

Genetic resources collections of leafy vegetables (lettuce, spinach, chicory, artichoke, asparagus, lamb's lettuce, rhubarb and rocket salad): composition and gaps

R. van Treuren · P. Coquin · U. Lohwasser

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Abstract Lettuce, spinach and chicory are generally considered the main leafy vegetables, while a fourth group denoted by 'minor leafy vegetables' includes, amongst others, rocket salad, lamb's lettuce, asparagus, artichoke and rhubarb. Except in the case of lettuce, central crop databases of leafy vegetables were lacking until recently. Here we report on the update of the international *Lactuca* database and the development of three new central crop databases for each of the other leafy vegetable crop groups. Requests for passport data of accessions available to the user community were addressed to all known European collection holders and to the main collection holders located outside Europe. Altogether, passport data of 17,530 accessions from a total of 129 collections were collected. The four separate databases were made available on line via a common entry page accessible at <http://documents.plant.wur.nl/cgn/pgr/LVintro/>.

Based on a literature study, an analysis of the gene pool structure of the crops was performed and an inventory was made of the distribution areas of the species involved. The results of these surveys were related to the contents of the newly established databases in order to identify the main collection gaps. Priorities are presented for future germplasm acquisition aimed at improving the coverage of the crop gene pools in *ex situ* collections.

Keywords Chicory · Crop database · Germplasm availability · Lettuce · Minor leafy vegetables · Spinach

Introduction

Leafy vegetables constitute a highly variable group of crop plants, which are grown for their edible leaves, which are rich sources of minerals and vitamins. Lettuce, spinach and chicory are generally considered the main leafy vegetables because of the worldwide human consumption and economical importance. For these three crops combined, the world production quantity in 2008 reached 38.11 million tons on a harvested area of 1.95 million hectares (FAO 2010). Other leafy vegetables include, amongst others, rocket salad, lamb's lettuce, asparagus, artichoke and rhubarb (Lebeda and Boukema 2001). This fourth group is denoted by 'minor leafy vegetables', which refers more to the level of attention these crops have received

R. van Treuren (✉)
Centre for Genetic Resources, The Netherlands (CGN),
Wageningen University and Research Centre,
P.O. Box 16, 6700 AA Wageningen, The Netherlands
e-mail: Robbert.vanTreuren@WUR.NL

P. Coquin
Variety Study Division, Domaine de la Boisselière,
GEVES, 49250 Brion, France

U. Lohwasser
Department of Genebank, Leibniz Institute of Plant
Genetics and Crop Plant Research, Corrensstrasse 3,
06466 Gatersleben, Germany

in the past, rather than to their market impact. For example, the worldwide harvested area of asparagus was 1.39 million hectares in 2008, which exceeds the value of 1.06 for lettuce and chicory combined (FAO 2010). Furthermore, crops such as rocket salad and lamb's lettuce have increased in production because of their use in processed products, such as mixed salad packages that have received growing interest from consumers during the last two decades.

European breeding companies, next to companies located in the United States, have a strong position in the seed market of leafy vegetables. As in many vegetable crops, breeding activities in leafy vegetables are mainly directed to quality characters and improvement of the resistance against pests and diseases. Currently, resistance to lettuce downy mildew (*Bremia lactucae* Regel) and lettuce aphid [*Nasonovia ribisnigri* (Mosley)] are the main characters in lettuce breeding (Lebeda et al. 2007; Mou 2008), while resistance to spinach downy mildew is of main interest in the improvement of spinach cultivars (Correll et al. 2003; Morelock and Correll 2008). The prime characters in chicory breeding vary among the different species and subspecies. The two main species within the genus *Cichorium* are *C. endivia* L., including ssp. *endivia* (cut and plain endive), and *C. intybus* L., including var. *foliosum* Hegi (leaf chicory, witloof) and var. *sativum* (Bisch.) Janch. (root chicory). For witloof and root chicory, the latter being used either for roasting or inulin production, breeding efforts focus on productivity and improvement of nutritional and taste components, in addition to the improvement of the resistance to pests and diseases (Doré and Varoquaux 2006). For cut and plain endive and for the Italian leaf chicory cultivar groups (e.g. Chioggia and Verona), adaptation to novel growing habits and resistance improvement to pests and diseases are the main characters of interest (Bellamy 2000). Only little is known about breeding activities for minor leafy vegetables. For lamb's lettuce one main aspect is to develop a strategy for resistance against downy mildew (*Peronospora valerianellae* Fuckel) (Pietrek and Zinkernagel 2002). Other breeding programmes are dealing with marker assisted selection for the identification of special agronomic traits in artichoke (Martin et al. 2008, 2010) and with hybrid breeding in asparagus (Scholten and Boonen 1996; Moreno et al. 2010). Particularly in the case of novel resistances, crop related wild relatives are increasingly being explored to find the characters of

interest (Hodgkin and Hajjar 2008). Efficient access to these genetic resources is therefore essential in modern plant breeding.

In addition to the presence of desired traits, the level of inter-fertility with cultivated plants is one of the key factors determining the usefulness of wild species in plant breeding. Species may be classified according to their position within the total gene pool following the principles of Harlan and de Wet (1971) who distinguished three categories of taxa based on the level of inter-fertility. The primary gene pool is formed by the cultivated and wild species that can be inter-crossed relatively easy, generally resulting in fertile offspring. The secondary gene pool consists of species that show only low levels of fertility with cultivated plants, although gene transfer is possible through considerable effort. The tertiary gene pool is constituted by species that usually do not display inter-fertility with cultivated plants, requiring techniques such as bridge crosses or embryo rescue to obtain some fertile offspring. Studies on species relationships within leafy vegetable crops have been performed predominantly for lettuce (for a review see Lebeda et al. 2007).

A survey of genetic resources collections of leafy vegetables in 1999 showed that approximately 23,000 accessions were conserved on a global scale (Van Hintum and Boukema 1999). In 2000, a central crop database for lettuce, called the international *Lactuca* database (ILDB), was developed (Stavelikova et al. 2002). However, availability of the included material to the user community was not considered. Until recently, databases for other leafy vegetables were lacking. Here we report on the recent update of the ILDB and the establishment of central crop databases for spinach, chicory and minor leafy vegetables, taking availability of the accessions into account. The composition of the databases is presented and analyzed with respect to species representation and coverage of the main distribution areas in order to identify important collection gaps that may serve as guidelines for future germplasm acquisition.

