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Genetic variation within and among some *Lactuca spp*. based on karyotype analysis

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ABSTRACT

This study aimed at assessing the relationships and genetic variation within and among *Lactuca* species based on karyological features, defined for 40 accessions represented 10 species. All the studied species were diploids with 2n = 18. Two pairs of satellites was observed in *L. sativa*, *L. serriola*, *L. saligna* and *L. indica* and one pair in *L. virosa*, *L. dregeana, L. perennis*, *L. altaica* and *L. viminea*. The cluster analysis of the collected data for the forty accessions demonstrated a significant inter and intra-specific differences for all variables measured. *L. sativa* and *L. serriola* had similar karyotypes, suggesting that *L. sativa* derived from *L. serriola*.

KEYWORDS

Karyotype;
Lactuca;
Asteraceae;
Genetic diversity.

INTRODUCTION

The genus *Lactuca* L. belongs to tribe Lactuceae, subfamily Cichorioideae, family Asteraceae. It includes 97 wild species of annual, biennial or perennial herbs with erect or ascending habit, which are predominantly self-pollinating[1,2]. It is distributed throughout the temperate and warm regions with 16 species in Europe, 12 species in America, 43 species in Africa and 51 species in Asia[3-5]. Some of these species are naturalized in Australia[4]. Genus *Lactuca* L. is variable from the ecological viewpoint and its species occupied various habitats including seashores, fields, highways, ditches, roadsides, waste places, ruderal habitats and woodland communities[4,6,7].

Wild *Lactuca* species have been divided into three main groups, on the basis of basic chromosome number[4]. The first relatively small group contains species with n = 8. This group is considered as more primitive and includes perennial species of Europe and the Himalayas. The second group comprises the majority of European and Mediterranean species, as well as species from the Middle East, Africa and India with n = 9. Species belonging to the gene pool of *L. sativa* L. and its closely related species (e.g. *L. serriola* L., *L. saligna* L., *L. virosa* L. and *L. altaica* Fisch. et Mey.) are included in this group. The third group, including North American species distributed from Canada to Florida, is characterized by n = 17 and somewhat geographically and genetically isolated.

Lindqvist[8] was the first to establish detailed *Lactuca* karyotypes from chromosome measurements. He found that the karyotypes of *L. sativa* and *L. serriola* were identical, *L. saligna* was slightly different from that of *L. sativa/serriola,* and *L. virosa,* had a distinct difference from *L. serriola, L. sativa and L.*

saligna. The banding patterns carried out by Koopman et al.[9] confirmed the finding of Lindqvist[8]. They showed that *L. sativa* and *L. serriola* had almost identical chromosome morphology and *L. saligna* differs slightly from them. *L. sativa*, *L. serriola* and *L. saligna* all had two satellite chromosome pairs, but *L. virosa* was quite distinct from the other species, indicating a closer relationship of *L. saligna* to *L. sativa/serriola* than to *L. virosa*.

The evolutionary relationships between *Lactuca sativa*, *L. serriola*, *L. saligna* and *L. virosa* were subjected to the study by Koopman and De Jong[10] using the karyotype and relative DNA content. They found no significant differences between the data of karyological characters and DNA content.

The aim of this study was to evaluate the genetic variation in accession of *Lactuca* spp. from diverse environments using karyological characters.

MATERIALS AND METHODS

Biological material

Forty accessions of nine *Lactuca* species and a hybrid between *Lactuca sativa* x *Lactuca serriola* belonging to three sections were obtained as a donation from the CGN (Centre for Genetic Resources, The Netherlands, P.O. Box 16, 6700 AA Wageningen, The Netherlands). The origin and the accession number of these accessions are recorded in TABLE 1.

Methods

For cytological preparations, one to two cm long roots of seven days old seedlings of each of the forty accessions were detached and pretreated in 0.002 M 8-hydroxyquinoline for 3-4 h. Roots were then washed briefly in water and fixed in a mixture of 3:1 (v/v) ethyle alcohol: glacial acetic acid for 24 h and kept in 75% ethanol in a refrigerator until use.

