and ORF3 about 200nt (Stevens et al. 2005).

Lettuce necrotic yellows virus (LNYV)

LNYV was first found in 1954 in Australia (Stubbs and Grogan 1963). LNYV is the type species of the genus *Cytorhabdovirus*, members of which are characterised by accumulation of enveloped virions, which is transmitted by *Hyperomyzus lactucae* L.

Lettuce plants naturally infected with LNYV acquire a dull green appearance, the young leaves developing bronzing and necrosis, especially along the veins, and older leaves become chlorotic or mottled and plants often die (Fry et al. 1972). Among the tested wild species are *L. serriola* and *L. saligna* (Table 3) and some accessions show a resistant response.

The LNYV genome consists of a monopartite, negative-sense, single-stranded RNA of 12–15 kb, which encodes five functionally conserved proteins (Dietzgen et al. 2006). The physical map of the LNYV genome is 3′ leader – N – P- 4b – M- G – L – 5′trailer, where N is the nucleocasid gene, P is phosphoprotein gene, 4b encodes a putative movement protein, M is the matrix protein gene, G is the glycoprotein gene and L is the polymerase gene (Wetzel et al. 1994).

Lettuce chlorotic virus (LCV)

Lettuce chlorotic virus (LCV) is a member of the rapidly emerging genus *Crinivirus*, family *Closteroviridae* (Duffus et al. 1996). It is transmitted by silverleaf whitefly *Bemisia tabaci* and *B. argentifolii* with about the same efficiency. This is a major difference between LCV and *Lettuce infectious yellows virus* (LIYV) since LIYV is transmitted very inefficiently by *B. argentifolii* (Davis et al. 1997; Wintermantel 2004).

LCV has a large bipartite RNA genome encoding several open reading frames (at least 13 ORFs). RNA1 encodes functions involved in virus replication, while RNA2 encodes up to 7 ORFs involved in virion assembly, vector transmission and other functions, many of which remain to be determined (German-Retana et al. 1999). Virions are encapsidated into long flexuous rods averaging between 650 to 900 nm in length.

Lettuce chlorotic virus resistance has only been assessed in *L. sativa* accessions.

Tomato spotted wilt virus (TSWV)

Tomato spotted wilt virus (TSWV) belongs to the genus *Tospovirus*, family *Bunyaviridae*, it is a one of the most widely spread plant viruses and the causal agent of economically important yield losses in many crops. TSWV was first found in 1915 in Australia (Brittlebank 1919). Since then, its known host range has increased to over 900 dicotyledonous and monocotyledonous plant species worldwide (Peters and Goldbach 1998). Many horticultural crops and weeds are hosts. TSWV is transmitted by several thrips species, of which the western flower thrips (*Frankliniella occidentalis*) is the most efficient vector (Hobbs et al. 1993). Infection reservoirs from which TSWV spreads to susceptible crops include nearby plantings of TSWV-susceptible crops, volunteer crop plants and weeds (Cho et al. 1989; Groves et al. 2002). TSWV is readily transmitted mechanically from sap of naturally infected plants. For manual inoculation *Nicotiana tabacum*, *N. glutinosa* and *N. bethamiana*, which develope large necrotic local lesions followed by systemic mosaic and necrosis are used to provide inoculum (Parella et al. 2003).

TSWV virions are 80–120 nm diameter, spherical, enveloped, and studded with surface projections composed of two glycoproteins G1 and G2. Virion composition is 5 % nuclei acid (RNA), 70 % protein, 5 % carbohydrate, and 20 % lipid. The genome consists of three negative or ambisense ssRNA species designated as S (2.9 kb), M (4.8 kb) and L (8.9 kb) (Parella et al. 2003).

Introgression of genes for resistance into to lettuce cultivars is a possible strategy for control of *Tomato spotted wilt virus*. However, screening of some wild *Lactuca* species (*L. serriola*, *L. virosa* and *L. floridana*)

have shown only susceptible reaction (Hobbs et al. 1993; Parella et al. 2003). Resistance was recorded only in one accession of *L. serriola* by Groves et al. (Groves et al. 2002; Table 3), but without any detailed specification.

Cucumber mosaic virus (CMV)

Cucumber mosaic virus (CMV) is the type species of the genus *Cucumovirus*, family *Bromoviridae*. CMV occurs worlwide and is a very important disease agent in temperate, tropic and subtropic regions of the world. It is a virus with a very wide host range including plants from approximately 365 genera and at least 85 families (Roossinck et al. 1999). CMV is an important pathogen of many vegetable crops and is the target of breeding programs for resistance. The *Cucumoviruses* are transmitted by aphids (especially *Myzus persicae*, *Aphis gossypii),* which ensures a multiplicity of inoculation sufficient to reliably establish infection. Symptoms of CMV infection in lettuce consist of leaf mottling, severe roughness of the leaf and occasional necrosis within the leaf tissue. Plants are usually stunted if infected at an early stage of development (Zitter and Murphy 2009).

CMV consists of three spherical particles, each approximately 28 nm in diameter (Zitter and Murphy 2009). The genome is divided into three plus-sense, single-stranded, RNA molecules, designated RNA 1, RNA 2 and RNA 3. Each RNA molecule is enclosed within a protective protein coat with each being a distinct single spherical-shaped particle. CMV contains five open reading frames (ORFs). These can be used for phylogeny estimation of the species of the *Cucumovirus* genus (indicating evolutionary histories for each RNA strongly supporting the occurrence of re-assortment in the evolutionary history of the genus (Roossinck 2002).

CMV resistance has been derived from *L. saligna* (Table 3), introgression of resistance in to lettuce was done by backcrossing with *L. sativa*. By the F7 generation of cultivar Montello×(Vanguard 75×*L. saligna* PI 261653) (Tamaki et al. 1995), and *Lactuca saligna*×*L. sativa* (Saladcrisp) (Provvidenti et al. 1980) the introgression was successful, and these lettuce lines are resistant to CMV.

Lettuce necrotic stunt virus (LNSV)

LNSV causes lettuce dieback, a disease resulting in stunting, necrosis, and lack of marketability in lettuce, it is likely that it has been present under the name brown blight since the 1920s (Wintermantel and Anchieta 2012). LNSV can infect lettuce through the soil in the absence of fungal vectors. Fields with high disease incidence are usually poorly drained and variation in soil salinity influences LNSV infection of lettuce (Wintermantel et al. 2003). Lettuce necrotic stunt virus is caused by several members of the soilborne virus family Tombusviridae, including the type member, *Tomato bushy stunt virus* (TBSV), and *Lettuce necrotic stunt virus* (LNSV) (Obermeier et al. 2001). Tombusviridae is a relatively large and diverse family of soil-borne viruses that have single-stranded, positive-sense, RNA (ribonucleic acid) genomes and that share morphological, structural, molecular and genetic features. Resistance against LNSV is conferred by *Tvr1*-a single, dominant gene that provides durable resistance (Grube et al. 2005b; Simko et al. 2009). Table 3 shows the resistance to LNSV of accessions of the wild species *L. serriola* (UC96US23; PI 491178; PI 271940), *L. virosa* (PI 273597; IVT 280) and *L. saligna* (PI 271940; PI 490999).

Bacterial pathogens

Corky root (Sphingomonas suberifaciens, formerly Rhizomonas suberifaciens)

Corky root of lettuce has been observed in several major lettuce producing areas of the world, including North America, Western Europe, Australia and New Zealand. The symptoms of disease are dark discolouration and longitudinal cracks on the taproot, penetrating to the cortical region and the disease causes slow progressive deterioration of the root system of infected plants and lettuce seedlings and plants wilt under water stress. In severly infested fields of California and Florida, yield losses from reduced head size can reach 30 % to 70 % (Mou et al. 2007); the reduced development of heads is correlated with reduced root growth. The pathogen most commonly isolated from diseased roots is the bacterium *Sphingomonas suberifaciens* (Yabuuchi et al. 1999), formerly *Rhizomonas suberifaciens* (van Bruggen 1997).

The use of resistant cultivars is the most efficient strategy to avoid economic losses (Mou 2011a). The first resistant lettuce cultivars Marquette, Montello and Green Lake developed by Sequiera (1970, 1978) were released from crosses with a resistant line PI 171669. This line was identified by Dickson (1963) as a local lettuce landrace from Turkey.

The resistance to corky root is conferred by a recessive allele (*cor*) at a single locus (Brown and Michelmore 1988), which is present in many modern crisphead lettuce cultivars, e.g. Bronco, Cannery Row, Glacier, Premiere, Misty Day, Sharp Shooter, Sniper (Mou et al. 2007), however there are few leaf lettuce varieties with this resistance (Mou 2011a). Recently two breeding lines 06–831 and 06–833 of greeen leaf lettuce were released from the cross between green leaf cultivar Waldmann's and the crisphead cultivar Glacier (Mou 2011a).

Brown and Michelmore (1988) identified resistant lines within the wild species *L. serriola*, *L. saligna*, *L. dentata*, *L. virosa* and *Lactuca* spp. (Table 4). Mou and Bull (2004) identified three *L. serriola* and one *L. virosa* accessions consistently resistant to corky root in growth chamber, greenhouse and field experiments (Table 4), and they demonstrated significant genotype by environment interactions for corky root severity. Moreover, none of these four resistant lines possessed the two molecular markers closely linked to the *cor* allele suggesting that they may be sources of a new resistance factor (Mou and Bull 2004).

Bacterial leaf spot (Xanthomonas campestris pv. vitians)

Bacterial leaf spot of lettuce caused by *Xanthomonas campestris* pv. *vitians* has been reported from different countries, since the beginning of the 20th century (Toussaint et al. 2012). On romaine cos type lettuce, symptoms typically appear at the leaf margin as water-soaked lesions which become black after a few days and may be surrounded by a chlorotic halo. Later on they enlarge and coalesce, and large necrotic areas on leaves may develop (Toussaint et al. 2012). Lesions may expand towards veins, resulting in V-shaped lesions. Small individual black spots on the leaf surface may also be observed (Sahin and Miller 1997). Seed collected from infected plants were found to be colonized by bacteria externally, but no bacteria were recorded from within the seed (Barak et al. 2002).

When the infection remains restricted to the older leaves, no economic losses occur, however, in severe epidemics, the inner leaves are infected and the lettuce is then unmarketable (Toussaint et al. 2012).

Populations of *X. campestris* pv. *vitians* can survive on lettuce plant debris and infect subsequent lettuce crops (Barak et al. 2001). The pathogen has also been

Table 4 Wild *Lactuca* spp. with resistance to bacterial pathogen causing Corky root

Name of pathogen	Source of resistance				
	Lactuca spp.	No. of accession/sample	Type of resistance	Remark	References
Sphingomonas suberifaciens (*Rhizomonas suberifaciens*)	*L. dentata*	PI 234204	n.k.		Brown and Michelmore (1988)
	L. saligna	PI 261653, PI 490999, PI 491204, PI 491206, UC83UK2, UC83US1	n.k.		Brown and Michelmore (1988)
	L. serriola	PI 2556665, PI 289064–1, PI 289064-2	n.k.		Brown and Michelmore (1988)
		PI 491096, PI 491110, PI 491239	n.k.	Resistant in grow chamber, greenhouse and field experiments	Mou and Bull (2004)
		PI 491249, PI 491250, PI 491251	n.k.		Brown and Michelmore (1988)
	L. virosa	PI 273597c	n.k.	Resistant in grow chamber, greenhouse and field experiments	Mou and Bull (2004)
		UC83UK1	n.k.		Brown and Michelmore (1988)
	Lactuca spp.	PI 274376	n.k.		Brown and Michelmore (1988)

recovered from leaves of several symptomless weed species collected around infested commercial fields, but not from weeds collected around previously infested fields during fallow periods. Thus, weeds may not be an important long-term source of *X. campestris* pv. *vitians*, possibly due to the lack of stable epiphytic populations on weedy plants (Barak et al. 2001). *Xanthomonas campestris* pv. *vitians* can infect not only cultivated lettuce but also the wild *Lactuca* species, *L. serriola* and *L. biennis*, and these species may serve as a reservoir for this pathogen (Toussaint et al. 2012).

The possibilities of chemical control of bacterial diseases on lettuce are limited (Toussaint et al. 2012) and so host resistance is the most likely means of controlling the disease. Several commercial cultivars of lettuce have been screened for resistance to this bacterial pathogen (Sahin and Miller 1997, Carisse et al. 2000) and activities aimed at the development of lettuce breeding lines resistant to bacterial leaf spot have been briefly reported (Anonymous 2005). However, the genetics of resistance to *X. campestris* pv. *vitians* and the response of wild *Lactuca* species to this bacterial pathogen have not yet been published.

Fungal pathogens

Downy mildew (Bremia lactucae)

Lettuce downy mildew (*Bremia lactucae*) has a very high economic impact on lettuce production (Crute 1992), and the study of its biology and epidemiology, sources of resistance, mechanisms and genetic control of resistance in *Lactuca* species, including germplasm evaluations have been a high priority of researchers and breeders in many countries (Lebeda et al. 2002, 2009a, b). The interaction between cultivars of *L. sativa* and *B. lactucae* is clearly race-specific (Crute and Johnson 1976; Lebeda 1984; Farrara and Michelmore 1987).