Materials and methods

Database development

The leafy vegetable crops that were addressed in the present paper followed the working group structure of

the European Cooperative Programme for Plant Genetic Resources (ECPGR). However, distinction between the different vegetable crop groups is not precisely defined. For example, the *Brassicaceae* are treated as a separate group although many cabbages are grown for their edible leaves, while rocket salad is considered a leafy vegetable although this crop is often included in *Brassica* collections. Dill is included in the Medicinal and Aromatic Plants working group although the leaves form one of the main use aspects of this herb. The crops considered by the leafy vegetables working group of ECPGR was established in 2000 during a meeting of the network coordinating group on vegetables. Lettuce, spinach and chicory were considered the main crops, while the minor leafy vegetables included, amongst others, rocket salad, lamb's lettuce, asparagus, artichoke and rhubarb (Lebeda and Boukema 2001).

To update the ILDB and to develop databases for spinach, chicory and minor leafy vegetables, it was aimed to include all European collections and the main collections located outside Europe. A request to provide accession information was directed to the members of the ECPGR working group on leafy vegetables (http://www.ecpgr.cgiar.org/Workgroups/Leafy_Vegetables/Leafy_Vegetables.htm), the holders of collections included in EURISCO (http://eurisco.ecpgr.org/home_page/home.php), the participants in the EU GENRES project "Leafy vegetables germplasm, stimulating use" (<http://documents.plant.wur.nl/cgn/pgr/leafyveg/>) and the main non-European collection holders listed in the Bioversity Directory of Germplasm Collections (http://www2.bioversityinternational.org/Information_Sources/Germplasm_Databases/Germplasm_Collection_Directory/). Collection holders that were not part of the aforementioned groups but who were included in the first version of the ILDB were also approached in the case of lettuce. Requests for data were accompanied by an Excel spreadsheet containing instructions for data submission and a format for information on 35 passport descriptors largely following the EURISCO descriptor list (http://eurisco.ecpgr.org/documents/eurisco_descriptors-update-feb2008.pdf). In the request for data it was emphasized to provide information only of accessions that in principle are available for distribution to users. Received data were corrected in case of obvious spelling and formatting errors, but otherwise remained untreated. No attempts were

made to verify the actual availability of the accessions, neither was the validity of the supplied data about taxonomy and geographic origin verified. Furthermore, the data were not checked for the existence of potential duplicate accessions within or between collections. Requesting and processing of the data was carried out between 2007 and 2009. Requests for data were repeated in case no response was received. In a few cases where no data were received for European collections, the data from EURISCO were downloaded and included in the database. For lettuce, the data from Iran in the previous version of the ILDB were maintained because of missing updates. The results presented in the present paper were based on the data extracted in March 2010 from the international leafy vegetables databases that can be accessed on line via <http://documents.plant.wur.nl/cgn/pgr/LVintro/>. Each of the four databases also include an overview of the contributors to the databases, together with links to the institutional websites and to the contact persons of the collections.

Wild species and distribution areas

In order to identify main collection gaps within the newly established databases an inventory was made of the crop related wild species and their distribution areas. The International Plant Names Index (IPNI; <http://www.ipni.org/index.html>) of September 2009 was used as the basis for the inventory. Species names were checked for synonymy and reassignment to other genera and reported distribution areas were collected using information provided by IPNI, GRIN Taxonomy for Plants (<http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl>), Flora Europaea (<http://rbgweb2.rbge.org.uk/FE/fe.html>), Mansfeld's World Database of Agricultural and Horticultural Crops (<http://mansfeld.ipk-gatersleben.de>), Tropicos (<http://www.tropicos.org/>) and the Cichorieae Portal (<http://wp6-cichorieae.e-taxonomy.eu/portal/>). For lettuce, the results of an earlier inventory (Lebeda et al. 2004) were included in the analyses.

To structure the lettuce gene pool we followed the classification described by Koopman et al. (1998; 2001) and by GRIN Taxonomy for Plants. This classification system is largely in line with the one described earlier by Zohary (1991). However, complete consensus about species relationships within the genus *Lactuca* is still lacking and discussed in more

detail by Lebeda et al. (2007). Hybrids and taxonomic classifications below the species level, such as subspecies and forms, were disregarded in the case of lettuce because of the size of the genus *Lactuca*. Also for chicory, species relationships within the genus *Cichorium* are still debated. Here we followed the chicory gene pool structure suggested by Kiers et al. (2000) and B. Desprez (pers. comm.). Concerning the gene pool structure of spinach usually three species are distinguished, namely cultivated spinach (*Spinacia oleracea* L.), which is inter-fertile with the wild species *S. tetrandra* Steven ex M. Bieb. (Sneep 1957) and *S. turkestanica* Iljin (Handke et al. 2000). For the group of minor leafy vegetables the analysis of the gene pool structure was restricted to rocket salad (*Diplotaxis* and *Eruca*) because of the relatively high number of accessions involved for this crop and because of the limited information about species relationships available for the other crops. In order to structure the gene pool of *Diplotaxis* and *Eruca* the crossing ability and inter-fertility between species in the entire family Brassicaceae was studied by means of a literature survey. Firstly, the relationship between species within the genus *Eruca* and within the genus *Diplotaxis* was investigated (Eschmann-Grupe et al. 2003; Warwick et al. 2007). Secondly, the relationship of each of these genera with other Brassicaceae genera and species was determined (Harberd and McArthur 1980; Warwick and Black 1993; Prakash et al. 1999; Snowdon et al. 2007; Warwick et al. 2009).

To summarize geographical information, countries were grouped together according to the regional subdivision of the United Nations Statistics Division (<http://unstats.un.org/unsd/methods/m49/m49regin.htm>) with the exception that for lettuce and spinach the Russian Federation was regarded to comprise Northern Asia instead of belonging to Eastern Europe, while Cyprus was considered to belong to Southern Europe instead of Western Asia.

