Characterization of *Lactuca* spp. germplasm by protein and molecular markers – a review

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ABSTRACT

The genus *Lactuca* L. belongs to one of the largest plant families, Asteraceae. *Lactuca* L. is represented by ca 100 species distributed in different geographical areas and ecological conditions. This is one of the reasons why this genus is characterised by very broad variation of different characters. Electrophoretic detection of some proteins (isozymes) has been applied to the study of genetic variability of *Lactuca* spp. individuals and populations. The development of molecular genetic methods (RFLP, Restriction Fragment Length Polymorphism; PCR methods: RAPD, Random Amplified Polymorphic DNA; AFLP, Amplified Fragment Length Polymorphism; minisatellites and microsatellites fingerprinting or SSR, Simple Sequence Repeats) and their application has contributed to the elucidation of various aspects related to the taxonomy, variability, biodiversity, genetics and breeding within the genus *Lactuca* L. Further potential application of these methods is discussed.

Keywords: genepools; genetic resources; lettuce; taxonomy; variability; biodiversity; isozymes; RFLP; RAPD; AFLP; microsatellites; STMS; SAMPL; PCR; AP-PCR

The genus *Lactuca* L. is represented by ca 100 species, they are widespread especially in temperate and warm areas in Europe, Asia, North America, Africa, and Australia (Lebeda et al. 2004a, b). Recent work on the taxonomy and relationships within the genus *Lactuca* L. is summarized in Kesseli and Michelmore (1996), Koopman et al. (1998, 2001), Koopman (1999), Lebeda and Astley (1999) and Lebeda et al. (2004b). The genus *Lactuca* L. belongs to the family Asteraceae, subfamily Cichorioideae, tribe Lactuceae, which includes 70 genera and 2300 species according to Tomb (1977), while Bremer et al. (1994) reported 98 genera and more then 1550 species. In recent classifications, the genus *Lactuca* is divided into seven sections (*Lactuca* /subsection *Lactuca* and *Cyanicae*/, *Phoenixopus*, *Mulgedium*, *Lactucopsis*, *Tuberosae*, *Micranthae* and *Sororiae*), and furthermore include two geographic clusters – African and North American (Table 1) (Feráková 1977, Rulkens 1987, Lebeda 1998, Lebeda and Astley 1999). However, the representation of taxa in germplasm collections is extremely non-uniform and relatively few (Lebeda and Astley 1999, Lebeda et al. 2004a).

In literature, most attention is paid to the cultivated lettuce (*L. sativa*). Lettuce is a member of the section *Lactuca*, subsection *Lactuca*, and diploid species with $n = 9$ chromosomes (Doležalová et al. 2002b). Other relatively well studied species of the same subsection are *L. serriola*, *L. virosa* and *L. saligna* (Lebeda et al. 2001b). Genetic resources of these species are considered as very important sources of many characteristics, including disease resistance, and play an irreplaceable role in lettuce breeding (Pink and Keane 1993, Ryder 1998, Lebeda et al. 2002, 2004a). The diversity and identity of taxa within the subsection *Lactuca* have been studied using protein marker techniques (Roux et al. 1985, Kesseli and Michelmore 1986, Mejia and Mc Daniel 1986, Cole et al. 1991, Vries de 1996, Lebeda et al. 1999, Doležalová et al. 2003b), analysis of restriction fragment length polymorphism (Kesseli et al. 1991, Vermeulen et al. 1994) and methods based on PCR reaction (Landry et al. 1987, Kesseli et al. 1994, Yamamoto et al. 1994, Hill et al. 1996, Witsenboer et al. 1997, Van de Wiel et al. 1998, 1999, Sicard et al. 1999, Jeuken et al. 2001, Koopman et al. 2001).

The aim of this contribution is to summarize recent knowledge of the application of protein and molecular marker techniques in the genus *Lactuca* in order to understand the taxonomy, phylogeny, genetic variation, germplasm maintenance and characterisation, and breeding as a basis for further research.

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PROTEIN MARKERS

Isozymes

Isozyme analysis has been used for over 60 years for various purposes in biology, e.g. to delineate phylogenetic relationships, to estimate genetic variability and taxonomy, to study population genetics and developmental biology and, to direct utilization in plant genetic resources management and plant breeding (Bretting and Widrlechner 1995, Staub and Serquen 1996). Isozymes were defined as structurally different molecular forms of an enzyme with, qualitatively, the same catalytic function. Isozymes originate through amino acid alterations, which cause changes in net charge, or the spatial structure (conformation) of the enzyme molecules and also, therefore, their electrophoretic mobility. After specific staining the isozyme profile of individual samples can be observed (Hadačová and Ondřej 1972, Vallejos 1983, Soltis and Soltis 1989).

