

KARYOLOGICAL STUDY ON SOME TAXA OF THE GENUS *LILIUM* L. (*LILIACEAE*)

HÜSEYİN İNCEER, SEMA HAYIRLIOĞLU-AYAZ AND OSMAN BEYAZOĞLU

*Karadeniz Technical University, Faculty of Sciences and Arts,
Department of Biology, 61080 Trabzon, Turkey*

Abstract

In the present study, the chromosome number and morphology of four *Lilium* L. (*Liliaceae*) taxa distributed in Northeast Anatolia, Turkey, were studied. Karyotype analysis was carried out on the following taxa: *L. carniolicum* Bernh subsp. *ponticum* var. *ponticum* (C.Koeb) Davis & Henderson and var. *artvinense* (Miscz) Davis & Henderson, *L. monadelphum* Bieb var. *szovitsianum* (Fischer & Ave-Lall.) Elwes and *L. kesselringianum* Miscz. The chromosome number was found $2n = 24$ in all taxa. The karyotype analysis showed that the chromosomes of these taxa consist of median, submedian, subterminal and terminal centromere.

Introduction

The genus *Lilium* L. (*Liliaceae*) includes up to 100 species widely distributed across Temperate Asia, Europe, and North America (Woodcock & Stearn, 1950; Syngé, 1980). According to the records in Flora of Turkey, the genus represented by 10 taxa and most of the species of this genus are distributed in North Anatolia (Davis, 1984).

A more detailed classification was proposed by Comber (1949) who defined seven sections on a combination of 15 traits. Particular importance was placed on seed weight and germination patterns, leaf arrangement, and the habit of the bulb and bulb scales in addition to the fact that floral characters were also considered (Smyth *et al.*, 1989).

The genus *Lilium* includes many beautiful ornamental species. Their cultivars are derived from intra- as well as interspecific hybridization and are now widely cultivated in the world. A recent large increase in the consumption of flowers and ornamental plants is now triggering demand for novel types of flowers in the lilies. For the production of such novel cultivars, interspecific hybridization is expected to be one of the most means (Mii *et al.*, 1994).

Lilium species are important for their economic value and used for cosmetic industry and medical science (Yılmaz & Korkut, 1998). Also Baytop (1984) and Zeybek & Zeybek (1994) reported that the genus has saponin glikozide used in the treatment of skin diseases.

The relevance of chromosomal information to the knowledge of the systematics and evolution of the genus *Lilium* was noted long ago. But, chromosomal data on Turkish populations are scarce. The aim of the present study is to provide karyological data for *Lilium* species. We also discuss some systematic and evolutionary aspects of the genus in light of the karyological data.

Material and Methods

Plant material

L. carniolicum Bernh subsp. *ponticum* var. *ponticum* and var. *artvinense*, *L. monadelphum* var. *szovitsianum* and *L. kesselringianum* were collected from the mountains of Northeast Anatolia, Turkey, between June and July in 1997 and 2000 (Table 1).

Karyotype analysis

Root tip meristems were obtained from germinating bulbs in pots. The root tip meristems were pre-treated with 0.05% colchicine for 3-4 h and then fixed in an ethanol-acetic acid (3:1), solution for at least 24 h at 4°C (Hayırhoğlu & Beyazoğlu, 1997). The root tips were hydrolyzed in 1 N HCl at 60°C for 13-15 min and then rinsed with tap water for a minimum of 5-6 min. Staining was carried out in Feulgen for 1 h. Squashing was done in 45% acetic acid and the preparations were mounted in Entellan.

Of each population, 20-25 specimens for each taxa were collected and of these more than 15 permanent slides were prepared. The well-spread 10 metaphase plates about 10 permanent slides were photographed with an Olympus BII-2 camera and drawn from permanent slides deposited at the Department of Biology, Karadeniz Technical University, Trabzon.

Karyotype analysis was performed according to Levan *et al.*, (1964).

Results and Discussion

The present study deals with chromosome number and morphology of the following species.

***L. carniolicum* Bernh subsp. *ponticum* var. *ponticum* (C.Koch) Davis & Henderson:**

It is an Euxine element. It grows on mountain meadows, bushy slopes, stream sides, often in shady places, on igneous substrata, usually above tree line at 1800-2400 m.