Cytological preparations were carried out using the Feulgen squash technique. For Feulgen staining, root tips were hydrolyzed in 1 N HCl at 60°C for 10-12 min, washed in distilled water and stained in leuco-basic fuchsin for at least 1 h. The terminal 1-2 mm of the root tips were squashed in a drop of 45% acetic acid on a clean slide. Cover slips were separated by the freeze-drying method. Samples were then dehydrated in absolute ethanol for 2-3 min. and made as permanent preparations by mounting in D.P.X. Slides were allowed to dry at room temperature for few days. Cells with good spreading of chromosomes were photographed using a Zeiss Ultraphoto microscope equipped with automatic camera. The nomenclature used for the description of the chromosome morphology was that proposed by Levan et al.[11]. The abbreviations m, sm, st, t and T designate metacentric, submetacentric, subtelocentric, acrocentric and telocentric chromosomes, respectively. Idiograms were drawn based on the means of centromeric indices and arranged in order of decreasing size.

Data analysis

For the numerical characterization of the karyotypes, the following parameters were calculated: (1) total chromosome length of the haploid complement (TCL); (2) mean chromosome length (CL); (3) mean centromeric index (CI). Comparisons of chromosome morphological features were made by arranging the chromosomes of each karyotype in pairs in order of their arm ratio and length as determined from the photographic prints. An idiogram for each sample was constructed using the total length of each pair of homologous chromosomes to represent the haploid chromosome number. The relative position of the centromere and their variation within the karyotype were expressed. A cluster analysis of the karyotype data was carried out to examine karyotype similarity among species and sections. A data matrix 40 OTUs (operational taxonomic units) × 6 variables was constructed. The TCL, CI, number of m, sm, and st chromosomes as well as the life cycle were considered. The SYSTAT ver. 7 program was used to standardize the data matrix, calculate the average taxonomic distance, and generate a phenogram. Clustering was performed using the unweighted pair-group method (UPGMA).

RESULTS

The studied species represent 3 sections; *Lactuca*, *Tuberosae* and *Phaenixopus*. Generally, the karyotypes of the studied accessions had a predominance of m chromosomes (TABLE 1). The somatic chromosomes of the three sections and their comparable idiogrames are illustrated in Figures 1 and 2. In section *Lactuca*, the most common formula was 10m+8sm. The second one was 12m+6sm, and some samples of

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this section were characterized by subtelocentric chromosomes such as the accessions of *L. virosa* and *L. dregeana* (TABLE 1). All accessions of section *Tuberosae* were characterized by the karyotype formula 6m+12sm (TABLE 1). In section *Phaenixopus*, two accessions had been studied, the karyotype formula contained m, sm and st chromosome. Satellites were detected in the two accessions.

TABLE 1 : Accession number, karyotype formula (KF), total length of the haploid complement (TCL), mean chromosome length (MCL), mean centromeric index (MCI), life cycle (CY), and satellite pairs (St P) of the studied *Lactuca* species. Metacentric (m), submetacentric (sm), subteleocentric (st), annual (a), biennial (b), perennial (p)

