

Analysis of Chromosome Number in Some *Allium* and *Silene* Wild Species with Ornamental Use

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Abstract

The present study analyses the number of somatic chromosomes in plant species with ornamental value, in Romanian indigenous flora, *Allium* (*A. flavum* L., *A. saxatile* Bieb.) and *Silene* (*S. compacta* Fischer., *S. supina* M.Bieb.). The biological material was identified and harvested in the South-Eastern part of Dobrogea (Tulcea and Constanța counties), area in the South-Eastern part of Romania, situated between the Danube and the Black Sea. Individuals from two populations of *Allium flavum* and *Allium saxatile*, respectively from Tulcea county (Turcoaia town) and Constanța county (from Cheile Dobrogei) were analyzed. In the case of *Silene compacta* and *Silene supina*, plants of one population in Tulcea county, Turcoaia town, were used. The aim of the analysis was the quantification and comparison of the somatic chromosomes of plants from the same species but different populations, as is the case of the two *Allium* species, but also to relate them to results from specialty literature, reported by others. The chromosome number was determined in the roots of the small plants that were obtained from seeds collected in their natural habitat. Except the case of *Silene supina* species, where only diploid forms were identified ($2n=24$), when in specific literature tetraploid forms are mentioned ($2n=48$), found in other areas, all other analyzed taxa register similar data to that reported in other geographic regions. Respectively, $2n=16$ in the case of *Allium flavum* and *Allium saxatile* and $2n=24$ in the case of *Silene compacta*.

Keywords: *Allium flavum*, *Allium saxatile*, chromosome numbers, *Silene compacta*, *Silene supina*, wild flora of Romania

Introduction

Romanian flora boasts a wealth and diversity due to geographical, climatic, edaphic and topographic factors specific to SE Europe in general, and the territory of Romania in particular. Studies regarding the chromosomal number in taxa from different ecological areas of Romania have previously been registered by Tăcină (1980) for endemic species (*Hepatica transilvanica*) and by Băra *et al.* (2003) for rare species of Romanian flora (*Allium flavum* ssp. *tauricum*, *Acanthus balcanicus*, *Asphodeline lutea*, *Dianthus spiculifolius*, *Digitalis ferruginea*, *Galanthus elwesii*, *Helleborus odoratus*, *Laburnum alpinum*, *Peltaria alliacea*, *Petrorhagia saxifraga*, *Prunus tenella*, *Alyssum borzeanum*, *Angelica arhangolica*, *Dianthus nardiformis*, *Globularia punctata*, *Paeonia tenuifolia*, *Paliurus spina christi*, *Polemonium coeruleum*, *Silene nutans* ssp. *dubia*, *Veratrum nigrum* etc.). Boșcaiu *et al.* (1999) studied the perennial polyploid taxa of *Cerastium* from Romania and other European countries, while Baltisberger and Widmer (2009) analyzed from a karyologic point of view *Achillea*, *Antennaria*, *Centaurea*, *Gnaphalium*, *Pilosella*, *Podospermum*, *Campanula*, *Dianthus*, *Silene*, *Geranium*, *Nepeta*, *Stachys*,

Teucrium, *Nigritella*, *Setaria*, *Aconitum*, *Consolida*, *Ranunculus*, *Geum montanum*, *Potentilla*, *Veronica* species from different Romanian regions. Similar research was done by Mraz and Szlag (2004) in the case of *Hieracium* and *Pilosella*, Brullo *et al.* (1996) in *Allium fuscum*, while Jacobsen and Ownbey (1977) and Weimarck (1971) (quoted by Krahulcova, 2003), analyzed *Allium flavum* and *Hierochloë australis*.

Dobrogea represents one of the most important Romanian areas in view of its flora and habitat diversity, due to its many ecosystems ranging from steppe, silvo-steppe, forest to aquatic zones, lakes and sea (Sălăgeanu *et al.*, 1973). In this area, around 2000 taxa are found, among which 20 endemic species and over 200 rare plants (Anastasiu and Negrean, 2006). *Allium* genus (Alliaceae) comprises of approximately 750 species (Stearn, 1992, quoted by Yusaf, 2004), spread all over the Northern hemisphere, from arid subtropical to boreal regions (Fritsch and Friesen, 2002). In the indigenous flora of Romania, a number of 22 species were recorded and 19 in Dobrogea (Oprea, 2005). *Allium flavum* L. is a geophytic perennial plant, originating from the specific temperate climate area of Europe. In Romania, *A. flavum* is sporadically met in arid, rocky regions, in

meadows and shrubberies, from the steppe area until the beech tree level (Ciocârlan, 2000). *Allium saxatile* Bieb. originating from the Pontic - Balkan area, is rarely met in Romania, mainly on rocky hills of the steppe and silvo-steppe in Dobrogea (Ciocârlan, 2000).