The chromosome number of this taxon is $2n=24$. Chromosome 1 is median and chromosome 2 is submedian-centromeric. Chromosomes 9, 10 and 11 are subterminal and chromosomes 3, 4, 5, 6, 7, 8 and 12 are terminal-centromeric. No satellite is present on the karyotype of this species (Table 2, Fig 1a & 2a). Nucleolus organizing region (NOR) is present on the chromosomes 2 and 4. Chromosomal features of this taxon has been determined in this study firstly.

***L. carniolicum* Bernh subsp. *ponticum* var. *artvinense* (Misz) Davis & Henderson:**

It is an endemic and Euxine element. It grows in *Picea-Rhododendron* forest, dense scrup and woodlands, below tree line at 1500-1800 m.

The chromosome number of this taxon is $2n=24$. Chromosome 1 is median and chromosome 2 is submedian. Chromosomes 8 and 11 are subterminal centromeric. Chromosomes 3, 4, 5, 6, 7, 9, 10 and 12 are terminal centromeric. The satellite is present on short arm of chromosome 7. NORs are present on the chromosomes 4, 6 and 8 (Table 3, Fig. 1b & 2b). Chromosomal features of this species have been determined in this study for the first time.

Table 1. Localities of *Lilium* L. taxa studied.

| Taxa | Localities | Voucher |
|--|---|-----------|
| <i>L. carniolicum</i> subsp. <i>ponticum</i> var. <i>ponticum</i> | A8 Rize: İkizdere to ispir, Sivrikaya Köyü, 1850 m. | İnceer 3a |
| <i>L. carniolicum</i> subsp. <i>ponticum</i> var. <i>artvinense</i> | A8 Rize: İkizdere, Çamlık Köyü 1500 m. | İnceer 4 |
| <i>L. monadelphum</i> var. <i>szovitsianum</i> | A7 Trabzon: Zigana Pass, 1800 m. | İnceer 5 |
| <i>L. kesselringianum</i> | A9 Artvin: Yalnız çam Dağları 1800 m. | İnceer 96 |

Table 2. Chromosomes types, chromosome length and arm ratio of *Lilium carniolicum* subsp. *ponticum* var. *ponticum*.

| Chromosome pairs | C (µm) | L (µm) | S (µm) | SAT (µm) | L/S | I (µm) | R % | Centromeric position |
|------------------|--------|--------|--------|----------|-------|--------|-------|----------------------|
| 1 | 14.85 | 8.65 | 6.20 | - | 1.39 | 41.75 | 11.85 | median region |
| 2 | 13.50 | 9.00 | 4.50 | - | 2.0 | 33.33 | 10.77 | submedian region |
| 3 | 12.45 | 11.27 | 1.18 | - | 9.55 | 9.47 | 9.94 | terminal region |
| 4 | 10.90 | 9.62 | 1.28 | - | 7.5 | 11.74 | 8.70 | terminal region |
| 5 | 10.50 | 9.2 | 1.3 | - | 7.0 | 12.38 | 8.38 | terminal region |
| 6 | 9.93 | 9.13 | 0.80 | - | 11.41 | 8.05 | 7.92 | terminal region |
| 7 | 9.77 | 8.95 | 0.82 | - | 10.91 | 8.39 | 7.80 | terminal region |
| 8 | 9.26 | 8.52 | 0.74 | - | 11.51 | 7.99 | 7.39 | subterminal region |
| 9 | 8.99 | 7.49 | 1.50 | - | 4.99 | 16.68 | 7.17 | subterminal region |
| 10 | 8.81 | 7.60 | 1.21 | - | 6.28 | 13.73 | 7.03 | subterminal region |
| 11 | 8.46 | 7.21 | 1.25 | - | 5.76 | 14.77 | 6.75 | subterminal region |
| 12 | 7.82 | 7.00 | 0.82 | - | 8.53 | 10.48 | 6.24 | terminal region |

C: Total chromosome length, L: Long arm length, S: Short arm length, L/S: Arm ratio, SAT: Satellite, I: Centromere index, R: Relative length.