Section	No.	Species	Origin	A. No.	KF	TCL	MCL	MCI	CY	St P
	A	*L. sativa*	Czechoslovakia	CGN11424	12m+ 6sm	6.7	0.74	0.4	a	2
	B	*L. sativa*	Netherlands	CGN04706	12m+ 6sm	6.7	0.74	0.4	a	2
	C	*L. sativa*	USA	CGN04888	10m+ 8sm	6.75	0.75	0.39	a	2
	D	*L. sativa*	China	CGN05048	10m+ 8sm	10.75	1.19	0.40	a	2
	E	*L. sativa*	France	CGN04512	10m+ 8sm	10.9	1.2	0.4	a	2
	F	*L. sativa*	USA	CGN05182	10m+ 8sm	10.8	1.2	0.39	a	2
	G	*L. sativa*	France	CGN04566	10m+ 8sm	9.65	1.07	0.38	a	2
	H	*L. sativa*	Spain	CGN05835	10m+ 8sm	9.65	1.07	0.38	a	2
	I	*L. sativa*	Argentina	CGN04557	10m+ 8sm	9.6	1.07	0.37	a	2
	J	*L. sativa*	Afghanistan	CGN04761	10m+ 8sm	9.6	1.07	0.38	a	2
	K	*L. sativa*	Turkey	CGN04744	10m+ 8sm	9.65	1.07	0.39	a	2
	L	*L. sativa*	France	CGN04832	10m+ 8sm	9.65	1.07	0.39	a	2
	M	*L. sativa*	Turkey	CGN04742	10m+ 8sm	9.7	1.08	0.38	a	2
	N	*L. sativa*	Italy	CGN10956	10m+ 8sm	9.75	1.08	0.39	a	2
	O	*L. sativa*	Union of Soviet	CGN04926	10m+ 8sm	10.75	1.19	0.37	a	2
	P	*L. sativa*	China	CGN11387	10m+ 8sm	10.85	1.21	0.38	a	2
Lactuca	Q	*L. sativa*	USA	CGN04546	10m+ 8sm	10.8	1.2	0.37	a	2
	R	*L. serriola*	Egypt	CGN04776	10m+ 8sm	8.8	0.98	0.43	a, b	2
	S	*L. serriola*	Egypt	CGN04770	10m+ 8sm	8.85	0.98	0.43	a, b	2
	T	*L. serriola*	Germany	CGN16210	10m+ 8sm	8.85	0.98	0.43	a, b	2
	U	*L. sat x L.ser serriola*	Egypt	CGN05115	10m+ 8sm	8.75	0.97	0.43	a, b	2
	V	*L. sat x L.ser serriola*	Egypt	CGN05981	10m+ 8sm	8.8	0.98	0.43	a, b	2
	W	*L. saligna*	Turkey	CGN13330	12m+ 6sm	8.25	0.92	0.4	a, b	2
	X	*L. saligna*	BGR	CGN13375	12m+ 6sm	8.3	0.92	0.4	a, b	2
	Y	*L. saligna*	Greece	CGN13327	12m+ 6sm	8.2	0.91	0.41	a, b	2
	Z	*L. saligna*	Spain	CGN05327	12m+ 6sm	8.2	0.91	0.41	a, b	2
	AA	*L. saligna*	Portugal	CGN10883	12m+ 6sm	8.2	0.91	0.4	a, b	2
	AB	*L. virosa*	Italy	CGN05332	8m+6sm+4st	11.55	1.28	0.37	b	1
	AC	*L. virosa*	France	CGN05145	4m+10sm+4st	11.5	1.28	0.35	b	1
	AD	*L. virosa*	Spain	CGN13352	4m+10sm+4st	11.5	1.28	0.354	b	1
	AE	*L. dregeana*	Italy	CGN04790	10m+6sm+2st	10.85	1.21	0.39	a, b	1
	AF	*L. dregeana*	France	CGN05805	10m+6sm+2st	10.8	1.2	0.39	a, b	1
	AG	*L. perennis*	Switzerland	CGN09321	12m+6sm	11.05	1.23	0.39	p	1
	AH	*L. perennis*	France	CGN13299	12m+6sm	11.1	1.23	0.39	p	1
	AI	*L. altaica*	Union of Soviet	CGN15711	10m+ 8sm	9.75	1.08	0.39	a, b	1
	AJ	*L. indica*	Indonesia	CGN14312	6m+ 12sm	10.3	1.14	0.35	a, b	2
Tuberosae	AK	*L. indica*	China	CGN13392	6m+ 12sm	10.25	1.14	0.35	a, b	2
	AL	*L. indica*	China	CGN20713	6m+ 12sm	10.35	1.15	0.36	a, b	2
Phaenixopus	AM	*L. viminea*	Union of Soviet	CGN16202	6m+10sm+ 2st	9.9	1.1	0.35	b	1
	AN	*L. viminea*	France	CGN14301	6m+10sm+ 2st	9.95	1.1	0.35	b	1

