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A karyological investigation on the two varieties of *Galanthus fosteri* Baker (*Amaryllidaceae)*

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Abstract

In this study, the chromosome numbers and morphology of the populations of *Galanthus fosteri* Baker var. *fosteri* and *Galanthus fosteri* var. *antepensis* Zeybek and Sauer distrubuting in various geographic regions of Turkey have been investigated. In the karyological investigations, the structural polymorphizm (SAT-chromosome) have been established in the chromosomes of *G. fosteri* var. *fosteri* and *G. fosteri* var. *antepensis.* Although the diploid chromosome numbers (2n=24) of these varieties are similar, the chromosome morphology of their are significantly different.

Key words: *Galanthus fosteri*, Karyotype analysis, Amaryllidaceae

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Galanthus fosteri Baker (*Amaryllidaceae*)'nin iki varyetesi üzerinde karyolojik bir araştırma

Özet

Bu çalışmada, Türkiye'nin farklı coğrafik bölgelerinde yayılış gösteren *Galanthus fosteri* Baker var. *fosteri* and *Galanthus fosteri* var. *antepensis* Zeybek and Sauer populasyonlarının kromozom sayıları ve morfolojisi incelenmiştir. Karyolojik incelemelerde, *G. fosteri* var. *fosteri* and *G. fosteri* var. *antepensis* kromozomlarında yapısal polimorfizm (SAT-kromozom) tespit edilmiştir. Bu varyetelerin diploid kromozom sayısı (2n=24) aynı olmasına rağmen, onların kromozom morfolojileri önemli derecede farklıdır.

Anahtar Kelimeler: *Galanthus fosteri*, Karyotip analizi, Amaryllidaceae

1. Introduction

Galanthus L. (Snowdrops) is represented by many species in Anatolia, Caucasus, Thrace, East Mediterranean countries and in the vicinity of the Black Sea (Kamari, 1982; Zeybek, 1988). The species of *Galanthus* L. genus have important features because of their use as ornamental plant and including various alkoloid (galanthamin) in their bulbs. The galanthamin alkoloid was the first time isolated from the bulbs of *G. woronowii* Los. Today, galanthamin is used for treatment of some vessel diseases, poliomyelitis and skeleton muscles. The bulbs of some of the wide spread Turkish *Galanthus* species have been investigated pharmaceutically for their alkoloid content (Zeybek, 1983; 1988).

Galanthus fosteri var. *fosteri* and *Galanthus fosteri* var. *antepensis* are bulbous ephemeroid geophytes of this family (Figures 1 and 2). They are highly decorative in early spring species.

The karyological studies have revealed a polymorphizm among different karyotypes and SAT-chromosomes have been reported to be important in the variation (Zeybek, 1988; Zeybek and Sauer, 1994; Şenel et al., 2002). A valuable information has been put forward on the structure of chromosomes, importance of B-chromosomes and the origin of the species through the karyological studies carried out on the taxa of *Galanthus*, distributed around Russia, Caucasus and Black Sea (Sveshnikova, 1975; Sveshnikova and Grif, 1981; Zeybek and Sauer, 1994). In Bulgaria

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(Popova, 1972) and Greece (Kamari, 1982; Papanicolaou and Zacharof, 1983) karyological investigations on this genus have been made and descriptions of the new taxa have been given by evaluating the karyotypes cytotaxonomically (Kamari, 1982). Karyological investigations on *Galanthus* in Turkey populations have been made by Zeybek (1983), Zeybek and Sauer (1994) and Şenel et al. (2002). The karyological studies on plants which are valuable as economic and ornamental plants are very important. Also, these studies can be used in taxonomical studies on these species.

The aim of the present study is to determine the number and morphological properties of chromosomes in Turkey populations of the two varieties.

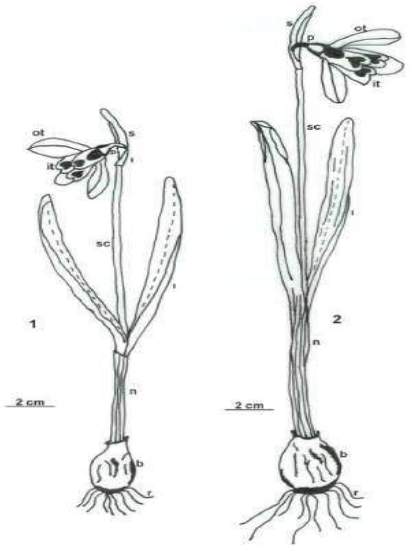
2. Materials and methods

The samples of *G. fosteri* var. *fosteri* have been collected from the localities listed below:

A5 Amasya: Akdağ, Doğantepe, Kayacık Village scrubs areas, 950 m, March 20, 2004, Kandemir, with the collection number 178 (Figure 3). (Karyotype analysis has been done the population in this localities)

A5 Amasya: Akdağ, Ziyaret Village, Alanoğlu site-Adatepe scrubs areas, 1450 m, March 23, 2004, Kandemir, with the collection number 179 (Figure 3).

