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Analysis of wild *Lactuca* accessions: conservation and identification of redundancy

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Abstract

Germplasm accessions of wild *Lactuca* species are maintained worldwide in *ex situ* collections as gene reservoirs for quality and disease resistance traits for cultivated lettuce. Accessions of 12 *Lactuca* species from 6 genebanks were compared via morphological characterization and AFLP (Amplified Fragment Length Polymorphism)-based profiling to estimate the extent of duplication. A method of assessing redundancy within very similar, but not identical accessions, is proposed, based on 352 polymorphic AFLP products. Seven duplication groups showed a high level of AFLP similarity, and one pair of *Lactuca saligna* accessions displayed identical AFLP profiles. In several cases, the morphological assessment indicated that a taxonomic reclassification of accessions was necessary. Candidate duplicates were identified using population parameters and inter- and intra-accession variability. The implications of these findings on the conservation of wild species are discussed.

Keywords: AFLP; duplication; plant genetic resources;

Introduction

The genus *Lactuca* comprises about 100 species. Cultivated lettuce (*Lactuca sativa* L.) is the best known member of this genus since it represents a common food species. Wild *Lactuca* species are considered to be an important source of disease resistance genes, and this has driven the need to establish *ex situ* collections of the wild species. During this process, accessions of other *Lactuca* species have become commonplace in many genebank collections (Lebeda *et al.*, 2004). The number of wild *Lactuca* accessions within collections has increased not only by the incorporation of newly collected material but also by the exchange of seeds among genebanks and other donors, thus partially duplicating specific genotypes. The appropriate maintenance of germplasm collections following current international standards demands considerable financial and human resources. Since these resources are limited, there is a need to avoid redundancy. To reduce redundancy, the degree of duplication among accessions needs first to be estimated. This is facilitated both by the development of crop-specific databases and by the application of molecular techniques.

Intensive research has been conducted in recent years to identify duplicate accessions and gather the information needed to rationalize germplasm collections. While the identification of lettuce duplicates has up to now relied on a combination of RAPD (Random Amplification of Polymorphic DNA) and morphological data, in other crop species, such as rice, barley and cabbage, the

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Table 1. Accessions of wild *Lactuca* used for morphological and AFLP analyses

Species (original botanical name in *Lactuca* database)	DG	Genebank acronym and location	Accession number
L. aculeata Boiss. & Kotschy ex Boiss.	1	CGN, Wageningen, NLD	CGN09357
	2	CGN, Wageningen, NLD	CGN15692
	4	RICP, Olomouc, CZK	RICP09H5801119
L. altaica Fisch. et C. A. Mey.	8	CGN, Wageningen, NLD	CGN15711
	9	HRIGRU, Wellesbourne, UK	HRI4955
	9	HRIGRU, Wellesbourne, UK	HRI4956
	9	RICP, Olomouc, CZK	RICP09H5800939
L. dentate (Thunb.) C. B. Rob.	23	CGN, Wageningen, NLD	CGN11404
	24	RICP, Olomouc, CZK	RICP09H5800942
L. dregeana DC.	26	CGN, Wageningen, NLD	CGN04790
	26	RICP, Olomouc, CZK	RICP09H5800961
	26	USDA-ARS, Salinas, USA	PI273574aLET
	26	USDA-ARS, Salinas, USA	PI273574bLET
	26	USDA-ARS, Salinas, USA	PI273574LET
	26	USDA-WG, Pullman, USA	PI273574WG
	27	RICP, Olomouc, CZK	RICP09H5801191
	27	CGN, Wageningen, NLD	CGN05805
	28	RICP, Olomouc, CZK	RICP09H5801320
L. indica L.	33	CGN, Wageningen, NLD	CGN13393
	33	RICP, Olomouc, CZK	RICP09H5800964
L. livida Boiss. et Reut.	42	HRIGRU, Wellesbourne, UK	HRI4972
	42	HRIGRU, Wellesbourne, UK	HRI4981
	42	HRIGRU, Wellesbourne, UK	HRI4979
	42	RICP, Olomouc, CZK	RICP09H5800944
	42	RICP, Olomouc, CZK	RICP09H5800943
	43	USDA-ARS, Salinas, USA	PI273585LET
	43	USDA-WG, Pullman, USA	PI273585WG
	44	RICP, Olomouc, CZK	RICP09H5801127
	45	RICP, Olomouc, CZK	RICP09H5801128
L. perennis L.	66	CGN, Wageningen, NLD	CGN10884
	66	USDA-ARS, Salinas, USA	PI274415LET
	66	USDA-WG, Pullman, USA	PI274415WG
L. quercina L.	82	CGN, Wageningen, NLD	CGN14220
	83	RICP, Olomouc, CZK	RICP09H5801131
L. saligna L.	119	CGN, Wageningen, NLD	CGN05329
	119	RICP, Olomouc, CZK	RICP09H5801059
	119	HRIGRU, Wellesbourne, UK	HRI6382
	119	USDA-ARS, Salinas, USA	PI261653LET
	126	CGN, Wageningen, NLD	CGN09311
	131	IPK, Gatersleben, DEU	LAC239
	131	RICP, Olomouc, CZK	RICP09H5801061
	131	CGN, Wageningen, NLD	CGN13300
	135	CGN, Wageningen, NLD	CGN13371
	140	CGN, Wageningen, NLD	CGN15705
L. serriola L.	8	CGN, Wageningen, NLD	CGN15737
	84	CGN, Wageningen, NLD	CGN05808
	84	CGN, Wageningen, NLD	CGN11402
	84	RICP, Olomouc, CZK	RICP09H5801190
	126	CGN, Wageningen, NLD	CGN09279
	304	CGN, Wageningen, NLD	CGN04770
	304	CGN, Wageningen, NLD	CGN04769
	305	CGN, Wageningen, NLD	CGN04776
	305	CGN, Wageningen, NLD	CGN04775
	305	CGN, Wageningen, NLD	CGN04774
	305	HRIGRU, Wellesbourne, UK	HRI5093
	305	RICP, Olomouc, CZK	RICP09H5801199
	305	RICP, Olomouc, CZK	RICP09H5801200
	305	USDA-WG, Pullman, USA	PI251245WG
	312	CGN, Wageningen, NLD	CGN04929
	312	IPK, Gatersleben, DEU	LAC160