Of the 100 wild *Lactuca* species described (Lebeda et al. 2004b) only 14 are definitely known as natural hosts of *B. lactucae* (Lebeda et al. 2002). *L. serriola*, is the most common wild *Lactuca* spp. occurring around the world (Lebeda et al. 2004b), and could be an important weedy host. However, except for the Czech Republic, there is no detailed information on the natural occurrence of lettuce downy mildew and its epidemiological impact on this species (Lebeda et al. 2002, 2008a). There is only limited knowledge of virulence variation of *B. lactucae* in wild pathosystems (Lebeda et al. 2008a). Only isolates originating from natural populations of *L. serriola* have been investigated for specific virulence variation (Lebeda et al. 2002, 2008a; Lebeda and Petrželová 2004). Generally, *B. lactucae* isolates from wild pathosystem are characterized in terms of v-factors mostly matching *Dm* genes or R-factors located or derived from *L. serriola* (Lebeda and Petrželová 2004).

Currently, searching for new sources of resistance and genes suitable for practical lettuce breeding (Lebeda and Zinkernagel 2003a; Beharav et al. 2006; Petrželová and Lebeda 2011; Petrželová et al. 2011; van Treuren et al. 2013) is considered to be very important. Accessions of *L. serriola* (reported as PI 91532 but subsequently shown to be PI 104584 and PI 167150) originating from Russia and Turkey were used in the 1930s in the USA as sources of resistance against *B. lactucae* (Lebeda et al. 2002). These sources created the breeding pool for a new generation of lettuce cultivars (Imperial 410, Calmar, Valmaine) for outdoor cropping which were introduced in the 1940s and 1950s (Whitaker et al. 1958). All of these cultivars have race-specific resistance (Table 5).

In Europe, the utilization of wild *Lactuca* germplasm was based on two different strategies (the Netherlands and Great Britain). In the 1950s, genes originating from old German and French cultivars of *L. sativa* were used mostly (Crute 1992). At the end of the 1960s in the Netherlands an interspecific hybrid between *L. sativa* (cv. Hilde) and an accession of *L. serriola,* described as H×B, Hilde×*L. serriola* was released. Resistance derived from this material was assigned to the race-specific gene *Dm*11 (Lebeda et al. 2002) (Table 5).

In the 1970s and 1980s other sources of resistance to *B. lactucae,* derived from *L. serriola* with resistance genes (factors) described as *Dm*16 and R18 (Table 5), were used in the Netherlands. All of these genes have been used frequently in breeding programs in Europe during the last 20 years. However, resistance based on these genes is no longer effective against many *B. lactucae* isolates (Lebeda and Zinkernagel 2003b). From the end of the 1980s there was increasing interest (esp. in the Netherlands and U.K.) for the utilization of resistance located in the hybrid line *L. serriola* (Swedish)×*L. sativa* (Brunhilde) and line CS-RL (Lebeda and Blok 1991) was derived from this material. This line was highly resistant for a long time. However recently a new race overcoming the resistance was described (Lebeda and Zinkernagel 2003a).

Table 5 Examples of race-specific resistance genes (*Dm*) or factors (R) located or derived from *Lactuca serriola* (modified according to Lebeda et al. 2002, 2007c)

Dm gene (R-factor)	*L. serriola* accession (line)	Origin	Occurrence in *L. sativa* cultivars[a]	Linkagegroup
*Dm*5	PI 167150	Turkey	Valmaine	2
*Dm*5/8+10	PI 91532	USSR	Sucrine	2
	PI 167150	Turkey		
*Dm*8	PI 91532	USSR	Avoncrisp	2
			Calmar	
			Salinas	
*Dm*6	PI 91532	USSR	Sabine	1
*Dm*7	LSE/57/15	UK	Great Lakes	3
			Mesa 659	
*Dm*11	IVT	[b]	Capitan	3
	Wageningen			
*Dm*15	PIVT 1309	Netherlands	[c]	1
*Dm*16	LSE/18	Czechoslo-vakia	Saffier	1
			Titania	
*Dm*7+10+13	PI 114512	Sweden	Vanguard	
	PI 114535	UK		
PI 125819		Afghanistan		
(+*L.virosa* PI 125130)		Sweden		
R17	LS 102	France [b]	[c]	2
R18	LS 17	France [b]	Mariska	1
R19 (R18+[b])	CS-RL	Sweden	Libusa	1
	LJ88356		Miura	
*Dm*7+R23	CGN 5153	USSR (Krym)[c]		3,5[b]
R24+R25	CGN14255	Hungary	[c]	3,5
R24+R26	CGN14256	Hungary	[c]	3,4
R24+R27	CGN14270	Hungary	[c]	3,4[b]
R24+R28	CGN14280	Hungary	[c]	3,[b]
R24+R29	PI 491178	Turkey	[c]	3,[b]
R30	PI 491229	Greece	[c]	1
R[b] (+modi-fiers,probably RNS)	PI 281876	Iraq	[c]	[b]

[a] only selected examples;

[b] not known or unclear;

[c] *Dm* gene or R-factor not yet located in *L. sativa* cultivar(s).

RNS race-nonspecific

L. saligna and *L. virosa* may possess novel and very interesting resistance to *B. lactucae* (Lebeda et al. 2002). As a result of studies in the 1990s a new lettuce cultivar Titan (Sluis & Groot) with the race-specific gene *Dm*6 plus resistance derived from *L. saligna* (pers. comm., K. Reinink, Rijk Zwaan, the Netherlands) was released in the Netherlands (Lebeda et al. 2002). However, this resistance is no longer effective (Lebeda and Zinkernagel 2003a, b). Recently, a very intensive program of lettuce breeding based on introduction of newly located sources and genes of resistance from *L. serriola, L. saligna* and *L. virosa* was developed in the USA (Michelmore et al. 2005). Also new sources of resistance were located in wild *Lactuca* spp. originating mostly from the Middle East (Beharav et al. 2006; Petrželová et al. 2011).

The effectiveness of the expression of some *Dm* genes located in *L. serriola* can be dependent on environmental factors. Judelson and Michelmore (1992) showed that resistance (assessed as the absence of sporulation) based on *Dm*6, *Dm*7, *Dm*11, *Dm*15, and *Dm*16 became less effective or ineffective at temperatures below 10 °C. The ecological and epidemiological consequences of this effect are not known (Lebeda et al. 2002).

The occurrence of race-specificity in other wild *Lactuca* species and related genera has not been analysed in detail. However, recent analyses (Lebeda et al. 2002) have shown that the occurrence of race-specific resistance in wild *Lactuca* species is a common phenomenon. In the section Lactuca, all of the species studied express race-specificity after inoculation with isolates of *B. lactucae* from *L. sativa* and *L. serriola.* The presence of race-specific resistance in *L. saligna* was described as questionable because most of the screened accessions exhibited complete or incomplete resistance at both the seedling and adult stage (Lebeda et al. 2002; Beharav et al. 2006), and recent results (Petrželová et al. 2011) showed that *L. saligna* may possess non-host resistance. A race-specific response was also confirmed in some species from other sections of the genus *Lactuca* (*L.viminea, L. tatarica, L. quercina, L. indica, L. biennis*) (Lebeda et al. 2002) and there is clear evidence of the occurrence of a race-specific response in some species of related genera (e.g. *Cicerbita, Mycelis*) (Lebeda et al. 2002).

Other types of resistance (race-nonspecific, field, non-host; for detailed description see Lebeda et al. (2002)) of *Lactuca* species against *B. lactucae* are not as well understood. There is only limited information available about race-nonspecific resistance in wild *Lactuca* spp. germplasm. The presence of race-nonspecific resistance has only been reported in *L. serriola* (Lebeda et al. 2002). Currently only two *L. serriola* accessions can be considered as potential sources of this type of resistance. It was recognised that accessions PI 281876 and PI 281877 at the seedling stage were infected by some *B. lactucae* isolates. However, the intensity of sporulation was mostly very low and in some interactions was followed by expression of a necrotic response (Lebeda 1986). Current thoughts are that this resistance is based on some major gene(s) and modifiers (Lebeda et al. 2002). *L. serriola* (PI 281876) has been used frequently in practical breeding programs (Lebeda and Pink 1998).

The most comprehensive experiments focused on field resistance of wild *Lactuca* spp. were carried out by Lebeda (1990). In total, thirty-one accessions of four *Lactuca* species (*L. serriola, L. saligna, L. aculeata, L. indica*/syn. *L. squarrosa*/) and one *L. serriola*×*L. sativa* hybrid (line CS-RL) were studied in 3 years of field experiments. The disease incidence was significantly different across species and accessions. *L. saligna, L. aculeata* accessions and the *L. serriola*×*L. sativa* hybrid were free of infection during the observation period. This reaction implies the presence of effective unknown R-factors (Lebeda et al. 2002) in these genotypes. In the *L. serriola* accessions, significant differences in the level of field resistance were observed (Lebeda 1990). Some accessions were highly susceptible (e.g. PI 204753, PI 253468, PI 273596, PI 273617, PI 274359), in contrast, accessions PI 281876 and PI 253467 were free of disease symptoms (again implying the presence of effective unknown R-factors). However, the possible race-nonspecific resistance in PI 281876 is also likely to be expressed as field resistance (Lebeda 1990).

Nonhost resistance should be very effective, durable and not influenced by changes of environmental conditions (Lebeda et al. 2002). It was hypothetized that some *L. saligna* accessions may possibly possess nonhost resistance (Lebeda 1986). Recent experimental results with new highly virulent isolates of *B. lactucae* originating from *L. sativa* have not confirmed the presence of race-specific resistance in *L. saligna* (Lebeda and Zinkernagel 2003a). However, recent findings indicate that, at least some *L. saligna* accessions possess race-specific resistance factors (Jeuken and Lindhout 2002), in addition to possible non-host resistance to *B. lactucae* (Beharav et al. 2006; Petrželová et al. 2011).

There is only limited information available on the histological, cytological, biochemical and molecular background of resistance to lettuce downy mildew in *L. sativa* and wild *Lactuca* species. Some basic ideas and conclusions related to this subject were summarized by Lebeda et al. (2002, 2006, 2008b), Jeuken and Lindhout (2002, 2004). Data obtained in histological studies of resistance in wild *Lactuca* spp. suggest there are a wide range of resistance mechanisms in *Lactuca* spp. against *B. lactucae* (Lebeda et al. 2008b).

Powdery mildew (Golovinomyces cichoracearum)

Lettuce powdery mildew is considered as a disease of increasing importance (Lebeda and Mieslerová 2011).

The ascomycete *Golovinomyces cichoracearum* V.P. Gelyuta (previously *Erysiphe cichoracearum* DC. s.str. (Lebeda and Mieslerová 2011)) is the predominating powdery mildew species, however, another powdery mildew species, *Podosphaera fusca* was collected and described on *Lactuca sativa* in Korea (Shin et al. 2006). Great progress in the research of the taxonomy, distribution and biology of lettuce powdery mildew (*Golovinomyces cichoracearum sensu stricto*) has been achieved during the last 15 years (Lebeda and Mieslerová 2011).

Natural hosts of powdery mildew include *L. muralis, L. perennis, L. quercina, L. serriola, L. saligna, L. sibirica, L. viminea*, *L. virosa* (Lebeda 1985a, b; Lebeda and Mieslerová 2011), and *L. aculeata* (Lebeda unpubl.). One of the most common species in Europe is *L. serriola* (prickly lettuce) which also could be considered as a common host of *G. cichoracearum*. Substantial variation in expression of the degree of infection between different sites and/or populations was recognized. It was concluded that *L. serriola* could act as a reservoir of inoculum for lettuce infection (Lebeda et al. 2012c, 2013).

Lebeda (1985c) demonstrated in a set of 25 lettuce (*L. sativa*) cultivars substantial differences in disease severity. Only two cultivars (Amanda Plus, Bremex) were free of natural infection.

The screening of more than one hundred accessions of wild representatives of the genus *Lactuca* (*L. aculeata, L. dentata, L. perennis, L. saligna, L. serriola, L. tatarica, L. tenerrima, L. viminea, L. virosa*) under conditions of natural infection by *G. cichoracearum* revealed high variability in resistance (Lebeda 1985b, 1994). The accessions of *L. serriola* were attacked most severely and *L. saligna* showed highly variable levels of resistance, while the lowest levels of infection were found in accessions of *L. virosa, L. viminea, L. tenerrima* and *L. tatarica.* In some species (e.g. *L. saligna, L. serriola*) the interaction with the pathogen is probably based on race-specific resistance (Lebeda and Mieslerová 2011; Lebeda et al. 2012c, 2013) (Table 6).

Some *L. saligna* accessions are potentially useful sources of resistance, especially where they carry resistance to *Bremia lactucae* as well (Lebeda 1985b). *L. virosa* could be also considered as a suitable donor of resistance; however its resistance seems to depend on a certain stage of the ontogenetic development (Lebeda 1985a).