Only a few articles have been published (Table 2), focusing on the study of *Lactuca* spp. using isozymes analysis. Three of these works are focused on *Lactuca* species (Roux et al. 1985, Lebeda et al. 1999, 2001a), one on *L. sativa* (cultivated lettuce) (Mejia and Mc Daniel 1986) and four studying the genetic variability of cultivated lettuce and wild *Lactuca* species (Kesseli and Michelmore 1986, Cole et al. 1991, Vries de 1996, Mizutani and Tanaka 2003). These publications summarized the application of isozyme techniques for the identification of genetic variability among cultivars and wild populations of *Lactuca* spp. (*L. aculeata*, *L. serriola*, *L. saligna*, *L. virosa*), and the determination of the genetic and phylogenetic relationships of *Lactuca* spp. Isozyme analysis is a good tool for the study of inter- and intra-species diversity. The results showed a lower level of intra-species than inter-species diversity.

Isozyme variation was used to characterize levels of variation and the systematic relationships of wild and cultivated *Lactuca* populations by Kesseli and Michelmore (1986). *L. sativa* is generally assumed to have a progenitor similar to *L. serriola* (Lindqvist 1960). Isozyme data suggest a polyphyletic origin of *L. sativa* (Vries de 1996). Roux et al. (1985) used isozyme data to show that *L. aculeata* is a part of the *L. serriola* complex, confirming their genetic closeness with *L. sativa*, and also reported that *L. saligna* and *L. virosa* are very distinct from the others that create this section (Table 1).

Enzymes regulating the metabolism of reactive oxygen were characterised in expanded lettuce

Table 1. The taxonomy of wild *Lactuca* spp. and classification to the sections, subsections and geographic clusters with examples of most important species (Feráková 1977, Rulkens 1987, Lebeda 1998, Lebeda and Astley 1999)

Family	Asteraceae			
Subfamily	Cichorioideae			
Tribus	Lactuceae			
Subtribus	Lactucinae			
Genus	Lactuca			
Section		I.	*Lactuca* subsect. *Lactuca*	*L. aculeata*, *L. altaica*, *L. azerbaijanica*, *L. dregeana*, *L. georgica*, *L. livida*, *L. saligna*, *L. sativa*, *L. serriola* f. *serriola* and f. *integrifolia*, *L. virosa*
			Lactuca subsect. *Cyaniacae*	*L. graeca*, *L. perennis*, *L. tenerrima*
		II.	*Phaenixopus*	*L. acanthifolia*, *L. longidentata*, *L. viminea*
		III.	*Mulgedium*	*L. sibirica*, *L. taraxacifolia*, *L. tatarica*
		IV.	*Lactucopsis*	*L. aurea*, *L. quercina*, *L. watsoniana*
		V.	*Tuberosae*	*L. indica*, *L. raddeana*
		VI.	*Micranthae*	*L. sororia*, *L. undulata*
	Geographic clusters	African cluster		*L. capensis*, *L. dregeana*, *L. homblei*
		North American cluster		*L. biennis*, *L. canadensis*, *L. floridana*, *L. graminifolia*, *L. ludoviciana*

(*Lactuca sativa*) leaf tissue (Bestwick et al. 2001). Catalase (CAT), superoxide dismutase (SOD) and lipoxygenase (LOX) activities were assessed following inoculation with *Pseudomonas syringae* pv. *phaseolicola* and a related *hrpD* mutant. They concluded that lettuce cells undergoing a hypersensitive reaction experience a prolonged oxidative stress, primarily through an increase in pro-oxidant activities initially occurring in the absence of enhanced antioxidant activities.

The above summary of results suggests that isozyme markers display a high level of polymorphism in *Lactuca* spp. (Table 2), and can be useful for the characterisation of variability and the determination of taxonomic relationships and species identity. However, the polymorphism of closely related species was relatively low thus limiting the resolution of some problems related to these relationships (Kesseli et al. 1991). For this reason there has been a tendency to search for new sensitive methods to eliminate the disadvantages of isozyme techniques.