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Table 1. *Continued*

Species (original botanical name in *Lactuca* database)	DG	Genebank acronym and location	Accession number
	313	CGN, Wageningen, NLD	CGN04930
	313	IPK, Gatersleben, DEU	LAC162
	567	CGN, Wageningen, NLD	CGN10979
	948	CGN, Wageningen, NLD	CGN04804
	948	USDA-ARS, Salinas, USA	PI289064LET
	948	USDA-ARS, Salinas, USA	PI289064bLET
	948	USDA-ARS, Salinas, USA	PI289064cLET
	948	USDA-ARS, Salinas, USA	PI289064dLET
	948	USDA-ARS, Salinas, USA	PI289064eLET
	948	USDA-WG, Pullman, USA	PI289064WGa = green leaves PI289064WGb = light green leaves PI289064WGa = red leaves
	1088	CGN, Wageningen, NLD	CGN04796
	1088	RICP, Olomouc, CZK	RICP09H5801206
L. tatarica (L.) C. A. Mey.	1220	RICP, Olomouc, CZK	RICP09H5800967
	1220	CGN, Wageningen, NLD	CGN09390
L. virosa L.	937	CGN, Wageningen, NLD	CGN13325
	937	USDA-ARS, Salinas, USA	PI271938LET
	1088	USDA-ARS, Salinas, USA	PI274901LET
	1088	USDA-WG, Pullman, USA	PI274901WG

DG, duplication group; NLD, The Netherlands; CZK, Czech Republic; USA, United States of America; DEU, Germany.

AFLP technique has been preferred, since it delivers more robust markers than does RAPD (Spooner *et al.*, 2006). AFLP has been employed in lettuce to analyse phylogenetic relationships and population structure, but not as yet for the detection of genotypic duplicates. In the course of an EU-funded project (www.gene-mine.org), duplicate accessions of wild *Lactuca* species were identified based on passport data (especially identical collection number ID and identical donor), and then by a morphological trait analysis. Since passport data can be erroneous and environmental conditions can influence morphology, and since the latter is not always sufficient to differentiate between closely related materials, a sample of accessions from defined duplication groups has now been genotyped by AFLP.

Material and methods

The identification of duplication groups

The study sample comprised 78 accessions from 12 *Lactuca* species, provided by 6 genebanks (Table 1). Putative duplicate accessions of wild *Lactuca* species were identified based on passport data held at the Centre for Genetic Resources (CGN) in Wageningen, The Netherlands, and by a search of the *Lactuca* database ILDB (The International Lactuca Database, www.plant.wageningen-ur.nl/cgn/ildb). Where passport data were scarce and the number of accessions limited, all accessions of a species were included. This exercise resulted in the identification of 33 duplication groups. Morphological trait analysis was then carried out for the putative duplicate accessions or 'duplication groups' at the Department of Botany of Palacký University (PU) and the Gene Bank Department of the Research Institute of Crop Production (RICP) in Olomouc, Czech Republic.

Morphological characterization

Twenty-five seeds per accession were sown in sterile Agroperlite (EP AGRO, PERLIT Ltd, Šenov u Nového Jičína, Czech Republic), to produce 16 vigorous individuals per accession. At the 5–7 fully developed leaf stage, the plants were transplanted into containers filled with garden soil and cultivated under standard greenhouse conditions (day/night temperature range, 18–30/13–16°C). Drip irrigation and chemical protection against powdery mildew and spider mites were provided. The visual assessment of plants was performed at various developmental stages. Twenty quantitative and qualitative characters were assessed (Doležalová *et al.*, 2003), eight of which were informative to define similarity/dissimilarity (Table 2). Based on the vegetative and generative characteristics, the accessions were then taxonomically verified (Feráková, 1977; Dostál, 1989; Iwatsuki *et al.*, 1995).