Results

Lettuce

Survey of the International Plant Names Index revealed a total number of 538 *Lactuca* species, of which 357 referred to synonyms and basionyms,

whereas for another 51 the taxonomic status and their belonging to the genus *Lactuca* was questionable. Of the remaining 130 species, 20 are generally considered to be part of the lettuce gene pool (Table 1).

Accession data were collected from a total of 30 collections, including contributions from Armenia, Iran and the United States. Altogether, the data of 11,643 accessions were included in the ILDB. The main part of the ILDB is constituted by landraces, cultivars, research lines and breeding materials of cultivated lettuce (*Lactuca sativa* L.) represented with 10,198 accessions (88%). A highly unbalanced distribution was observed for species belonging to the crop related wild gene pool as 96% of the accessions are covered by only three species, namely *L. serriola* L., *L. saligna* L. and *L. virosa* L. Eight species, including *L. azerbaijanica* Rech. f. and *L. scarioloides* Boiss. that are part of the primary lettuce gene pool, even lack a single accession in the ILDB. Concerning the primary gene pool, *L. serriola* L. with 987 accessions is the only wild species that is well represented (Table 1). The category “unknown and other taxa” included 167 accessions, comprising specimens of *L. biennis* (Moench) Fernald, *L. canadensis* L., *L. dentata* Makino, *L. dissecta* D. Don, *L. floridana* (L.) Gaertn., *L. homblei* De Wild., *L. indica* L., *L. livida* Boiss. et Reut., *L. perennis* L., *L. raddeana* Maxim. and *L. tenerrima* Pourret, a number of hybrids and undetermined *Lactuca* species, and a few samples from the genera *Chondrilla*, *Cicerbita*, *Mycelis* and *Steptorhamphus* that are related to *Lactuca* (results not shown).

The reported geographic distribution area of the species of the lettuce gene pool and the degree of coverage by accessions in the ILDB is also presented in Table 1. Accessions of cultivated lettuce have been collected from all regions of the world, the majority originating from Europe and Asia and relatively few from Africa. All the main horticultural types, i.e. ‘butterhead’, ‘crisphead’, ‘cutting’, ‘cos’, ‘stalk’, and ‘Latin lettuce’, are well represented in the ILDB with a wide variety in geographic origin (results not shown). The more primitive horticultural type ‘oil-seed lettuce’, which has a restricted cultivation in the Middle East, is represented by six *L. sativa* L. accessions, all originating from Egypt. No data on geographic origin was provided for 3,063 accessions of *L. sativa* L. (30%).

Table 1 Number of accessions of *Lactuca* species of the primary, secondary and tertiary (separated by vertical lines) lettuce gene pool included in the international *Lactuca* database (ILDB) per reported geographic region within the total distribution area

Region	<i>L. sativa</i> L.	<i>L. aculeata</i> Boiss.	<i>L. altaica</i> Fish. et C.A. Mey.	<i>L. azerbaijanica</i> Rech. f.	<i>L. dregeana</i> DC.	<i>L. georgica</i> Grossh.	<i>L. scariolooides</i> Boiss.	<i>L. serriola</i> L.	<i>L. satigna</i> L.	<i>L. virosa</i> L.
Europe	5,702							458	37	67
East	1,107							119	6	2
North	406							19	3	3
South	2,096							116	27	23
West	2,093							204	1	39
Asia	1,029	2	1	0		0	0	344	48	15
Central	8		0			0		77	3	
East	152		0					0		
North	83		0			0		24	4	7
South	108	0	0	0		0	0	9	0	1
Southeast	14									
West	664	2	1			0	0	234	41	7
Africa	64				0			14	0	0
Central	1									
East	3							1		0
North	58							13	0	0
South	1				0			0		
West	1									
America	322							16	0	1
Caribbean	12									
Central	3							0	0	
North	281							16	0	0
South	26							0	0	1
Oceania	18							0	0	0
Unknown	3063	0	6	0	4	1	0	155	18	58
Total	10,198	2	7	0	4	1	0	987	103	141

Table 1 continued

Region	<i>L. acanthifolia</i> (Willd.) Boiss.	<i>L. aurea</i> (Sch.Bip. ex Vis. et Pančić) Stebbins	<i>L. longidentata</i> Moris	<i>L. orientalis</i> Boiss.	<i>L. quercina</i> L.	<i>L. sibirica</i> Benth. ex Maxim.	<i>L. taraxacifolia</i> Schumach. et Thonn.	<i>L. tatarica</i> C.A. Mey.	<i>L. viminea</i> (L.) J. Presl et C. Presl	<i>L. watsoniana</i> Trel.
Europe	0	0	0	2	2	1	2	10	0	
East	0	0	2	0	0	0	0	2		
North	0	0	0	0	0	1	0			
South	0	0	0	0	0	0	2	4	0	
West	0	0	0	0	0	0	2	4		
Asia	0	0	0	0	2	0	4	7		
Central			0	0	0	0	0	0		
East			0	0	0	0	0			
North			0	0	0	0	1	0		
South			0	0	0	0	0	0		
Southeast			0	0	0	0	0			
West	0	0	0	0	2	0	3	7		
Africa			0	0	0	0	0	0		
Central										
East										
North			0	0				0		
South										
West							0			
America							0			
Caribbean										
Central										
North							0			
South										
Oceania										
Unknown	0	0	0	0	0	0	0	2	3	
Total	0	0	0	0	4	1	8	20	0	

Reported regions not represented in the ILDB are denoted by '0'. Totals per continent are denoted in bold

The majority of wild species of the primary gene pool have their distribution area in Asia, with the exception of *L. dregeana* DC. that is restricted to South Africa and *L. serriola* L. that can be found on all continents (Table 1). For *L. serriola* L., being the only wild species of the primary gene pool that is well represented in the ILDB, relatively few samples were collected from Africa (14) and America (16), and none from Oceania. Like *L. serriola* L., also *L. saligna* L. and *L. virosa* L., constituting the secondary gene pool, show a cosmopolitan distribution. The predominance of accessions of these species from Europe and Asia in the ILDB was even more clear-cut than observed for *L. serriola* L. as no accessions from Africa and Oceania are included and America is only represented by a single sample of *L. virosa* L. The ten species of the tertiary gene pool are mainly distributed in Europe and Asia, and are represented by low numbers of accessions, or not at all, in the ILDB.