A5 Amasya: Akdağ, Vermiş-Lokman Hekim Mausoleum, scrubs areas, 1000 m, March 23, 2004, Kandemir, with the collection number 180 (Figure 3).



Figures 1, 2. General appearences of *G. fosteri* var. *fosteri* and *G.fosteri* var. *antepensis* (r: root, b: bulb, n: neck, l: leaf, sc: scape, s: spatha, p: pedicel, ot: outher tepal, it: inner tepal)

The samples of *G. fosteri* var. *antepensis* have been collected from the localities listed below:

C6 Gaziantep: Bahçe, Acarobası Village scrubs areas, 1250 m, February 20, 2004, Kandemir, with the collection number 181 (Figure 3).

Somatic chromosomes have been studied from actively dividing root-tip cells obtained from natural populations. The chromosome counts and morphology have been carried out during the mitotic phase and processed according to the following squash technique (Ozkan et al., 2001; Şenel et al., 2002). The root-tips have been pretreated in α-monobromonapthalene solution for 14 h at 4°C and then fixed in acetic acid-alcohol (1:3). After washing in alcohol they have been hydrolyzed in 1N HCl for 10-12 minutes at 60°C in an oven. The root-tips have been stained with the leuco-basic fuchsin for 1 hour and squashed in 45 % acetic acid. Karyotype analysis has been performed according to the method described by Naranjo et al. (1986). Permanent slides for karyotype analysis for each variety have been prepared from at least ten well-spreaded metaphase cells. The photographs of the preparations have been taken with a Nicon microscope. The karyograms have been drawn from the metaphase. Measurements have been made on each pair of mitotic chromosomes.

3. Results

3.1. Galanthus fosteri var. *fosteri*: The chromosome number of this variety is 2n=24 (10M+8ST+6SM) (Figures 4 and 5). The karyotype of this variety consists of 5 pairs of median (M), 4 pairs of subterminal (ST) and 3 pairs of submedian (SM) chromosomes. The 1st, 2nd, 9th, 11th and 12th chromosomes are median, the 3th, 5th, 6th and 7th chromosomes are subterminal and the 4th, 8th and 10th chromosomes are submedian centromers (Figure 4 and Table 1). Satellite is present

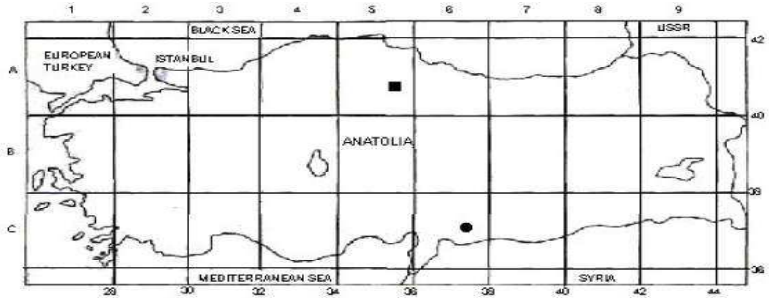


Figure 3. The distribution in Turkey. :*G. fosteri* var. *fosteri* , : *G fosteri* var. *antepensis*

on the short arm of the 5th chromosome of this variety. Chromosome lengths range from 5.5 to 22 µm. Longest arm is 12.5 µm and shortest arm is 2 µm. The lenght and arm ratio of each chromosome are presented in Table 1.

3.2. *Galanthus fosteri* var. *antepensis* : The chromosome number of this variety is 2n=24 (10M+10ST+4SM) (Figures 6 and 7). The karyotype of this variety consists of 5 pairs of median (M), 5 pairs of subterminal (ST) and 2 pairs of submedian (SM) chromosomes. The 1st, 8th, 10th, 11th and 12th chromosomes are median, the 2nd, 3th, 4th, 5th and 6th chromosomes are subterminal and the 7th and 9th chromosomes are submedian centromers (Figure 6 and Table 2). Satellite is present on the short arm of the 6th chromosome. Total chromosome lengths are about 5.5 to 20µm. Longest arm is 11.5 µm and shortest arm is 2 µm. The karyotype details, including chromosomes lenght and arm ratio, are shown Table 2.