AFLP analysis

Seventy-eight accessions with 20 individual plants each were grown in the greenhouse, and genomic DNA was

Table 2. Eight most discriminatory morphological characteristics that determine similarity/dissimilarity of *Lactuca* accessions

Organ of plant	Descriptor name (descriptor number)
Rosette leaves	Entire rosette leaf shape of blade in outline (1.3.2)
	Divided rosette leaf – depth of incisions (1.3.3)
	Shape of apex (1.3.4)
Cauline leaves	Entire cauline leaf – shape of blade in outline (1.3.6)
	Divided cauline leaf – depth of incisions (1.3.7)
	Cauline leaf – shape of apex (1.3.8)
Flower and inflorescence	Flower head – number of ligules in head (1.4.1)
	Flower head – colour of ligules (1.4.2)

Numbers in parentheses according to Doležalová *et al.* (2003).

isolated in 96-well plates from leaves according to Doyle and Doyle (1990), but modified for a robotic, liquid-handling system. AFLP procedure was performed according to the following modifications: genomic DNA (100 ng) was simultaneously restricted and ligated with appropriate adapters (Table 3), with 5 U *Eco* RI, 1 U *Mse* I (both from New England Biolabs, Frankfurt, Germany), 0.2 pmol *Eco* RI adapters, 2.0 pmol *Mse* I adapters, 2.0 pmol NaCl, 50 μg/ml BSA, 1 × ligase buffer and 0.2 U ligase (Invitrogen, Karlsruhe, Germany). Restriction/ligation products were diluted ten times in TE buffer. Preselective amplification was performed in two steps: first with primers with two bases, and then with three selective bases (Table 3). Diluted, restricted and ligated DNA (3.5 μl) was added to 10 pmol *Eco* RI and *Mse* I primers, 200 pmol dNTP, 2.25 nmol $Mg(OAc)_2$, 1 × PCR buffer and 0.3 U *Taq* polymerase (Eppendorf, Hamburg, Germany). After each PCR, the template was again diluted at a ratio of 1:50 in TE. For the selective amplification, three primer combinations were used (Table 3). The products of the three selective amplifications were pooled and fragment analysis was performed on the MegaBACE 1000 sequencer (Amersham Biosciences Europe, Freiburg, Germany), following its genotyping protocol.

Table 3. List of primers and adaptors used

Adaptors	Sequences
Eco RI	5′-CTCGTAGACTGCGTACC-3′
	3′-AATTGGTACGCAGTC-5′
Mse I	5′-GACGATGAGTCCTGAG-3′
	3′-TACTCAGGACTCAT-5′
Preamplification primers	
Eco RI + 1 primer	E00 + A
Mse I + 1 primer	M00 + C
Mse I + 2 primer	M00 + CT
Amplification primers	
Eco RI + 3 primer	E00 + ACA
Mse I + 4 primer	M00 + CTAT
Mse I + 4 primer	M00 + CTTC
Mse I + 4 primer	M00 + CTTT

Primer information kindly provided by Keygene N.V., Wageningen, The Netherlands.

Data analysis

A binary matrix was created from genotyping data (peak present/absent) with Fragment Profiler 1.2 software (Amersham Biosciences). The number of polymorphic loci, Nei's original measures of genetic identity and genetic distance and genetic diversity (G_{st}) were calculated using POPGENE software v1.32 (Yeh and Boyle, 1997). Jaccard's coefficient of similarity, the neighbour-joining (NJ) tree and unweighted pair group method with arithmetic mean (UPGMA) tree were calculated by NTSYS 2.1 software (Rohlf, 2002). The NJ tree was used for multiple-species analysis, since different species can have different evolutionary rates, and UPGMA was used for identifying within-species duplication. The same software was used to perform principal coordinate analysis (DCENTER and EIGENVEC procedures). An analysis of molecular variance (AMOVA) was performed with WINAMOVA 1.55 software package (Excoffier *et al.*, 1992). Variance components were tested for significance by a non-parametric re-sampling approach using 1000 permutated datasets. For random choice of plants in testing influence of plant number reduction on variance components, a table of 2000 random digits was used (Weir, 1996).

Results

AFLP and morphological analysis of examined accessions

In total, 357 peaks in the range from 70 to 415 base pairs were identified, with a G_{st} value of 0.49. The number of polymorphic fragments per accession generated by one primer combination ranged from 0 (only monomorphic fragments, detected in two duplicates) to 66. Within some of the species, the morphological test indicated probably taxonomic misidentification (Table 4), based on comparisons with herbarium specimens. Most of these errors were confirmed from the AFLP analysis (Sretenović Rajičić and Dehmer, 2008). Pending

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Table 4. Taxonomic re-determination within the set of wild *Lactuca* spp. after morphological characterization

Accession number	Donor	Donor identification[a]	Determined as
HRI4955	HRIGRU	*L. altaica*	Primitive *L. sativa*
HRI4956	HRIGRU	*L. altaica*	Primitive *L. sativa*
RICP09H5800939	RICP	*L. altaica*	Primitive *L. sativa*
RICP09H5800942	RICP	*L. dentata*	Oilseed lettuce
PI273574LET	USDA-ARS	*L. dregeana*	*L. sativa*
PI273574WG	USDA-WG	*L. dregeana*	Light seeds, *L. sativa* Dark seeds, *L. dregeana*
PI273574bLET	USDA-ARS	*L. dregeana*	*L. sativa*
HRI4972	HRIGRU	*L. livida*	*Lactuca* sp.
HRI4979	HRIGRU	*L. livida*	*Lactuca* sp.
HRI4981	HRIGRU	*L. livida*	*Lactuca* sp.
RICP09H5800943	RICP	*L. livida*	*Lactuca* sp.
RICP09H5800944	RICP	*L. livida*	*Lactuca* sp.
PI273585LET	USDA-ARS	*L. livida*	*Lactuca* sp.
PI273585WG	USDA-WG	*L. livida*	*Lactuca* sp.
RICP09H5801127	RICP	*L. livida*	*L. dregeana*
RICP09H5801128	RICP	*L. livida*	*L. dregeana*
CGN14220	CGN	*L. quercina*	Absent
RICP09H5801131	RICP	*L. quercina*	*L. sativa* × *L. serriola*
CGN10979	CGN	*L. serriola*	*L. serriola* and *L. dregeana*
CGN04796	CGN	*L. serriola*	*L. dregeana* × *L. serriola*
PI274901LET	USDA-ARS	*L. virosa*	*L. dregeana* × *L. serriola*
PI274901WG	USDA-WG	*L. virosa*	*L. dregeana* × *L. serriola*
RICP09H5801206	RICP	*L. serriola*	*L. dregeana* × *L. serriola*

[a] Original botanical name in *Lactuca* database.

reclassification, we have retained the existing labelling of the accessions.