Spinach

In the International Plant Names Index only 14 *Spinacia* species were reported, of which seven were found to be synonyms and four were reassigned to the genus *Atriplex*. Therefore, the gene pool of spinach is quite small, consisting of cultivated spinach (*Spinacia oleracea* L.) and the wild species *S. tetrandra* Steven ex M. Bieb. and *S. turkestanica* Iljin that are interfertile with each other. Data of 2,017 accessions were collected from a total of 25 collections located mainly in Europe, with the exception of Armenia, Azerbaijan and the United States.

The international spinach database includes the data of 1,769 accessions of cultivated spinach originating from a wide variety of geographic regions (Table 2). As was observed for cultivated lettuce, the majority of accessions of cultivated spinach originated from Europe and Asia. The wild species are poorly represented in the database including only 12 accessions of *S. tetrandra* Steven ex M. Bieb. and 14 of *S. turkestanica* Iljin. Although for each of these species a single accession was included with Germany as documented origin country, their distribution areas are restricted to Asia (Uotila 1997). The database contains 222 accessions (11%) with missing taxonomic data.

Chicory

Thirty seven *Cichorium* species were found in IPNI including 32 synonyms. The chicory gene pool appeared quite limited in terms of number of different species as it is represented by the two cultivated species *C. endivia* L. and *C. intybus* L. and the three wild species *C. calvum* Sch. Bip., *C. spinosum* Jacq. and *C. botae* De Flers (Table 3). Due to the very low fertility resulting from interspecific crosses and the very diverse use aspects of the cultivated types, the species *C. endivia* L. (cut and plain endive) and *C. intybus* L. (root chicory and leaf chicory, the latter including witloof and the Italian chicory cultivar groups such as Chioggia and Verona) are separated in the primary and secondary gene pool, respectively. *C. endivia* L. is a self-compatible annual, while *C. intybus* L. is a predominantly self-incompatible biennial to perennial species (Rick 1953; Eenink 1981; Cichan 1983).

In the international chicory database *C. endivia* L. and *C. intybus* L. are represented with 585 (34%) and 947 accessions (55%), respectively. The taxonomic status of 184 accessions (11%) was found to be unknown. The primary gene pool also includes the wild subspecies *C. endivia* L. ssp. *pumilum* (Jacq.) Cout. (9 accessions) and the wild species *C. calvum* Sch. Bip. that is not represented in the ICDB. The secondary gene pool also includes the wild species *C. spinosum* Jacq. that has also been regarded as a subspecies of *C. intybus* L. and that is lacking from the ICDB. The tertiary gene pool is constituted by *C. botae* De Flers that occurs in Saudi Arabia and Yemen and that is completely infertile with *C. endivia* L. and *C. intybus* L.. No accessions of this species occur in the ICDB. Altogether the data of 1,716 accessions were collected from 21 collections located mainly in Europe. Accessions from the United States represent less than 5% of the ICDB. The main part of the ICDB is formed by *C. intybus* L. var. *foliosum* Hegi (36%), *C. endivia* L. ssp. *endivia* (32%) and *C. intybus* L. var. *intybus* (11%) that are mainly landraces and cultivars of European origin.

Minor leafy vegetables

The database of the minor leafy vegetables (IMDB) consists of 163 accessions of artichoke, 356 of

Table 2 Number of accessions of *Spinacia* species belonging to the primary spinach gene pool included in the international spinach database (ISDB) per reported geographic region within the total distribution area

	<i>S. oleracea</i> L.	<i>S. tetrandra</i> Steven ex M. Bieb.	<i>S. turkestanica</i> Iljin	Unknown
Europe	636	1	1	96
East	79			23
North	82			25
South	158			10
West	317	1	1	38
Asia	741	8	5	95
Central	4		4	1
East	133			37
North	20	1	1	10
South	111	0	0	16
Southeast	1			1
West	472	7	0	30
Africa	10			5
Central				
East	3			
North	7			5
South				
West				
Americas	14			26
Caribbean				
Central	1			
North	13			26
South				
Oceania	3			
Unknown	365	3	8	
Total	1,769	12	14	222

Reported distribution areas not represented in the ISDB are denoted by '0'. Totals per continent are denoted in bold

asparagus, 77 of lamb's lettuce, 326 of rhubarb, and 1,232 of rocket salad, with a total number of 2,154 accessions (Table 4). A gap analysis was carried out for rocket salad, which was studied separately for the genera *Eruca* and *Diplotaxis* that both are used as salad (Table 5). In the International Plant Names Index 50 records were found for *Eruca* and 112 for *Diplotaxis*. For *Eruca*, 46 records could be ascribed to synonyms and basionyms or displayed a questionable status. Only four names are accepted, of which *Eruca sativa* Mill. together with *E. vesicaria* (L.) Cav. that is synonymous to *E. sativa* Mill., and *Eruca pinnatifida* (Desf.) Pomel, which is sometimes described as a subspecies of both other species represent the primary gene pool of *Eruca*, whereas the others are wild species that are not crossable with the species of the primary gene pool (Table 5A). For *Diplotaxis*, 14 accepted names could be recognized of

which *D. tenuifolia* (L.) DC. (Table 5B) and *D. muralis* (L.) DC. (Table 5C) are used as cultivated crops in addition to their use as wild plants. *D. tenuifolia* (L.) DC. can also be found in the secondary gene pool of *Eruca*, and *D. harra* (Forssk.) Boiss. belongs to the tertiary gene pool of *D. muralis* (L.) DC. An important position is occupied by many *Brassica* species as they occur in the secondary and tertiary gene pool of *Eruca* as well as of *Diplotaxis*, while most of the other species of the genus *Diplotaxis* and *Erucastrum* are closely related but not involved in the gene pool.

