

Zhaneta ZEKAJ (TROJANI)*, Lefter KASHTA**

CYTOLOGICAL DATA ABOUT *LEUCOJUM AESTIVUM* L. FROM SHKODRA LAKE SURROUNDINGS

Përmbledhje: *Leucojum aestivum* L. është e njohur si specie e përhapur në zona të lagëta në Europë dhe Turqi. Kjo specie bimore autoktone është përdorur që prej disa dekadash në mjekësinë bullgare dhe ndofta prej shekujsh në Europën Juglindore, prej përmbajtjes së alkaloidit galantaminë. Përveç përhapjes së kësaj specie, janë paraqitur gjithashtu dhe disa të dhëna citologjike. U studiua numri i kromozomeve $2n=22$ dhe karakteristikat morfologjike të kariotipit. U përcaktuan formula Levan, kariograma dhe idiograma e kromozomeve për këtë citotip. Meqenëse kjo specie është gjetur në mjedise të lagëta në rrethinat e Liqenit të Shkodrës dhe në disa lokalitete të tjera në Shqipëri, është propozuar si kandidat e Listës së Kuqe të Shqipërisë.

Fjalë kyçe: *Leucojum aestivum*, përhapja, kromozome, citotip, kariograma, Liqeni i Shkodrës

Abstract: *Leucojum aestivum* L. is known as a species of wetlands distribution in Europe and Turkey. This indigene plant species has been used since several decades in Bulgarian medicine and maybe for centuries in countries of southeastern Europe, because of alkaloid galantamine content. Along with distribution for this species some cytological data are also presented. The chromosome number $2n=22$ and the morphological characteristics of the karyotype have been studied. Formula Levan, karyogram and idiogram of chromosomes for this cytotype studied have been determined. Since the species it is found in wet meadows in surroundings of Shkodra Lake and in few other localities in Albania, as candidate of Red List of Albania is proposed.

Key words: *Leucojum aestivum*, distribution, chromosomes, cytotype, karyogram, Shkodra Lake

* Zhaneta Zekaj (Trojani), Department of Biotechnology, Faculty of Natural Sciences, University of Tirana

** Lefter Kashta, Museum of Natural Sciences, Faculty of Natural Sciences, University of Tirana

INTRODUCTION

The genus *Leucojum* (Amaryllidaceae) is distributed in Europe and in most of the Mediterranean region. Three species occur in the Balkans: well known European species *L. vernum* and *L. aestivum* and recently described *L. ionicum* Kit Tan, Mullaj, Sfikas & Strid, endemic of the Western Greece and South Albania (Tan *et al.*, 2004).

There are two species of genus *Leucojum* distributed in Albania: *L. aestivum* L. and *L. ionicum* Kit Tan, Mullaj, Sfikas & Strid.

Informations from different studies about *L. aestivum* species in the Balkan are found for Albania (Vangjeli *et al.*, 1995), Serbia (Jovanović *et al.*, 2009), Bulgaria (Ignatova *et al.*, 2006), Greece (Bareka *et al.*, 1998), Macedonia (Sopova & Sekovski, 1982), Turkey (Senel, 2002).

In Albania *L. aestivum* is the only member of this genus distributed in Shkodra Lake surroundings and in few other localities in Albania: near Kruja (J. Vangjeli, personal communication), near Ohrid lake (Markgraf, 1927), Mamurras (Markgraf, 1931), Buna Delta (M. Rakaj, personal communication) and Voskopoja (L. Shuka, personal communication).

The habitats of *L. aestivum* are wet or periodically flooded lowland forests and meadows, mostly marsh-like, water-soaked terrain near rivers, river islands and swamps.

In Albanian area botanical characteristics of *L. aestivum* species are described by Demiri, 1962; Demiri, 1979. It is not included as threatened species in the Red List of Plants of Albania, although distribution of *Leucojum aestivum* in the country is limited.

This species is a bulbous plant with very important medicinal uses, because of its rich alkaloid *galantamine* content. *Leucojum aestivum* is an important source of pharmacologically active alkaloids that, so far, have been obtained only from natural populations (Ignatova *et al.*, 2006). The aim of this study is a karyological analysis of plants from Shkodra population.

MATERIALS AND METHODS

Plant material was collected from surroundings of Shkodra Lake, in the small island in the upper part of Buna River (Geographical coordinates: N 42° 03' 0.01", E 019° 29' 46.2").

The morphological characteristics of the karyotype have been studied.

For the karyological study, root tips were pretreated with 0.5 % colchicine at room temperature and fixed in ethanol: acetic acid (3: 1) for about 24 h at 4 °C. (Darlington & La Cour, 1960). The root meristem was stained with orcein acetic and hidrolised with pectinase and cellulose solution. The root tips were squashed in 45 % acetic acid, and after dehydration in 96 % ethanol; permanent slides were obtained through mounting in Entellan.

Slides were examined under an Olympus photomicroscope and photographs were taken with the same microscope with photo camera Paralux. The karyograms were drawn from mitotic metaphase. Measurements were made on each pairs of mi-

totic chromosomes. For the analysis of the karyotypes, five well spread metaphase plates from population were measured, using the image analysis software Microgiel.

The lengths of the short (S) and long (L) arms, and the satellites (Sat) were measured. The chromosome types were classified according to Levan *et al.*, 1964. The chromosomes are arranged in the idiograms in a descending order. The elaborate dates were drawn with computer program Cromo II (Pavone & Salmeri, 1993).