MOLECULAR MARKERS

RFLP (Restriction Fragment Length Polymorphism)

Restriction Fragment Length Polymorphism (RFLP) is a method based on the cleavage of DNA by restriction endonuclease at specific nucleotide sequences. Size fractionation is achieved by gel electrophoresis and, after transfer to a membrane by Southern blotting, fragments of interest are identified by hybridisation with radioactive labelled probe. Different sizes or lengths of restriction fragments are typically produced when different individuals are tested. Such a polymorphism can by used to distinguish plant species, genotypes and, in some cases, individual plants (Karp et al. 1998).

RFLP has been used for the study of *Lactuca* spp. (Landry et al. 1987, Kesseli et al. 1991, 1994, Vermeulen et al. 1994). Kesseli et al. (1991) used RFLP analysis for the determination of variation in *Lactuca* spp. and origin of cultivated lettuce (*L. sativa*) (Table 3). Sixty-five accessions representing the morphological and geographical diversity of cultivated *L. sativa* and wild species of the subsection *Lactuca* were examined. *L. sativa* was represented by different morphotypes (butterhead, looseleaf, cos, latin types and crisphead) and wild *Lactuca* spp. by five species (*L. serriola*, *L. saligna*, *L. irosa*, *L. perennis* and *L. indica*). As expected, most of the diversity was distributed between populations and little was within populations. RFLP data showed that *L. sativa* is closely related to *L. serriola*, but not to any of the other species involved in this study (*L. saligna*, *L. virosa*, *L. perennis* and *L. indica*). The types of cultivated *L. sativa* formed distinct clusters what proving that RFLP could be suitable for studies of intraspecific variation and among closely related taxa. This study also confirmed numerous genetic differences characteristic for each lettuce morphotype suggesting a polyphyletic origin of *L. sativa* (Kesseli et al. 1991).

A set of RFLP genetic markers provided the opportunity to develop a detailed genetic map of lettuce, for use in selection studies, and the identification and organization of plant genomes with practical utilisation in plant breeding. The cross, Calmar × Kordaat, was developed as the source of the segregating population to construct a genetic linkage map of lettuce using RFLP markers (Landry et al. 1987). RFLP markers proved to be powerful tools in the identification of individuals, in the segregation analysis of progenitors and the evolution of diversity in lettuce germplasm collections. The limitations of this method are primarily that it is labour intensive and expensive, these being the main reasons to look for new methods. The development of polymerase chain reaction (PCR) for amplifying DNA led to a revolution in the application of molecular methods and a range of new technologies has been developed, which can overcome many of technical limitations of RFLPs.

RAPD (Random Amplified Polymorphic DNA)

RAPD is a PCR-based technology. The method is based on enzymatic amplification of target or random DNA segments with arbitrary primers. Each product is derived from a region of the genome that contains two short segments in inverted orientation, on opposite strands that are complementary to the primer. The amplification products are separated on agarose gels in the presence of ethidium bromide and view under ultraviolet light (Jones et al. 1997).

The RAPD technique has been used in lettuce for evaluating variation within accessions and to establish differences between lines of apparently closely related populations in germplasm collections (Waycott and Fort 1994). They analysed ten populations of butterhead and one of crisphead lettuce with 13 primers. Nine out of the 10-butterhead lines were visually very similar and homogeneous, but the 10th line was highly heterogeneous. Seven out of 10 lines showed a within-line genetic purity of 96% or more. Thus, most of the lines could easily be identified using only 8–10 primers. A dendrogram of the RAPD data confirmed the close relationship of the nine butterhead lines

Table 2. Survey of protein marker methods and isozyme systems used for *Lactuca* spp. characterization