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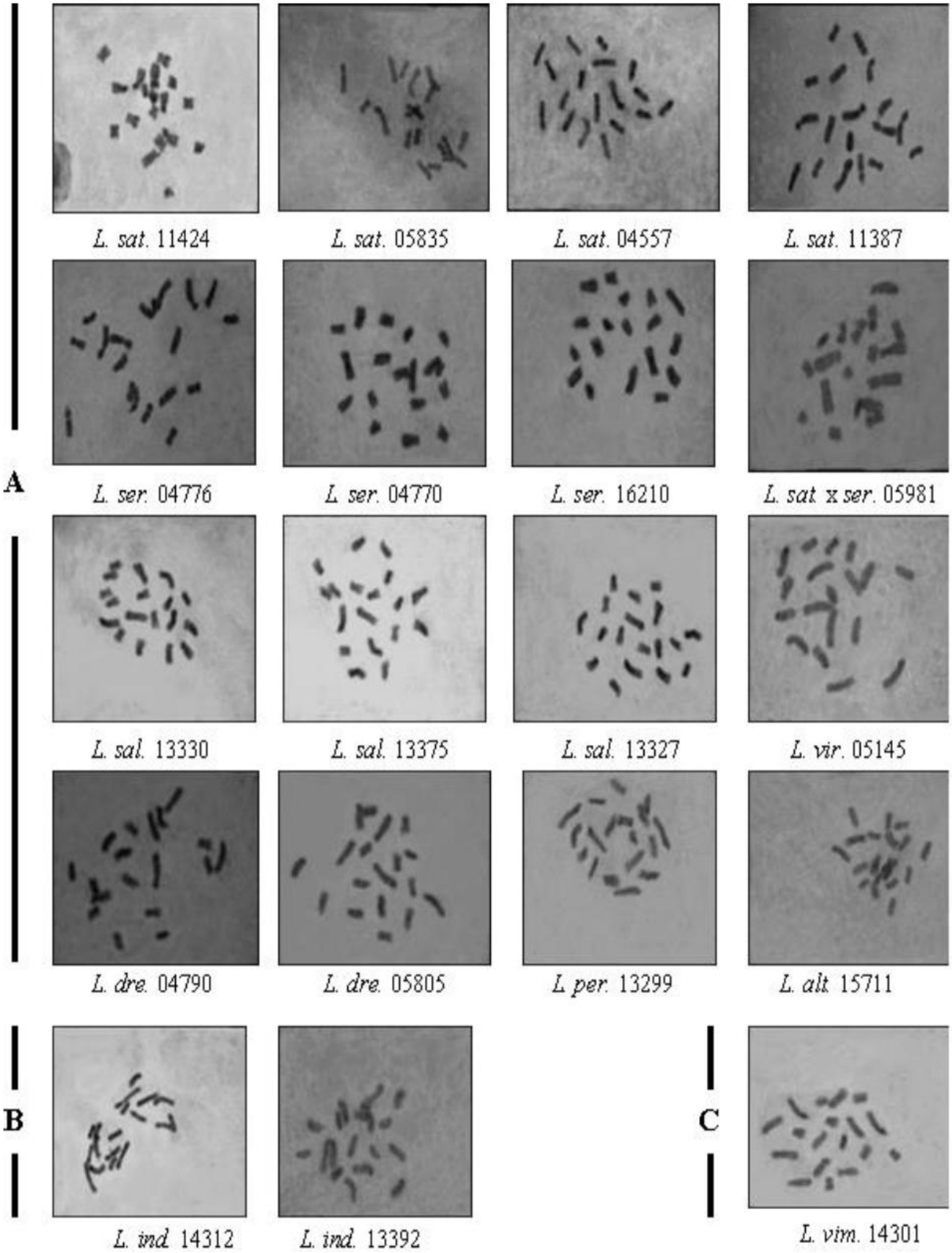

Figure 1 : The somatic chromosomes of some of the studied accessions in the sections: (A) *Lactuca*, (B) *Tuberosae*, and (C) *Phaenixopus*.