Table 3. Chromosomes types, chromosome length and arm ratio of *Lilium carniolicum* subsp. *ponticum* var. *artvinense*.

| Chromosome pairs | C (µm) | L (µm) | S (µm) | SAT (µm) | L/S | I (µm) | R % | Centromeric position |
|------------------|--------|--------|--------|----------|-------|--------|-------|----------------------|
| 1 | 13.75 | 8.00 | 5.75 | - | 1.39 | 41.81 | 11.56 | median region |
| 2 | 13.25 | 9.25 | 4.00 | - | 2.31 | 30.18 | 11.14 | submedian region |
| 3 | 10.50 | 9.50 | 1.00 | - | 9.50 | 9.52 | 8.83 | terminal region |
| 4 | 10.38 | 9.38 | 1.00 | - | 9.38 | 9.63 | 8.73 | terminal region |
| 5 | 10.26 | 9.38 | 0.88 | - | 10.65 | 8.57 | 8.63 | terminal region |
| 6 | 9.75 | 9.00 | 0.75 | - | 12.00 | 7.69 | 8.20 | terminal region |
| 7 | 9.63 | 8.63 | 1.00 | 0.88 | 8.63 | 10.38 | 8.10 | terminal region |
| 8 | 9.38 | 7.38 | 2.00 | - | 3.69 | 21.32 | 7.89 | subterminal region |
| 9 | 8.50 | 8.00 | 0.50 | - | 16.00 | 5.88 | 7.15 | terminal region |
| 10 | 8.38 | 7.38 | 1.00 | - | 7.38 | 11.93 | 7.05 | terminal region |
| 11 | 8.25 | 7.00 | 1.25 | - | 5.60 | 15.15 | 6.94 | subterminal region |
| 12 | 6.88 | 6.25 | 0.63 | - | 9.92 | 9.16 | 5.79 | terminal region |