All the available genebank accessions were included in the analyses for the five rarely collected species (*Lactuca aculeata*, *Lactuca dentata*, *Lactuca dregeana*, *Lactuca livida* and *Lactuca quercina*). The genetic diversity within these species can be illustrated in PCO plots (Fig. 1). Within *L. livida* (Fig. 1a), accessions RICP09H5801127, RICP09H5801128 and HRI4979 differed from the others. The former two are morphologically *L. dregeana* (Table 4), while among the remaining *L. livida* accessions, some duplicates were found. More diversity was detected in *L. dregeana* (Fig. 1b): accession RICP09H5800961 was very outlying, and PI273574bLET and PI273574WG probably need to be taxonomically re-identified. The third mislabelled accession PI273574LET was grouping within *L. dregeana* species. The accessions of *L. aculeata*, *L. dentata* and *L. quercina* were genetically dispersed (Fig. 1c). Most of the diversity (more than 80%) is contained within the first two principal components (Fig. 1).

Redundancy determination

Seventeen accessions (covering *L. livida*, *Lactuca saligna* and *Lactuca serriola*) formed seven groups (Fig. 2; a more detailed analysis is given elsewhere; Sretenović Rajičić and Dehmer, 2008). Coefficients of similarity among those 17 accessions are presented in Table 5. Only one pair of accessions (HRI6382 and PI261653LET) showed 100% similarity on the basis of AFLP profiling.

An additional layer of redundancy was determined by investigating how many distinct genotypes are present within any one accession or duplication group (Table 6). All plants that displaying the same AFLP profile were scored as an identical genotype, and these were arrayed in duplication-group-specific phenograms (Fig. 3). Genotypic variability, which should relate to accession diversity, differs widely among the duplication groups. For example, a minimum of five distinct genotypes were found within duplication group 119, and up to 18 in duplication groups 304/305 (Table 6).

Within duplication group 119, CGN05329 and RICP09H5801059 formed a pair of highly similar accessions (Nei's coefficient of genetic identity 0.999; Table 5). If the basis for duplication reduction is to eliminate all but one member of groups of accessions that have the same genotype, then accession RICP09H5801059, with three of the four genotypes present in accession CGN05329, is redundant and should be discarded. Similarly, HRI6382 and PI261653LET contain the same genotype, and one of these should be eliminated. Same approach is used for all duplication groups. Overall, therefore, one accession is probably redundant in each of the groups 42/43, 131 and 304/305,

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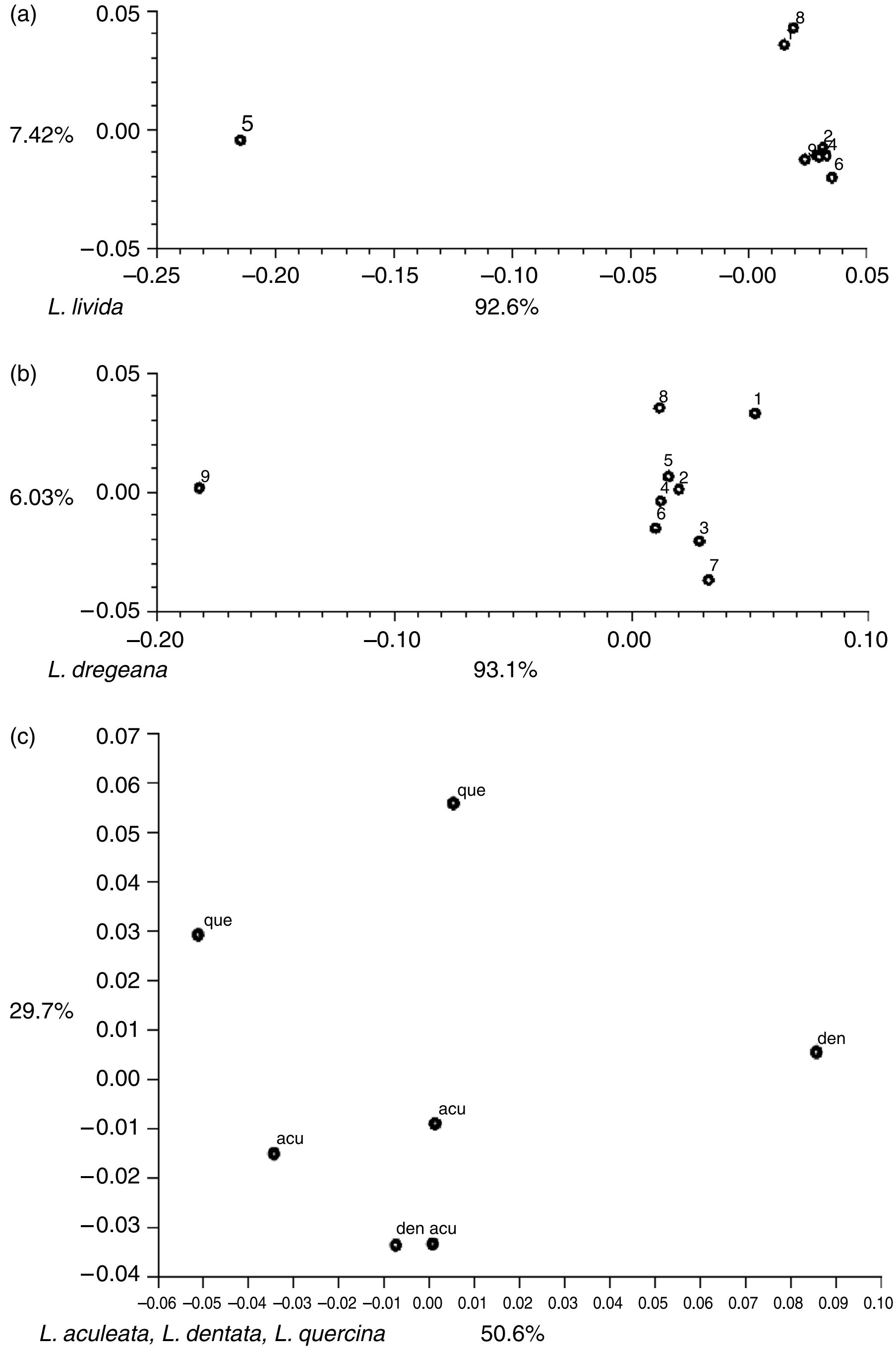

