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Giemsa C-banding Analysis of *Sternbergia lutea* (L.) Ker-Gawl. ex Sprengel and *S. sicula* Tineo ex Guss. from Turkey

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Karyosystematic characteristics of *Sternbergia* Waldst. and Kit. species in Greece were studied by Kamari and Artelari (1990) and Artelari and Kamari (1991). Özhatay (1983) described the chromosome numbers and karyotypes as well as taxonomy of *Sternbergia* species in Turkey.

Karyotype and C-banding analysis of species is important to identify individual chromosomes and genomic relationships among related species (Gill and Kimber 1974, Hadlaczky and Belea 1975, Yu *et al.* 1991). However, banding characteristics of *Sternbergia* are still unknown. *Sternbergia*, a small genus of Mediterranean region, is represented by six species in Turkey (Davis 1984). These are *S. lutea*, *S. sicula*, *S. fischeriana*, *S. candida*, *S. clusiana* and *S. colchiciflora*. Two of these, *S. lutea* and *S. sicula*, were analysed in terms of Feulgen and Giemsa C-banding karyotype. Results were discussed in cytological and taxonomical point of view.

Materials and methods

Two taxa of *Sternbergia*, *S. sicula* Tineo ex Guss. and *S. lutea* (L.) Ker-Gawl. ex Sprengel, were collected from different parts of Turkey (Table 1). Root tips (5–10 mm) obtained from potted bulbs were pretreated for 3 hr in 0.05% colchicine and fixed in 3 : 1 absolute alcohol : glacial acetic acid for overnight. They were hydrolysed in 1 N HCl at 60°C for 12–13 min and stained in Feulgen. Squashes were made in 1% lactopropionic orcein.

For Giemsa C-banding, root tips were hydrolysed in 45% acetic acid for 20 min at 60°C.

Table 1. Localities and chromosome numbers of *Sternbergia* populations used for karyological studies.

Species	Locality	Collector number (year)	Number of bulbs examined	Chromosome number (2n)
S. lutea	C1 İzmir: Selçuk, Belevi village, Kozpınarı district, 80 m, open field	H. Duman 5681 (1994)	30	33
	C1 Aydin: Aydın-Çine road, Koçarlı junction, 100 m, open field	H. Duman 5682 (1994)	15	22
	C2 Muğla: Fethiye, Fethiye-Ovacık road, 50–200 m, bushes, calcareous, stony places	H. Duman 5687 (1995)	10	22
	C3 Konya: Beyşehir, Kurucaova, 1150 m, open field	H. Duman 5685 (1995)	10	22
S. sicula	C2 Muğla: Marmaris, Taşlıca village, 200 m, calcareous, rocky places	H. Duman 5695 (1995)	20	22

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Squashes were made in 45% acetic acid. Slides were treated in 5% $Ba(OH)_2$ for 5 min at 55°C, washed in tap water and submerged in 2×SSC at 55–60°C for 1 hr. Slides stained for 10–30 min in 2% Giemsa at pH 6.8 were rinsed and air-dried. In both Feulgen and Giemsa staining, slides were mounted in entellan. Preparations were examined and photographs were taken using Olympus BH2 microscope and C-35 AD camera, on Agfapan APX film at 25 ASA.

Measurements were based on both unbanded and C-banded mitotic metaphases. Detailed karyotypes are based on a minimum of five cells. Chromosomes were classified according to the nomenclature of Levan *et al.* (1964). The satellites of nucleolar chromosomes were also measured and considered in determining the total chromosome length and arm ratio. In the