The genera *Eruca* and *Diplotaxis* are represented in the IMDB with high numbers of accessions belonging to the primary gene pool and with even higher numbers of secondary and tertiary gene pool accessions, the latter being due to the involvement of *Brassica* species. The native distribution of cultivated

Table 3 Number of accessions of *Cichorium* species belonging to the primary, secondary and tertiary (separated by vertical lines) chicory gene pool included in the international chicory database (ICDB) per reported geographic region within the total distribution area

	<i>C. endivia</i> L. sp. <i>endivia</i>	<i>C. endivia</i> L. sp. unknown	<i>C. endivia</i> L. sp. <i>pumilum</i> (Jacq.) Cout.	<i>C. calvum</i> Sch. Bip.	<i>C. intybus</i> L. var. <i>intybus</i>	<i>C. intybus</i> L. var. <i>foliosum</i> Hegi	<i>C. intybus</i> L. var. <i>sativum</i> (Bisch.) Janch.	<i>C. intybus</i> L. var. unknown	<i>C. spinosum</i> Jacq.	<i>C. botae</i> De Flers	Unknown
Europe	326	13	3		126	427	41	42	0		121
East	2				25	2	10	11			3
North	4				7	2					
South	131	9	3		49	158	4	17	0		26
West	189	4			45	265	27	14			92
Asia	8	1	3	0	3	5	2	10		0	7
Central											
East	4										
North					1	2	2	3			
South	1		2		2			6			5
Southeast											
West	3	1	1	0		3		1		0	2
Africa	6		0					1			2
Central											
East	1							1			1
North	5		0								1
South											
West											
Americas	21	15			1	6	6	6			1
Caribbean											
Central											
North	14	15			1	6	4	5			
South	7						2	1			1
Oceania											
Unknown	186		3		62	187	20	2			53
Total	547	29	9	0	192	625	69	61	0	0	184

Reported distribution areas not represented in the ICDB are denoted by '0'. Totals per continent are denoted in bold

Table 4 Number of accessions of species related to artichoke (A), asparagus (B), lamb's lettuce (C), rhubarb (D) and rocket salad (E) included in the international minor leafy vegetables database (IMDB)

Species	IMDB	Species	IMDB
(A) Artichoke (<i>Cynara</i>)—163 accessions			
<i>C. alba</i> DC.	1	<i>C. humilis</i> L.	3
<i>C. cardunculus</i> L.	131	<i>C. scolymus</i> L. ^a	27
<i>C. cornigera</i> Lindl.	1		
(B) Asparagus (<i>Asparagus</i>)—356 accessions			
<i>A. acutifolius</i> L.	8	<i>A. maritimus</i> Mill.	1
<i>A. africanus</i> Lam.	2	<i>A. officinalis</i> L.	224
<i>A. albus</i> L.	2	<i>A. oligoclonos</i> Maxim.	1
<i>A. aphyllus</i> L.	1	<i>A. pastorianus</i> Webb et Berthel.	1
<i>A. arborescens</i> Willd. ex Schult. et Schult. f.	1	<i>A. plumosus</i> Baker ^b	1
<i>A. asparagoides</i> (L.) Druce	4	<i>A. pseudoscaber</i> Grecescu	1
<i>A. brachyphyllus</i> Turcz.	1	<i>A. racemosus</i> Willd.	8
<i>A. bucharicus</i> Iljin	1	<i>A. scoparius</i> Lowe	2
<i>A. caspicus</i> Hohen.	1	<i>A. scandens</i> Thunb.	2
<i>A. cochinchinensis</i> (Lour.) Merr.	3	<i>A. setaceus</i> (Kunth) Jessop	9
<i>A. dauricus</i> link	3	<i>A. stipularis</i> Forssk.	2
<i>A. declinatus</i> L.	3	<i>A. tenuifolius</i> Lam.	1
<i>A. densiflorus</i> (Kunth) Jessop	19	<i>A. umbellatus</i> Link	2
<i>A. falcatus</i> L.	4	<i>A. verticillatus</i> L.	14
<i>A. gonocladus</i> Baker	1	<i>A. virgatus</i> Baker	4
<i>A. laricinus</i> Burch.	4	<i>A. sp.</i>	24
<i>A. litoralis</i> Steven	1		
(C) Lamb's lettuce (<i>Valerianella</i>)—77 accessions			
<i>V. carinata</i> Loisel.	1	<i>V. lusitanica</i> Font Quer	1
<i>V. coronata</i> (L.) DC.	2	<i>V. pumila</i> (L.) DC.	4
<i>V. dentata</i> (L.) Pollich	2	<i>V. rimosa</i> Bastard	2
<i>V. eriocarpa</i> Desv.	2	<i>V. sp.</i>	4
<i>V. locusta</i> (L.) Laterr.	59		
(D) Rhubarb (<i>Rheum</i>)—326 accessions			
<i>R. altaicum</i> Losinsk.	3	<i>R. palmatum</i> L.	12
<i>R. australe</i> D. Don	6	<i>R. rhabarbarum</i> L.	162
<i>R. compactum</i> L.	3	<i>R. rhaponticum</i> L.	23
<i>R. crispum</i> Hort. ex G. Don	1	<i>R. ribes</i> L.	2
<i>R. emodi</i> Wall. ex Meisn. ^c	1	<i>R. tanguticum</i> (Maxim. ex Regel) Maxim. ex Balf.	3
<i>R. hybridum</i> Murray	6	<i>R. tataricum</i> L. f.	7
<i>R. macrocarpum</i> Losinsk.	1	<i>R. tibeticum</i> Maxim.	2
<i>R. maculatum</i> C. Y. Cheng et T. C. Kao	1	<i>R. undulatum</i> L. ^d	1
<i>R. maximowiczii</i> Losinsk.	3	<i>R. webbianum</i> Royle	1
<i>R. moorcroftianum</i> Royle	1	<i>R. wittrockii</i> C. E. Lundstr.	2
<i>R. officinale</i> Baill.	13	<i>R. sp.</i>	72
E. Rocket salad—1,232 accessions			
<i>Bunias erucago</i> L.	2	<i>Diplotaxis sp.</i>	41
<i>Diplotaxis acris</i> (Forssk.) Boiss.	1	<i>Eruca pinnatifida</i> (Desf.) Pomel	13
<i>Diplotaxis assurgens</i> (Delile) Gren.	15	<i>Eruca sativa</i> Mill.	495