Anthracnose (Microdochium panattoniana)

Anthracnose (shothole disease, ringspot) caused by *Microdochium panattoniana* (Berl.) Sutton, Galea & Price is manifested as small circular brown spots primarily on the lower leaf blades. The centers of these spots dry and fall out. Lesions on the midrib become necrotic, sunken, and elongated. The initial infection may be soilborne or seedborne, the anthracnose conidia are spread in lettuce crops by splashes of rain or irrigation water (Galea et al. 1986). Lettuce ringspot causes serious damage of lettuce crops in the southern states of Australia, in California and throughout Europe especially under cool wet conditions when application of fungicides is difficult (Galea and Price 1988). Sources of resistance have been identified in wild *Lactuca* species *L. angustana, L. livida, L. perennis, L. serriola, L. saligna,* and *L. virosa* (Ochoa et al. 1987; Galea and Price 1988) (Table 7).

Stemphylium leaf spot (Stemphylium spp.)

Symptoms of *Stemphylium botryosum* f. *lactucum* Wallr. on lettuce leaves are small, round, and brown spots, which may appear sunken because the tissue becomes necrotic (Netzer et al. 1985). This fungal disease has been reported in many parts of the world (Raid 1997), but it has relatively small economic impact. The only known source of resistance to the disease is an unspecified line of *L. saligna* collected in Israel (Table 7). Resistance is controlled by two genes, with one allele dominant Sm_1 for resistance and the other recessive sm_1 (Netzer et al. 1985).

Sclerotinia drop-lettuce drop (Sclerotinia spp.)

Two fungal species, *Sclerotinia minor* and *Sclerotinia sclerotiorum* cause sclerotinia drop of lettuce, one of the most widespread and destructive disease worldwide in lettuce production (Purdy 1979; Subbarao 1998). Both *S. minor* and *S. sclerotiorum* survive mainly as sclerotia in soil. *S. minor* primarily infects lettuce by direct eruptive germination of soilborne sclerotia. This mode of infection is less frequent in *S. sclerotiorum*. The primary inoculum source of *S. sclerotiorum* is airborne ascospores from carpogenic germination of sclerotia (Abawi and Grogan 1979). *Sclerotinia* is difficult to control with cultural methods (Lebeda et al. 2007c), and it is difficult to elaborate

Table 6 Sources of resistance in wild *Lactuca* species to lettuce powdery mildew

Name of pathogen	Source of resistance			
	Lactuca spp.	No. of accession/sample	Type of resistance	References
Golovinomyces cichoracearum	*L. aculeata*	LAC/92/2	n.k.	Lebeda (1985a)
	L. dentata	PI 234204	n.k.	Lebeda (1985a)
	L. perennis	09318, 09319, 09323	n.k.	Lebeda (1994)
	L. saligna	LSA/92/1, LSA/92/2	n.k.	Lebeda (1985a)
	L. saligna	05282, 05304, 05306, 05308, 05309, 05311, 05313, 05314, 05315, 05318, 05319, 05320, 05322, 05323, 05326, 05330, 05895, 09311, 09313	n.k.	Lebeda (1994)
	L. saligna	09-H58-1013	race-specific	Lebeda et al. (2013)
	L. serriola	PI 255665	n.k.	Lebeda (1985a)
	L. serriola	PI 273617	race-specific	Lebeda et al. (2013)
	L. tatarica	09389, 09390	n.k.	Lebeda (1994)
	L. tenerrima	09386, 09387, 09388	n.k.	Lebeda (1994)
	L. viminea	09326	n.k.	Lebeda (1994)
	L. virosa	LVIR/26, LVIR/57/1	n.k.	Lebeda (1985a)
	L. virosa	04678, 04679, 04680, 04681, 04682, 04683, 04954, 04955, 04956, 04963, 04964, 04970, 04972, 05020, 05077, 05145, 05148, 05266, 05268, 05270, 05283, 05331, 05332, 05333, 05793, 05794, 05816, 05869, 05941, 05978, 09315, 09316, 09364, 09365, PI 271938	n.k.	Lebeda (1994)
	L. virosa	LVIR/50	race-specific	Lebeda et al. (2012c)

protocols of resistance screening (Grube and Ryder 2004).

Extensive evaluation of lettuce germplasm has been carried out either for resistance to *S. sclerotiorum* (Chupp and Sherf 1960; Elia and Piglionica 1964; Whipps et al. 2002) or *S. minor* (Abawi et al. 1980; Subbarao 1998; Grube and Ryder 2004) but no complete resistance has been identified, and it is unknown whether resistance to the two species is correlated (Lebeda et al. 2007c). Wild *Lactuca* species were included in these tests but the numbers of accession was relatively low (Abawi et al. 1980; Whipps et al. 2002).

An accession of primitive oilseed lettuce *L. sativa* (PI 251246) may have partial resistance to *Sclerotinia* sp. infection (Whipps et al. 2002; Hayes et al. 2010), but this is very likely associated with its primitive growth habit (Grube 2004). *L. dentata* (PI 234204, later named as *Sonchus oleraceus* (Doležalová et al. 2004; Lebeda et al. 2007c)), *Lactuca* sp. (PI 274376) and *L. serriola* (PI 271938) were shown to be highly resistant to *S. minor* (Abawi et al. 1980), and the latter accession was also resistant to *S. sclerotiorum* (Whipps et al. 2002) (Table 7). In the *S. sclerotiorum*-infested field experiments, three *L. virosa* accessions (SAL 012, IVT 280 and IVT 1398) demonstrated high levels of resistance (Table 7), although further analysis is needed to determine the role of the slow bolting/biennial nature of *L. virosa* in resistance (Hayes et al. 2010).

Verticillium wilt (Verticillium dahliae)

Verticillium wilt, is a relatively newly recognised lettuce disease caused by the soilborne fungus *Verticillium dahliae* Kleb. It was reported for the first time in a lettuce crop in the Pajaro Valley (California) in 1995 (Subbarao et al. 1997; Bhat and Subbarao 1999), in 1999 it was first observed on lettuce in the Salinas Valley (Atallah et al. 2011), in 2006 in northern Italy (Garibaldi et al. 2007). In 2009, this disease appeared in commercial fields in Japan (Usami et al. 2012). Losses of up to 100 % may occur in head lettuce: smaller losses occur in other lettuce types (Lebeda et al. 2007c).

V. dahliae was first isolated from *L. serriola* during a field survey carried out in Crete in 1992–2000 (Ligoxigakis et al. 2002). Disease symptoms and recovery of *V. dahliae* are known in wild *L. serriola* and other *L. serriola*–like species, *L. saligna*, and *L. virosa* (Hayes et al. 2009).

Table 7 Resistance of wild *Lactuca* species to fungal pathogens

Name of pathogen	Source of resistance				
	Lactuca spp.	No. of accession, sample	Type of resistance	Remark	References
Anthracnose (*Microdochium panattoniana*)					
	L. angustana	PI 190906			Ochoa et al. (1987)
	L. livida	PI 273585		re-determined as *Lactuca* sp. (Doležalová et al., 2004; Lebeda et al. 2007c)	Ochoa et al. (1987)
	L. perennis	PI 273594			Ochoa et al. (1987)
		PI 274415			Ochoa et al. (1987)
		PI 274378			Ochoa et al. (1987)
	L. saligna	LAG1	immune	coll. in Hillston, NSW	Galea and Price (1988)
		UC83US1	resistant to races A, B, C, D, E		Ochoa et al. (1987)
		PI 273482			Ochoa et al. (1987)
		UC83UK2			Ochoa et al. (1987)
		PI 490999			Ochoa et al. (1987)
	L. serriola	LAG2	highly resistant	coll. from Zoology Reserve, La Trobe University	Galea and Price (1988)
		PI 274372			Ochoa et al. (1987)
		PI 274457			Ochoa et al. (1987)
		PI 289064			Ochoa et al. (1987)
	L. virosa	PI 271939			Ochoa et al. (1987)
		PI 273579			Ochoa et al. (1987)
		PI 274375			Ochoa et al. (1987)
Stemphylium botryosum f. *lactucum*					
	L. saligna	not specified material	two genes, dominant Sm_1 and recessive sm_1	collected in Israel	Netzer et al. (1985)
Sclerotinia sclerotiorum					
	L. serriola	PI 271938		this accession is probably *L. virosa*	Whipps et al. (2002)
	L. virosa	SAL 012			Hayes et al. (2010)
		IVT 280			Hayes et al. (2010)
		IVT 1398			Hayes et al. (2010)
S. minor					
	L. dentata	PI 234204		re-determined as *Sonchus oleraceus* (Doležalová et al., 2004; Lebeda et al. 2007c)	Abawi et al. (1980)
	L. serriola	PI 271938		this accession is probably *L. virosa*	Abawi et al. (1980)
	Lactuca sp.	PI 274376		USDA ARS GRIN, re-determined as *Sonchus oleraceus*	Abawi et al. (1980)
Verticillium dahlie					
	L. virosa	IVT 280			Grube et al. (2005a)

Two pathogenic races of *V. dahliae* were described, and currently race 2 predominates as a result of worldwide cultivation of lettuce cultivars resistant to race 1 (Hayes et al. 2006, 2007a, b, 2011b; Atallah et al. 2011). Despite widespread screening, complete resistance to race 2 has yet to be identified (Grube et al. 2005a; Attalah et al. 2011). Partial resistance to race 2, in the form of reduced disease incidence or delayed expression of symptoms, has been found in four PI accessions *L. sativa* (Hayes et al. 2011a).

The *L. virosa* accession IVT 280 (Table 7) and some other accessions have shown high levels of resistance in field tests (Grube et al. 2005a).

Fusarium wilt (Fusarium spp.)

Fusarium wilt was first reported on lettuce in Japan in 1955 (Matuo and Matahashi 1967), but it was not until many years later that its widespread occurrence and potential for economic damage was fully recognized (Fujinaga et al. 2003; Hubbard and Gerik 1993; Garibaldi et al. 2004). The disease is caused by *Fusarium oxysporum* f. sp. *lactucae* n.f. (same as f. sp. *lactucum* (Hubbard and Gerik 1993; Fujinaga et al. 2003). Three races of the fungus have been identified (Fujinaga et al. 2003). It is a disease of the root vascular system. As one of several wilting diseases exhibiting yellowing and wilting of leaves and stunting and plant death, the principal diagnostic symptom is a reddish brown discoloration of the cortex and upper crown (Matheron and Koike 2003). Higher temperatures tend to increase the severity of fusarium wilt in lettuce (Scott et al. 2010).

Resistance sources have been identified for races 1 and/or 2, but not for race 3 (Garibaldi et al. 2004; Tsuchiya et al. 2004). All sources are cultivars of the various lettuce types.

Pythium wilt (Pythium spp.)

Soilborne pathogens *Pythium tracheiphylum* and *P. uncinulatum* were reported as causing vascular wilt and stem rot of lettuce in Italy in 1965 (Matta 1965) and subsequently in other parts of Europe (Blok and van der Plaats-Niterink 1978), North America (Tortolero and Sequeira 1978), Australia (Kumar et al. 2007) and Japan (Matsuura et al. 2010). Yield reductions up to 30 % have been recorded (Davis et al. 1995). In spite of the economic importance of these pathogens, the screening of wild *Lactuca* species for resistance is not reported.

Nematodes

Root-knot nematode (Meloidogyne spp., incognita, hapla, javanica, enterolobii)

Nematodes occuring on lettuce can be classified into 23 genera: *Aphelenchoides* (da Silveira 1990), *Meloidogyne* (e.g. Viaene and Abawi 1996, Blancard 2011), *Pratylenchus* (Moretti et al. 1981; Mani et al. 1997), *Rotylenchulus*, *Tetylenchus*, *Tylenchorhynchus* (e.g. Radewald 1969a; Philis 1995; Koenning et al. 1999; Kohl 2011; Pedroche et al. 2012), *Helicotylenchus* (Anwar and McKenry 2012), *Criconemoides*, *Heterodera*, *Hoplolaimus*, *Paratylenchus* (Machado and Inomoto 2001; Bao and Neher 2011), *Paratrichodorus* (Boydston et al. 2004), *Hemicycliophora* (Chitambar 1993; Blancard 2011), *Rotylenchoides*, *Tylenchus*, (Addoh 1971), *Longidorus* (Radewald 1969b, c; MacGowan 1982; Huang and Ploeg 2001), *Mesocriconema* (DAFF 2012), *Nacobbus*, *Paralongidorus*, *Xiphinema* (Sikora and Fernández 2005), *Aglenchus* (Ökten 1988), *Belonolaimus* (Chitambar 2007), *Radopholus* (Ferris 2013), *Merlinius* (Bridge 1976).

However the most important nematodes with documented impact on lettuce growth and yield include the needle nematode (*Longidorus africanus*), root-knot nematode (*Meloidogyne* spp.), root lesion nematode (*Pratylenchus penetrans*), and the spiral nematode (*Rotylenchus robustus*) (Davis et al. 1997). There are several other nematodes associated with lettuce in the field-stubby root nematode *Paratrichodorus minor*, the reniform nematode *Nacobbus aberrans*, and stunt nematodes *Tylenchorhynchus clarus* and *Merlineus* spp. (Davis et al. 1997).