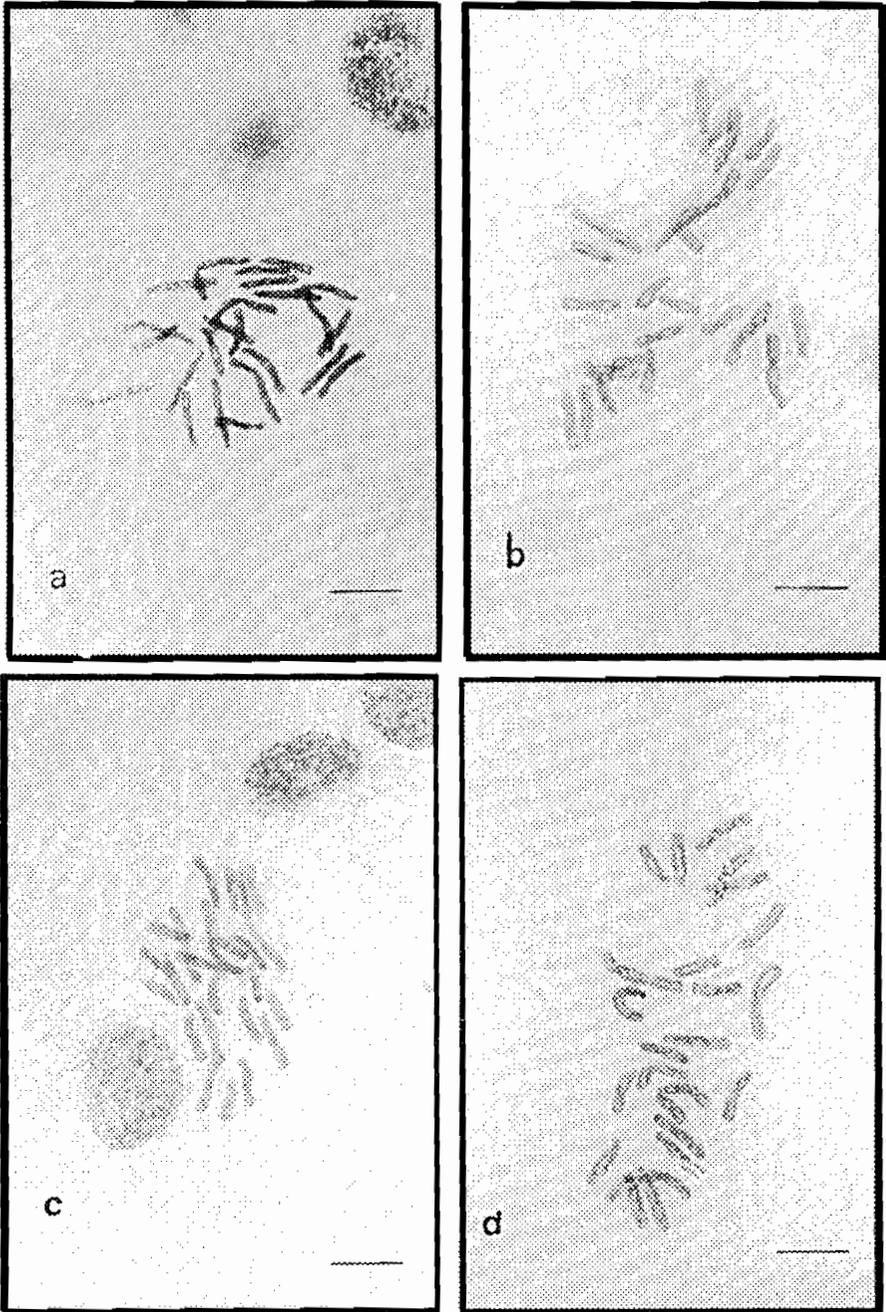


Fig. 1. Mitotic metaphase chromosomes of *Lilium* L. taxa studied.

a. *L. carniolicum* subsp. *ponticum* var. *ponticum*; b. *L. carniolicum* subsp. *ponticum* var. *artvinense*;
c. *L. monadelphum* var. *szowitsianum*; d. *L. kesselringianum* Bar: 10 μ m.

L. monadelphum Bieb var. *szovitsianum* (Fischer & Ave-lall.) Elwes: It is an Euxine element. It grows on edge of forests (*Fagus*, *Picea*), scrup (*Rhododendron*, *Corylus*) at 1700-1800 m.

The chromosome number of this taxon is $2n=24$. Chromosomes 1 and 2 are median-centromeric. Chromosomes 3, 4, 5, 8, 9, 10 and 12 are terminal centromeric. Chromosomes 6, 7 and 11 are subterminal centromeric. The satellite is present on the long arms of the chromosome 5. NORs are present on the chromosome 5 and 10 (Table 4, Fig. 1c & 2c).

Table 4. Chromosomes types, chromosome length and arm ratio of *Lilium monadelphum* var. *szovitsianum*.

| Chromosome pairs | C (μm) | L (μm) | S (μm) | SAT (μm) | L/S | I (μm) | R % | Centromeric position |
|------------------|---------------------|---------------------|---------------------|-----------------------|-------|---------------------|-------|----------------------|
| 1 | 12.13 | 7.38 | 4.75 | - | 1.55 | 39.16 | 11.45 | median region |
| 2 | 11.88 | 7.25 | 4.63 | - | 1.57 | 38.97 | 11.21 | median region |
| 3 | 9.38 | 8.63 | 0.75 | - | 11.50 | 8.0 | 8.85 | terminal region |
| 4 | 9.13 | 8.63 | 0.5 | - | 17.26 | 5.48 | 8.62 | terminal region |
| 5 | 8.76 | 8.13 | 0.63 | 0.6 | 12.90 | 7.19 | 8.27 | terminal region |
| 6 | 8.26 | 7.13 | 1.13 | - | 6.30 | 13.68 | 7.80 | subterminal region |
| 7 | 8.13 | 7.0 | 1.13 | - | 6.19 | 13.90 | 7.70 | subterminal region |
| 8 | 8.01 | 7.13 | 0.88 | - | 8.10 | 10.99 | 7.56 | terminal region |
| 9 | 7.88 | 7.25 | 0.63 | - | 11.50 | 7.99 | 7.44 | terminal region |
| 10 | 7.75 | 7.0 | 0.75 | - | 9.33 | 9.67 | 7.32 | terminal region |
| 11 | 7.5 | 6.25 | 1.25 | - | 5.0 | 16.67 | 7.08 | subterminal region |
| 12 | 7.13 | 6.63 | 0.5 | - | 13.26 | 7.01 | 6.73 | terminal region |

L. kesselringianum Miscz.: This is an Euxine element. It grows on wooded slopes, meadows at edge of *Picea* forest, igneous bare spots and cornfields at 1450-2400 m.