Method	*Lactuca* spp.	Enzyme systems	Goal of work	References
Electrofocusing	*L. sativa*, *L. serriola*, *L. saligna*, *L. aculeata*, *L. virosa*	EST	characterization of *L. sativa* and related species by electrofocusing of esterases	Roux et al. (1985)
Isozymes	*L. sativa*, *L. serriola*, *L. saligna*, *L. virosa*	ACO, ACP, ADH, ALD, ALO, ALP, AMY, ASO, CAT, DIA, ENP, EST, FDP, FUM, GALD, GPD, GPT, GDH, GOT, GLS, GAPD, HXK, ICD, LAC, LAD, LAP, LED, MDH, MDR, ME, PER, PGD, PGI, PGM, PHP, SHDH, SCD, SOD, TPI, TYR, XDH	genetic variability and phylogenetic study	Kesseli and Michelmore (1986)
Isozymes	*L. sativa*	ACP, ADH, EST, GOT, ICD, LAP, MDH, ME, PGD	electrophoretic characterisation of lettuce cultivars	Mejia and Mc Daniel (1986)
Isozymes	*L. sativa*, *L. serriola*, *L. saligna*, *L. virosa*	ADH, DIA, EST, GOT, IDH, LAP, MDH, PGD, PGI, SHDA	characterization of wild populations of four *Lactuca* spp. by 10 enzyme systems by using polyacrylamide gradient gel electrophoresis	Cole et al. (1991)
SDS-electrophoresis	*L. sativa*, *L. serriola*, *L. saligna*, *L. virosa*		characterization and identification of *L. sativa* cultivars and wild relatives	Vries de (1996)
Isozymes	*L. aculeata*, *L. altaica*, *L. canadensis*,*L. dregeana*, *L. indica*, *L. perennis*, *L. saligna*, *L. sativa*, *L. serriola*, *L. taraxacifolia*, *L. tatarica*, *L. tenerrima*, *L. virosa*, *L. viminea*	ACP, ADH, EST, PER	characterization germplasm collection of *Lactuca* spp. by 4 enzyme systems, confirmation of taxonomy determination	Lebeda et al. (1999, 2001a), Doležalová et al. (2003b)
Isozymes	*L. sativa*, *L. serriola*, *L. saligna*, *L. virosa*, *L. indica*	ADH, DIA, EST, FDH, GDH, GOT, ICD, MDH, ME, PGI, PGM, PGD, SADH, SOD	characterization of polymorphism, segregation analysis of the F_2 progeny between *L. sativa* and *L. serriola*, linkage analysis, genetic markers for lettuce breeding	Mizutani and Tanaka (2003)

ACO (aconitase); ACP (acid phosphatase); ADH (alcohol dehydrogenase); ALD (aldolase); ALO (aldehyde oxidase); ALP (alkaline phosphatase); AMY (amylase); ASO (ascorbate oxidase); CAT (catalase); DIA (diaphorase); ENP (endopeptidase); EST (esterase); FDH (formate dehydrogenase); FDP (fructose-1,6-diaphorase); FUM (fumarase); GALD (galactose dehydrogenase); GPD (glucose-6-phosphate dehydrogenase); GPT (glucose-1-phosphate transferase); GDH (glutamate dehydrogenase); GOT (glutamate oxaloacetate transaminase); GLS (glutamate syntetase); GAPD (glyceraldehyde-3-phosphate dehydrogenase NAD, glyceraldehyde-3-phosphate dehydrogenase NADP); HEX (hexokinase); ICD (isocitrate dehydrogenase); LAC (laccase); LAD (lactose dehydrogenase); LED (leucine dehydrogenase); LAP (leucine aminopeptidase); MDH (malate dehydrogenase NAD^+); ME (malic enzyme $NADP^+$); MDR (menidione reductase); PER (peroxidase); PGD (6-phosphogluconate dehydrogenase); PGI (phosphoglucoisomerase); PGM (phosphoglucomutase); PHP (phosphorylase); SADH (shikimic acid dehydrogenase); SHDG (shikimate dehydrogenase); SCD (sucinate dehydrogenase); SOD (superoxide dismutase); TPI (triosephosphate isomerase); TYR (tyrosinase); XDH (xanthinine dehydrogenase)

vs the heterogeneous tenth line. Moreover, it was able to sequester among the nine visually similar lines with a different origin and pedigree.

Twelve lettuce (*L. sativa*) varieties were identified using AP-PCR (Arbitrary Primed PCR) (Yamamoto et al. 1994). Twenty one RAPD primers and 8 sequence-specific primers were used for amplifying four specific DNA fragments. Of the amplified fragments of *L. sativa* 47% was polymorphic and all varieties were differentiated. Some of the PCR fragments were variety or morphotype specific, which could be used as indicators for morphotype-selection. The dendrogram derived showed well differentiated clusters of crisphead, leaf and butterhead types.

Two resistances to downy mildew derived from *L. serriola* were characterized genetically and mapped using RAPD markers and codominant SCAR (Sequence Characterized Amplified Region) markers (Maisonneuve et al. 1994). Montesclaros et al. (1997) investigated the interaction between two different potyviruses (turnip mosaic virus TuMV and lettuce mosaic virus LMV) and resistant cultivars of *L. sativa*. The resistance loci were characterized at the genetic level by mapping them relative to molecular markers (RAPD).