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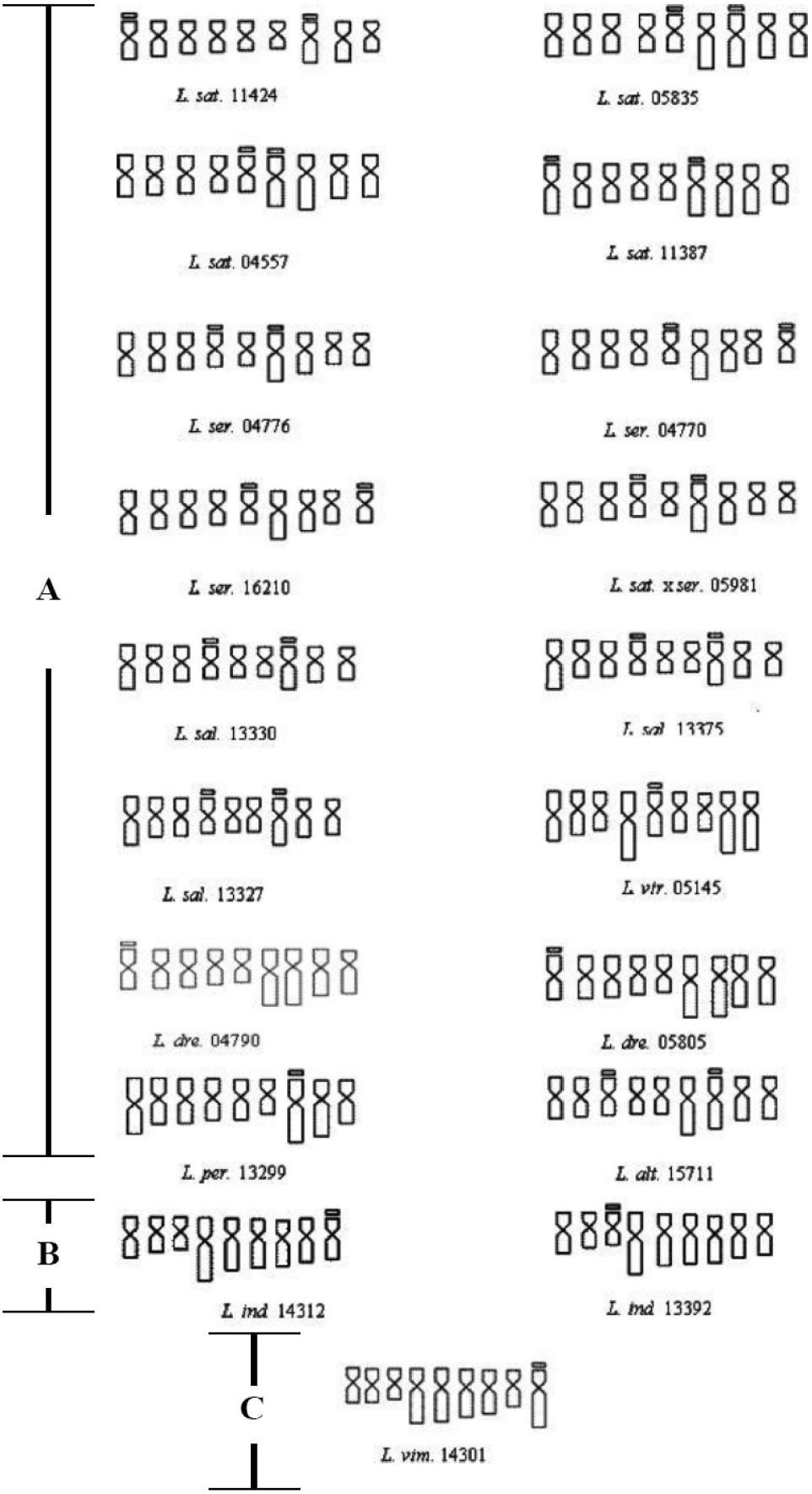

Figure 2 : The idiograms of the somatic chromosomes of the accessions in Figure 1.