Silene genus (Caryophyllaceae) has over 700 species in the Northern hemisphere in Europe, Asia and North of Africa (Bari, 1973; Greuter 1995 quoted by Sheidai et al., 2009). In the Romanian spontaneous flora approximately 40 species were identified 29 in Dobrogea (Oprea, 2005). *Silene compacta* Fischer. is an annual species, of Pontic - Mediterranean origin. In Romania it is met rarely on the rocky coasts from the North of Dobrogea (Ciocârlan, 2000; Oprea, 2005). *Silene supina* M.Bieb. (syn. *Silene spergulifolia* (Wild) M.Bieb.) is considered rare in Romania and is usually encountered in stony areas in Tulcea, Mehedinți, Caraș Severin (Ciocârlan, 2000).

The aim of this article is to update information referring to the number of chromosomes in Romanian wild plants with ornamental use. Although there is already a number of cytogenetic studies regarding the Romanian indigenous flora, an update of the information is necessary either for confirming the existing data, either for identifying new possible forms, especially when, at the same species, variations of the chromosome number appear, determined by specific conditions of the origin area of the plants.

Materials and methods

The analysis regarding the chromosome number was done for species of *Allium* and *Silene*: *Allium flavum* L., *Allium saxatile* Bieb., *Silene compacta* L., *Silene supina* Bieb. The observations were done on populations originating from different areas, except the *Silene compacta* and *Silene supina* species, where the seeds were collected from only one population.

The number of chromosomes was determined from the roots of the young plants obtained from seeds harvested from their natural habitat, Dobrogea, area situated in the South-Eastern part of Romania (Fig. 1). Harvesting of seeds was done during 2009 - 2010.

Allium flavum L. has ornamental use due to its yellow flowers, grouped in umbelliferous inflorescences that bloom in July-August (Fig. 2). Seeds were harvested from two populations from the area of Măcin mountains (Turcoaia town, Tulcea county) and in Cheile Dobrogei (Constanța county).

Allium saxatile Bieb. is ornamental through its globular umbelliferous inflorescences, with white pinkish flowers. Flowering takes place in June-September (Fig. 3). The biological material (seeds) was harvested from two populations, respectively from the area of Măcin mountains (Turcoaia town, Tulcea county) and from Cheile Dobrogei.

Geographical characteristics (lat. N, long. E, approximate altitude) of settlements where the *Allium* taxa originate from are presented in Tab. 1.

The ornamental elements of *Silene compacta* Fischer. are the pink flowers, grouped in a compact capituliform inflorescence (Fig. 4), that bloom in summer (June-Au-

Tab. 1. Origin areas of *Allium flavum* and *Allium saxatile* populations

Species	Origin	Geographical position	Altitude (m)
<i>Allium flavum</i> L.	Turcoaia (Tulcea county)	45.10 N 28.18 E	290
	Cheia (Cheile Dobrogei – Constanța county)	44.50 N 28.41 E	120
<i>Allium saxatile</i> Bieb.	Turcoaia (Tulcea county)	45.10 N 28.18 E	290
	Cheia (Cheile Dobrogei – Constanța county)	44.50 N 28.41 E	120



Fig. 1. Natural habitats of studied taxa (Romania)

(<http://www.world-geographics.com/maps/europe/map-of-romania/>)



Fig. 2. *Allium flavum* (<http://www.harperleyhallfarmnurseries.co.uk/image/cache/data/Allium%20flavum-500x500.jpg>)

gust). Seeds from a population in Măcin mountains, Turcoaia town (Tulcea county) were harvested. *Silene supina* M.Bieb. has a branched stem, while the flowers are grouped in inflorescences, with a short pedicel, a long, cylindrical calyx and white bifurcate petals (Fig. 5). The seeds were harvested from plants from o population in Măcin mountains, Turcoaia town (Tulcea county).

