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Karyological Characteristics of *Lilium ledebourii* Boiss and *Lilium longiflorum* Thunb.

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Summary The karyological characteristics of two *lilium* species were investigated by aceto-ferric-hematoxylin staining. Chromosome characteristics, including the number and length of the chromosomes, length of their long and short arms, length of the total set of chromosomes, the arm ratio index and relative lengths of chromosome, were measured based on averages for five different metaphase cells. Both species are diploid ($2n=2x=24$). The karyotype of *Lilium ledebourii* consisted of 1 pair of metacentric, 4 pairs of submetacentric, 3 pairs of acrocentric and 4 pairs of subtelocentric chromosomes. The karyotype of *Lilium longiflorum* was comprised of 1 pair of metacentric, 4 pairs of acrocentric and 7 pairs of subtelocentric chromosomes. Chromosomes 5 and 7 in *Lilium ledebourii* and chromosomes 6 in *Lilium longiflorum* had a satellite. Karyotypes were classified as 3A by Stebbins classification.

Key words Chromosome, Karyotype, *Lilium ledebourii*, *Lilium longiflorum*.

Lilium (Liliaceae) species and hybrids contribute significantly as floricultural crops. They represent one of the three major bulb crops in the commercial market (Robinson and Firozabady 1993). Lilies consist of *ca.* 80 species distributed mainly in the northern hemisphere across Eurasia and the North American continent.

South-East Asia (China, the Korean peninsula and Japan) and North America are important distribution centers for the lily, with 61 and 21 species, respectively (Van Tuyl and Boon 1997). The number of native European and Caucasian (Eurasian) species is *ca.* 10 (Woodcock and Stearn 1950). The haploid number of chromosomes at 12 is very constant throughout the whole genus. Natural species are mostly diploid ($2n=2x=24$). However, triploid ($3n=3x=36$) and sterile forms are reported in some genotypes. The latter include the *Longiflorum*, *Asiatic* and *Oriental* hybrids that are of profound commercial importance and, accordingly, are most widely cultivated. *Lilium longiflorum* Thunb. cultivars, for example, are popular in Japan, the USA and in many European and Asian countries (Nhut 1998).

Lilium longiflorum Thunb. ($2n=2x=24$) is an important species for flower production and also as a parent in interspecific hybridization programs (Asano 1980, Beattie and White 1993, Van Creij *et al.* 1993). The genus *Lilium* L. contains about 85 species classified into seven sections (De Jong 1974). The genome size of *Lilium* species is one of the largest in the plant kingdom. The 2C value

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is about 72 pg in *L. longiflorum* Thunb. (Bennett and Smith 1976).

Lilium ledebourii Boiss is one of the most important ornamental lily species. It has a high floriculture performance and is considered valuable as a genetic resource for cold tolerance and disease resistance improvement. In addition, *L. ledebourii* with its attractive large raceme of 2–15 flowers is widely used in ornamental breeding programs. The wild population of this species is continuously decreasing in nature because of the cutting of the plants and the removal of underground organs (Khosh-khui and Azadi 2007).

The large size of lily chromosomes makes them convenient for cytological analyses. Nonetheless, their morphology (length and centromere position) is highly conserved within and between species. Therefore, only a few chromosomes are recognizable on the basis of the traits mentioned above (Lim *et al.* 2001, Marasek and Orlikowska 2003).

Cytogenetic studies provide highly valuable information about the kinship relations and systematic and genetic diversity, which are important for plant genetics, breeding and conservation. Such studies yield important and reliable tools in systematics and for assessment of evolutionary relationships and genetic variation (Lewis 1980, Bauchan and Hossein 1998, Sessions 1996). In recent years the genus *Lilium* has been the subject of karyological studies; among the well known are Stewart (1947), Smyth *et al.* (1989) and Purwantoro and Koba (1998). Smyth *et al.* (1989) described the C and NOR-banding patterns for 20 *Lilium* spp. and reported similar patterns for *L. regale* and *L. sulphureum* and for *L. formosanum* and *L. longiflorum*. They concluded that because of the rapid change of C-banding patterns in *Lilium*, the usefulness of these patterns in classification is limited to the identification of closely related species of this genus. Purwantoro and Koba (1998) reported the variability in number of rRNA loci and activity of rRNA genes in *L. formosanum*, *L. longiflorum* and *L. X formolongi* by fluorescence *in situ* hybridization (FISH) and silver staining.