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In section *Lactuca*, the total chromosome length (TCL) and the mean value (MCL) of chromosome length were ranged from 6.7 to 0.74 in *L. sativa* CGN11424 from Czechoslovakia to 11.55, 1.28 in *L. virosa* CGN05332 from Italy. Section *Lactuca* showed the highest centromeric index (CI) within the studied sections. In section *Tuberosae*, the total chromosome length (TCL) was ranged from 10.25 in *L. indica* CGN13392 (China) to 10.35 in *L. indica* CGN20713 (China). The two accessions of *L. viminea* which belongs to section *Phaenixopus* had TCL 9.9 for CGN16202 (from Union Soviet) and 9.95 for CGN14301 (TABLE 1).

The dendrogram constructed on the basis of karyotype characters showed two clusters (Figure 3). The first cluster comprised all accessions characterized by the presence of subtelocentric chromosomes. These accessions belong to two accessions of *L. viminea* in section *Phaenixopus*, the two accessions of *L. dregeana* in section *Lactuca* and the three accessions of *L. virosa* in section *Lactuca*. The second cluster included the rest of studied accessions. This cluster comprised two groups. The first group included the two perennial accessions of *L. perennis*, the two annual accessions of *L. sativa* (CGN11424 and CGN04706) and all accessions of *L. saligna*. The second group was separated into 2 subgroups. All accessions of *L. indica* were delimited in one subgroup. The second subgroup included the rest of studied accessions of *L. sativa*, *L. altaica* and all accessions of *L. serriola* and *L. sativa x L. serriola*.