The chromosome number of this species is $2n=24$. Chromosomes 1 and 2 are median-centromeric. Chromosomes 3, 4, 5, 7, 8 and 9 are terminal centromeric. Chromosomes 6, 10, 11 and 12 are subterminal centromeric. The satellite is present on the short arms of the chromosome 6. NORs are present on the chromosome 2 and 10 (Table 5, Fig. 1d & 2d).

Table 5. Chromosomes types, chromosome length and arm ratio of *Lilium kesselringianum*.

| Chromosome pairs | C (μm) | L (μm) | S (μm) | SAT (μm) | L/S | I (μm) | R % | Centromeric position |
|------------------|---------------------|---------------------|---------------------|-----------------------|-------|---------------------|-------|----------------------|
| 1 | 14 | 8.0 | 6.0 | - | 1.33 | 42.86 | 12.23 | median region |
| 2 | 11.5 | 7.0 | 4.5 | - | 1.56 | 39.13 | 10.05 | median region |
| 3 | 11.18 | 9.88 | 1.3 | - | 7.6 | 11.63 | 9.77 | terminal region |
| 4 | 10.75 | 9.5 | 1.25 | - | 7.6 | 11.63 | 9.39 | terminal region |
| 5 | 9.5 | 8.75 | 0.75 | - | 11.66 | 7.89 | 8.30 | terminal region |
| 6 | 9.38 | 7.25 | 2.13 | 0.88 | 3.40 | 22.71 | 8.19 | subterminal region |
| 7 | 9.25 | 8.25 | 1.0 | - | 8.25 | 10.81 | 8.08 | terminal region |
| 8 | 9.01 | 8.13 | 0.88 | - | 9.24 | 9.77 | 7.87 | terminal region |
| 9 | 8.38 | 7.88 | 0.5 | - | 15.76 | 5.97 | 7.32 | terminal region |
| 10 | 7.88 | 6.5 | 1.38 | - | 4.7 | 17.51 | 6.88 | subterminal region |
| 11 | 7.63 | 6.25 | 1.38 | - | 4.53 | 18.09 | 6.67 | subterminal region |
| 12 | 6.01 | 4.88 | 1.13 | - | 4.32 | 18.80 | 5.25 | subterminal region |