Kesseli et al. (1994) compared the levels of polymorphism of two types of molecular markers, RFLP and RAPDs, as detected between two cultivars of lettuce in the construction of a genetic linkage map. From 1008 probes derived from cDNA, 10% were polymorphic and 9% could be mapped. Similar results were obtained with 180 probes derived from genomic DNA, 11% were polymorphic, which could all be mapped. Fifty primers were tested. Errors in scoring bands were similar for both techniques. RFLP and RAPD markers showed similar distributions throughout the genome, both identified similar levels of polymorphism. RAPD loci, however, were identified more rapidly. On the other hand, RAPD markers were found to be easy to perform by different laboratories, but reproducibility was not achieved to a satisfactory level (Jones et al. 1997) and, therefore, the method was utilized less for routine identifications.

RAPD marker diversity was used also applied for diversity studies within and among some other Asteraceae species (Esselman et al. 2000).

AFLP (Amplified Fragment Length Polymorphism)

More recently, a new PCR-based technique has been developed, named amplified fragment length polymorphism (AFLP), which is essentially intermediate between RFLPs and PCR. AFLP is a DNA fingerprinting technique, which detects DNA restriction fragments by means of PCR amplification. 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).

The AFLP marker technique (Table 3) was used to determine the phylogenetic relationships between *Lactuca* spp. (Hill et al. 1996). Genetic distances based on AFLP data were estimated for 44 morphologically different varieties of cultivated *L. sativa* and 13 accessions of the wild *Lactuca* species (*L. serriola*, *L. saligna*, *L. virosa*, *L. perennis* and *L. indica*). A total of 320 polymorphic AFLP loci were identified using only three pairs of primers. The clustering analysis showed that all tested species clustered as distinct units (*L. serriola*, *L. saligna*, *L. virosa*, *L. perennis* and *L. indica*). The accessions of *L. serriola* formed a cluster on the sister branch of the *L. sativa* complex. This result is consistent with *L. serriola* being the likely progenitor species of *L. sativa* (Lindqvist 1960, Kesseli et al. 1991). Also, the 44 accessions of *L. sativa* were subdivided as discrete branches according to morphotypes (butterhead, crisphead, romaine and looseleaf). The AFLP data were compared to RFLP data derived from analysis of the same 56 accessions *Lactuca* spp. (Kesseli et al. 1991). Although AFLP and RFLP data resulted in similar dendrograms, the overall genetic distance between taxa was generally higher with RFLP markers. The AFLP markers were also used for the elucidation of the relationships between *Lactuca* species and species from related genera (Koopman et al. 2001). An AFLP data set comprised of 95 accessions from 20 species of *Lactuca* and related genera was generated using two primer combinations (E35/M48, E35/M49). The results did not show a distinction among the *serriola*-like species (*L. sativa*, *L. serriola*, *L. dregeana*, and *L. altaica*) and the authors postulated that these species are conspecific. However, the *serriola*-like species, *L. aculeata* occupyied a clearly separate position. The subsection *Lactuca* as a group is well defined by this data, but the positions of *L. saligna* and *L. virosa* as related species to the *serriola*-like species remains unclear. The close relationship between the sect. *Mulgedium* species *L. tatarica* and *L. sibirica* was corroborated. The AFLP marker technique was used also to construct an integrated interspecific AFLP map of lettuce based on two *L. saligna* × *L. sativa* F_2 populations (Jeuken et al. 2001).

Jeuken and Lindhout (2002) developed an F_2 population based on a resistant *L. saligna* × susceptible *L. sativa* cross. This F_2 population was fingerprinted with AFLP markers and tested for

Table 3. Survey of molecular marker methods used for *Lactuca* spp. characterization