Fig. 1. PCO plots of individual accessions from five *Lactuca* species. (a) *Lactuca livida* accessions. 1, RICP09H5801127; 2, RICP09H5800943; 3, HRI4981; 4, RICP09H5800944; 5, HRI4979; 6, HRI4972; 7, PI273585LET; 8, RICP09H58001128; 9, PI273585WG. (b) *Lactuca dregeana* accessions. 1, PI273574bLET; 2, PI273574LET; 3, RICP09H5801191; 4, PI273574aLET; 5, CGN04790; 6, RICP09H5801320; 7, CGN05805; 8, PI273574WG; 9, RICP09H5800961. (c) acu, *Lactuca aculeata* (CGN09357, RICP09H5800942, CGN15692); den, *Lactuca dentata* (RICP09H5800942, CGN11404); que, *Lactuca quercina* (RICP09H5801131, CGN14220).

while in group 119, two of the four accessions should be conserved, and in group 312, none of the accessions are redundant (Table 6).

In all the groups except 119, within-accession variation is higher than that between groups. With a reduction in plant number from 20 to 10, the number of identifiable genotypes was reduced by at least one in four of the five groups, whereas this only occurred once when plant number was reduced from 20 to 15 (Table 7).

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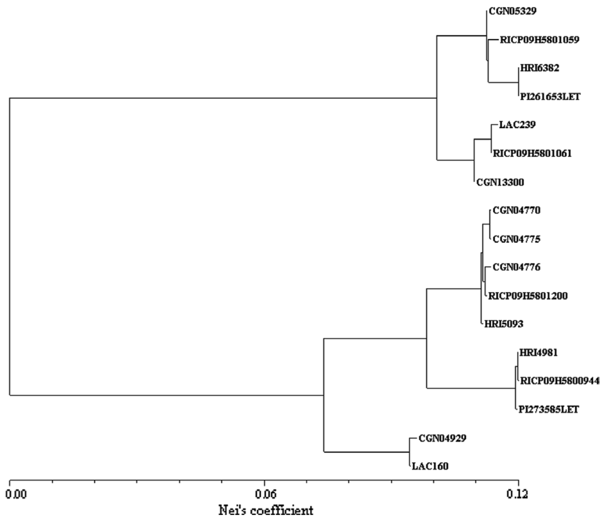

Fig. 2. Duplication groups found after AFLP analyses presented on the NJ tree with Nei's genetic distance coefficient.

Discussion

Rarely collected species

We have particularly attempted to examine in detail some of the more rarely collected *Lactuca* species. Those species labelled as *L. livida* appear to form three separate gene pools. Duplication within one of these has most likely occurred as a result of exchange of materials among genebanks. Duplication due to exchange was also expected for the other rarely collected *Lactuca* species, but there was no evidence for this. In *L. dentata*, the two accessions were distant enough from one another not to be considered as duplicates. The grouping of *L. dregeana* accessions indicates that they most probably did not result from the exchange of the same material. Some accessions of *L. dentata* and *L. quercina* thought to be duplicates proved to be genotypically quite distinct from one another.