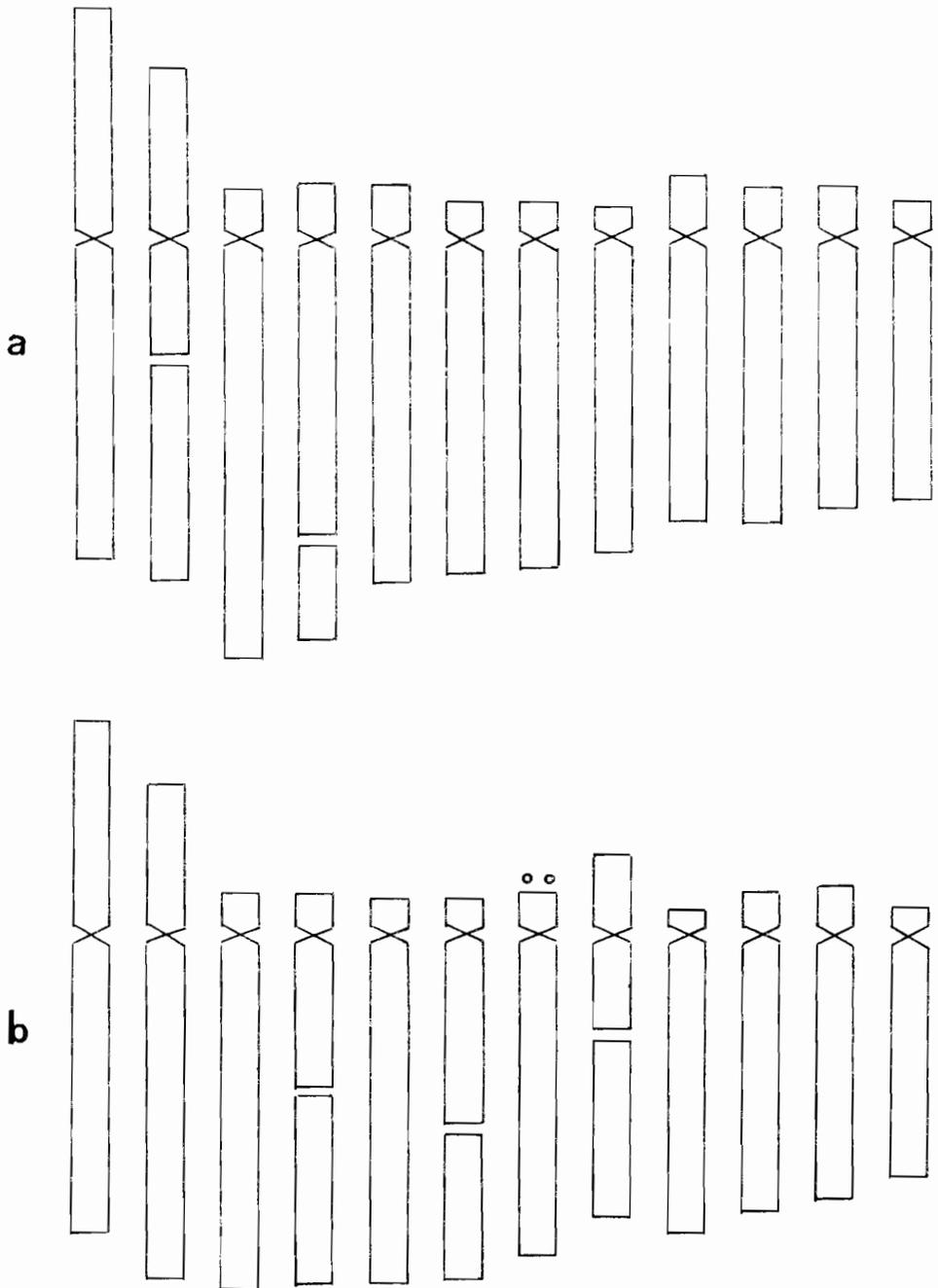


Fig. 2. Haploid idiograms of *Lilium* L. taxa studied.

a. *L. carnolicum* subsp. *ponticum* var. *ponticum*; b. *L. carnolicum* subsp. *ponticum* var. *artvinense*;
 c. *L. monadelphum* var. *szowitsianum*; d. *L. kesselringianum*

The chromosome number and karyological features of *L. carniolicum* subsp. *ponticum* var. *ponticum* and *L. carniolicum* subsp. *ponticum* var. *artvinense* are being reported for the first time. Previously, the chromosome number of other two species (*L. monadelphum* var. *szovitsianum* $2n=28-32$, *L. kesselringianum* $2n=24$) have been reported by Fedorov (1969). According to our observations all taxa investigated are diploid with $2n=2x=24$ chromosomes. The karyotypes of these taxa consist of chromosomes with median, submedian, subterminal and terminal centromeres (Table 2-5). These findings are in agreement with reports of the chromosome numbers and karyotypes for other species of the genus *Lilium* (Stewart, 1947; Lighty, 1960; İnceer *et al.*, 1999). Asona (1983) reported that the chromosome complement of diploid hybrid species in *Lilium* usually consists of two pairs of larger submedian and 10 pairs of subterminal chromosomes. Smyth *et al.*, (1989) also noted that 20 species of *Lilium* have a similar basic karyotype and the chromosome number of taxa is $2n=2x=24$. According to İnceer *et al.*, (1999), four taxa of *Lilium* have similar chromosome complements with $2n=24$ chromosomes.

Stewart (1947) and Smyth *et al.*, (1989) noted that the karyotypes of the genus *Lilium* have secondary constrictions (NORs). In present paper, studied four *Lilium* taxa have NOR at least two chromosome pairs. Jackson (1971) and Greilhuber (1984) reported that the karyotype often used to deduce and clarify interspecies relationships. But, this has been of little help in *Lilium* because all species examined to date have $2n=24$, with two large metacentric chromosomes and ten smaller acrocentric chromosomes (Stewart, 1947; Lighty, 1960). Even so, the number and location of nucleolar organizing regions within the karyotype has been of some use. Although quite diverse overall, closely related species often have NORs at the same locations (Stewart, 1947).