The number of papers reporting occurrence of nematode infection/diseases on lettuce wild relatives is limited. The majority of reports are based on field observation of nematode infection on *L. serriola* (Table 8). There are just two reports related to identification of resistant accessions to root-knot nematode (*M. hapla*) in a larger germplasm collections. Abawi and Robinson (1991) evaluated 85 genotypes (including accessions of *L. serriola*, *L. virosa* and *L. saligna*) in two greenhouse tests, and in a recent study Kaur and Mitkowski (2010) analysed 494 lettuce accessions, including 36*L. serriola*, 7*L. virosa* and 8*L. saligna* genotypes. Genotypes with moderate to highly resistant reaction to *M. hapla* inoculation were observed in these studies (Table 8). The occurrence of root-knot nematodes on

Table 8 Resistance of wild *Lactuca* species to nematodes

Name of nematode	Source of resistance				
	Lactuca spp.	No. of accession/sample	Type of resistance	Remark	References
Meloidogyne hapla	*L. serriola*	3738 (M-HR)	n.k.	GRS	Abawi and Robinson (1991)
					Kaur and Mitkowski (2010)
	L. saligna	PI 281876, PI 491000, 261653, PI 273582 (M-HR)	n.k.	GRS	Abawi and Robinson (1991)
					Kaur and Mitkowski (2010)
	L. virosa	PI 273579, PI 271938, PI 273597 (M-HR)	n.k.	GRS	Abawi and Robinson (1991)
					Kaur and Mitkowski (2010)
M. incognita	*L. serriola*	n.s.	n.k.	FO, highly susceptible species	Gaskin (1958)
				WGT, highly susceptible to race 1	Gharabadiyan et al. (2012)
	L. saligna	n.s.	n.k.		Rich et al. (2010)
	L. saligna var. *runcinata*	n.s.	n.k.	WGT, host species	Gowda et al. (1995)
M. javanica	*L. serriola*	n.s.	n.k.	WGT, highly susceptible	Gharabadiyan et al. (2012)
Paratrichodorus allius	*L. serriola*	n.s.	n.k.	host species for *P. allius* (vector for tobacco rattle virus)	Boydston et al. (2004)
					Mojtahedi et al. (2003)
Pratylenchus neglectus	*L. serriola*	n.s.	n.k.	WGT	Vanstone and Russ (2001)
P. thornei	*L. serriola*	n.s.	n.k.	WGT	Vanstone and Russ (2001)

Explanation of terms used in the table: *FO* field observation; *GRS* germplasm resistance screening in greenhouse; *M-HR* moderate-highly resistant genotype; *n.k.* not known; *n.s.* not specified; *WGT* weed greenhouse testing

weed hosts was investigated by Gowda et al. (1995), who reported light root gall intensity and small size of galls indicating resistance to *Meloidogyne incognita*. Davis and Venette (2004) considered *Meloidogyne falax* as potential risk (potential hosts) for several threatened and/or endangered wild *Lactuca* species (*L. floridana*, *L. hirsuta*, *L. tatarica* var. *pulchella*).

The information on the genetic basis of nematode resistance in wild *Lactuca* species is missing. In cultivated lettuce the resistance genes for *Meloidogyne* appears to be under control of a single gene locus, with predominantly additive gene action (for *M. incognita* races 1, 2, 3 and 4, and *M. kabanica*) (Gomes et al. 2000; Maluf et al. 2002; Cavalhi Filho et al. 2008). On the other hand de Carvalho et al. (2011) proved that two different genes are involved in control of resistance to *M. incognita* race 1 in lettuce cultivars Grand Rapids and Salinas-88. Further, they reported that lines with higher levels of nematode resistance than either Grand Rapids or Salinas-88 could be selected in the F4 generation of the cross between these resistant parental lines indicating that the two parental cultivars possess different genetic factors for resistance.

There are also reports on transgenic lettuce linies bearing tomato root-knot resistance gene *Mi-1* (Zhang et al. 2010) and the linkage of tomato resistance genes to root-knot nematode (*Meloidogyne* spp) to leaf mold (*Cladosporium fulvum*) (Dickinson et al. 1993; Jones et al. 1993).

Insects and mites

There are a number of aphid species, occuring both on cultivated lettuce and its wild relatives, these belong to the following genera-*Acyrthosiphon*, *Aulacorthum*, *Aphis*, *Dysaphis*, *Hyperomyzus*, *Macrosiphum*, *Myzus*, *Nasonovia*, *Neomyzus*, *Pemphigus*, *Protrama*, *Sitobion*, *Trama*, *Uroleucon* (Blackman and Eastop 2000), *Eucarazzia* (Stoetzel 1985) and *Rhopalosiphum* (Sangün and Satar 2012). However, we present here a review of papers using wild lettuce species as parents for resistance breeding to three aphid species-the green lettuce aphid (*Nasonovia ribisnigri*), the potato aphid (*Macrosiphum euphorbiae*) and lettuce root aphid (*Pemphigus bursarius*) and the leafminer (*Liriomyza langei*). There are other important aphid species (e.g. *Myzus persicae*, the green peach aphid, possibly the most important leaf-feeding pest on lettuce because of its ability to transmit several important viruses - see above), however no information related to resistance screening studies (or breeding) using wild lettuce species has been published so far. For a detailed survey of occurence of various aphid species on other *Lactuca* spp. (see Blackman and Eastop 2000).

Nasonovia ribisnigri Mosley [the „green lettuce aphid"(GLA) or „currant-lettuce aphid"(CLA)] is commercially the most important lettuce pest (Martin et al. 1996) with a worldwide distribution (Blackman and Eastop 2000). It colonizes the interior of the lettuce head, making its control difficult both with contact insecticides (Liu 2004) and biological control (Mackenzie and Vernon 1988). The use of resistant cultivars is therfore the best option to protect lettuce from this pest. Resistance to biotype 0 (Nr:0) was first reported in *Lactuca virosa* accession IVT 280 (Eenink et al. 1982a,b) and characterized as complete (i.e. virtually no aphids survived), and genetically dominant to the partial resistance found in *L. virosa* accession IVT 273. Complete and partial resistances to Nr:0 were conditioned by two alleles, *Nr* (complete resistance) and *nr* (partial resistance). McCreight and Liu (2012) proposed the following system of allelic designation: $Nr{:}0^{C}$ for complete resistance and $Nr{:}0^{P}$ for partial resistance, with their relationships: $Nr{:}0^{C}$ (in IVT 280, 'Barcelona')>$Nr{:}0^{P}$ (in PI 491093)>*nr* (in susceptible genotypes) (McCreight and Liu 2012).

Subsequently, resistance in IVT 280 was successfully transferred to lettuce by a bridging cross to *L. serriola* (e.g. Eenink et al. 1982a, b; van der Arend et al. 1999). There are a large number of modern lettuce cultivars with GLA resistance e.g. cvs. Barcelona, Campionas, Dynamite, Elenas, Fortunas, Irina, Krinas, Veronas, 83-67RZ (van der Arend et al. 1999; Liu and McCreight 2006) and in 2010 there were 88 comercially available lettuce varieties in Australia (McDougall and Troldahl 2010), produced by several breeding companies including: RijkZwaan, Nunhems, South Pacific Seeds, Lefroy Valley, Seminis Vegetable Seeds, Terranova Seeds. However, this widespread deployment of a single dominant gene for resistance has exerted a high selection pressure for the emergence of a resistance breaking phenotype and several *N. ribisnigri* populations feeding on resistant cultivars were detected in 2007. These were subsequently characterized as a resistance-breaking biotype of *N. ribisnigri* designated Nr:1 (Sauer 2008). This biotype has spread throughout the European continent (Cid et al. 2012).

Wild progenitors of cultivated lettuce appear to be a valuable source of resistance to GLA, as evident from the results of recent large germplasm screenings. McCreight (2008) identified two new potential sources for resistance to Nr:0 in *L. serriola* acc. PI 491093 (partial resistance) and *L. virosa* PI 274378 (complete resistance). This was a result of a large greenhouse screening of 1203 lettuce accessions (included 7*L. perennis*, 18*L. saligna*, 125*L. serriola*, and 6*L. virosa* accessions). Sixty-four *L. serriola* and *L. virosa* accessions (see Table 9) resistant to Nr:1 were reported in CGN germplasm collection (the Center for Genetic Resources, the Netherlands) (Anonymous 2008). For some of these (CGN13361, CGN16266, CGN16272), all five replicate plants were resistant while in other accessions (CGN04757, CGN04930, CGN04973) resistance segregated in the tested plants. Several accessions were resistant to both Nr:0 and Nr:1 (Anonymous 2008). Dominant resistance to Nr:0 and Nr:1 was also reported to have been found in the *L. serriola* accession 10G.913571 by Thabuis et al. (2011).

More recently, Cid et al. (2012) performed two tests: a greenhouse screening of 264 lettuce accessions including 40 accessions closely related to *L. sativa* (3*L. perennis*, 6*L. virosa*, 13*L. serriola* and 18*L. sativa*×*L. serriola*) and laboratory screening of 40*L. virosa* accessions against both *N. ribisnigri* biotypes (Nr:0, Nr:1) and against a clone of *M. euphorbiae*. Three *L. virosa* accessions showed (Table 9) resistance against *N. ribisnigri*, two (CGN16272 and CGN13361) partial resistance to the Nr:1 biotype of *N. ribisnigri* and to *M. euphorbiae*. While near complete resistance to *M.*

Table 9 Resistance of wild *Lactuca* species to aphids

Name of aphid	Source of resistance		Type of resistance	Remark	References
	Lactuca spp.	No. of accession/sample			
Liriomyza langei	*L. serriola*	PI 491178, PI 491181, PI 274901	R		Mou and Liu (2003, 2004)
	L. saligna	PI 490999, PI 261653-1, PI 509525	R		Mou and Liu (2003, 2004)
	L. virosa	PI 273597, PI 274375, PI 274901	R		Mou and Liu (2003, 2004)
Macrosiphum euphorbiae	*L. serriola*	n.s.	n.k.		Blackman and Eastop (2000)
					Barbosa (1998)
	L. virosa	CGN13355	HR		Cid et al. (2012)
		CGN16272, CGN13361	PR		Cid et al. (2012)
Nasonovia ribisnigri	*L. serriola*	PIVT 252	S		Eenink and Dieleman (1983)
		PI 491093	R, psr		McCreight (2008)
					McCreight and Liu (2012)
		CGN04757, W6 21998	PR		Cid et al. (2012)
	L. virosa	PI 274378	R, psr		McCreight (2008)
		PIVT 273	PR		McCreight and Liu (2012)
					Eenink and Dieleman (1983)
		PIVT 275, PIVT 278, PIVT 280, PIVT 714, PIVT 731, PIVT 72723	CR		McCreight and Liu (2012)
					Eenink and Dieleman (1983)
		CGN13361	PR		Cid et al. (2012)
		CGN16272	PR	R to Nr:1	Cid et al. (2012)
		CGN13355, CGN16266, W6 23867	CR		Cid et al. (2012)
		CGN05148, CGN21399	CR	R to Nr:1	Cid et al. (2012)
		CGN13361, CGN16266, CGN16272	R	R to Nr:0, Nr:1	Anonymous (2008)
	L. virosa ×*L. serriola*	F1 (F1 (73723×255)×F1 (255×Suzan)) ×F1 (F1(254×280)×1037)	CR		Eenink et al. (1982b)
					Reinink and Dieleman (1989)
	L. perennis	CGN10885, PI 274378	CR, PR		Cid et al. (2012)
Pemphigus bursarius	*L. serriola*	001562, HRIGRU1606, HRIGRU1573, HRIGRU7145	R		Cole et al. (1991)
	L. saligna	006186, 001627, HRIGRU1630	R		Cole et al. (1991)
	L. virosa	n.s.	HR		Cole et al. (1991); Ellis et al. (2002)
	L. perennis	n.s.	HR		Ellis et al. (2002)

Explanation of terms used in the table: *CR* completely resistant; *HR* highly resistant; *PR* partially resistant; *R* resistant; *S* susceptible; *n.k.* not known; *n.s.* not specified; *psr* potential source of resistance

euphorbiae was found in CGN13355 but this was susceptible to *N. ribisnigri* (Cid et al. 2012).

The „lettuce root aphid"(LRA), *Pemphigus bursarius* (L.) is one of several aphid species that feed on cultivated lettuce (Blackman and Eastop 2000) and can cause severe damage to crops (Dunn, 1959). It is considered as an occasional pest of lettuce crops but can also cause severe losses in lettuce seed production. Additionally, it is able to colonise a variety of non-crop species, largely within the Compositae (Dunn 1959; Alleyne and Morrison 1977). This aphid is regarded as holocyclic, alternating annually between sexual reproduction on a primary woody host, poplar (*Populus nigra*, L.) and parthenogenesis on its secondary hosts such as lettuce (see Dunn 1959; Miller et al. 2003, 2008). Lettuce root aphid is one of the first examples of successful insect resistance breeding in vegetable crop (Reinink 1999). As the most effective control of root aphids, highly resistant cultivars eliminating the aphid colonisation on lettuce roots. This resistance is controlled by one or two

genes as described by Ellis et al. (1994, 2002). At least one of these genes is not allelic to the existing *Lra* gene, which can be linked to downy mildew resistance gene DM6 (e.g. linked in cv. Avonscrisp but not in cv. Lakeland). There is only a single paper focussed on LRA resistance screening in germplasm collections by Ellis et al. (2002). This describes the testing of 55 *Lactuca* spp. accessions for resistance to *P. bursarius* and the identification of extremely high levels of resistance in accessions of the wild species *L. saligna*, *L. perennis*, *L. virosa*, and in the variety Grand Rapids. Cole et al. (1991) described the use of allozyme analysis to detect bands related to LRA resistance in a screening of four *Lactuca* species (*L. serriola*, *L. virosa*, *L. saligna* and primitive *L. sativa*, eight accessions per species). Out of the forty samples tested, only ten accessions were resistant to colonisation by the pest (*L. serriola*—001562, HRIGRU1606, HRIGRU1573, HRIGRU7145; *L. saligna*—006186, 001627, HRIGRU1630 and *L. sativa* 006779, 001886, 006612).