Redundancy determination

The use of molecular markers to determine the redundancy in a germplasm collection is not a trivial activity. Small differences between (and within) accessions can be expected to arise as a result of a number of reasons. First of all, there can be an error, noise, in the genotyping. For example, when duplicate samples were employed to check the robustness of the DNA profiles in an AFLP-based diversity analysis of *Populus nigra*, the identity level was from 96 to 100% (Winfield *et al.*, 1998). But still diversity that was not yet mendeled out and point mutations can cause small differences to

Table 5. Nei's coefficient of genetic identity (above diagonal) and genetic distance (below diagonal) among 17 highly similar accessions

Accessions	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)
CGN05329 (1)	0	0.999	0.993	0.993	0.970	0.973	0.978	0.800	0.801	0.798	0.800	0.801	0.817	0.815	0.797	0.796	0.797
RICP09H5801059 (2)	0.001	0	0.991	0.991	0.968	0.971	0.975	0.797	0.798	0.795	0.797	0.798	0.814	0.812	0.794	0.793	0.795
HRI6382 (3)	0.007	0.009	0	1.000	0.978	0.980	0.984	0.790	0.795	0.788	0.790	0.791	0.810	0.807	0.787	0.787	0.788
PI261653LET (4)	0.007	0.009	0.000	0	0.978	0.980	0.984	0.790	0.795	0.788	0.790	0.791	0.810	0.807	0.787	0.787	0.788
LAC239 (5)	0.030	0.032	0.023	0.023	0	0.999	0.995	0.798	0.802	0.795	0.796	0.798	0.810	0.807	0.794	0.794	0.796
RICP09H5801061 (6)	0.028	0.030	0.020	0.020	0.001	0	0.997	0.800	0.804	0.797	0.798	0.800	0.813	0.810	0.796	0.796	0.797
CGN13300 (7)	0.023	0.026	0.016	0.016	0.005	0.003	0	0.801	0.805	0.799	0.801	0.802	0.819	0.816	0.798	0.797	0.799
CGN04770 (8)	0.223	0.227	0.235	0.235	0.226	0.224	0.222	0	0.996	0.999	0.998	0.997	0.942	0.943	0.965	0.965	0.965
CGN04776 (9)	0.222	0.226	0.230	0.230	0.221	0.219	0.217	0.004	0	0.996	0.998	0.997	0.946	0.947	0.962	0.962	0.963
CGN04775 (10)	0.226	0.229	0.238	0.238	0.229	0.227	0.224	0.001	0.004	0	0.998	0.998	0.941	0.942	0.966	0.966	0.966
RICP09H5801200 (11)	0.224	0.227	0.236	0.236	0.228	0.226	0.222	0.003	0.002	0.002	0	0.999	0.944	0.945	0.964	0.964	0.964
HRI5093 (12)	0.222	0.226	0.234	0.234	0.226	0.224	0.221	0.003	0.003	0.002	0.001	0	0.942	0.943	0.967	0.967	0.968
CGN04929 (13)	0.202	0.206	0.210	0.210	0.210	0.208	0.200	0.060	0.056	0.061	0.058	0.060	0	0.998	0.934	0.934	0.932
LAC160 (14)	0.205	0.209	0.214	0.214	0.214	0.211	0.204	0.059	0.055	0.060	0.057	0.058	0.002	0	0.937	0.937	0.935
HRI4981 (15)	0.227	0.231	0.240	0.240	0.231	0.229	0.226	0.036	0.039	0.035	0.036	0.033	0.069	0.065	0	0.999	0.999
RICP09H5800944 (16)	0.228	0.231	0.240	0.240	0.230	0.229	0.226	0.036	0.039	0.035	0.037	0.033	0.069	0.066	0.000	0	0.999
PI273585LET (17)	0.226	0.230	0.238	0.238	0.229	0.227	0.225	0.035	0.037	0.035	0.036	0.033	0.071	0.067	0.001	0.001	0

Numbers on the top of each column correspond to the accessions labels given at the beginning of each row.

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Table 6. Genotypes within the duplication groups (DG) determined by AFLP analysis

Accession name	Species	DG	Genotypes																	
CGN05329	*L. saligna*	119	119-1 (9)	119-2 (4)	119-3 (4)		119-5 (2)													
RICP09H5801059			119-1 (3)	119-2 (2)	119-3 (13)															
HRI6382						119-4 (20)														
PI261653LET						119-4 (20)														
LAC239	*L. saligna*	131	131-1 (15)																	
RICP09H5801061			131-1 (12)		131-3 (4)		131-5 (4)													
CGN13300			131-1 (2)	131-2 (2)	131-3 (2)	131-4 (2)		131-6 (4)	131-7 (2)	131-8 (6)										
CGN04770	*L. serriola*	304	304-1 (8)		304-3 (6)			304-6 (2)		304-8 (2)										
CGN04776		305		304-2 (4)			304-5 (2)					304-10 (2)	304-11 (2)	304-12 (2)		304-14 (5)	304-15 (2)			304-18 (1)
CGN04775			304-1 (6)		304-3 (10)						304-9 (2)									
RICP09H5801200			304-1 (3)		304-3 (3)	304-4 (3)												304-16		
HRI5093			304-1 (2)		304-3 (6)				304-7 (6)					304-12 (2)	304-13 (2)				304-17 (2)	
CGN04929	*L. serriola*	312	312-1 (9)	312-2 (4)		312-4 (3)	312-5 (3)													
LAC160			312-1 (8)	312-2 (6)	312-3 (4)			312-6 (2)												
HRI4981	*L. livida*	42	42-1 (6)	42-2 (4)	42-3 (4)	42-4 (4)														
RICP09H5800944		43	42-1 (3)	42-2 (3)	42-3 (3)	42-4 (4)	42-5 (4)													
PI273585LET			42-1 (1)	42-2 (5)	42-3 (4)		42-5 (1)	42-6 (1)	42-7 (2)	42-8 (1)	42-9 (1)	42-10 (1)								

Numbers of individuals per genotype are indicated in parentheses.