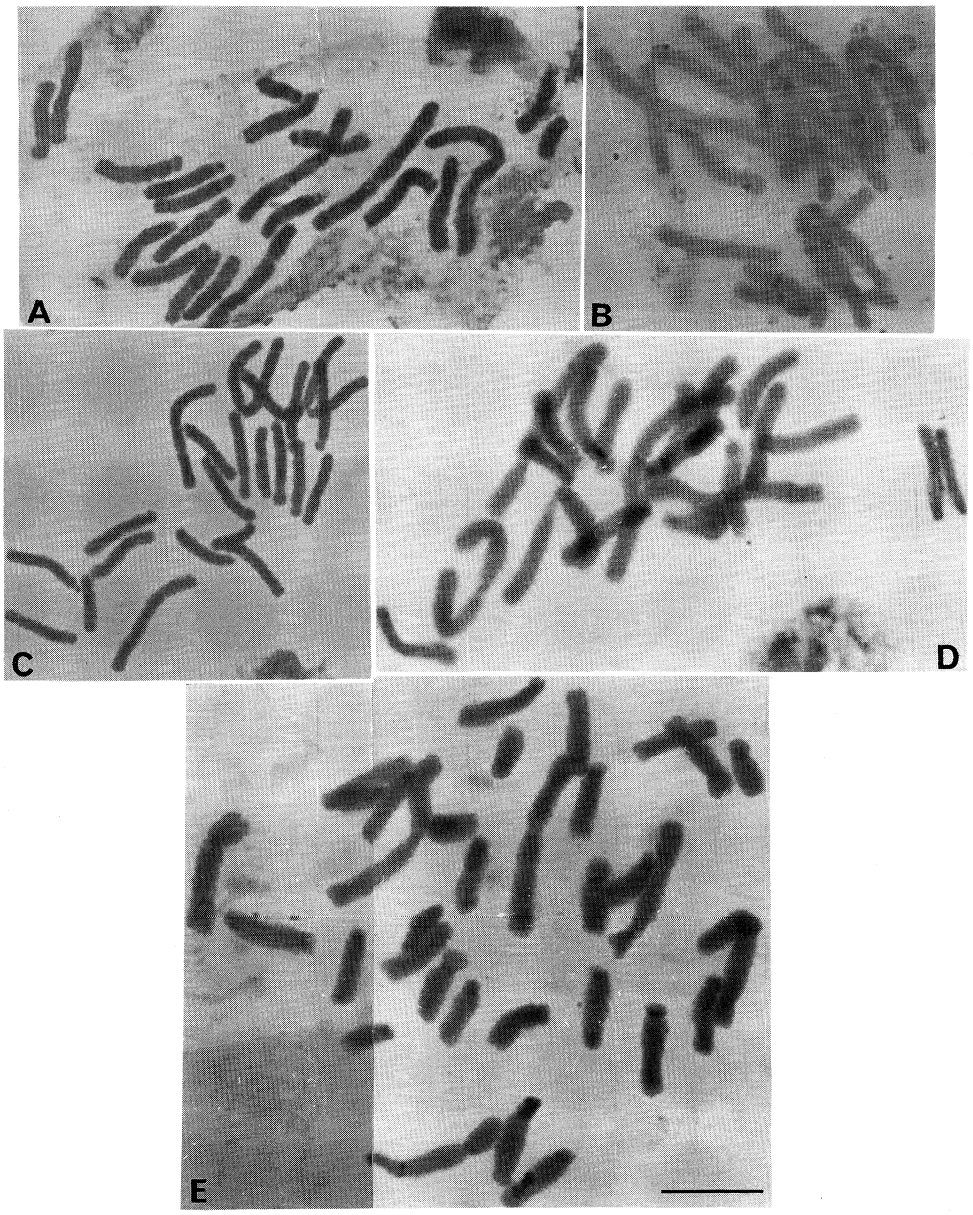


Fig. 1. Mitotic complements of *Sternbergia.* Feulgen/orcein stained preparations in *S. lutea* (A) and *S. sicula* (C). C-banded preparations in diploid *S. lutea* (B), *S. sicula* (D) and triploid *S. lutea* (E). Bar=10 μm.

karyotypes, the chromosomes were arranged in order of decreasing length.