Leafminer (*Liriomyza langei* Frick) is a major insect pest of many important agricultural crops including lettuce (Mou and Liu 2003, 2004; Lebeda et al. 2007c). Succesfull breeding for resistance to leafminer in lettuce was reported by Mou and Ryder (2010). However, studies exploring genetic variation of leafminer resistance in lettuce germplasm, including wild progenitors, are limited (Mou and Liu 2003, 2004). Also the mechanism of leafminer resistance in lettuce is unknown (Mou and Liu 2004). Mou and Liu (2003) performed screening of 46 *Lactuca* accessions, including 2 accessions of *L. serriola*, 1 acc. of *L. saligna*, and 1 acc. of *L. virosa*. High levels of resistance were discovered in these wild genotypes. In a subsequent study by Mou and Liu (2004) fifty-four lettuce genotypes and 232 F2 plants of crosses were evaluated for leafminer resistance, again a significantly lower occurence of leafminers were found on the accessions of the wild species (*L. serriola*, *L. saligna*, and *L. virosa*) compared to the cultivars.

Approaches to exploitation of wild *Lactuca* spp. in lettuce resistance breeding

Interspecific hybridization

Autogamy is the predominating breeding system within the genus *Lactuca*, especially in the marginal parts of its distribution area (Feráková 1977). Stebbins (1957) estimated a higher occurrence of allogamy in the centre of distribution. Lindqvuist (1960) proved experimentally that all species belonging to the "*serriola*" group were self-fertile. Spontaneous cross-pollination occurs through activity of various insect from the Hymenoptera and Diptera groups (Feráková 1977). In the commercial seed production of *L. sativa*, up to 5 % of cross-pollination has been observed (George 1999). Hybridization can occur not only within one species, but also between species. *Lactuca altaica* hybridizes spontaneously with *L. saligna* and *L. serriola*; *L. aculeata* hybridizes with *L. sativa* and with *L. serriola*; *L. serriola* hybridizes with *L. dregeana* (Zohary Zohary 1991). The close relationship of *serriola*-like species *L. serriola*, *L. dregeana*, *L. altaica*, and *L. aculeata* to *L. sativa* is supported not only by the same chromosome number but also by molecular (AFLP) markers (Koopman et al. 1998, 2001), and by DNA content (Koopman 2002).

Hybridization data on the species belonging to the different sections or groups of the genus *Lactuca* are limited to *L. viminea* and *L. tatarica*. Groenwold (1983) reported partly fertile hybrids between *L. viminea* (section *Phoenixopus*) and *L. virosa* (section *Lactuca*).

Natural hybridization

D'Andrea et al. (2008) proved in a field experiment conducted in Switzerland that natural hybridization between lettuce and *L. serriola* occurred up to the maximal distance tested (40 m), and hybridization rates varied between 0 to 26 %, decreasing with distance. More than 80 % of the wild plants produced at least one hybrid at within 1 m and 4 to 5 % at 40 m. In sympatric crop-wild populations, cross-pollination between cultivated lettuce and its wild relative has to be seen as the rule rather than the exception for short separation distances. However, in the northern parts of Europe, where expansion of prickly lettuce (*L. serriola*) took place, only a few putative hybrids with *L. sativa* were found. So, very probably mechanisms other than crop/wild gene flow, such as those connected to the human activities around building and transport are more likely explanations for this phenomenon (Hooftman et al. 2009; Uwimana et al. 2012a).

The phenotype of putative natural interspecific hybrids was recorded for primarily self- pollinated *Lactuca* species acquired during collecting missions in natural habitats: *Lactuca serriola* (× *L. sativa*) acquired from natural populations *L. serriola* f. *serriola* in northern

Moravia in the year 1995 (Křístková et al. 2012). The hybrid character of plants *L. aculeata* (× *L. serriola*) from a natural population *L. aculeata* collected in Israel in 2005 was confirmed by allozyme markers (Lebeda et al. 2012b). Hybrid characteristics of *L. saligna* (× *L. serriola*) were observed on plants raised from *L. saligna* achenes collected in Jordan in 2007. Putative hybrid plants showed a low level of self-fertility (Křístková et al. 2012). The molecular profile of plants of *L. serriola* collected in Israel in 2009 suggests natural hybridization of *L. serriola* (× *L. saligna*) (Křístková et al. 2012).

The phenotype of interspecific hybrids was recorded also on several *Lactuca* spp. accessions obtained from world germplasm collections. Species *L. sativa*, *L. serriola*, *L. saligna*, *L. dregeana* and *L. virosa* probably participated in the hybridization (Doležalová et al. 2007).

Managed hybridization

Hybridization experiments have been used to: reveal evolutionary relations among *Lactuca* species (de Vries 1990) and aspects of domestication process of lettuce; serve as a base for plant genetic resources management (van de Wiel et al. 2010) and the practical application in breeding programmes; and recently for the assessment of ecological risk of transgenes (Giannino et al. 2008; Hooftman et al. 2011).

Three approaches are employed in order to prevent self-pollination and to perform sexual hybridization: manual removal of anthers, spraying with water of the self-pollen from the stigma prior the cross-pollination, and the exploitation of male-sterile lines as pollen recipients (Davey and Anthony 2011). Examples of interspecific hybridization with potential economic impact are given in this paper below in following chapters.

Hybridization experiments of lettuce with *L. serriola* and QTL analysis identified genomic regions with major QTL effects important for breeding programmes and plant transformation (Hartman et al. 2012, 2013a). Hartman et al. (2013b) show that there is a high likehood in lettuce for novel crop-wild hybrids to arise with a higher fitness than the wild parent through combination of heterosis, linkage and transgressive segregation.

The genomic analysis of plants derived from the hybridization of *L. sativa*×*L. serriola* and their backrosses proved that the domesticated parent contributed QTLs with either a positive or a negative effect on plant vigour, and there are genomic locations where transgenes could be preferably located to mitigate (reduce) their persistance in natural populations in occassional crop-wild hybridization (Uwimana et al. 2012b, c).

Interspecific hybrids between the species with a low sexual compatibility have been obtained by using a bridging species (e.g. *L. serriola*) i.e. crossing one parent to the bridging species and then crossing the resultant F1 with the other parent (Thompson and Ryder 1961; Eenink et al. 1982b; Lebeda et al. 2007c).

Cell and tissue culture

Tissue culture based procedures for the regeneration of fertile plants from tissue explants, cells and isolated protoplasts technologies are the basis for their exploitation in lettuce improvement. Techniques and approaches used for in vitro culture of lettuce have been reviewed thoroughly (e.g. Michelmore and Eash 1986; Alconero 1988; Pink and Keane 1993; Davey et al. 2007a, b; Lebeda et al. 2007c), however there is no recent reports of tissue culture technologies exploring wild *Lactuca* species in lettuce improvement.

In vitro rescue of immature embryos was used successfully for sexual hybridization between *L. sativa* and *L. virosa* (Maisonneuve et al. 1995; Maisonneuve 2003).

Protoplast fusion permitted the regeneration of somatic hybrids between *L. sativa* and either *L. tatarica* or *L. perennis* (Chupeau et al. 1994; Maisonneuve et al. 1995). Somatic hybrids between cultivated lettuce and *L. virosa* were produced by protoplast electrofusion (Matsumoto 1991). Hybrids had normal flower morphology, but all were sterile. *L. indica* (section *Tuberosae*) can be somatically hybridized with *L. sativa* to produce a viable callus (Mizutani et al. 1989).

So far, fertile interspecific hybrids have only been produced between species *L. sativa* (section *Lactuca*) and *L. tatarica* (section *Mulgedium*) by using somatic hybridization (Chupeau et al. 1994; Maisonneuve et al. 1995).

Transformation

During the last 10 years, there are numerous reports on the gene introduction into lettuce by transformation (Davey et al. 2002, 2007a, b; Klocke et al. 2010). *Agrobacterium* inoculation of leaf explants is a universal approach for inducing genes into lettuce, and although this approach has been focused, to date, on *L. sativa*, wild *Lactuca* species could be exploited in a similar way (Davey and Anthony 2011).

Transformation of the plastid genome has several advantages over conventional nuclear transformation, mainly by the high level of transgene expression in chloroplasts, this is opening new possibilities for metabolic engineering, resistance management, and production of biopharmaceuticals (Davey and Anthony 2011). Plastid transformation was applied on the lettuce cv. 'Cisco' (Kanamoto et al. 2006) and cv. 'Grand Rapid' (Pileggi et al. 2001).

There are two ways that transgenic approaches could allow better access to the tertiary gene pool (*L. virosa*, *L. saligna* and others). One way would be through directly transforming *L. sativa* with characterized genes from wild species. The second method is less direct, and would be used in conjunction with somatic hybridization techniques. Transformation would be used to create a universal hybridizer by combining dominant antibiotic resistance and recessive albinism markers in the same genotype (Chupeau et al. 1994). Another potentially valuable use of this technology is to increase or decrease the expression of endogenous genes, or genes already present within cultivated lettuce.

Construction of transgenic plants follows various aims, e.g. production of vaccines, ascorbic acid, tocopherols, increase of iron uptake, decrease of nitrate accumulation, as reviewed by Davey and Anthony (2011) and Davey et al. (2002), Lebeda et al. (2007c), to induce the herbicide resistance (McCabe et al. 1999; Mohapatra et al. 1999; Torres et al. 1999; Dubois et al. 2005) or to delay leaf senescence (McCabe et al. 2001).

Transgenic male-sterile plants are valuable as recipient of donor pollen during hybridization (Curtis et al. 1996; Takada et al. 2007).

Viral genes to confer LMV resistance have been introduced into lettuce (Dinant et al. 1993; Dinant 1997; Gilberton, 1996). Kawazu et al. (2009, 2010) generated lettuce plants resistant to LBVaV, MLBVV by introducing coat protein in anti-sense orientation.

The cloning of genes (*Dm3*) for resistance to the lettuce downy mildew (*Bremia lactucae*) performed by Okubara et al. (1997) have led to clarification of the molecular basis of resistance to this pathogen,

Despite the potential value of some of these traits, no transgenic lettuce has yet been commercialized. In some cases the transgenes did not have the expected desirable effects on the plant phenotype possibly due to geen silencing mechanisms such as methylation (Dinant et al. 1993; McCabe et al. 1999). However, the main reasons for the lack of commercial application of GM technology in lettuce is due to the currently high costs associated with the regulatory procedures for release of a transgenic cultivar and a worldwide lack of public acceptance of the technology in a fresh vegetable crop. Biotechnology-derived vegetables, including lettuce, will only succeed if clear advantages and safety are demonstrated to both growers and more importantly to consumers (Dias and Ortiz 2012a, b).

Mutations

Induced mutation has led to the creation of lettuce lines with interesting traits, like dwarfing, early flowering, male sterility or herbicide tolerance (Mou 2011b). Lettuce mutants are useful in genomic studies of resistance to *Bremia lactucae*, and for gene cloning; combined with genomic advances and new technologies like TILLING (targeted induced local lesions in genome), mutagenesis is becoming more useful tool for lettuce breeders (Mou 2011b).

Known examples of lettuce cultivars issued from exploitation of wild *Lactuca* spp.

Lettuce breeders have increased genetic diversity and achieved disease resistance through crossing cultivated lettuce with non-cultivated or wild lettuce types (Mikel 2007). Breeding aims, strategies and issues have been reviewed by Ryder (2001). Reviews of wild *Lactuca* species used in the lettuce breeding and description of breeding approaches and methods were given by Lebeda et al. (2007c), Mou (2008), and Davey and Anthony (2011).

Lebeda and Blok (1991) reported downy mildew resistance in the hybrid of *L. sativa*×*L. serriola*. More details about this and the historical consequences about the influence of *L. serriola* on lettuce resistance breeding to *B. lactucae* were summarized by Lebeda et al. (2002).

Lactuca saligna is known to produce, hybrids with *L. sativa* and *L. serriola* when used as the female parent (Pink and Keane 1993). *L. saligna* was crossed to a cultivated iceberg type by R.W. Robinson (Provvidenti et al. 1980) who developed the cultivar 'Salad Crisp' from that cross. Jeuken and Lindhout (2004) developed backcross inbred lines in which chromosome segments from *L. saligna* were introgressed into cultivated lettuce and used for genetic analysis of resistance to *Bremia lactucae*.