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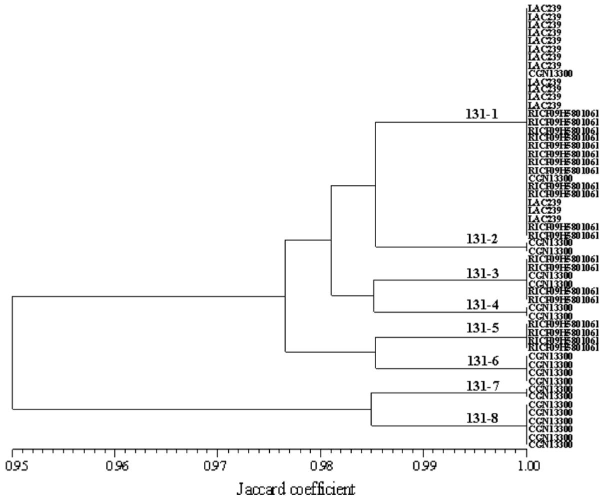

Fig. 3. Phenogram of genotypes found in duplication group 131, based on Jaccard's (1908) coefficient of similarity, as an example for genotypes existing within duplication groups. Genotype labels are on the branches.

occur. This was shown by Waycott and Fort (1994) who used morphological analysis and RAPDs to identify duplicates within *L. sativa*, leading to similarity coefficients between nearly identical accessions of >92%.

However, since *Lactuca* species are mostly inbreeding with only sparse evidence of spontaneous hybrids (Lindqvist, 1960), it may be tempting to define as duplicates those accessions showing 100% similarity, as has been done for some autogamous and clonal crops (Virk *et al.*, 1995; McGregor *et al.*, 2002).

The question remains: what to do with accessions that are very similar but not identical. The proposed approach is to analyse the genotypes within duplication groups prior to a decision about redundancy. The accessions that are more diverse, i.e. within which more genotypes can be observed, should be retained unless there are indications that contamination has occurred. In case duplicates are identical (have the same fingerprint), the most original one according to genebank documentation, should be maintained.

From the 78 accessions that were studied, grouped into 22 duplication groups identified on the basis of passport and morphological analysis, 17 within 7 duplication groups (approximately 21% of the analyzed material) presented a similarity coefficient above 0.995. In total, only five pairs of accessions showed identical genotypes (6% of the analyzed material) and therefore could easily be considered as redundant. This allows a first reduction

Table 7. Analyses of molecular variance in duplication groups: cases with different numbers of plants analysed

Duplication group	Variance component	$n = 20$	$n = 15$	$n = 10$
131 *L. saligna*	Variance between accessions	0.34 (39.88%)	0.29 (37.96%)	0.28 (42.22%)
LAC239	Variance within accessions	0.51 (60.12%)	0.48 (62.04%)	0.38 (57.78%)
RICP09H5801016	No. of genotypes	8	8	7
CGN13300	*P*	<0.001	<0.001	<0.001
304/305 *L. serriola*	Variance between accessions	0.29 (31.25%)	0.29 (30.60%)	0.28 (29.85%)
CGN04770	Variance within accessions	0.65 (68.75)	0.65 (69.40%)	0.66 (70.15%)
CGN04775	No. of genotypes	18	18	16
CGN04776	*P*	<0.001	<0.001	<0.001
RICP09H5801200				
HRI5093				
119 *L. saligna*	Variance between accessions	0.98 (81.56%)	0.97 (78.72%)	0.94 (82.13%)
CGN05392	Variance within accessions	0.22 (18.44%)	0.26 (21.28%)	0.21 (17.87%)
RICP09H5801059	No. of genotypes	5	5	4
HRI6382	*P*	<0.001	<0.001	<0.001
PI261653LET				
312 *L. serriola*	Variance between accessions	0.08 (13.18%)	0.12 (20.03%)	0.10 (18.13%)
CGN04929	Variance within accessions	0.51 (86.82%)	0.49 (79.97%)	0.47 (81.87%)
LAC160	No. of genotypes	6	5	4
	P	<0.001	<0.001	<0.001
42/43 *L. livida*	Variance between accessions	0.02 (3.18%)	0.02 (2.28%)	0.008 (0.96%)
HRI4981	Variance within accessions	0.78 (96.82%)	0.81 (97.72%)	0.88 (99.04%)
RICP09H5800944	No. of genotypes	10	10	10
PI273585LET	*P*	<0.001	0.021	0.23

Reduction in number of plants has been performed randomly by choosing 10 or 15 plants (*n*, number of plants), according to the random numbers from the 'tables of 2000 random digits' (Weir, 1996). Analyses have been performed with the two hierarchical levels: between accessions and within accessions belonging to a certain duplication group. *P* values are derived from permutation tests and present probability of observing larger variance components at random.

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from 78 to 73 accessions (6.4%). In the case of *L. serriola*, the most common wild *Lactuca* species, 7 out of 28 accessions (25.0%) were highly similar to others and one (3.6%) was identical to another. Much stronger tendency towards duplication was found in samples labelled as *L. livida*, where three out of seven accessions (42.8%) were highly similar and one was identical (14.3%). These results imply considerable redundancy in the tested material.

Implications for genebank management and conservation

The presented molecular findings allow some recommendations about wild *Lactuca* conservation in genebanks. First of all, given the number of wrongly classified material, the taxonomic status of all accessions should be verified by experts in this field. Second, to avoid further duplication of genebank material, the global diversity across genebanks should be assessed prior to the planning of future collection activities, as was also suggested by Guarino *et al.* (1995).