Results

Karyotypes

Analysis of the somatic metaphases in two taxa of *Sternbergia* indicated that *S. sicula* is diploid with 2n=22 chromosomes (Fig. 1C) while *S. lutea* has both diploid and triploid numbers, with 2n=22 (Fig. 1A) and 2n=33, respectively. Karyotypic data for each taxa are presented in Table 2.

The first pair of chromosomes of each taxa are metacentric with arm ratios (r) 1.23 in *S. lutea* and 1.18 in *S. sicula.* Second pairs are submetacentric and their arm ratios are 2.87 and 2.81 in *S. lutea* and *S. sicula* respectively. Rest of the chromosomes are subtelocentric and acrocentric in both taxa but their order show little difference. Chromosomes 3 and 4 are acrocentric and their arm ratios range between 7.51 and 9.97. Fifth pairs are also the same as subtelocentric with arm ratios 3.17 and 3.79 in *S. lutea* and *S. sicula*, respectively. In *S. lutea*, both Chromosomes 6 and 7 are acrocentric and arm ratios are 7.57 and 14.13. In *S. sicula*, Chromosome 6 is subtelocentric (r=6.66) and Chromosome 7 is acrocentric (r=9.16). While Chromosome 8 is subtelocentric (r=6.05) in *S. lutea*, acrocentric (r=7.90) in *S. sicula.* Ninth Chromosome is subtelocentric in *S. lutea* and acrocentric in *S. sicula*, with arm ratios 5.01 and 8.89, respectively. In both taxa, Chromosome 10 is acrocentric; r=7.74 in *S. sicula* and 9.37 in

Table 2. Measurements (μm) of somatic chromosomes of two taxa in the genus *Sternbergia*

Chromosome pair no	Chromosome arms		Satellite length	Total length	Arm ratio (r) (L/S)	Relative length (%)	Centromeric position* (chromosome designation)
	long arm (L)	short arm (S)					
S. lutea							
1	10.93	8.92	—	19.85	1.23	17.45	m
2	11.21	3.90	—	15.15	2.87	13.31	sm
3	9.69	1.21	—	10.90	8.01	9.58	t
4	9.09	1.21	—	10.30	7.51	9.05	t
5	7.37	2.32	—	9.69	3.17	8.52	st
6	8.03	1.06	—	9.09	7.57	7.99	t
7	8.48	0.60	—	9.08	14.13	7.99	t
8	7.27	0.60	0.60	8.48	6.05	7.45	st
9	6.82	1.36	—	8.18	5.01	7.19	st
10	7.12	0.76	—	7.88	9.37	6.93	t
11	3.94	1.21	—	5.15	3.26	4.53	st
Total length of complement: 113.75							
S. sicula							
1	9.68	8.20	—	17.88	1.18	17.88	m
2	9.68	3.44	—	13.12	2.81	13.12	sm
3	8.76	0.90	—	9.66	9.73	9.66	t
4	7.78	0.78	—	8.56	9.97	8.56	t
5	6.68	1.76	—	8.44	3.79	8.44	st
6	7.00	0.75	0.30	8.04	6.66	8.04	st
7	6.96	0.76	—	7.72	9.16	7.72	t
8	6.64	0.84	—	7.48	7.90	7.48	t
9	6.58	0.74	—	7.32	8.89	7.32	t
10	5.88	0.76	—	6.64	7.74	6.64	t
11	3.96	1.20	—	5.16	3.30	5.16	st
Total length of complement: 100.02							

*m=median (metacentric); sm=submedian (submetacentric); st=subterminal (subtelocentric); t=terminal (acrocentric).