Figure 4. Microphotograph of somatic metaphase chromosomes of *G. fosteri* var. *fosteri*

Table 1. The chromosome types, chromosome length and arm ratio of *G. fosteri* var. *fosteri*

Chromosome pairs	Total Length	Long arm length	Short arm lenght	Arm ratio	AT	Centromeric index I=(S/C) 100	Centromere position
1	22	11	11	1.		50	Medi
2	21	10.5	10.5	1.		50	Medi
3	15	12.5	2.5	1.		16	Subte
4	12.5	9	3.5	5.		28	Subm
5	11	8.5	2.5	2.		22	Subte
6	10	8	2	3.		20	Subte
7	9	7	2	4.		22	Subte
8	8.5	6	2.5	3.		29	Subm
9	7	3.5	3.5	3.		50	Medi
10	6.5	4	2.5	1.		38	Subm
11	6	3	3	1		50	Medi
12	5.5	3	2.5	1.		45	Medi

*: Satellite

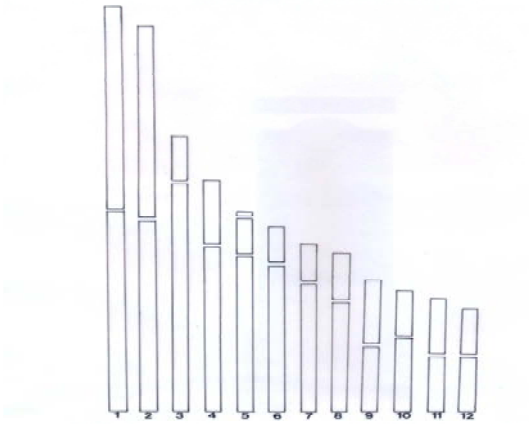


Figure 5. Idiogram of chromosome complement of *G.fosteri* var. *fosteri* at mitotic metaphase

Figure 6. Microphotograph of somatic metaphase chromosomes of *G. fosteri* var. *antepensis*

Table 2. The chromosome types, chromosome length and arm ratio of *G. fosteri* var. *antepensis*

Chromosome pairs	Total Length (C) µm	Long arm length (L) µm	Short arm lenght (S) µm	Arm ratio R=L/S	AT	Centromeric index I=(S/C) 100 µm	Centromere position
1	20	10	10	1.		50	Median
2	14.5	11.5	3	3.		20	Subter
3	12.5	9.5	3	3.		24	Subter
4	11.5	9.0	2.5	3.		21	Subter
5	10.5	8	2.5	3.		23	Subter
6	10	8	2	4.		20	Subter
7	9.5	7	2.5	2.		26	Subme
8	8	4	4	1.		50	Median
9	6.5	4	2.5	1.		38	Subme
10	6	3	3	1.		50	Median
11	6	3	3	1.		50	Median
12	5.5	3	2.5	1.		45	Median

4. Conclusions

Galanthus is a very ornamental genus and of potential medicinal value for its alkoloid content. They are formely widely distrubuted in Turkey but its area has been considerably reduced as a result of destruction of its primary habitats and through gathering of the flowers and bulbs (Zeybek, 1988; Ekim et al., 1991).

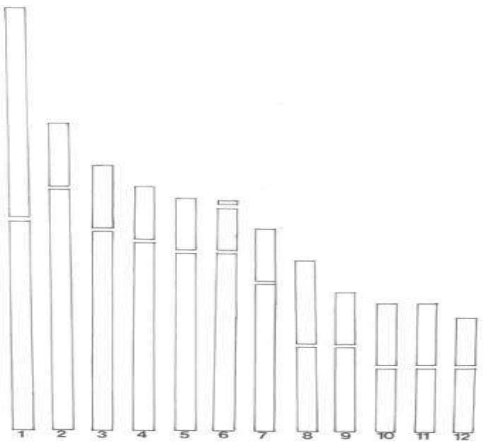


Figure 7. Idiogram of chromosome complement of *G. fosteri* var. *antepensis* at mitotic metaphase.

The essential *Galanthus* species growing in Turkey, i.e. *G. elwessi* Hooker fil., *G. ikariae* Baker, *G. nivalis* L. subsp. *cilicicus* (Baker) Gottlieb-Tannenhain, *G. fosteri* Baker and *G. gracilis* Celak are exported (Ekim et al.,,1991; Budnikov and Kricsfalusy, 1994; Kandemir et al., 1997).