Method	*Lactuca* spp.	Goal of work	References
RFLP	*L. sativa* (Calmar × Kordaat cross)	a construction of lettuce genetic map	Landry et al. (1987)
	L. sativa, *L. saligna*, *L. serriola*, *L. virosa*, *L. perennis*, *L. indica*	study of relationships between cultivated lettuce and five related wild *Lactuca* spp.; study on the origin of cultivated lettuce (*L. sativa*)	Kesseli et al. (1991)
	L. sativa, *L. serriola*, *L. saligna*, *L. virosa*, *L. alpina*, *L. perennis*, *L. tatarica*	study of relationships among *Cichorium* species and related genera of the tribe *Lactuceae*	Vermeulen et al. (1994)
	L. sativa (Calmar × Kordaat cross)	a construction of genetic map of *L. sativa*	Kesseli et al. (1994)
RAPD	*L. sativa* (Calmar × Kordaat cross)	a construction of genetic map of *L. sativa*	Kesseli et al. (1994)
	L. sativa	DNA polymorphisms in 12 lettuce (*L. sativa*) varieties were identified by amplification with 21 arbitrary RAPD primers	Yamamoto et al. (1994)
AFLP	*L. sativa*, *L. serriola*, *L. saligna*, *L. virosa*, *L. perennis*, *L. indica*	study genetic relationships in *Lactuca* spp.	Hill et al. (1996)
	L. sativa, *L. saligna*	an integrated interspecific AFLP map of lettuce based on two *L. sativa* × *L. saligna* F_2 populations	Jeuken et al. (2001)
	L. sativa, *L. serriola*, *L. dregeana*, *L. altaica*, *L. aculeata*, *L. saligna*, *L. virosa*, *L. tenerrima*, *L. perennis*, *L. tatarica*, *L. sibirica*, *L. quercina*, *L. viminea*, *L. indica*	study of species relationships in *Lactuca* spp.	Koopman et al. (2001)
Micro-satellites	*L. sativa*, *L. serriola*, *L. saligna*, *L. virosa*, *L. perennis*, *L. indica*	identification, genetic localization, and allelic diversity SAMPL in lettuce and wild relatives (*Lactuca* spp.)	Witsenboer et al. (1997)
	L. sativa, *L. serriola*, *L. saligna*, *L. virosa*	variety identification in lettuce cultivars (*L. sativa*) and discriminate between cultivated lettuce and wild relatives	Van de Wiel et al. (1998)
	L. sativa, *L. serriola*, *L. saligna*, *L. virosa*	distinguishing lettuce cultivars and screening diversity of genetic resources	Van de Wiel et al. (1999)
ITS-1 DNA sequence	*L. sativa*, *L. serriola*, *L. aculeata*, *L. dregeana*, *L. saligna*, *L. altaica*, *L. virosa*, *L. tenerrima*, *L. perennis*, *L. tatarica*, *L. sibirica*, *L. quercina*, *L. viminea*, *L. indica* Other related species: *Mycelis muralis*, *Steptorhampus tuberosus*, *Cicerbita plumieri*, *C. alpina*, *Prenanthes purpurea*, *Chondrilla juncea*, *Taraxacum officinale*, *Sonchus asper*, *Cichorium intybus*	phylogenetic relationships among *Lactuca* species and related genera; specification position of *L. altaica;* delimitation of genus *Lactuca*; the taxonomic position of *Cichorium*	Koopman et al. (1998)

resistance to two *Bremia lactucae* races (NL 14 and NL 16). QTL mapping revealed a qualitative gene (*R39*) involved in the race-specific resistance and three QTLs (*RBQ1*, *RBQ2* and *RBQ3*) involved in the quantitative resistance to this pathogen.

Johnson et al. (2000) used F_2 and F_3 families of lettuce (*L. sativa*) and *L. serriola* for genotyping using codominantly scored AFLP markers distributed throughout the genome. Composite interval mapping was used to analyze marker-trait associations. Quantitative trait loci were identified for differences between wild and cultivated lettuce in root architectural traits and water acquisition.

From the above data it is evident that AFLP markers are useful in genetic studies, such as biodiversity evaluation, analysis of germplasm collections, genotyping of individuals and genetic distance analyses. The availability of many different restriction enzymes and corresponding primer combinations provides a great deal of flexibility, enabling the direct manipulation of AFLP fragment generation for defined applications (e.g. polymorphism screening, QTL analysis, genetic mapping). However, the major disadvantage of AFLP markers is that these are dominant markers.