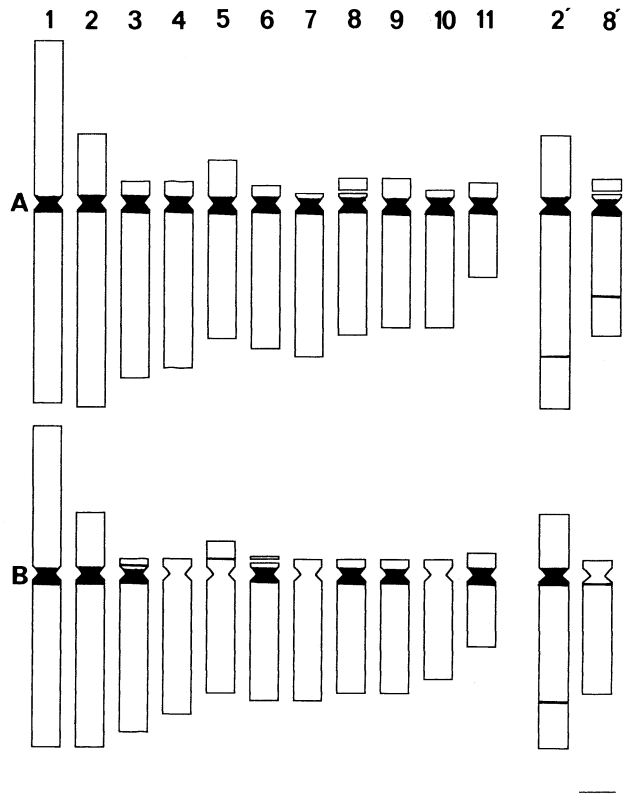


Fig. 2. Idiograms of C-banded chromosomes in *Sternbergia lutea* (A) and in *S. sicula.* (B). Bar=2 μm.

S. lutea. Chromosome 11 is subtelocentric with arm ratios 3.26 and 3.30 in *S. lutea* and *S. sicula*, respectively.

A satellite is observed on the short arms of Chromosome 6 in *S. sicula* and Chromosome 8 in diploid *S. lutea* but only on one of the homologous. In addition, satellited short arm in *S. lutea* is smaller than those in its homologous.

In triploid *S. lutea*, 2n=33 chromosomes were identified as coincident with diploid in chromosome morphology and measurements, in general. However, satellite is observed on the short arm of one representative of Chromosome 5, unlike diploid *S. lutea.*

C-banding

In *S. lutea*, all chromosomes showed centromeric C-bands (Figs. 1B, 2A). Interstitial bands are also detected on the long arm of Chromosomes 2 and 8 but only on one of the homologous.

In *S. sicula*, centromeric C-bands are detected on six pairs of chromosomes: 1, 2, 3, 6, 9 and 11 (Figs. 1D, 2B). The centromeric band on Chromosome 8 was replaced by a proximal band on the long arm in its homologous. One of the second chromosome pair carries an interstitial C-band on the long arm. Chromosome 5 shows no centromeric but an interstitial band on the short arm. Additional interstitial band is observed on the short arm of Chromosome 3. C-band is not observed on Chromosomes 4, 7 and 10 in *S. sicula.* In triploid *S. lutea*, centromeric C-band on the second chromosome was observed only on the short arms of two chromosomes but not on the long (Fig. 1E). All the other bands were the same with diploid.