The present study indicates that all taxa have similar chromosome complements. The karyotypes of two varieties of *L. carniolicum* subsp. *ponticum* are similar. Likewise, the karyotype of *L. monadelphum* var. *szovitsianum* is related to *L. kesselringianum*. These karyotypes are in good agreement with the systematic relationships of investigating taxa within the genus *Lilium*. Besides, the karyotype characteristics of these species could be correlated with the external morphological characteristics. Phylogenetic relationships among the *Lilium* species will be widely determined in the future by revision and monograph studies. Our investigations on this subject are still continuing.

References

- Asona, Y. 1983. Random distribution of the chromosome pairings in interspecific hybrids of *Lilium*. *Cytologia*, 48, 803-809.
- Baytop, T. 1984. *Türkiye'de Bitkiler ile Tedavi*. İstanbul Üniversitesi Yayınları, İstanbul.
- Comber, H.F. 1949. A new classification of the genus *Lilium*. R. Hort. Soc. Lily Year Book, 13: 86-105.
- Davis, P.H. 1984. *Flora of Turkey and East Aegean Islands*. Edinburgh University Press, Edinburgh, 8: 279-284.
- Fedorov, A.N. 1969. *Chromosome numbers of flowering plants*. Leningrad.
- Greilhuber, J. 1984. *Chromosomal evidence in taxonomy*. In: Heywood, V.H., Moore, D.M., *Current concepts in plant taxonomy*, London, 157-180.
- Hayırlıoğlu, S. and O. Beyazoğlu. 1997. Chromosome numbers in species of *Alchemilla* L., belong to the series Sericeae Bus. and Pubescentes Bus. (section *Alchemilla* Rothm.) in Turkey. *Caryologia*, 50: 77-84.
- Jackson, R. C. 1971. The karyotype in systematics. *Ann. Rev. Ecol. Syst.*, 2: 327-368.

- İnceer, H., O. Beyazođlu and S. Hayırhođlu-Ayaz. 1999. Karyotype analysis of some *Lilium* L. (*Liliaceae*) species from Turkey. *Pak. Journal of Botany*, 31: 315-321.
- Levan, A., K. Fredga and A.A. Sandberg. 1964. Nomenclature for centromeric position on chromosomes. *Hereditas*, 52: 201-220.
- Lighty, R.W. 1960. *Cytological and interspecific hybridization studies in Lilium L. and their significance for classification*. Ph.D. Thesis, Cornell University.
- Mii M., Y. Yuzawa, H. Suetomi, T. Motegi and T. Godo. 1994. Fertile plant regeneration from protoplasts of a seed-propagated cultivar of *Lilium x formolongi* by utilizing meristematic nodular cell clumps. *Plant Science*, 100: 221-226.
- Smyth, D.R., K. Kongsuwan and S. Wisudharom. 1989. A survey of c-band patterns in chromosomes of *Lilium* (*Liliaceae*). *Pl. Syst. Evol.*, 163: 53-69.
- Stewart, R.N. 1947. The morphology of somatic chromosomes in *Lilium*. *Amer. J. Bot.*, 34: 9-26.
- Synge, P.M. 1980. *Lilies*. London, Batsford.
- Woodcock, H.B.D. and W.T. Stearn. 1950. *Lilies of the world*. London, Country life.
- Yılmaz, R. and B. Korkut. 1998. *Zambak (Lilium L.) yetiřtiriciliđinde deđişik harç kullanımının çiçeklenmeye etkileri*. I. Ulusal süs bitkileri kongresi, Yalova.
- Zeybek, N. and U. Zeybek 1994. *Farmasötik Botanik Kapalı Tohumlu Bitkiler (Angiospermae) Sitematiđi ve Önemli Maddeleri*. Ege Üniversitesi Eczacılık Fakültesi Yayınları, Ege Univ. Basımevi, Bornova.

(Received for publication 30 July 2001)