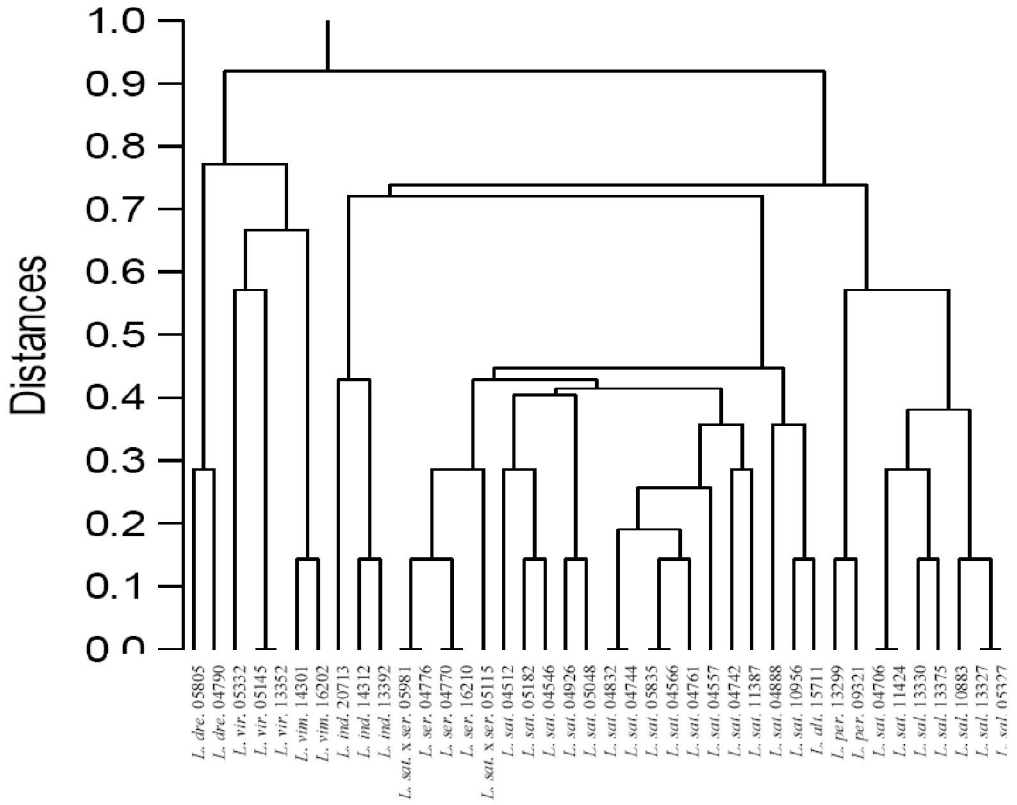

Figure 3 : Dendrogram showing the phenetic relationships among the studied species of *Lactuca* using average linkage method.

DISCUSSION

The relationships and genetic diversity within and among *Lactuca* species were assessed using karyological features. The karyological features (total chromosome length, chromosome index, number of metacentric chromsome, number of subteleocentric chromsome, nature of life cycle) of 40 accessions belonging to 10 species, located in three sections were considered in this study. Like most species of *Lactuca*, all the studied accessions were diploids with 2n = 18. The chromosome numbers of the species agree with those recorded previously[4,12-15]. In addition to this numerical chromosome constancy, species displayed uniformity in chromosome morphology, most chromosomes

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were either metacentric or submetacentric.

Our results were in agreement with the conclusions of Lindqvist[8], Chatterjee and Sharma[16], Haque and Godward[17] and Koopman and De Jong[10] who found that *L. sativa* and *L. serriola* had similar karyotypes. This data indicated that the two species were closely related. The karyotype of *L. virosa*, described by Lindqvist[8] and Koopman and De Jong[10] as containing more asymmetric chromosomes compared to that of *L. sativa/serriola* was confirmed by our results.

The conclusion of Lindqvist[8] and Koopman and De Jong[10] that *L. saligna* karyogram showed chromosomes that were shorter and more unequal in length compared to those of *L. sativa/serriola* was supported by our results. Also, in accordance with the observations of Lindqvist[8] and Koopman and De Jong[10], two pairs of satellites were observed in *L. sativa*, *L. serriola*, *L. saligna* and *L. indica* and one pair in *L. virosa*, *L. dregeana*, *L. perennis*, *L. altaica* and *L. viminea*.

The variation in mean number of visible satellites among the accessions can be explained by differences in the state of despiralization of the secondary constrictions in part of the nucleolar organizing chromosomes. If the constrictions are completely condensed, the microsatellites remain tightly attached to the chromosome and therefore become invisible. The extent of despiralization of the secondary constriction reflects metabolic activity of that region rather than polymorphisms for the satellite and so makes the number of visible microsatellites inappropriate for using as a taxonomic parameter[10,18-20].

Stebbins[21] assumed a predominant evolutionary trend towards increasing asymmetry in the karyotype. Although opposite trends occur in specific genera[10,21-26], the trends towards increasing asymmetry are particularly obvious within the Compositae, tribe Cichorieae (including *Lactuca*)[27-29].

Since all the studied accessions of *Lactuca species* have 18 chromosomes, differences in their karyotypes can be ascribed to processes which do not influence the chromosome number, such as rearrangement within the chromosome arms, pericentric inversions and unequal translocations. Lindqvist[8] found no multivalents in interspecific hybrids within the subsect. *Lactuca*. Therefore he concluded that the differences in chromosome structure among the species of subsect. *Lactuca* originated in pericentric inversions rather than in translocations. Since this is a process driving a primary trend towards increasing asymmetry, therefore the most asymmetric of the karyotypes in our study (karyotypes of *L. sativa* and *L. virosa*) can be considered the most derived. Alternatively, gradual deletions and/or duplications of repetitive sequences, and so of some heterochromatin classes may contribute to the shift of centromeres.