Crosses between *L. sativa* as one parent and *L. virosa* as the other parent are made with a great difficulty, resulting in low seed set, unviable seeds, stunted plants and/or sterile hybrids (Lindqvist 1960). Viable hybrid plants were obtained only when *L. serriola* was used as a bridging species (Thompson and Ryder 1961; Eenink et al. 1982b). In 1958, the cultivar Vanguard was developed from a cross between a *L. sativa*×*L. serriola* which was then crossed to *L. virosa*. However, with some manipulations, crosses have been made and have led to development of cultivars (Ryder 1999).

During at least the last two decades there has been an increasing private sector breeding effort in lettuce cultivar development with a concomitant decrease in public sector breeding in many countries. In the USA the legal protection of cultivars is accomplished by their registration supplemented by their pedigree which are made publically available (Mikel 2007), in contrast to lettuce cultivars bred in Europe where such pedigrees are not released in to the public domain.

The pedigree analysis of 328 proprietary and publicly developed lettuce cultivars registered in the USA from 1970 through to 2004 showed that 1 % of these cultivars were developed from interspecific crosses (Mikel 2007). Three wild *Lactuca* species, *L. serriola*, *L. saligna* and *L. virosa* were involved in this process (Mikel 2007). A further analysis of pedigree history of 146 lettuce cultivars registered in the U.S. by Plant Variety Protection and/or utility patent of the era from 2000 through 2010 have led to the determination of a coefficient of parentage among these cultivars (Mikel 2013). Among three crisphead lettuce ancestors, cultivars 'Vanguard' and 'Salinas' descend from the interspecific cross of *L. sativa* with *L. virosa* and *L. serriola*. Among these three cultivars, 'Vanguard' is the major ancestor contributing 23.8 % of the genes to crisphead lettuce. Cultivars 'Vanguard' and 'Salinas' followed by the cultivar 'Calmar' (which has no *L. virosa* in its ancestry) were determined as elite programme cultivars, frequently used in lettuce breeding (Mikel 2007). Of the 37 progenitor cultivars, breeders at public institutions and private companies in the USA developed 31 new cultivars in the period of 1970–2004, and ten of these progenitors of today's lettuce germplasm were developed before 1960 (Mikel 2007). The cultivar 'Salinas' was frequently crossed with romaine lettuce types and the romain parental cultivar 'Paris Island Cos' was crossed with leaf types contributing to romaine and leaf lettuce genetic diversity (Mikel 2013).

Pedigree analysis of 146 lettuce cultivars registered in the USA in the period 2000–2010 demonstrated that leaf lettuce cultivars were more genetically diverse than romaine and crisphead cultivars (Mikel 2013). The lower diversity among romaine and crisphead cultivars is due to recurrent recycling of related cultivars in breeding programmes (Mikel 2013). This however, means that the percentage of cultivars possessing genes from wild species given above are likely to be underestimates as many modern day cultivars will have gained such genes through this recycling of parental cultivars most of which will have Vanguard and/or Salinas in their pedigrees both of which possess *L. virosa* genes.

Conclusions and future prospects

Lettuce (*Lactuca sativa*) is one of the oldest domesticated plants and vegetable crops (Hancock 2012). However, our knowledge about the origin, process of domestication, diversification and spread of lettuce around the globe is still quite fragmentary (Lebeda et al. 2007c). The genus *Lactuca* L. comprises approximately 100 wild species, however, detailed information about the biogeography and ecobiology of most of these species is not available (Lebeda et al. 2004b, 2007c, 2009a, 2012a). The taxonomy of wild *Lactuca* species and related genera is currently unclear (Lebeda et al. 2007c; Funk et al. 2009), and needs more detailed elaboration at the level of the genus, involving all known described species (Lebeda et al. 2007c, 2009a). Also the collection, conservation and evaluation of wild *Lactuca* germplasm is incomplete. Most (ca 90 %) of the currently available wild *Lactuca* genetic resources in world genebank collections is represented by accession of only three species (*L. serriola*, *L. saligna*, *L. virosa*) originating mostly from Europe and from the primary center of origin (Lebeda et al. 2004a, 2007c, 2009a, b). Although some collections have been made for example in the territory of North America (Lebeda et al. 2011, 2012a) in general, field studies and collecting activities have been reduced and neglected in the last few decades (Lebeda et al. 2009a). In some areas of the world (Africa, Asia) local landraces are still grown but the breeding and widespread marketing of modern cultivars often on a regional basis by multinational companies is leading to a loss of genetic diversity and local adaptation in the crop (Lebeda et al. 2007c). For many traits (e.g. *Bremia* resistance) extensive screening for new

resistance genes/factors in gene bank accessions of the cultivated crop has led to to a diminshing return in terms of new resistances. This is leading to an increas in the use of wild *Lactuca* species germplasm as sources of new beneficial alleles for a range of valuable characters for future breeding progress (Lebeda and Boukema 2005).

Lettuce is one of the main horticultural crops where a strategy of wild related germplasm exploitation and utilization in breeding programmes is most commonly used with very high practical impact. During the last 70 years, the genus *Lactuca* has been intensively studied with respect to exploitation of wild relatives in commercial lettuce breeding (Pink and Keane 1993; Lebeda et al. 2007c; Mou 2008). In the last three decades, significant progress has been made in germplasm enhancement and the introduction of novel traits into cultivated lettuce, mostly from the primary lettuce gene-pool, however more recently the secondary and tertiary gene-pools have been accessed, particularly for disease and insect resistances (Lebeda et al. 2007c, 2009a). Unfortunately, the process of resistance breeding is complicated because the variation in host plant resistance is mirrored by the diversity (emergence of new different strains, pathotypes and races) within pathogen/pest populations (Lebeda et al. 2007c). Nevertheless, as is evident from this review, wild *Lactuca* germplasm are being increasingly studied and used in lettuce breeding. Valuable sources of resistance have been located in many accessions of different *Lactuca* species and successfully introduced to recent commercial lettuce cultivars. However, despite the progress that has been made, there are still many questions and problems which must be solved by close cooperation between plant scientists, ecologists, plant pathologists, geneticists and lettuce pre-breeders and breeders.

Current areas of weakness are the lack of detailed floristic, bio-geographic and ecologic delimitation of the distribution of known *Lactuca* spp., and few recent collecting and exploration missions, especially to the areas of high species richness and diversity (e.g. South Africa and Asia) (Lebeda et al. 2009a). Such activities need to be linked with detailed observations and recording of the occurrence of diseases and pests on weedy growing *Lactuca* species (e.g. Lebeda et al. 2008a, 2011, 2012a,2013) to provide a better understanding of host-pathogen/pest interactions in natural habitats and aid the exploitation of wild *Lactuca* germplams in lettuce resistance breeding.

Another relatively under researched area is the agro-ecological interface (Burdon and Thrall 2008), i.e. interactions between wild (weedy growing *Lactuca* spp.) and crop (lettuce, *Lactuca sativa*) pathosystems. For *Lactuca* spp. and lettuce this has only really been considered for interactions with *Bremia lactucae* (Lebeda and Petrželová 2004; Lebeda et al. 2002, 2008a) and *Golovinomyces cichoracearum* (Lebeda and Mieslerová 2011; Lebeda et al. 2012c, 2013). This type of knowledge may yield a better understanding about the maintenance of a dynamic balance (homeostasis) between hosts and pathogens in wild pathosystems which could inform deployment staregies for resistance genes in the cultivated crop as well as identify the potential risks of pathogen transfer from wild to crop pathosystems from the viewpoint of potential breakdown of resistance introduced to lettuce from wild *Lactuca* progenitors as demonsrated by the example of *Lactuca serriola*/*L. sativa*-*Bremia lactucae* interactions studied by Lebeda et al. (2008a). In addition the potential transfer of genes from lettuce to weedy growing *Lactuca* species (Hartman et al. 2012; Uwimana et al. 2012a, b) should be investigated from the viewpoint of stability of both the wild and cultivated systems.

From this review, and previous reports (Lebeda et al. 2007c, 2009a), it is clearly evident that wild *Lactuca* germplasm are highly valuable sources of genetic variation and resistance to different biotic stresses. However, it is also evident that current knowledge about these interactions is fragmentary covering only a limited part of the potential variation in the host-pathogen/pest interaction. This can be addressed by screening large collections of well defined wild *Lactuca* germplasm for resistance to the most important lettuce pathogens and pests, followed by detailed genetical studies of the inheritance of resistance. Where knowledge of pathogen effectors is available these can be substituted for the pathogen isolates, with the aim of finding recognition factors which recognise effectors which are highly conserved within the pathogen population. However, again knowledge of the variation in effectors in wild *Lactuca* spp/pathogen interactions is necessary to inform this strategy.

One of the main goals of current lettuce breeding is multiple disease and pest resistance. A detailed characterisation of wild *Lactuca* germplasm resistances can contribute to this goal. At least some *Lactuca* accessions have been shown to possess multiple resistances which can be introduced to cultivated lettuce and

combined with agronomically important traits. The use of wild *Lactuca* spp. as sources of resistance has been hampered in the past by loss of quality characteristics, this has been particularly problematic when 'poor' quality loci have been linked to resistance loci e.g. stunting linked to *Nasonovia* resistance. However, many lettuce breeding companies have now addressed this by instigating 'pre-breeding' programes which aim to produce resistant lines of sufficient quality to be included in a commercial lettuce breeding programme. The use of markers to select for recombinants where undesirable linkages have been broken has been a great step forward in this respect.

New technologies and knowledge offer new ways to screen germplams collections for novel alleles. Currently 52 phenotypic loci that confer resistance in lettuce to 8 diseases (*Bremia lactucae*, *Sphingomonas suberifaciens*, *Microdochium panattonianum*, *Fusarium oxysporum* and *Verticillium dahliae*, lettuce root aphid, lettuce mosaic virus, and lettuce big vein) have been reported as being mapped in lettuce. For some of these diseases candidate genes have been identified and causality demonstrated for a subset of them using RNAi (Truco et al. 2013). This opens up a new more efficient strategy for assessing the value of *Lactuca* germplasm through resequencing to identify allelic variation to provide a library of alleles which can then be tested against the variation present in the pathogen either in the form of diverse isolates or where available, effectors. Those alleles which determine resistance against a broad spectrum of the variation in individual pathogens can then be combined using MAS to produce advanced breeding lines with multiple resistance (Michelmore 2013, pers. commun.).

In addition to the combination and pyramiding of race-specific resistance genes/factors (Lebeda et al. 2002; Pink 2002), exploitation of non-host resistance in lettuce breeding remains a challenge (Lebeda et al. 2002). In relation to lettuce non-host resistance was hypothetised for first time in the response of *Lactuca saligna* to infection by *Bremia lactucae* (Lebeda 1986). *L. saligna* is sexually compatible with *L. sativa* (Lebeda et al. 2007c). The concept of non-host resistance in *L. saligna* was later studied in detail from a mechanistic and population viewpoint (see e.g. Lebeda et al. 2002, 2008b; Petrželová et al. 2011), as well as a genetical viewpoint (Jeuken et al. 2008, 2009; Zhang et al. 2009). This resistance is hypothesised to be durable (Lebeda et al. 2002). However, the use of this type of resistance in lettuce breeding is still at the early stages and requires further research to develop the tools and knowledge for its exploitation (Jeuken 2012).

Future developments in methodology of interspecific hybridization, as well as transfer of resistance genes are playing important role in accessing the genetic variation present in wild *Lactuca* germplasm collections (Lebeda et al. 2007c; Davey and Anthony 2011). Both conventional breeding and genetic manipulation approaches will be very likely applied for the improvement of the crop. Conventional breeding augmented by marker assisted selection will continue to play a key role in the introgression of genetic material from wild *Lactuca* species into cultivated lettuce, particularly where quantitaitive resistance under the control of several loci is involved or where the aim is to pyramid several resistance genes. Somatic hybridization will enable novel nuclear-cytoplasmic combinations, and the introgression of beneficial alleles from wild *Lactuca* species which are sexually incompatible with lettuce (Davey and Anthony 2011). Improvements in transformation technologies are opening up the possibilities of deploying resistance genes in multilines to provide a more durable resistance crop phenotype as described by Pink and Puddephat (1999) and Pink (2002).

It is also evident that our knowledge of the mechanisms of resistance in various *Lactuca*-pathogen/pest interactions is limited. Only in a few plant-pathogen interactions (e.g. *Lactuca-Bremia lactucae*) is more detailed information about the type and mechanism of resistance, including features of inheritance available (Lebeda et al. 2002, 2008b). For many lettuce/disease interactions the basic information about the type of resistance (non-host versus host, race-specific vs. race-nonspecific, field or durable resistance etc.) is not available. Again this knowledge will improve our efficiency in exploiting the *Lactuca* genepool for lettuce crop improvement and allow the combination of different resistance mechanisms in a single cultivar in order to provide a potentially more durable resistance phenotype.

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5. CONCLUSIONS

Resistance gene resources of the genus *Lactuca* against downy mildew (*Bremia lactucae*) and their genetic variability

The first part of this Ph.D. thesis provides interesting insights into the genetic variability of *L. serriola* populations originating from completely different eco-geographical areas, Sweden and Slovenia. Verification of the taxonomic status of the plants showed that *Lactuca serriola* f. *serriola* is predominant in both countries. The putative relationships among the analyzed individuals were visualized using 7 EST-SSR loci and 257 AFLP markers, Bayesian clustering and construction of a Neighbor-Network. The higher genetic variability in the Slovenian samples was observed. This study showed that *L. serriola* populations originating from various eco-geographical conditions differ significantly in their genetic background, which is also reflected in the geographic patterns of their phenotypic features.