To our knowledge, there is no published report on chromosome number and morphology of *Lilium ledebourii* Boiss. Thus, the karyological characteristics of *Lilium ledebourii* Boiss and *Lilium longiflorum* were researched in the present study to complete our knowledge about these biologically and commercially important species.

Materials and methods

In order to study the kariological characteristics of *Lilium ledebourii* and *Lilium longiflorum*, the scales were cultured on Murashige and Skoog's basal medium supplemented with NAA ($1\,mg\,L^{-1}$) and BA ($0.5\,mg\,L^{-1}$) for induction of roots directly from scales. Roots of 1.5–2 cm in length were selected and pretreated with colchicine 0.05% for 3 h. They were then washed in distilled water and immediately transferred into Lewitscky fixative [1 : 1 v/v 10% formaldehyde and 1% chromic acid (Smyth *et al.* 1989)] solution and kept for 24 h at 4°C (Hayirlioglu and Beyazoglu 1997). Next, they were washed for 3 h under a running tap water flow. The root tips were hydrolyzed by NaOH 1 N for 8 min in a water bath at 60°C and then stained with ferric hematoxylin solution for 16 h at room temperature. To complete softening of the root tissue, the root tips were treated in cytase enzyme for 2 h (Asghari-Zakaria *et al.* 2002). Metaphase cells were observed with a Leica (Leica Co.) microscope model Gallen III. Karyological characteristics of chromosome length, length of their large and short arms, the arms ratio index and relative length of the chromosomes were recorded based on averages for five metaphase cells as measured by Micromeasure software. For karyogram preparation, chromosomes were arranged according to descending order of length and named according to Levan *et al.* (1964) nomenclature.

Results and discussion

Both *L. ledebourii* and *L. longiflorum* were diploid with $2n=2x=24$. Chromosomes 5 and 7 in

Table 1. Analysis of variance for the karyological characteristics of *L. ledebourii* and *L. longiflorum*.

S.O.V.	*df*	Short arm	Long arm	Length of chromosome	Arm ratio index
Species	1	124.59**	232.12**	16.59 ns	217.55**
Chromosome	11	104.68**	104.64**	222.29**	115.48**
Species×chromosome	11	15.58**	19.64**	7.64 ns	20.03**
Error	96	4.49	8.01	11.08	7.06
CV (%)		18.05	12.78	12.39	19.03

ns, * and **: not significant, significant at 5% and 1%, respectively.

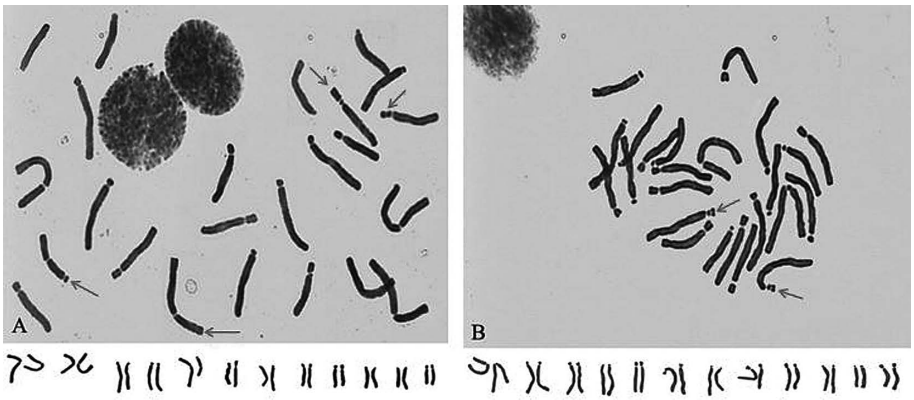

Fig. 1. Metaphase spreads (upper panels) and karyogram of chromosomes (lower panels) for *Lilium ledebourii* (A) and *Lilium longiflorum* (B).

Lilium ledebourii and chromosome 6 in *Lilium longiflorum* each had a satellite. Based on Levan *et al.* (1964) method, the karyotype formula was determined to be 2m+8sm+6ac+8st for *L. ledebourii* and 2m+8ac+14st for *L. longiflorum*. This result is consistent with previous reports on *L. longiflorum* (Noda 1991, Lim *et al.* 2001, Inceer *et al.* 2002). Analysis of variance (Table 1) indicated that the long and short arm lengths and the arms ratio index were significantly different between the *L. ledebourii* and *L. longiflorum*. The metaphase spreads and karyograms of these two species were shown in Fig. 1. The total length of chromosomes of *L. longiflorum* (330 μm) was higher than that of *L. ledebourii*.