Discussion

Sternbergia lutea and *S. sicula* are classified as two different species in the Flora of Turkey (Davis 1984). The character used to define them in species category is limited to the width of leaves which is 7–12 μm in *S. lutea* and 2–6 μm in *S. sicula.* However, they are confused with each other especially in early coming into leaf. Some midform of these species are also detected in nature. Taxonomic classification of plant species is initially based on morphological characteristics. Because these features vary and sometimes difficult to observe, other taxonomic criteria such as chrmosome number, chromosome morphology and C-banding have been developed. The results obtained in Feulgen and C-banding karyotypes showed that these two species are also strongly similar cytologically. All the members of *S. sicula* investigated are characterised by a uniform number of 2n=22 chromosomes which confirms previous reports from Turkey (Özhatay 1983) and Greece (Kamari and Artelari 1990, Artelari and Kamari 1991). *S. lutea* has both diploid and triploid chromosome number that is reported for the first time from Turkey. Analysis of these two taxa showed a general homogeneity in karyotypes, total chromosome lengths and arm ratios (Table 2). However, there are some differences from the previous studies (Özhatay 1983, Kamari and Artelari 1990, Artelari and Kamari 1991). Analysis following Giemsa C-banding performed for the first time to these taxa reveals distinct centromeric bands in both *S. lutea* and *S. sicula.* All chromosomes carry C-band in diploid and triploid *S. lutea.* In *S. sicula*, while Chromosomes 1, 2, 3, 6, 8, 9 and 11 displayed C-bands, Chromosomes 4, 7 and 10 did not. Heteromorphism detected in maize (Rayburn *et al.* 1985), *Scilla sibirica* (Vosa 1973) and *Lathyrus aphaca* (Ünal *et al.* 1995) is also observed in *S. lutea* and *S. sicula* being on the same chromosomes that are 2 and 8. These morphologic and cytologic similarities between *S. lutea* and *S. sicula* led us to make a decision about their classification category. *S. sicula* should be a subspecies of *S. lutea.*

Summary

Karyotype and C-banding analyse have been performed to *Sternbergia lutea* and *S. sicula* from Turkey. While *S. lutea* was found to be diploid (2n=22) and triploid (2n=33), *S. sicula* proved to be diploid with 2n=22. Chromosome lengths in *S. lutea* and *S. sicula* are 19.85–5.15 μm and 17.88–5.16 μm, respectively. While all chromosomes in *S. lutea* showed centromeric C-band, in *S. sicula*, eight chromosomes carry constitutive heterochromatin, being centromeric on seven and interstitial on one. Three chromosomes, 4, 7 and 10, in *S. sicula*, show no C-band. Based on these results, the relationships between the two taxa are discussed.

Key words: C-banding, Giemsa, Karyotype, *Sternbergia lutea*, *S. sicula*, Turkey.

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References

Artelari, A. and Kamari, G. 1991. The genus *Sternbergia* (*Amaryllidaceae*) in Greece: taxonomy and karyology. II. Bot. Chron. **10**: 239–251.

Davis, P. H. (ed.) 1984. Flora of Turkey and East Aegean Islands, V. 8, pp. 360–364.

Gill, B. S. and Kimber, G. 1974. Giemsa C-banding and the evolution of wheat. Proc. Natl. Acad. Sci. (USA) **71**:

4086–4096.

Hadlaczky, G. Y. and Belea, A. 1975. C-banding in wheat evolutionary cytogenetics. Plant Sci. Lett. **4**: 85–88.

Kamari, G. and Artelari, R. 1990. Karyosystematic study of the genus *Sternbergia* (*Amaryllidaceae*) in Greece. I. South Aegean Islands. Willdenowia **19**: 367–388.

Levan, A., Fredga, K. and Sandberg, A. A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas **52**: 201–220.

Özhatay, N. 1983. Türkiyenin *Sternbergia* türleri üzerinde sitotaksonomik çalışmalar. Tübitak VII. Bilim Kongresi, Biyoloji Seksiyonu Tebliğleri 117–124.

Rayburn, A. L., Price, H. J., Smith, J. D. and Gold, J. R. 1985. C-band heterochromatin and DNA content in *Zea mays*. Amr. J. Bot. **72**: 1610–1617.

Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M. and Webb, D. A. 1968. Flora Europea, V. 2. Cambridge University Press, Cambridge.

Ünal, F., Wallace, A. J. and Callow, R. S. 1995. Diverse heterochromatin in *Lathyrus*. Caryologia **48**: 47–63.

Vosa, C. 1973. Heterochromatin recognition and analysis of chromosome variation in *Scilla sibirica*. Chromosoma, **43**: 269–273.

Yu, H., Liang, G. H. and Kofoid, K. D. 1991. Analysis of C-banding chromosome patterns of *Sorghum*. Crop. Sci. **31**: 1524–1527.