In conclusion, the accessions of the same species showed a great uniformity in Karyotype formulae and quantitative analysis. At the interspecific level, quantitative and qualitative data allowed the differentiation of several of the studied accessions.

REFERENCES

[1] R.C.Thompson, T.W.Whitaker, G.W.Bohn, C.Horn, C.W.Van Horn; Proceedings of the American society for Horticultural Science, **72**, 403-409 **(1958)**.
[2] R.H.Sammour, M.A.Hamoud, A.S.Haidar; Cytologia, **56**, 289-291 **(1991)**.
[3] A.Lebeda, I.Doležalová, D.Astley; Genetic Research and Crop Evolution, **48**, 121-133 **(2001a)**.
[4] Doležalová, E.Křístková, A.Lebeda, V.Vinter; Horticultural Science in Prague, **29**, 56-83 **(2002)**.
[5] R.H.Sammour; Feddes Repertorium, **105**, 191-196 **(1994)**.
[6] A.Lebeda, I.Doležalová, E.Křístková, B.Mieslerová; Genetic Research and Crop Evolution, **48**, 153-164 **(2001b)**.
[7] R.H.Sammour; FABIS Newsletter, **18**, 30-32 **(1987)**.
[8] K.Lindqvist; Hereditas, **46**, 75-151 **(1960)**.
[9] W.Koopman, J.De Jong, I.De Vries; Plant Systematics and Evolution, **185**, 249-257 **(1993)**.
[10] W.Koopman, J.De Jong; Acta Botanica Neerlandica, **45**, 211-222 **(1996)**.
[11] A.Levan, K.Fredga, A.A.Sandberg; Hereditas, **52**, 201-220 **(1965)**.
[12] A.Lebeda, D.Astely; In: A.Lebeda, E.Kristkova, (Eds); Eucarpia leafy vegetables '99, Palacky Univ. Olomouc, CR, 81-94 **(1999)**.
[13] R.H.Sammour; Bot.Bull.Acad.Sin., **38**, 171-177 **(1994)**.
[14] R.H.Sammour, A.E.Z.Mustafa, S.Badr, W.Tahr; Acta Agric.Slovenica, **88**, 33-43 **(2007)**.
[15] R.H.Sammour, A.E.Z.Mustafa, S.Badr, W.Tahr; Acta Bot.Croat., **66**, 1-13 **(2007)**.

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[16] T.Chatterjee, A.Sharma; Genetica, **40**, 577-590 **(1969)**.
[17] M.Haque, M.Godward; Cytologia, **5**, 725-738 **(1985)**.
[18] R.H.Sammour, M.A.Hamoud, S.A.A.Alla; Bot. Bull.Acad.Sin., **34**, 37-42 **(1993)**.
[19] R.H.Sammour; Feddes Repertorium, **105**, 283-286 **(1994)**.
[20] R.H.Sammour; Thesis (Ph.D.), Ph D Thesis, Tanta University, Tanta, Egypt, **(1985)**.
[21] G.Stebbins; Edward Arnold, London, **(1971)**.
[22] K.Jones; Advanced Botany, **6**, 119-194 **(1978)**.
[23] R.H.Sammour, M.A.Hamoud, A.S.Haidar; Cytologia, **56**, 289-291 **(1991)**.
[24] M.A.Hamoud, R.H.Sammour, S.A.Abdalla; Science International (Lahore), **6**, 255-260 **(1994)**.
[25] R.H.Sammour; Plant Varieties and Seeds, **12**, 11-210 **(1999)**.
[26] R.H.Sammour; Turk.J.Bot., **29**, 177-184 **(2005)**.
[27] E.Babcock; University of California Publications in Botany, **21**, 1-197 **(1947)**.
[28] G.Stebbins, J.Jenkins, M.Walters; University of California Publications in Botany, **26**, 401-430 **(1953)**.
[29] R.H.Sammour, A.R.El-Shanoshoury; Bot.Bull. Academica Sinica, **23**, 185-190 **(1992)**.