Table 4 continued

Species	IMDB	Species	IMDB
<i>Diplotaxis berthautii</i> Braun-Blanq. et Maire	2	<i>Eruca vesicaria</i> (L.) Cav.	171
<i>Diplotaxis brachycarpa</i> Godr.	3	<i>Eruca</i> sp.	7
<i>Diplotaxis brevisiliqua</i> (Coss.) Mart.-Laborde	3	<i>Erucaria cakiloidea</i> O. E. Schulz	1
<i>Diplotaxis catholica</i> (L.) DC.	16	<i>Erucaria erucarioides</i> Müll. Berol.	1
<i>Diplotaxis cossoniana</i> (Reut. ex Boiss.) O. E. Schulz	1	<i>Erucaria hispanica</i> Gaertn.	8
<i>Diplotaxis cretacea</i> Kotov	3	<i>Erucaria microcarpa</i> Boiss.	1
<i>Diplotaxis eruroides</i> (L.) DC.	31	<i>Erucaria ollivieri</i> Maire	1
<i>Diplotaxis glauca</i> O. E. Schulz	2	<i>Erucaria pinnata</i> (Viv.) Tackholm et Boulos	1
<i>Diplotaxis gomez-campo</i> Mart.-Laborde	1	<i>Erucastrum abyssinicum</i> R. E. Fr.	1
<i>Diplotaxis gracilis</i> O. E. Schulz	2	<i>Erucastrum arabicum</i> Fisch. et C. A. Mey.	1
<i>Diplotaxis griffithii</i> Hook. f. et Thomson	2	<i>Erucastrum brevirostre</i> (Maire) Gomez-Campo	2
<i>Diplotaxis harra</i> (Forssk.) Boiss.	31	<i>Erucastrum canariense</i> Webb et Berthel.	1
<i>Diplotaxis hirta</i> (Chev.) Rustan et L. Borgen	1	<i>Erucastrum cardaminoides</i> (Webb ex Christ) O. E. Schulz	7
<i>Diplotaxis ibicensis</i> (Pau) Gomez-Campo	7	<i>Erucastrum elatum</i> (Ball) O. E. Schulz	8
<i>Diplotaxis ilorcitana</i> (Sennen) Aedo, Mart.-Laborde et Munoz Garm.	4	<i>Erucastrum gallicum</i> (Willd.) O. E. Schulz	6
<i>Diplotaxis muralis</i> (L.) DC.	24	<i>Erucastrum ifniense</i> Gomez-Campo	2
<i>Diplotaxis ollivieri</i> Maire	2	<i>Erucastrum leucanthum</i> Coss. et Durieu	10
<i>Diplotaxis siettiana</i> Maire	2	<i>Erucastrum littoreum</i> (Pau et Font Quer) Maire	6
<i>Diplotaxis siifolia</i> Kunze	25	<i>Erucastrum nasturtifolium</i> (Poir.) O. E. Schulz	20
<i>Diplotaxis simplex</i> (Viv.) Spreng.	13	<i>Erucastrum pachypodium</i> (Chiov.) Jonsell	1
<i>Diplotaxis tenuifolia</i> (L.) DC.	49	<i>Erucastrum rifanum</i> (Emb. et Maire) Gomez-Campo	4
<i>Diplotaxis tenuisiliqua</i> Delile	26	<i>Erucastrum strigosum</i> (Thunb.) O. E. Schulz	1
<i>Diplotaxis villosa</i> Boulos et W. Jallard	1	<i>Erucastrum varium</i> (Durieu) Durieu	33
<i>Diplotaxis viminea</i> (L.) DC.	3	<i>Erucastrum virgatum</i> C. Presl	38
<i>Diplotaxis virgata</i> (Cav.) DC.	68	<i>Erucastrum</i> sp.	7
<i>Diplotaxis vogelii</i> O. E. Schulz	1	<i>Pseuderucaria teretifolia</i> O. E. Schulz	3

Cultivated species are denoted in bold

Upon request of the journal, authorities were added to the species name without validation in cases where author names are not provided in the IMDB

^a Listed separately in the IMDB although generally considered to belong to *C. cardunculus* L.

^b Listed separately in the IMDB although generally considered to belong to *A. setaceus* (Kunth) Jessop

^c Listed separately in the IMDB although generally considered to belong to *R. australe* D. Don

^d Listed separately in the IMDB although generally considered to belong to *R. rhabarbarum* L.

Eruca and *Diplotaxis* is Central, Southeastern and Southwestern Europe, Northern Africa and temperate Asia. These regions are all covered by accessions included in the IMDB. Moreover, the secondary and tertiary gene pools are well represented by the accessions included in the European *Brassica* database (Bras-EDB).

Discussion

Leafy vegetables databases

Crop databases are important information sources for the user community of genetic resources. Two such information systems coexist in Europe, i.e. the

Table 5 Gene pool structure of rocket salad presented separately for the cultivated species *Eruca sativa* (A) *Diplotaxis tenuifolia* (B) and *Diplotaxis muralis* (C), and the number of accessions included in the IMDB or the European *Brassica* database (Bras-EDB)

Gene pool	Species	Number of accessions in the IMDB or Bras-EDB
(A) <i>Eruca sativa</i>		
Primary	<i>Eruca sativa</i> Mill. and <i>E. vesicaria</i> (L.) Cav.	666
	<i>Eruca pinnatifida</i> (Desf.) Pomel	13
Secondary	<i>Diplotaxis tenuifolia</i> (L.) DC.	49
	<i>Brassica oleracea</i> L.	10,751
	<i>Brassica repanda</i> (Willd.) DC.	11
Tertiary	<i>Brassica oleracea</i> L.	10,751
	<i>Brassica rapa</i> L.	3,622
	<i>Brassica juncea</i> (L.) Czern.	2,260
(B) <i>Diplotaxis tenuifolia</i>		
Primary	<i>Diplotaxis tenuifolia</i> (L.) DC.	49
Secondary	<i>Brassica rapa</i> L.	3,622
	<i>Brassica juncea</i> (L.) Czern.	2,260
	<i>Brassica nigra</i> (L.) W. D. J. Koch	366
Tertiary	<i>Erucastrium virgatum</i> C. Presl	38
	<i>Brassica oleracea</i> L.	10,751
	<i>Brassica elongata</i> Ehrh.	10
(C) <i>Diplotaxis muralis</i>		
Primary	<i>Diplotaxis muralis</i> (L.) DC.	24
Secondary	<i>Brassica juncea</i> (L.) Czern.	2,260
	<i>Brassica napus</i> L.	5,480
	<i>Brassica rapa</i> L.	3,622
Tertiary	<i>Diplotaxis harra</i> (Forssk.) Boiss.	31
	<i>Erucastrium gallicum</i> (Willd.) O. E. Schulz	6