The next topic was to study a biodiversity of *Lactuca aculeata* and resistance variation to *Bremia lactucae*. On the basis of research of *L. aculeata* from the Turkey, Jordan and Israel, the significant difference was found among the studied samples. Molecular data reflect the geographical origin, i.e., the clustering of samples according to their country of origin. The genetic structure and diversity were analyzed using 8 EST-SSR loci and 287 AFLP markers. Our results confirmed a previous determination of three putative hybrids of *L. aculeata* × *L. serriola*. Moreover, we also detected at least 6 additional hybrid samples showing certain proportion of *L. serriola*-like genotype. The part of the samples of *L. aculeata* were screened for their response to five *B. lactucae* races (Bl: 17, Bl: 18, Bl: 24, Bl: 27, Bl: 28). The results from screening for resistance demostrated that *L. aculeata* could be an additional and interesting race-specific source of resistance against *B. lactucae*. It is useful for *Lactuca sativa* resistance breeding.

The third topic is focused on the morphological and genetic structure of three closely-related, primarily autogamous wild *Lactuca* species - *L. serriola*, *L. aculeata* and *L. saligna*, which were collected in Israel. The taxonomic status of the individual plants was morphologically validated during greenhouse multiplication. The genetic structure and diversity were analyzed using 11 EST-SSR loci and 230 AFLP markers. The results showed that although these species have the predominantly self-pollinating character, the populations were not morphologically

and genetically uniform. The each population represent a unique combination of genotypes, which differ from other populations of the same species. The highest diversity was observed in *L. saligna*, the lowest in *L. aculeata* populations. The Network analysis clearly separated samples according to their taxonomic determination, which reflect the gene diversity as well as genetic distance values among the three species.

In the fourth and fifth part, the results of intensive research of the genus *Lactuca* L. are summarized.

The fourth part includes knowledge of variation in reactions to pathogens (including viral pathogens, oomycete and fungal pathogens) and pests (incl. nematodes, insects and mites), and the exploitation of wild *Lactuca* germplasms in lettuce resistance breeding.

The last part of the results summarizes the current knowledge of wild *Lactuca* L. species in taxonomy, biogeography, gene-pools, germplasm collection. Genetic diversity is characterized at the level of phenotypic and phenological variation, variation in karyology and DNA content, biochemical traits, and protein and molecular polymorphism. Challenges and plans for the future scientific research are discussed. The future studies should be focused on: improving knowledge of the mechanism of resistance in various *Lactuca*-pathogen/pest interactions; detection of new sources of resistance; using wild *Lactuca* spp. such as *L. saligna* and *L. serriola* as durable sources of resistance; exploiting the *Lactuca* genepool for lettuce improvement; molecular markers linked to resistance genes as a useful tool for selection during *L. sativa* breeding process.

6. SOUHRN (SUMMARY, in Czech)

Rezistence genových zdrojů rodu *Lactuca* vůči plísni salátové (*Bremia lactucae*) a jejich genetická variabilita

První část předkládané disertační práce pojednává o genetické variabilitě populací *L. serriola* pocházejících ze dvou odlišných částí Evropy – Švédska a Slovinska. Taxonomické ověření vzorků potvrdilo, že v obou zemích převládá *L. serriola* f. *serriola*. Variabilita mezi jednotlivými populacemi byla hodnocena pomocí 7 EST-SSR a 257 AFLP markerů. Vzájemné vztahy mezi analyzovanými vzorky byly vizualizovány pomocí dvou přístupů, Bayesovské shlukovací analýzy a Neighbor-Network diagramu. Vyšší genetická variabilita byla pozorována u slovinských vzorků. Tato studie poukazuje na fakt, že populace *L. serriola* pocházející z odlišných ekogeografických podmínek se významně liší i ve své genetické výbavě, což se odráží i v jejich fenotypových vlastnostech.

Dalším tématem bylo studium genetické variability pomocí 8 EST-SSR a 287 AFLP markerů u vzorků *Lactuca aculeata* pocházejících z Turecka, Jordánska a Izraele, včetně testování rezistence vůči plísni salátové (*Bremia lactucae*). Výsledky molekulárních analýz potvrdily zeměpisný původ vzorků. Podle námi dosažených výsledků se potvrdila i dřívější determinace hybridních vzorků *L. aculeata* × *L. serriola* včetně detekce nejméně 6 dalších vzorků, které vykazovaly taktéž podobnost s genotypovými profily *L. serriola*. Část vzorků *L. aculeata* byla použita k testování rezistence vůči pěti rasám *B. lactucae* (Bl: 17, Bl: 18, Bl: 24, Bl: 27, Bl: 28). Výsledky studia rezistence vykazovaly rasově-specifické reakce, a je tedy pravděpodobné, že *L. aculeata* by mohla být nositelem nových zdrojů rasově-specifické rezistence využitelné ve šlechtění kulturního salátu *L. sativa*.

Třetí téma disertační práce je zaměřené na hodnocení morfologické a genetické variability tří blízce příbuzných, převážně samosprašných, planě rostoucích druhů *L. serriola*, *L. aculeata* a *L. saligna*, které byly sesbírány na území Izraele. Taxonomické hodnocení jednotlivých vzorků bylo morfologicky ověřeno přemnožením ve skleníkových podmínkách. Genetická stuktura a diverzita byla analyzována pomocí 11 EST-SSR a 230 AFLP markerů. Výsledky ukázaly, že i když jsou tyto druhy převážně samosprašné, populace nebyly morfologicky ani geneticky uniformní a představovaly jedinečné kombinace genotypů, které se lišily

od jiných populací stejného druhu. Největší diverzita byla pozorována u populací *L. saligna*, nejmenší u populací *L. aculeata*. Síťový diagram zřelně oddělil vzorky na základě jejich taxonomického určení, genetické diverzity a genetické vzdálenosti mezi těmito třemi druhy.

V následujících kapitolách jsou shrnuty výsledky a dosavadní poznatky intenzivního výzkumu rodu *Lactuca* L.

Čtvrtá část shrnuje poznatky na reakce planých druhů rodu *Lactuca* k patogenním organismům (zahrnující virové patogeny, oomycety a houbové patogeny) a škůdcům (hlístice, hmyz a roztoči), včetně využití planých genetických zdrojů rodu *Lactuca* ve šlechtění na odolnost proti těmto chorobám a škůdcům.

Poslední, pátá část, kromě výše zmíněného, shrnuje dosavadní poznatky intenzivního výzkumu planých druhů rodu *Lactuca* L. v oblasti: taxonomie; biogeografie; konzervační strategie; v hodnocení genetické diverzity na fenotypové, fenologické úrovni; v karyologické proměnlivosti; v obsahu DNA, biochemických vlastností či molekulárního polymorfismu. Jsou zde uvedeny také směry a plány budoucího výzkumu, který by se měl dále zaměřit na prohloubení znalostí mechanismů rezistence u interakcí *Lactuca*-patogen/škůdce; na detekci nových zdrojů rezistence; využití planých druhů *Lactuca* spp. např. *L. saligna* a *L. serriola,* jako zdrojů trvalé rezistence; na využití genofondu rodu *Lactuca* L. a molekulárních markerů rezistence ve šlechtění *L. sativa*.

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Resistance gene resources of the genus *Lactuca* against downy mildew (*Bremia lactucae*) and their genetic variability

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Content

1. Introduction

The genus *Lactuca*, in the broad sense, is considered as a very large and heterogeneous group included in the family Asteraceae. It comprises wild species of annual, biennial or perennial herbs growing in throughout the temperate and warm regions in Europe, America, Africa and Asia (Lebeda et al., 2001). From the ecological viewpoint, the genera is very variable and its species occupies various habitats.

Several viral, bacterial and fungal pathogens, such as yellows virus, turnip mosaic potyvirus, *Microdochium*, *Rhizomonas* (corky root disease), *Bremia* (downy mildew), *Golovinomyces* (powdery mildew) (Lebeda et al. 2014, 2015) are one of the most important problems affecting lettuce. Breeding for resistance is a major activity of most lettuce improvement programmes, and there is an increasing need for information and methods to accelerate the development of new disease-resistant cultivars (Lebeda et al., 2007a; Michelmore and Wong, 2008). Modern breeding methods of cultivated lettuce are based on utilization of wild related species (e.g. *L. serriola*, *L. saligna* and *L. virosa*) and progenitors. The study of genetic variability within a wild *Lactuca* species is vital for plant breeders because of its importance for selecting germplasm included in a breeding program. The study of genetic diversity is essential to receive information about propagation, domestication, which can be used in breeding programs and for conservation of genetic resources of *Lactuca* spp.

Molecular methods have become an essential part of most studies on genetic diversity extend and distribution and in the analyses of breeding system, bottlenecks and other key features affecting genetic diversity patterns. However, it is important, to understand that different markers have different properties and will reflect different aspects of genetic diversity (Karp and Edwards, 1995). Thus, it is likely that molecular methods (e.g. AFLPs, SSRs, ...) are most useful for evaluation of genetic diversity, for estimating a gene flow, genetic drift and

degree of outbreeding. Therefore, information generated using different PCR-based molecular markers can provide valuable information on a number of practical issues of germplasm management, including the classification of accessions by known allelic constitution, detection of redundancy in collections or the detection of genes influencing economically important traits. These markers have been successfully applied in *Lactuca* research. The studies related to use of molecular markers (AFLP, SSR) in *Lactuca* spp. germplasm collections have been reviewed by Dziechciarková et al. (2004) and Lebeda et al. (2014; see Ph.D. thesis).

During the last three years, the AFLPs and SSRs methods have been used in study of genetic diversity of *L. aculeata* populations from the Near East (Jemelková et al., 2015) and in study of population structure of three predominantly self-pollinating wild *Lactuca* species (*L. serriola*, *L. saligna* and *L. aculeata*) collected from Israel (Kitner et al., 2015). These publications are part of this Ph.D. thesis. The microsatellites were also used in D'Andrea et al. (2017) for evaluation of interpopulation diversity and the recent range expansion process of *L. serriola* in Europe.

2. Aims of the thesis

- Process available literature relating to the topic;

- Testing resistance with inoculation tests under laboratory conditions and evaluation a variability of the resistance of the wild *Lactuca* genetic resources to lettuce downy mildew (*Bremia lactucae*);

- The analysis of a genetic variability of the *Lactuca* species using microsatellite and AFLP markers;

- Genetic resources of wild *Lactuca* L. species and their exploitation in lettuce breeding – critical analysis.

3. Material and methods

Plant material

Original seed materials were collected across the wide geographic range in Sweden, Slovenia, Turkey, Jordan and Israel. The regeneration of plants from the collected seeds followed standard multiplication protocols for wild *Lactuca* species (Lebeda et al., 2007b). The plants were cultivated in the greenhouse of the Department of Botany, Palacký University in Olomouc, and the taxonomic status of each accession was verified to its morphology.

Isolates of *Bremia lactucae*

For *Lactuca aculeata* resistance testing, the isolates of *Bremia lactucae* were used. The isolates represent the officially denominated races of *B. lactucae* with known virulence patterns (Van Ettekoven and Van der Arend, 1999; Van der Arend et al., 2006). These isolates originated from cultivated lettuce (*L. sativa*), and they are maintained by the Department of Botany (Palacký University in Olomouc, Czech Republic) in the collection of microorganisms (http://botany.upol.cz).

Molecular methods

The genomic DNA was extracted from fresh leaf tissue using an Innu-PREP Plant DNA Kit (Analytik Jena AG, Jena, Germany) following the manufacturer's protocol and using the CTAB method (Kump and Javornik, 1996). DNA quality and quantity were determinated by agarose gel electrophoresis and by use of a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Delaware, USA).

For microsatellite genotyping, EST-SSR markers were used. The primers pairs were selected according to their high diversity shown in previously published

papers (Simko, 2009; Riar et al. 2011). Amplification of SSRs was performed according to newly developed protocol (15 μl PCR reactions contained 7 ng/μl of DNA template, 5 U/μl of Taq polymerase, 10 μM of both primers, reaction Buffer, and 10 mM dNTPs) and also according to Majeský et al. 2012. The number of cycles and the annealing temperatures were adjusted for each locus, in oder to obtain unambiguously scorable products. The length of the SSR alleles was scored based on their migration relative to the molecular weight size markers 30-330bp AFLP® DNA ladder (Invitrogen, Carlsbad, California, USA).

The AFLP analyses were carried out according to Vos et al. (1995) protocol with modifications, and the AFLP fragment detection according to Kitner et al. (2008, 2012). The selective primer combinations, with two to four selective nucleotides, were chosen to generate the AFLP profiles.

The products of amplification were separated on 6%, 0,4-mm-thick denaturing polyacrylamide gel (0.5x TBE buffer) using the T-REX (Thermo Scientific Owl Separation Systems, Rochester, NY, USA) sequencing gel electrophoresis apparatus. Then, silver staining was used for the visualisation of the PCR products.