Duplication analysis can hint at problems in genebank management. Reproduction cycles with suboptimal regeneration and maintenance conditions might cause slight deviations in the genetic structure of accessions. If comparison of material regenerated at different sites shows that the diversity after regeneration changed at only one site, then the maintenance system of that respective site should be examined more closely.

Reduction of redundancy improves the cost efficiency of conservation, but will also introduce the risk of losing low frequency but potentially important diversity (Van Hintum *et al.*, 1996; Van Treuren *et al.*, 2001). With 20 plants in the sample, the probability of observing a genotype that occurs with a frequency of 0.10, 0.05 or 0.01 is 0.88, 0.64 and 0.18, respectively. If the number of plants is reduced to 15, these probabilities decrease to 0.79, 0.54 and 0.14, respectively. Similar considerations are valid in regard to the number and kind of markers applied: diversity of important traits might not be sampled by the marker system used; the higher the number or polymorphism of the marker system used the larger the chance of detecting differences between accessions. However, as noted before, differences between and within accessions are expected to occur, and decisions about redundancy have to be based on the scale of these differences.

On top of this are economic considerations; does the investment in the redundancy analysis pay off in terms of savings of capacity or increased access? Redundancy that exists in wild *Lactuca* germplasm consumes significant capacity available for the preservation of these accessions. Tracing and reducing such redundancy can, however, consume even more capacity. When appropriate data are available for reduction of redundancy, this should, obviously, be done. However, investments in tracing these redundancies should be weighed against the saving resulting from these investments.

In any case, it is therefore important to avoid duplication of germplasm prior to the inclusion of accessions in the genebank, whenever possible.

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References

Doležalová I, Krístková E, Lebeda A, Vinter V, Astley D and Boukema IW (2003) Basic morphological descriptors for genetic resources of wild *Lactuca* spp. *Plant Genetic Resources Newsletter* 134: 1–9.

Dostál J (1989) *Nová květena ČSSR, 2. díl*. Prague: Academia, pp. 1112–1115.

Doyle JJ and Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus* 12: 13–15.

Excoffier L, Smouse PE and Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479–491.

Feráková V (1977) *The Genus* Lactuca *L. in Europe*. Bratislava: Komenský University Press.

Guarino L, Ramanatha Rao V and Reid R (1995) *Collecting Plant Genetic Diversity, Technical Guidelines*. Rome, Italy: International Plant Genetic Resources Institute.

Iwatsuki K, Yamazaki T, Boufford DE and Ohba H (1995) *Flora of Japan*. vol. IIIb. Tokyo: Kodansha.

Jaccard P (1908) Nouvelles rescherches sur la distribution florale. *Bulletin de la Société Vaudoise des Sciences Naturelles* 44: 223–270.

Lebeda A, Doležalová I and Astley D (2004) Representation of wild *Lactuca* spp. (Asteraceae, Lactuceae) in world genebank collections. *Genetic Resources and Crop Evolution* 51: 167–174.

Lindqvist K (1960) The origin of cultivated lettuce. *Hereditas* 46: 319–349.

McGregor CE, Van Treuren R, Hoekstra R and Van Hintum ThJL (2002) Analysis of the wild potato germplasm of the series *Acaulia* with AFLPs: Implications for *ex situ* conservation. *Theoretical and Applied Genetics* 104: 146–156.

Rohlf FJ (2002) *NTSYSpc: Numerical Taxonomy System, ver. 2.11a*. Detauket, NY: Exter Publishing, Ltd., pp. 381–391.

Sretenović Rajičić T and Dehmer KJ (2008) Analysis of wild *Lactuca* genebank accessions and implication on wild species conservation. In: Maxted, Ford-Lloyd, Kell, Iriondo, Dulloo, Turok (eds) *Crop Wild Relative Conservation and Use*. CABI Publishing, pp. 429–436.

Spooner D, Van Treuren R and De Vicente MC (2006) Molecular markers for genebank management. IPGRI Technical Bulletin No. 10. International Plant Genetic Resources Institute, Rome, Italy.

Van Hintum ThJL, Boukema IW and Visser DL (1996) Reduction of duplication in a *Brassica oleracea* germplasm collection. *Genetic Resources and Crop Evolution* 43: 343–349.

Van Treuren R, Van Soest LJM and Van Hintum ThJL (2001) Marker-assisted rationalisation of genetic resource collections: a case study in flax using AFLPs. *Theoretical and Applied Genetics* 103: 144–152.

Virk PS, Newbury HJ, Jackson MT and Ford-Lloyd BV (1995) The identification of duplicate accessions within a rice germplasm collection using RAPD analysis. *Theoretical and Applied Genetics* 90: 1049–1055.

Waycott W and Fort SB (1994) Differentiation of nearly identical germplasm accessions by a combination of molecular and morphologic analyses. *Genome* 37: 577–583.

Weir BS (1996) *Genetic Data Analyses II*. Sunderland, MA: Sinauer Associates, Inc.

Winfield MO, Arnold GM, Cooper F, Le Ray M, White J, Karp A and Edwards KJ (1998) A study of genetic diversity in *Populus nigra* subsp. *betulifolia* in the Upper Severn area of the UK using AFLP markers. *Molecular Ecology* 7: 3–10.

Yeh FC and Boyle TJB (1997) Population genetic analysis of co-dominant and dominant markers and quantitative traits. *Belgian Journal of Botany* 129: 157.