EURISCO web catalogue and the central crop databases (CCDBs), both developed and maintained under the auspices of the European Cooperative Programme for Plant Genetic Resources (Van Dooi-jeweert and Menting 2010). The advantage of the EURISCO web catalogue is that the information is supposed to be regularly updated by national focal points via national inventories. However, EURISCO is limited to European collections, does not provide access to the collections and does not contain characterization and evaluation data. Moreover, germplasm availability is not a criterion for uptake of accessions in the EURISCO database. This criterion did play a role in the establishment of the CCDBs for leafy vegetables by asking collection holders only to provide data of accessions that in principle are available for distribution to potential users. Nevertheless, availability cannot be guaranteed in each case as this depends on the reliability of the provided information and because accessions may

temporarily be unavailable due to depletion of seed stocks. In order to facilitate the request for germplasm by users, links are provided to the institutional websites and databases, while also the contact details of the curators are presented in the leafy vegetables databases. In addition, access is provided to characterization, evaluation and utilization data allowing identification of the accessions with the characters of interest. Moreover, the uptake of accession data was not limited to European collections in order to provide a more complete overview of what's available in the world. Unfortunately, thus far we did not succeed to include the data from a few non-European collections with potential relevance to the leafy vegetables databases, such as those from China with 684 lettuce and 321 spinach accessions, respectively (http://icgr.caas.net.cn/cgris_english.html).

The data of a total number of 17,530 accessions were included in the four leafy vegetables databases combined. However, the number of distinct germplasm

accessions can be expected to be considerably lower due to the presence of duplicates both within and between collections. For example, a duplication study based on passport data showed an average of 12% redundancy within four main lettuce collections, whereas only from 33 to 54% of the accessions appeared unique to single collections (Van Hintum 2000). Recently, the collection of cultivated lettuce of the Centre for Genetic Resources, the Netherlands (CGN) was rationalized using passport information and morphological and molecular data, revealing 198 redundant accessions corresponding to 13% of the collection (Van Treuren et al. 2010).

The provided passport information was checked for obvious spelling and formatting errors, but no validation of the provided data was carried out. However, taxonomic data may not always be accurate, as for example was shown in a morphological study on 78 wild *Lactuca* accessions revealing 23 taxonomic misclassifications (Sretenović Rajičić et al. 2008). Inaccuracy may also apply to geographic information when donor and origin data are confounded. For instance, this may be observed for material received from botanical gardens when accessions are given origin data identical to the country in which the donor is located, although the collection site was actually unknown (e.g. Van de Wiel et al. 2010). In such cases, in which more detailed collecting information is missing, data quality could be improved by removal of the country of origin. Also in the present study dubious origin data were observed as, for example, accessions of *S. tetrandra* Steven ex M. Bieb. and *S. turkestanica* Iljin were reported to originate from Germany although the distribution areas of these wild relatives of spinach are supposed to be restricted to Asia. In general, actual distribution areas may deviate from those currently reported because regions may have been insufficiently explored or because the occurrence of species may have altered due to climatic and/or habitat changes. Continuous enlargement of the distributional range has for example been reported for *Lactuca serriola* L. (D'Andrea et al. 2009).

Priorities for acquisition

The general aim of genebanks is to develop collections that represent the genetic diversity of a crop gene pool as widely as possible with a minimum of

redundancy (Frankel and Brown 1984). Since the total diversity of a crop gene pool can be considered the widest possible diversity, a collection gap could be defined as that part of the total diversity within a crop gene pool that is not represented in current genebank accessions. Obviously, conserving the total diversity of a crop gene pool is impossible. Moreover, since this diversity is generally not quantified it is also unclear how representative a collection is for the total genetic diversity of a crop gene pool. In the absence of adequate genetic data, priorities for acquisition may be directed to a species or a part of its known distribution area that is not, or only poorly, represented in a germplasm collection. In general, gap analysis aims to examine the extent to which taxa, locations or traits are conserved using relevant information such as taxonomic, distribution and ecological data (Maxted et al. 2008; Ramírez-Villegas et al. 2010). Based on the data collected in the present study, the following priorities for acquisition for leafy vegetables were identified.

Lettuce

The first priority concerns the wild species of the primary gene pool that are currently lacking or severely underrepresented in germplasm collections, i.e. *L. aculeata* Boiss., *L. altaica* Fisch. et C.A. Mey., *L. azerbaijanica* Rech. f., *L. dregeana* DC., *L. georgica* Grossh. and *L. scarioloides* Boiss. Because of their more restricted distribution areas, these species could be sampled less extensively as has been carried out for *L. serriola* L. The second priority involves enlargement of the number of accessions of *L. sativa* L. and *L. serriola* L. from North Africa. Relatively few samples have been collected from this area, although the Mediterranean region is considered an important part of their distribution area. The latter also applies to *L. saligna* L. and *L. virosa* L. that constitute the secondary gene pool. Since specimens of these species from North Africa are completely lacking from the ILDB, acquisition of *L. saligna* L. and *L. virosa* L. accessions from this region is the third priority. Also from America and Oceania relatively few samples of *L. serriola* L., *L. saligna* L. and *L. virosa* L. have been collected, but these regions are expected to harbour less genetic diversity due to their relatively late introduction to these continents.

The species of the tertiary gene pool are either lacking or represented poorly in the ILDB. As long as the usefulness of these species in plant breeding is minimal or not fully understood, the presence of a few specimens from the main distribution area of each of these species is considered sufficient for collection development. Therefore, introduction of tertiary gene pool species that are currently lacking in the ILDB is considered the fourth priority. The fifth priority concerns the uptake of a few specimens of the remaining *Lactuca* species that are currently absent in collections. Access to this germplasm will enable investigation of their position in the lettuce gene pool and their relevance in plant breeding, after which their representation in germplasm collections can be adjusted accordingly.