Screening for resistance

Pathogen races were maintained and multiplied on seedlings of *L. sativa* cvs. 'Cobham Green' and 'Hilde', which also served as susceptible controls in the resistance tests. Thirty seeds in three replications per individual sample were sown on moistened filter paper in plastic boxes. Plants were inoculated at the stage of fully expanded cotyledon leaves, and incubated in a growth chamber as described by Lebeda and Petrželová (2010). Data on sporulation intensity were recorded in two-day intervals, 6-14 days after inoculation, using a 0-3 scale (Dickinson and Crute, 1974). Intensity of sporulation was expressed as a percentage of the maximum possible scores according to Townsend and Heuberger (1943). The reaction of a particular *L. aculeata* sample was considered

as susceptible, if the sporulation intensity was more than 30 %, and at least half of the tested seedlings showed a degree of infection of 2 or 3 (Lebeda and Petrželová, 2010). Differentiation of resistance phenotypes (R-phenotypes) of individual *L. aculeata* samples was used for examination of variation in resistance patterns to *B. lactucae* within and among populations of *L. aculeata*.

Data analysis

Allele frequency and polymorphism were evaluated in each SSR locus. AFLP fragments were checked visually, and only clear and unambiguous bands were scored for their presence (1) or absence (0) across all samples. For SSR data, the proportion of polymorphic loci (P%), number of private alleles (PA), observed and expected heterozygosity (H_O and H_E) were performed using GenAlEx 6/6.5 software (Peakall and Smouse, 2006, 2012). The mean number of alleles per locus (A) were calculated in Arlequin 3.5 (Excoffier and Lischer, 2010). The number of different genotypes (N_G), the number of samples with a heterozygous constitution (N_{HET}), and the maximal number of heterozygous loci (N_{HETmax}) were calculated manually. R- and F- statistics were computed with SPAGEDI (Hardy and Vekemans, 2002). Analysis of molecular variance (AMOVA) and population pairwise comparisons were computed in Arlequin 3.5. For AFLP data, the polymorphic rate (PLP%) was calculated manually; the number of private bands (PA), the proportion of polymorphic loci (P%), and gene diversity (H_E) were calculated using GenAlEx 6/6.5 software (Peakall and Smouse, 2006, 2012). To determination the strength of association between matrices of genetic distances for a given marker (AFLP and EST-SSR) and the geographic distance for each population was used the Mantel test (based on Cavalli-Sforza and Edwards chord distance and Dice's similarity coefficient) calculated in Populations 1.2.32 (Langella, 2002), and in FreeTree (Pavlíček et al.,1999).

Phylogenetic analysis

To elucidate genetic relationships within and among the analyzed samples, a Neighbor-Network based on Dice's similarity coefficient (D) was constructed in SplitsTree 4 (Huson and Bryant, 2006). The Nexus input file for SplitsTree was exported from GenAlEx after transformation of SSR genotypes into a binary matrix, which was merged with the AFLP binary data. The robustness were tested by bootstrap analysis with 1,000 bootstrap replicates. For analysis of the population structure, a Bayesian clustering approach was used as implemented in STRUCTURE 2.2 (Falush et al., 2007) for combined SSR and AFLP binary data (K in range 1-10 with ten replicate runs for each K, 100,000 burn in iterations followed by 1,000,000 MCMC iterations). For the graphical interpretation of clustering CLUMPP (Jacobson and Rosenberg, 2007) and DISTRUCT (Rosenberg, 2004) software were used.

4. Summary of results

4.1. Genetic and morphological variability of *Lactuca serriola* populations

In this tudy, EST-SSR loci and AFLP markers were used to characterize the genetic variability of 121 samples of the common weed, *L. serriola* L. (prickly lettuce), representing 53 populations originating from Sweden and Slovenia. The seed materials, originating from different habitats, were regenerated and taxonomically validated at the Department of Botany, Palacký University in Olomouc, Czech Republic. The morphological characterizations of the collected plant materials classified all 121 samples as *L. serriola* f. *serriola*; one sample was heterogeneous, and *L. serriola* f. *integrifolia* was also presented. Based on 7 EST-SSR loci and 257 AFLP markers, Bayesian clustering and construction of a Neighbor-Network, the putative relationships among the analyzed individuals were visualized. The higher genetic variability was observed in the Slovenian samples. Under the Bayesian approach, the best partitioning (according to the most frequent signals) was resolved into three groups. While the absence of an admixture or low admixture was detected in the Slovenian samples, and in the majority of the Swedish samples. A significant admixture was detected in the profiles of five Swedish samples collected near Malmö, which bore unique morphological features of their rosette leaves. The Neighbor-Network divided samples into 6 groups, each consisting of samples coming from a particular country. This study showed that *L. serriola* populations originating from various eco-geographical conditions differed significantly in their genetic background, which also reflected in the geographic patterns of their phenotypic features.

4.2. Biodiversity of *L. aculeata* germplasm including the resistance variation to *Bremia lactucae*

In total, seventy two *Lactuca aculeata* and three *Lactuca serriola* samples originating from natural populations of these species in Turkey, Jordan, and Israel were analyzed by 8 EST-SSR loci and 287 AFLP markers. Neighbor-Network and Bayesian clustering were used for visualisation of the differences among the analyzed *L. aculeata* and *L. serriola* samples, and to confirm hybrid origin (*L. aculeata* × *L. serriola*) of three samples (343-8A, 343-8B, 54/07) previously indicated by their morphological traits. Molecular data reflect the geographical origin, i.e., the clustering of samples according to their country of origin. Samples from neighbouring parts of Jordan and Israel expressed similar genetic characteristics, indicating the possibility of migration or artificial introduction of plant material. Forty-one *L. aculeata* samples were screened for their response to five *Bremia lactucae* races (Bl: 17, Bl: 18, Bl: 24, Bl: 27, and Bl: 28). *L. aculeata* samples were most frequently susceptible to races Bl: 18, Bl: 24, Bl: 27, Bl: 28; and least susceptible to Bl: 17. No highly efficient source of resistance was detected. However race-specific reaction patterns were frequently recorded, indicating the possible presence of some race-specific resistance factors/genes in the studied samples of *L. aculeata*.

4.3. Genetic and morphological variability of the wild *Lactuca* species in natural populations in Israel

In this study, 11 EST-SSR loci and 230 AFLPs markers were used to analyze genetic structure and diversity of three predominantly self-pollinating wild *Lactuca* species (*Lactuca serriola*, *L. saligna*, and *L. aculeata*). Studied seeds were collected from individual plants in northern Israel, along a line transect, and

two populations per each *Lactuca* species. The distance between neighboring plants sampled for seeds varied from 1.5 to 37 m. The transect length at single sites ranged from 47.2 to 151.8 m. The taxonomic status of 67 individual plants was morphologically validated during greenhouse multiplication. The results showed that although these species have the predominantly self-pollinating character, the populations were not morphologically and genetically uniform and each population could represent a unique combination of genotypes, which differed from other populations of the same species. The highest diversity was observed in *L. saligna*, the lowest in *L. aculeata* populations. The Network analysis clearly separated samples according to their taxonomic determination, also reflecting the gene diversity as well as genetic distance values among the three species.

4.4. Wild *Lactuca* genetic resources – summary of the intensive research

This study is focused on the results of intensive research of the genus *Lactuca* L. First part includes knowledge about variation in reaction to pathogens (including viral pathogens, oomycete and fungal pathogens) and pests (incl. nematodes, insects and mites), and the exploitation of wild *Lactuca* germplasms in lettuce resistance breeding. The second part summarizes the results, in addition to the abovementioned, current knowledge of wild *Lactuca* L. species taxonomy, biogeography, gene-pools and germplasm collection. Genetic diversity is characterized at the level of phenotypic and phenological variation, variation in karyology and DNA content, biochemical traits, and protein and molecular polymorphism. Challenges and plans for the future research are discussed. The future studies should be focused on: improving knowledge of the mechanism of resistance in various *Lactuca*-pathogen/pest interactions; detection of the new sources of resistance; using wild *Lactuca* spp. such as *L. saligna* and *L. serriola*

as durable sources of resistance; efficiency in exploiting the *Lactuca* genepool for lettuce improvement; molecular markers linked to resistance genes as aids in selection during *L. sativa* breeding process.

5. Conclusions

The present work focused on five various topics related to the *Lactuca* spp.-germplasm, their genotypic and phenotypic variation, ecogeography, resistance to pathogens and pests, as well as utilization in lettuce breeding. The first on is the development of genetic variability of 53 Swedish and Slovenian population *L. serriola*, two marginal areas of natural distribution this species. The genetic variability between populations was evaluated with 7 EST-SSR loci and 257 AFLP markers. The mutual relationships among the analyzed samples were visualized using two approaches, Neighbor-Network diagram and Bayesian clustering method.

In the next part are summarized the results of genetic variability of 69 samples *L. aculeata*, 3 samples of *L. serriola* and 3 putative hybrids *L. aculeata* × *L. serriola,* coming from Turkey, Jordan and Israel, including data from screening (41 samples of *L. aculeata*) for response to lettuce downy mildew (*Bremia lactucae*). The genetic structure and diversity were analyzed using 8 EST-SSR loci and 287 AFLP markers. Our results confirmed a previous determination of three putative hybrids *L. aculeata* × *L. serriola*. Moreover, we also detected at least 6 additional hybrid samples showing certain proportion of *L. serriola*-like genotype. The results from studies of resistence demostrated race-specific reaction patterns, indicating the possible presence of some race-specific resistance factors/genes in the studied samples of *L. aculeata*.

The third part describes the results obtained from study of population structure, including morphological/genetic variability in Israeli samples of *L. serriola*, *L. aculeata* and *L. saligna* (two populations per each *Lactuca* species). The genetic structure and diversity were analyzed using 11 EST-SSR loci and 230 AFLP markers. The results showed that although these species have the predominantly self-pollinating character, the populations were not morphologically and genetically uniform. The genetic variability in a population

increases at its periphery, due to the presence of plants with „non-indigenous“ alleles, which most likely come from migration and subsequent interpopulation or interspecific hybridization.

The other two parts are complementary and interconnected. The fourth part is focused on reaction of wild *Lactuca* genetic resources to diseases (including viral pathogens, oomycete and fungal pathogens) and pests (incl. nematodes, insects and mites). And the last part, except the aforementioned, summarizes the current knowledge of intensive research the wild *Lactuca* species in taxonomy, ecogeography, conservation strategy, karyology, molecular biology and in approaches to the use of wild *Lactuca* species in lettuce breeding programme to biotic factors.

6. Souhrn (Summary in Czech)

Rezistence genových zdrojů rodu *Lactuca* vůči plísni salátové (*Bremia lactucae*) a jejich genetická variabilita

Rod *Lactuca* L. zahrnuje více než 97 druhů, z nichž některé jsou pěstovány pro svůj hospodářský význam. Navzdory nezpochybnitelné důležitosti tohoto rodu přetrvává nedostatek informací o genetické diverzitě, fylogenetických vztazích, rezistenci vůči plísni salátové (*Bremia lactucae*) a využitelnosti planých druhů ve šlechtitelských programech. Tato disertační práce si kladla za cíl přispět k charakterizaci genetické struktury a diverzity planých druhů za pomocí molekulárních metod; včetně zhodnocení variability rezistence vůči plísni salátové.

V první části práce byla pro studium genetické variability populací *L. serriola* využita analýza 8 EST-SSR a 257 AFLP markerů. Tato studie poukazuje na fakt, že populace *L. serriola* pocházející z odlišných ekogeografických podmínek se významně liší nejen ve své genetické výbavě, ale i ve svých fenotypových vlastnostech. Dalším tématem bylo studium genetické variability pomocí 8 EST-SSR a 287 AFLP markerů u vzorků *L. aculeata* pocházejících z Turecka, Jordánska a Izraele, včetně testování rezistence vůči plísni salátové (*Bremia lactucae*). Výsledky molekulárních analýz potvrdily zeměpisný původ vzorků. Výsledky studia rezistence vykazovaly rasově-specifické reakce, a je tedy pravděpodobné, že *L. aculeata* by mohla být nositelem nových zdrojů rasově-specifické rezistence využitelných ve šlechtění kulturního salátu *L. sativa*. Třetí téma práce bylo zaměřené na hodnocení morfologické a genetické variability tří blízce příbuzných, převážně samosprašných, planě rostoucích druhů *L. serriola*, *L. aculeata* a *L. saligna*, které byly sesbírány na území Izraele. Genetická stuktura a diverzita byla analyzována pomocí 11 EST-SSR a 230 AFLP markerů. Výsledky ukázaly, že i když jsou tyto druhy převážně

samosprašné, populace nebyly morfologicky ani geneticky uniformní a představovaly jedinečné kombinace genotypů, které se lišily od jiných populací stejného druhu. Poslední dvě části shrnují výsledky a dosavadní poznatky intenzivního výzkumu planých druhů rodu *Lactuca* L. v oblasti: variability k patogenním organismům a škůdcům, včetně využití ve šlechtění na odolnost; v oblasti taxonomie; biogeografie; konzervační strategie; v hodnocení genetické diverzity na fenotypové, fenologické úrovni; v karyologické proměnlivosti; v obsahu DNA, biochemických vlastností či molekulárního polymorfismu.

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