Microsatellites

Microsatellites, also known as Simple Sequence Repeats (SSRs), are sections of DNA, consisting of tandemly repeating mono-, di-, tri-, tetra- or penta-nucleotide units that are arranged throughout the genomes of most eukaryotic species (Powell et al. 1996). Microsatellite sequences are especially suited to distinguish closely related genotypes; because of their high degree of variability, they are, therefore, favoured in population studies (Smith and Devey 1994) and for the identification of closely related cultivars (Vosman et al. 1992). Microsatellite polymorphism can be detected by Southern hybridisation or PCR. Van de Wiel et al. (1998) used oligonucleotides complementary to mini- and microsatellite sequences as probes in Southern hybridisation to detect polymorphism between cultivars of lettuce as well as accessions of *L. serriola*, *L. virosa* and *L. saligna*. The greater majority of this material has been characterized morphologically (Vries de and Raamsdonk van 1994). The rest of the material has also been characterized morphologically and analysed using ITS1 sequencing (Koopman et al. 1998). Fourteen microsatellite and three minisatellite motifs were tested for fingerprinting in *Lactuca* spp. The microsatellite array TCT proved to be the best for fingerprinting in cultivated lettuce and some of wild *Lactuca* species. DNA from 73 cultivars and accessions of *L. sativa*, *L. serriola* and *L. virosa* was digested with *Tag*I, Southern blotted and hybridised to $(TCT)_{10}$. In *L. sativa* and *L. serriola*, a pattern of two to three highly polymorphic bands was visible in the high molecular weight range. This sufficed to distinguish all the accessions and cultivars tested. In *L. virosa* and *L. saligna* more bands were visible and here too all the accessions tested could be distinguished. The TCT fingerprinting also appeared to be useful for testing the homogeneity of cultivars, however, it was not suitable for determining relationships among accessions. The level of polymorphism detected with this probe was too high.

Microsatellites can also be implemented as monolocus, codominant markers by converting individual microsatellite loci into PCR-based markers by designing primers from unique sequences flanking the microsatellite. Microsatellite containing genomic fragment have to be cloned and sequenced in order to design primers for specific PCR amplification. This approach was called sequence-tagged microsatellite site (STMS) (Beckmann and Soller 1990). Van de Wiel et al. (1999) isolated microsatellite-containing sequences from lettuce (*L. sativa*) by using enriched genomic libraries. Up to 55% of the clones contained a microsatellite, of which about half of these clones primers could be designed for PCR amplification of the microsatellite. In total, 28 primer set amplifying unambiguously scorable products, of which 26 showed polymorphism in a test set of six lettuce varieties. Practically all microsatellite-amplifying primer sets yielded products in *L. serriola*, but only half of the primer sets yielded products in more distant species *L. saligna* and *L. virosa*. The microsatellite loci isolated will be useful for distinguishing lettuce cultivars and for screening diversity of genetic resources.

Witsenboer et al. (1997) studied the potential of SAMPL (Selectively Amplified Microsatellite Polymorphic Locus) analysis in lettuce to detect PCR-based codominant microsatellite markers. SAMPL is a method of amplifying microsatellite loci using general PCR primers. SAMPL analysis uses one AFLP primer in combination with a primer complementary to microsatellite sequences (Witsenboer et al. 1997). Fifty-eight SAMPLs were identified and placed on the genetic map of lettuce. Seventeen were codominant. Forty-five cultivars of lettuce and five wild species of *Lactuca* were analysed to determine the allelic diversity for codominant SAMPLs. From 3 to 11 putative alleles were found for each SAMPL; 2–6 alleles were found within *Lactuca sativa* and 1–3 alleles were found among the crisphead genotypes, the most genetically homogeneous plant type of *L. sativa*. This allelic diversity is greater than that found for RFLP markers. Therefore, SAMPL analysis is more applicable to intraspecific than to interspecific comparisons.

Sicard et al. (1999) analysed diversity in wild and cultivated *Lactuca* germplasm using molecular markers derived from resistance genes of the NBS-LRR type. Three molecular markers, one microsatellite markers and two SCAR markers that amplified LRR-encoding regions, were developed from sequences of resistance gene homologs at the main resistance gene cluster in lettuce.

Microsatellites and AFLPs were also used to characterise the entire lettuce collection of the Centre for Genetic Resources (CGN, Wageningen) (Hintum van 2003).