Geographical characteristics of the settlement where *Silene* taxa originate from are presented in Tab. 2.

Chromosome analysis

In order to study the number of chromosomes, temporary preparates from radicular meristem (1-1.5 cm in length) of 3-7 days old plants were obtained. The seeds



Fig. 3. *Allium saxatile*

(http://farm2.staticflickr.com/1038/1347242925_88042bd8be.jpg)



Fig. 4. *Silene compacta* (original)



Fig. 5. *Silene supina* (original)

Tab. 2. Origin areas of *Silene compacta* and *Silene supina* populations

Species	Origin	Geographical position	Altitude (m)
<i>Silene compacta</i> Fischer.	Turcoaia (Tulcea county)	45.10 N 28.18 E	290
<i>Silene supina</i> M.Bieb.	Turcoaia (Tulcea county)	45.10 N 28.18 E	290

were set to germinate in Petri dishes, on moist filter paper, at room temperature (18-20°C). The stages of preparing the biological material were: prefixation, fixation, hydrolysis, staining and mounting the prepareate on slides. Prefixation of biological material was done in the morning, between 8-10, using colchicine 0.5%, for 30 minute. Fixation was obtained using Carnoy I (glacial acetic acid: ethylic alcohol 96° 1:3), for 24 hours, at 4°C. Hydrolysis was done with clorhydric acid 1N, at 60°C, for 14 min., following a rinsing of the material taken out of the fixator with distilled water. Staining was done using the Feulgen method (Țirdea and Leonte, 2003).

The samples were analyzed with Motic B1 optical microscope with a 100× lens, while the relevant images were captured with Motic video camera and processed with the specific software. The quantification of the number of chromosomes was performed in metaphasic cells from the total of 4000 cells analysed.

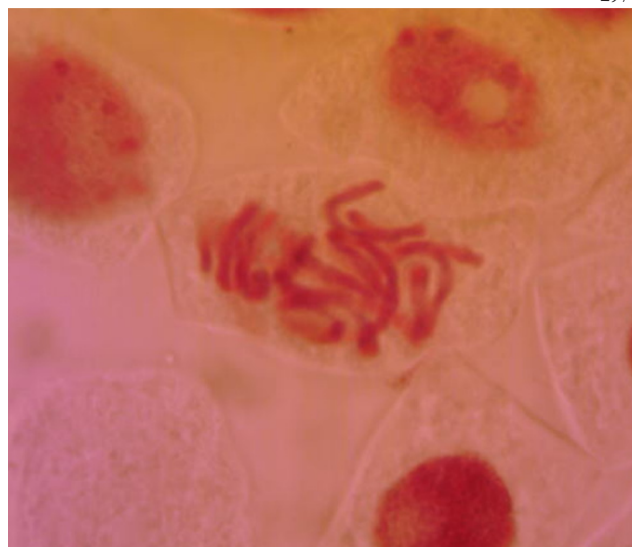
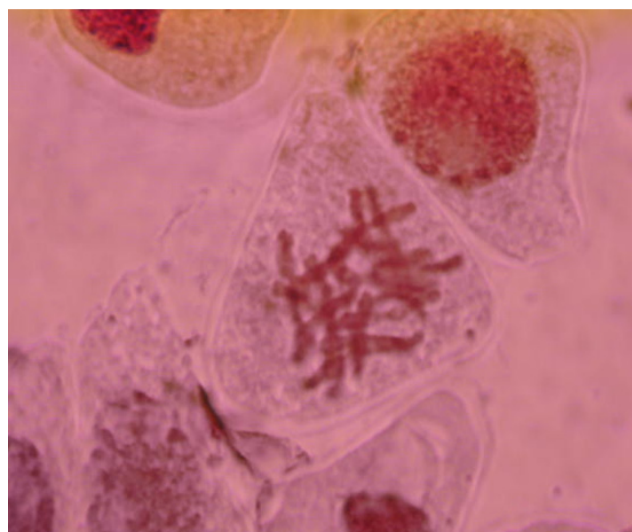
Results and discussion

Our analysis on the two populations of *Allium flavum* harvested in Dobrogea have confirmed the existence of diploid forms, $2n=16$ (Fig. 6).