The karyological study in this family has been attempted by a number of workers (Zeybek and Sauer, 1994; D'amato and Bianchi, 1999; Ito et al., 1999; Şenel et al.,2002). Although chromosome number of *G. fosteri* is given as 2n=24 in Flora of Turkey (Davis, 1984), chromosome numbers of these two varieties are not given. Also, the karyotypes of these varieties mentioned above have not been studied before in Turkey. In this paper, we have determined the chromosome number of these varieties as 2n= 24. The basic chromosome number of two varieties are x= 12. Ito et al. (1999) divided *Amaryllidaceae* into geographical clades according to their center of origin (namely The African Clade I, The African Clade II, The Cyrtanthus Clade, The American Clade, The Malaysia-Australian Clade, The Asian Clade and The Mediterranean Clade). In the Mediterrenean Clade comprises of three different tribes: *Pancratieae* (x=11), *Galantheae* (x= 7, 8,9, 11and 12) and *Narcisseae* (x= 7, 10 and 11) that includes a wide range of different basic chromosome numbers (Meerow, 1995). The genus of *Galanthus* L. distributed in Turkey and Caucasia are reported to have diploid with 2n=24 chromosomes number. Budbikov and Kricsfalusy (1994) and D'amato and Bianchi (1999) reported that *G. nivalis* showed 2n=24 chromosome number. Also, some species in Turkey and Caucasia are established to have different chromosome numbers (2n=24, 26, 48 ve 72)(Zeybek, 1983; Sveshnikova, 1975; Şenel et al., 2002). Although most of the *Galanthus* L. species in Caucasia is of the diploid form with 2n=24 chromosome, *G. lagodechianus* Kem-Nat. species is of the hexaploid form with 2n=72 chromosomes (Sveshnikova, 1975). Furthermore, diploid and tetraploid forms of *G. elwesii* subsp. *elwesii* have been observed in İzmir Yamanlar mountain, with 2n=24 chromosomes and Greece with 2n=48, respectively (Zeybek and Sauer, 1994). On the other hand, the diploid (2n=24) and triploid (2n=36) forms of *G. rizehensis* Stern (1956) have been obtained. In addition, it is reported by Şenel et al. (2002) that the chromosome number of *G. rizehensis* is as 2n= 26 in Turkey populations.

The karyological studies in *Galanthus* have revealed a polimorphism among different karyotypes SAT and accesory chromosomes (B- chromosomes) have been reported to be important in the variation (Zeybek, 1983; 1988; Zeybek and Sauer, 1994; Kamari, 1982; Şenel et al., 2002). In addition, D'amato and Bianchi (1999) reported that the occurence of accessory chromosomes is very common in *Galanthus.* In populations of *G. elwesii* in Rumania and Bulgaria, 0-3 B-chromosomes are obtained. In population of *G. gracilis, G. nivalis* subsp. *reginae-olgae, G. elwesii* subsp.*akmani* in Turkey 0-1B chromosomes are determined (Zeybek and Sauer, 1994). Also, in population of *G. nivalis* in the East Carpathians 0-1B are obtained (Budnikov and Kricsfalusy, 1994). In this study, the SAT chromosomes have been found whereas the accesory chromosomes have not been found in two varities.

Phylogenetic analyses of the genera *Leucojum* and *Galanthus* based on plastid (trnL-F and mat K) and nuclear (ITS) DNA sequences were done by Lledo' et al. (2004). The data was analysed separately and in combination, showing that the boundaries between the two genera are not appropriate. *Galanthus* is monophyletic but embedded in *Leucojum*. Also, DNA sequences are useful for comparing species and closely related genera. Similar chromosome morphology among the species of these genera is characteristic. Previous studies indicated that noncoding chloroplast DNA region, such as trnL-F, consistently yield low levels of variation. Briefly, it is necessary to analyse the plastid (trnL-F and matK) and nuclear (ITS) DNA sequences of the *Galanthus* species Mediterranean Clade to clarify the taxonomic states of two varieties.

The somatic chromosomes of two varieties of *G. fosteri* have been examined. There are some differences between the karyotypes of two varieties. These differences are total chromosome lengths, centromeric positions, arm ratio and centromeric index. number of these varieties as 2n= 24. *G. fosteri* var. *fosteri* and *G. fosteri* var. *antepensis* have 3 chromosome types. These types are classified as median centromeric, submedian centromeric and subterminal centromeric. The karyotype of *G. fosteri* var. *fosteri* consists of 5 pairs median, 4 pairs subterminal and 3 pairs

submedian centromeric chromosomes (Table 1). The karyotype of *G. fosteri* var. *antepensis* consists of 5 pairs median, 5 pairs subterminal and 2 pairs submedian centromeric chromosomes (Table 2). Also, some differences have been obtained in morphologic and anatomic properties of two varieties (Kandemir and Akçin, 2006). The morphological and anatomical differences between these two varieties are thought to be originated from the morphological structures of the chromosomes; for instance the differences of leaf apieces, spatha and pedicel length, the shapes of green spots in inner and outher surfaces of iner tepals, anther apieces, stigma and style properties, number of xylem arm in root anatomy, the thickness structure of cuticular layer in leaf anatomy, the number of cell layers in palizad and spongy parenchyma layers and stomatal index. The morphologic, anatomic and karyologic properties mentioned above are import in taxonomy of two varieties. Also, between these two varieties there are both geographical and reproduction isolation. Thus, their have been thought to be allopatric.

In sum, taking into account these differences, we are of the opinion that by changing the taxonomic status, these two varieties should be enhanced to subspeciescategory.

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