Stewart (1947) evaluated the chromosomal morphology of 37 lily species and reported that all 37 species were diploid. Marasek and Orlikowska (2003) reported 2 pairs of satellites in *L. henryi*, 3 pairs in *L. formolongi*, 5 pairs in *L. pumilum* and 5 satellites (2II+1I) in *L. candidum*. Among the cultivars of lily, 7 satellites (3II+1I) were reported in 'Alma Ata,' 6 (2II+2I) in 'Marco Polo,' 5 (2II+1I) in 'Expression' and 'Muscadet' and 4 (1II+2I) in 'Star Gazer' (Marasek and Orlikowska 2003). Wang *et al.* (2012) reported that four Lilium species (*L. regale*, *L. duchartrei*, *L. brownii* var. *viridium* and *L. leucanthum* var. *centifolium*) were diploid with chromosome numbers of 24.

In both species, the chromosomes were significantly different from each other in terms of chromosome length, relative chromosome length, length of long and short arms and the ratio of arm lengths. In *L. ledebourii*, chromosome length and relative length of the chromosome ranged from 19.06 μm and 5.99% in chromosome 12 to 36.94 μm and 11.62% in chromosome pair 1. In this species, the length of the long arm varied from 13.77 in chromosome pair 12 to 27.69 μm in chromosome pair 3. The longest and shortest values of the short arm length were for chromosome pairs 1 and 9, with averages of 15.33 and 1.87 μm, respectively. Meanwhile, chromosome pair 9,

Table 2. Chromosomal characteristics of *Lilium ledebourii*.

Chr.	Type	Short arm (μm)	Long arm (μm)	Length of chromosome (μm)	Relative length (%)	Arm ratio index	SAT (μm)
1	M	15.33±1.14	21.60±1.43	36.94±2.43	11.62±0.18	1.42±0.07	—
2	Sm	12.15±1.91	21.01±1.98	33.16±3.57	10.43±0.69	1.73±0.03	—
3	St	3.02±0.16	27.69±1.49	30.71±1.61	9.66±0.11	9.16±0.01	—
4	St	2.85±0.20	26.69±1.94	29.54±2.09	9.29±0.23	9.38±0.01	—
5	Sm	7.72±1.35	18.14±1.71	25.86±1.42	8.14±0.17	2.35±0.05	1.73±0.24
6	St	2.33±0.28	23.61±1.53	25.94±1.62	8.16±0.33	10.33±0.98	—
7	Ac	4.52±0.51	20.56±1.53	25.08±1.38	7.89±0.42	4.54±0.02	3.25±0.27
8	Sm	6.20±1.05	17.70±1.41	23.90±0.71	7.52±0.21	2.85±0.05	—
9	St	1.87±0.21	21.94±1.84	23.81±1.75	7.49±0.21	11.75±2.17	—
10	Ac	3.89±0.24	19.11±1.61	23.00±1.52	7.24±0.16	4.91±0.66	—
11	Ac	3.63±0.81	17.17±1.18	20.80±0.47	6.54±0.27	4.73±1.74	—
12	Sm	5.28±1.10	13.77±1.21	19.05±0.50	5.99±0.34	2.61±0.84	—

m: metacentric, ac: acrocentric, st: sub telocentric, sm: sub metacentric.

Table 3. Chromosomal characteristics of *Lilium longiflorum*.

Chr.	Type	Short arm (μm)	Long arm (μm)	Length of chromosome (μm)	Relative length (%)	Arm ratio index	SAT (μm)
1	M	15.59±2.89	25.16±0.83	40.76±2.92	12.32±0.65	1.61±0.04	—
2	St	3.52±0.69	27.69±0.64	31.21±0.31	9.43±0.22	7.86±1.08	—
3	St	2.91±0.14	27.18±0.61	30.09±0.54	9.09±0.15	9.35±0.61	—
4	St	2.57±0.66	26.50±0.91	29.06±0.67	8.79±0.09	10.32±2.54	—
5	Ac	4.29±0.53	24.12±0.17	28.41±0.67	8.59±0.11	5.62±0.64	—
6	St	2.34±0.50	25.52±0.60	27.86±1.05	8.42±0.13	10.89±2.19	1.38±0.24
7	St	2.57±0.57	22.56±0.74	25.13±0.65	7.60±0.14	8.79±1.87	—
8	Ac	3.65±0.27	23.05±1.08	26.70±0.84	8.07±0.17	6.30±0.90	—
9	Ac	3.83±0.25	21.01±0.41	24.84±0.35	7.51±0.21	5.48±0.47	—
10	St	1.86±0.25	21.65±0.72	23.51±0.62	7.11±0.12	11.67±1.25	—
11	Ac	3.56±0.37	18.60±0.46	22.16±0.57	6.70±0.21	5.23±0.85	—
12	St	1.66±0.07	19.35±0.45	21.01±0.48	6.35±0.08	11.70±0.45	—