Moreover, the analysis conducted on plants of *Allium saxatile* from Tulcea and Constanța led us to draw the conclusion that the plants are diploid, with chromosome number of $2n=16$ (Fig. 7).

The basic chromosome number for *Allium* genus is $x=7$, $x=8$ and even $x=9$, while polyploidy forms can have from $2x$ up to $16x$ (Bennett et al., 2000), the existence of a variable number of chromosomes being also possible (Baranyi et al., 1999; Fritsch and Friesen, 2002). The majority of Asian species are registered as tetraploid $2n=32$, while the European ones are recorded as diploid, $2n=16$ (Kawano and Nagai, 2005). Only one species has an octaploid form, *Allium monophyllum* (Fritsch and Astanova, 1998). A greater karyological stability is recorded in *Allium ursinum* and all bibliographical data mention its diploid form, $2n=14$ (Baranyi et al., 1999; Draghia et al., 2010; Karpaviciene, 2007).

In Europe, *Allium flavum* L. has a variable number of chromosomes, being registered in its diploid, as well as in its tetraploid form ($2n=16$, respectively $2n=32$), being characterized by a morphological diversity of homologue chromosomes. The existence of diploid and tetraploid populations, separated from a geographical as well as an

Fig. 6. Somatic metaphase in *Allium flavum* ($2n=16$) (original)Fig. 7. Somatic metaphase in *Allium saxatile* ($2n=16$) (original)

ecological point of view, has been confirmed by many authors. In some cases, B-chromosomes have been registered in diploid forms. Krahulcová (2003) reports the presence of diploid populations ($2n=16+2B$) of *Allium flavum* in the South of Moravia, synthetizing at the same time, the results obtained by other authors, from different areas of Europe: diploid forms ($2n=16$) identified in Albania ($2n=16+1B$: Baltisberger and Baltisberger, 1995; sec. Goldblatt and Johnson, 1998), Austria ($2n=16$, $2n=16+1-2B$: Dobeš and Vitek, 2000), France (Natarajan, 1978), Italy ($2n=16$, $2n=16+2B$: Capineri et al. sec. Goldblatt, 1984), Macedonia (Šopova, 1972 sec. Goldblatt, 1981), Serbia (Lovka, 1995), Romania (Jacobsen and Ownbey, 1977), Slovakia (Hindáková, 1970 sec. Májovský, Murín et al., 1987; Hrušovská-Osuská, 1988 sec. Májovský et al., 2000), Turkey ($n=8+1B_{II}$: De Sarker et al., 1997, $2n=16$: Özhatay, 1984 sec. Goldblatt, 1988); tetraploid forms ($2n=32$) identified in Mediterranean areas, in Spain (Ruiz

Rejon, 1976) and Montenegro (van Loon Kieft, 1980); both diploid and tetraploid forms: in Bulgaria (van Loon and van Setten, 1982) and in Greece (Strid and Franzén, 1981; Tzanoudakis, Vosa, 1988 sec. Goldblatt and Johnson, 1991; Karavokyrou and Tzanoudakis, 1991 sec. Goldblatt and Johnson, 1994).

The information regarding the karyotype of the *Allium saxatile* Bieb. species is insufficient, the specific literature having only data that mentions the presence of diploid plants, with the basic chromosomes number $x=8$ ($2n=2x=16$) (Ciocârlan, 2000; Ricroch et al., 2005)

The obtained results from the analyzed *Silene compacta* and *Silene supina* species ($2n=24$) are similar to those recorded by other authors, in the case of taxa from other areas.

The analysis of plants harvested from Măcin Mountains (Turcoaia town) (Tab. 3), have confirmed the results found in specific literature (Fig. 8 and 9).

Cytogenetic studies of *Silene* genus were applied to species and populations from different geographical areas: Turkey (Martin et al., 2008; Minareci et al., 2009; Ozturk et al., 2009; Yildiz et al., 2006, 2009), Iran (Gholipour and

Sheidai, 2010; Sheidai et al., 2008, 2009), Cypress (Yildiz and Gucl, 2006), Greece (Constantinidis et al., 2009), Bulgaria (Pavlova and Tocheva, 2004), North America (Popp and Oxelman, 2007) or other regions (Bari, 1973; Nersesian and Goukasian, 1995; Vogt and Oberprieler, 2009) as well as for Romanian species (Baltisberger and Widmer, 2009; Ciocârlan, 2000; Ironside and Filatov, 2005).