CONCLUSIONS

The genus *Lactuca* L. is widely distributed in the main worlds phytogeographical regions. Currently there is not a precise morphological taxonomic treatment of this genus (Lebeda and Astley 1999, Whitton et al. 1995, Doležalová et al. 2002a, 2003a, Lebeda et al. 2004a, b). Protein and molecular marker techniques have contributed to the clarification of various problems in the variability and biodiversity of this genus. Isozyme markers have been used to characterize the levels of variation and the systematic relationships of wild and cultivated lettuce (Kesseli and Michelmore 1986). However, they have basic limitations; the number of polymorphism is rather low for solving these complex relationships. RFLP markers can distinguish between most accessions of cultivated lettuce, except for sister lines from the same breeding population (Landry et al. 1987, Kesseli et al. 1991, 1994, Vermeulen et al. 1994). RAPDs appear to be able to distinguish between nearly identical germplasm accessions of cultivated lettuce (Waycott and Fort 1994). RFLP and RAPD markers showed similar levels of polymorphism. However, RAPDs have been shown to be poorly reproducible between different laboratories (Karp et al. 1997a), and are therefore less useful for routine identification purposes. AFLP markers are useful for measuring genetic diversity and the determination of genetic relationships within and among species. A major advantage of AFLP analysis is the short time required to assay large numbers of DNA loci. Another advantage of AFLP analysis is the effectively unlimited number of loci that can be assayed with different combinations of a relatively small number of oligonucleotide primers. Compared to other marker technologies, e.g. randomly amplified polymorphic DNA (RAPD), restriction fragment-length polymorphism (RFLP) or microsatellites, AFLP provides equal or greatly enhanced performance in terms of reproducibility, resolution, and time efficiency. However, the major disadvantage of AFLP markers are that high quality of DNA is needed, the high cost and they are dominant markers. Microsatellites were used for plant population studies (Ouborg et al. 1999), including *Lactuca* spp., but their usage for phylogenetic studies is limited.

All types of molecular marker methods were also used for the characterization of different resistance genes in lettuce (Irwin et al. 1999, Sicard et al. 1999, Chin et al. 2001, Jeuken and Lindhout 2002, Grube et al. 2003, Hand et al. 2003, Maisonneuve 2003, Mou and Ryder 2003, Nicaise et al. 2003, Ryder et al. 2003).

In general, molecular tools in plant genetic resource conservation are considered to be very important (Bretting and Widrlechner 1995, Ayad et al. 1997, Karp et al. 1996, 1997b, Treuren van 2001). There are international projects currently working on various aspects of the molecular characterization of *Lactuca* spp. and some other taxa of the Asteraceae. An international project, Gene-Mine, within the Fifth Framework Programme of the European Union is targeting the complex characterization of some European populations of *Lactuca serriola* (Jansen 2001, Doležalová et al. 2003a, b, Lebeda et al. 2004a, b). A Composite Genome Project (Michelmore et al. 2003) has generated 80000 Expressed Sequence Tags (ESTs) of lettuce and sunflower. An EU project will study the gene flow between crop and wild forms of lettuce and chicory in the context of GMO biosafety (Van de Wiel et al. 2003).

The main aim of this paper is to summarise information on the comprehensive molecular based research on the genus *Lactuca* spp. The methods discussed here (Tables 2 and 3), add to the wide potential of all previously used methods in plant variability research. Modern molecular and biochemical marker techniques must be applied in the broadest context of a complex view of taxonomy, biodiversity, ecobiology, population genetics and genetic resources management of *Lactuca* spp. (Vosman 1997, Van de Wiel et al. 1998, Lebeda et al. 1999, 2001a, b, Lebeda and Boukema 2001, Doležalová et al. 2002b, Lebeda et al. 2004b, c). By contrast, the interpretation of results based on single methods can lead to completely wrong conclusions (Doležalová et al. 2003a, b).

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ABSTRAKT

Charakterizace genových zdrojů *Lactuca* spp. pomocí proteinových a molekulárních markerů – studie

Rod *Lactuca* L. patří do nejrozsáhlejší čeledi rostlin Asteraceae. *Lactuca* L. je reprezentována přibližně sto druhy rozšířenými v různých geografických oblastech a ekologických podmínkách. To je také jeden z důvodů, proč je tento rod charakteristický velmi širokou variabilitou různých znaků. Elektroforetická detekce některých proteinů (izoenzymů) byla aplikována při studiu genetické variability jedinců a populací *Lactuca* spp. Vývoj molekulárně genetických metod (RFLP, Restriction Fragment Length Polymorphism; PCR metody: RAPD, Random Amplified Polymorphic DNA; AFLP, Amplified Fragment Length Polymorphism; minisatelity a mikrosatelity nebo SSR, Simple Sequence Repeats) a jejich aplikace přispěly k hodnocení různých aspektů týkajících se taxonomie, variability, biodiverzity, genetiky a šlechtění v rámci rodu *Lactuca* L. Předmětem diskuse je další potenciální využití těchto metod.

Klíčová slova: genové pooly; genové zdroje; salát; taxonomie; variabilita; biodiverzita; izoenzymy; RFLP; RAPD; AFLP; mikrosatelity; STMS; SAMPL; PCR; AP-PCR

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