Spinach

The wild species *S. tetrandra* Steven ex M. Bieb. and *S. turkestanica* Iljin, both belonging to the primary spinach gene pool, are severely underrepresented in current germplasm collections. Acquisition of samples of these species is therefore considered the highest priority. To improve the composition of its spinach collection, sampling of *S. turkestanica* Iljin in Tajikistan and Uzbekistan was carried out by CGN in 2008. This mission resulted in 66 sampled populations of *S. turkestanica* Iljin and two collected landraces of *S. oleracea* L. Currently, these samples are being regenerated, after which they will be included in CGN's spinach collection and made available to the user community. A collection mission to the Caucasus region to sample *S. tetrandra* Steven ex M. Bieb. is planned by CGN for 2011. Spinach is generally thought to have originated in Iran and to have been introduced early by the Arabs to North Africa from which it was spread later into Europe (Morelock and Correll 2008). Since only 18 samples of cultivated spinach from Iran and 7 from North Africa are currently included in the ISDB, more extensive sampling from these areas is considered the second priority. Also, relatively few samples of *S. oleracea* L. from America are included in the ISDB. However, spinach varieties have been introduced to the United States relatively late and nowadays hybrids are the major type of cultivars (Morelock and Correll 2008), which are difficult to maintain by genebanks.

Chicory

Because the breeding potential is limited for witloof (Bellamy et al. 1996; Demeulemeester et al. 1997) as well as for endives, varietal diversification in chicory is needed. The first priority concerns the enlargement of the number of accessions of *C. endivia* L. ssp. *endivia* from North Africa and from West Asia as these regions are underrepresented in the ICDB although they are important parts of the distribution area of *C. endivia* L. ssp. *endivia*, and may provide novel variation for growing habit and disease resistance to endive breeders. The second priority concerns the wild species of the primary and secondary gene pool which are either lacking (*C. calvum* Sch. Bip. and *C. spinosum* Jacq.) or underrepresented (*C. endivia* L. ssp. *pumilum* (Jacq.) Cout.) in the ICDB. In the latter case, the African distribution area is of particular interest because accessions from this region are absent. For all these taxa, reduced distribution areas and a limited use thus far by the breeding industry are the main reasons for the existence of collection gaps. The third priority concerns acquisition of *C. botae* De Flers, the single species constituting the tertiary gene pool. Accessions of *C. botae* De Flers are lacking from the ICDB because of their infertility with *C. endivia* L. and *C. intybus* L. and hence their limited use thus far by chicory breeders.

Minor leafy vegetables

Separate investigations of the gene pool were performed for rocket salad, lamb's lettuce, asparagus, artichoke and rhubarb, the minor leafy vegetables crops that currently constitute the IMDB. The gene pool of the genus *Eruca* is well represented. The primary gene pool, consisting of *E. sativa* and *E. pinnatifida*, is covered adequately by the accessions in the IMDB. The secondary and tertiary gene pool is also well represented due to the fact that they largely consist of *Brassica* species (Warwick et al. 2009) that have ample representation in collections. For the primary gene pool of the genus *Diplotaxis* similar results were observed. *D. tenuifolia* (L.) DC. and *D. muralis* (L.) DC. that both are used as edible crops are represented by a sufficient number of accessions in the IMDB. As in the case of *Eruca*, the secondary and part of the tertiary gene pool is

constituted by various *Brassica* species (Warwick et al. 2009). Therefore, no acquisition priorities were identified for rocket salad.

No clear-cut recommendations for acquisition could be given in the case of artichoke, lamb's lettuce, rhubarb and asparagus. The cultivated species of these crops are well represented in the IMDB, but the majority of the wild species are represented with a single or only a few accessions. The poor representation of the wild species could be interpreted as collection gaps. However, in most cases there are too few data on the relationship of these species with the cultivated crop, and hence on the crop's gene pool structure. For example, the wild allies of cultivated artichoke are not yet clearly identified (Sonnante et al. 2007). For lamb's lettuce, related species could be identified by AFLP markers (Muminovic et al. 2004) but information about crossing ability and inter-fertility within the genus is absent. A similar lack of knowledge exists for rhubarb. Relationships between wild and cultivated *Rheum* species were analyzed by AFLP markers (Suo et al. 2010) but insight in the gene pool structure is completely lacking. Limited information about species relationships for asparagus was obtained from breeding experiments. Inter-specific hybridization between wild and cultivated asparagus species has been studied in order to obtain asparagus lines improved for spear quality and resistance to drought, salt and fungal diseases (Alberti et al. 2004). *Asparagus maritimus* Mill. was found to be sexually compatible with the cultivated species *A. officinalis* L., and therefore can be considered to belong to the primary gene pool. Further information about inter-specific hybridization is presented by González Castañón and Falavigna (2008), but due to different results of the success of the crosses the gene pool structure of asparagus remained largely unclear.

With the exception of rocket salad, more research is needed to elucidate the gene pool structure of minor leafy vegetables crops. As long as the species relationship with the cultivated crop, and hence the importance of the species to the user community is unclear, recommendations for acquisition are considered inappropriate. It should also be noted that the minor leafy vegetables are actually more diverse than the five crops included in the present study, as the genera *Atriplex*, *Chenopodium*, *Chrysanthemum*, *Lepidium*, *Portulaca*, *Rumex*, *Taraxacum* and *Tetragonia*

also are considered to belong to this group (Lebeda and Boukema 2001). An inventory of the accessions of these genera has only just begun (Maggioni et al. 2010).

Concluding remarks

When more data about the relevance of the different elements of the crop gene pool, and the diversity contained therein, have become available, optimization of collection composition could be further elaborated by hierarchical structuring of the gene pools and assigning a relative importance to each of its different components. Comparison of the optimal distribution of the number of accessions with the actual distribution will then enable identification of under- and over-representation within a collection, after which the composition thereof can be improved accordingly (Van Treuren et al. 2009).

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