m: metacentric, ac: acrocentric, st: sub telocentric.

with an average of 11.75, had the highest arm ratio index and the minimum value of this ratio was obtained in chromosome 1 with an average of 1.40 (Table 2).

In *L. longiflorum*, chromosome length and relative length of the chromosome ranged from 21.01 μm and 6.35% in chromosome 12 to 40.76 μm 12.32% in chromosome pair 1 (Table 3). In this species, chromosome pair 2 has the longest long arm with an average of 27.68 μm, and the shortest long arm was observed in chromosome 11 with an average of 18.60 μm. In terms of the short arm length, the highest mean was recorded for chromosome 1 with an average of 15.59 μm, while chromosome 12 with an average of 1.66 μm had the lowest short arm length. In terms of the arm ratio, chromosome number 12 exhibited the highest mean with an average of 11.70 μm, and chromosome 1 showed the lowest arm ratio with an average of 1.61 μm (Table 3). In both species, the maximum differences between the arms of chromosome were observed in chromosome pair 3 and the minimum differences of arms length was related to chromosome pair 1.

The comparison of morphological details for chromosomes has enabled the identification of species, such as in the genera *Amaryllis* (Narain and Khoshoo 1968), *Alstroemeria* (Rustanius *et al.* 1991), *Clivia* (Ran *et al.* 1999) and *Brassica* (Cheng *et al.* 1995). Chromosome size *per se* may,

Table 4. Karyotype formula (KF), difference of the relative length between the longest and the shortest (DRL), proportion (%) of karyotype total form (TF%), ratio of the longest to the shortest chromosomes (R), relative length of the shortest chromosome (S%), total length and Stebbins symmetry class of karyotype (ST).

Species	KF	DRL	TF	R	%S	Total length	ST
Lilium ledebourii	1m+4sm+3ac+4st	5.62	21.64	1.94	5.99	317.83	3A
Lilium longiflorum	1m+4ac+7st	5.97	14.61	1.94	6.35	330.75	3A

however, be affected by growth conditions and may also depend on the duration of the analysis process (Heimburger 1962). Matern and Simak (1968) concluded that chromosomes are distinguishable on the basis of length if the average difference between the long and the short chromosome exceeds 8% of the lengths of these two chromosomes. Moreover, the risk of reversed classification was lower when the difference was at least 11% of the average lengths of the two chromosomes. A higher difference (>15%) in the length of the arms is necessary for accurate classification of the chromosomes into metacentric and submetacentric.

There was a significant (p<0.01) interaction between species and chromosomes in terms of the long and short arms length and the arm ratio index (Table 1), indicating that these characteristics can be used for differentiation of these species from each other. According to Stebbins symmetric index, both species were located on 3A group. Inceer *et al.* (2002) indicated from karyotype analyses of *L. carniolicum* and *L. monadelphum* that these species were in 3B and 3A group, respectively.

For the *L. ledebourii*, the total form of karyotype (TF%) was 21.64 which was higher than that of *L. longiflorums* (14.61%), indicating considerable differences between the two species. Accordingly, *L. ledebourii* had more karyotypic symmetry than *L. longiflorums* (Table 4). The difference between the relative length of the longest and the shortest chromosome (DRL) revealed that *L. longiflorum* species, in terms of the chromosome length, is more uniform than *L. ledebourii* (Table 4). Concerning these results, chromosome morphology can be used as a reliable marker to assess the variations among lily species.

Conclusion

To our knowledge, this is the first report on the karyological study of *L. ledebourii.* The two *Lilium* species studied were diploid with 24 chromosomes. In terms of karyotype asymmetry indices, there was a marked difference between *L. ledebourii* and *L. longiflorum*. Overall, there were clear karyotypic differences in morphology, symmetry and size of chromosomes between these species.

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