The majority of the references in specialty literature show that diploid species ($2n=2x=20$ or $2n=2x=24$) are more spread, then tetraploid forms ($2n=4x=48$), hexaploid ($2n=6x=72$) and that only some have a higher degree of polyploidy $2n=c.96$, 120 and 192 (Bari, 1973). Triploid forms are found in the *Silene fortunei* species (Heaslip, 1951, quoted by Sheidai, 2009). Furthermore, reports of $2n=18$ ($x=9$) in the case of *S. conica* and *S. lacera* (Spova and Sekovski, 1982, quoted by Sheidai et al., 2009) or $2n=46$ ($x=23$) for *S. firma* (Zhang, 1994, quoted by Sheidai et al., 2009). In Turkey, Ozturk et al. (2009) registers a different number of chromosomes in three *Silene* species: *Silene alba* subsp. *divaricata* $2n=24$, *Silene conoidea* $2n=20$ and $2n=24$ for *Silene otites*.

In the case of *S. compacta* and *S. supina*, the literature specifies that the basic number of chromosomes is $x=12$ and that diploid forms $2n=2x=24$ exist in populations from Dobrogea, Romania (Ciocârlan, 2000), Turkey (Bari, 1973; Yildiz and Cirpici, 1996, quoted by Pavlova, 2004) and Greece (Strid and Franzén, 1983). The results are different from the ones obtained by Pavlova and Tocheva (2004), that report tetraploid forms $2n=48$ chromosomes in populations of *S. supina* from Bulgaria (coast of the Black Sea).

Conclusions

The results obtained from the *A. flavum*, *A. saxatile*, *S. compacta* and *S. supina* taxa from populations located in the South-Eastern area of Romania (Dobrogea) are similar to the data reported for populations in other natural habitats. The two *Allium* species (*A. flavum* and *A. saxatile*) are diploid, with $2n=16$ chromosomes. The somatic number of chromosomes registered in *S. compacta* and *S. supina* species from Dobrogea (Turcoaia town) was $2n=24$.

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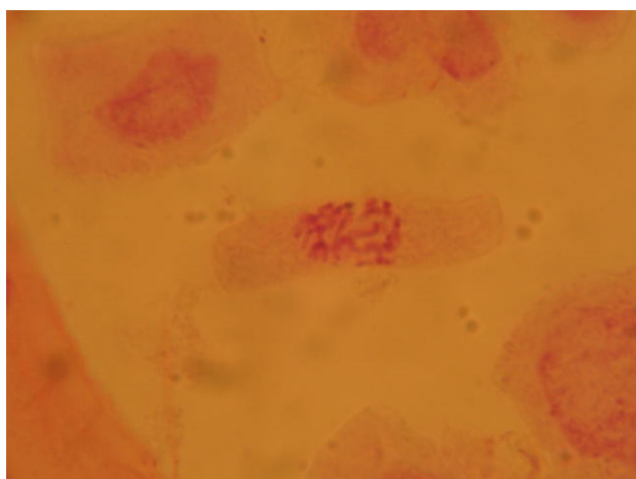


Fig. 8. Somatic metaphase in *Silene supina* ($2n=24$) (original)

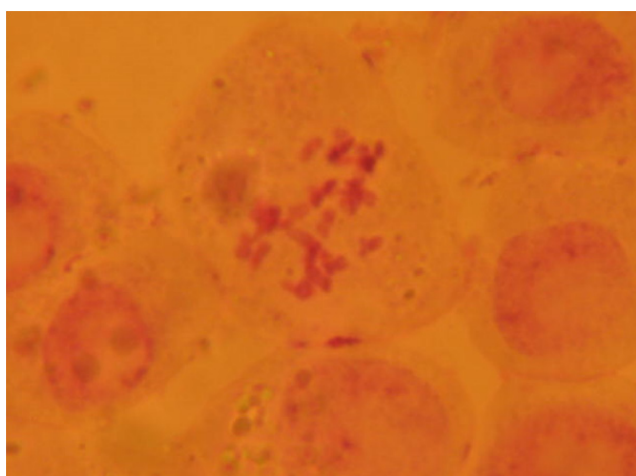


Fig. 9. Somatic metaphase in *Silene compacta* ($2n=24$) (original)

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