RESULTS AND DISCUSSION

In Shkodra region, *L. aestivum* occurs on wet meadows around the Shkodra Lake (Fig. 1) and on the upper part of the Buna River, near its outflow from lake, below „Rozafa” Castle. In a small island there is a dense population of *Leucojum aestivum*, which form the most typical associations of *Leucojo-Fraxinetum angustifolia* in Albania (Vangjeli *et al.*, 1997) (Fig. 2).

Only Shkodra population of *L. aestivum* L. was investigated karyologically in Albania. The karyotype of *L. aestivum* has been the subject of many karyological studies, most of which provide only the chromosome number of the species.

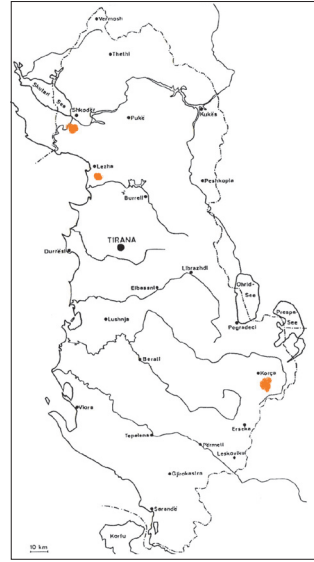


Figure 1. *Leucojum aestivum* L. distribution in Albania



Figure 2. Characteristic habitat of *Leucojum aestivum* L. in upper part of Buna River



Figure 3. Dense population of *Leucojum aestivum* L. in upper part of Buna River

The chromosome number of this species in metaphase of mitosis was $2n=22$ (Fig. 4).

A similar results was detected by some authors (Darlington & Wylie, 1955; Van Loon J. CHR. 1987; Goldblatt & Johnson, 1994; Bareka *et al.*, 1998; Marcucci & Tornadore, 1999; Senel *et al.*, 2002; Ignatova *et al.*, 2006). Rarely are registered some chromosomal plates with 24 chromosomes. Detailed karyological analysis in meiosis of our materials make present the haploid chromosome number $n=11$ (Fig. 5 and Fig 7b). This is realized through analysis of metaphase to meiotic chromosomes plate.

All studies show that the karyotype of *L. aestivum* consists of 11 mostly asymmetrical chromosome pairs. Similar conclusion has been conducted by other au-

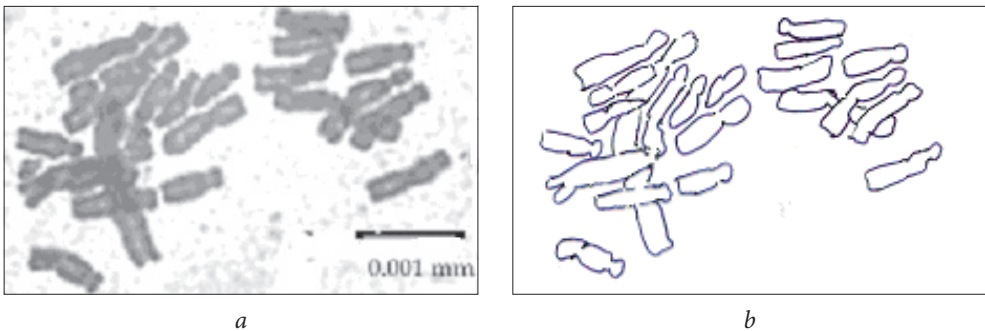


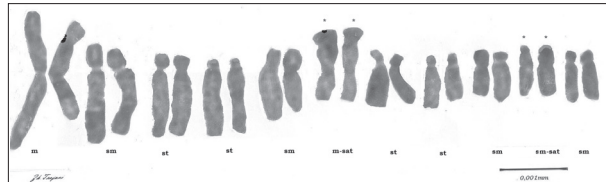
Figure 4. *Leucojum aestivum* L. photomicrograph and diagram of chromosomes plate in mitosis



Figure 5. *Leucожum aestivum* L. photomicrograph and diagram chromosomes in meiosis

Num:57 Taxon:*Leucожum aestivum*
 Scala: 100 Locus:Shkoder 2x = 22
 Formula di Levan:2m+2m⁰+9sm+7st+2st⁰

Id	Lu	Co	Tot	r	S	S1	T1	S2	T2
1	300	240	540	1.25					
2	220	200	420	1.10					
3	200	100	300	2.00					
4	270	70	340	3.86					
5	260	60	320	4.33					
6	255	55	310	4.64					
7	260	40	300	6.50					
8	240	40	280	6.00					
12	130	100	230	1.30			65TC		
11	120	90	210	1.33			70TC		
9	200	80	280	2.50					
17	120	60	180	2.00					
10	170	75	245	2.27					
14	170	50	220	3.40					
13	160	80	240	2.00					
15	160	80	240	2.00					
19	120	40	160	3.00			35TC		
20	120	40	160	3.00			35TC		
18	150	45	195	3.33					
22	140	50	190	2.00					
16	140	50	190	2.00					
21	140	50	190	2.00					



a

Figure 6. *Leucожum aestivum* L.: (a) – table and (b) – Photokaryogram

thors (Ignatova, 2006; Bareka *et al.*, 2003., D’Amato & Bianchi, 1999; Marcucci & Tornadore, 1999).

The karyotype of our studied population corresponds to the formula Levan:

$$2n = 2x = 2 m + 2 m^0 + 9 sm + 7 st + 2 st^0 = 22$$

The karyotype of this species consisted of 4 pair of median (m), 9 pair of submedian (s/m) and 9 pairs of subterminal (s/t) chromosomes (Fig. 6 a, b). Two pairs satellite chromosomes were present in the karyotype of this species (Fig 7. a, b). One

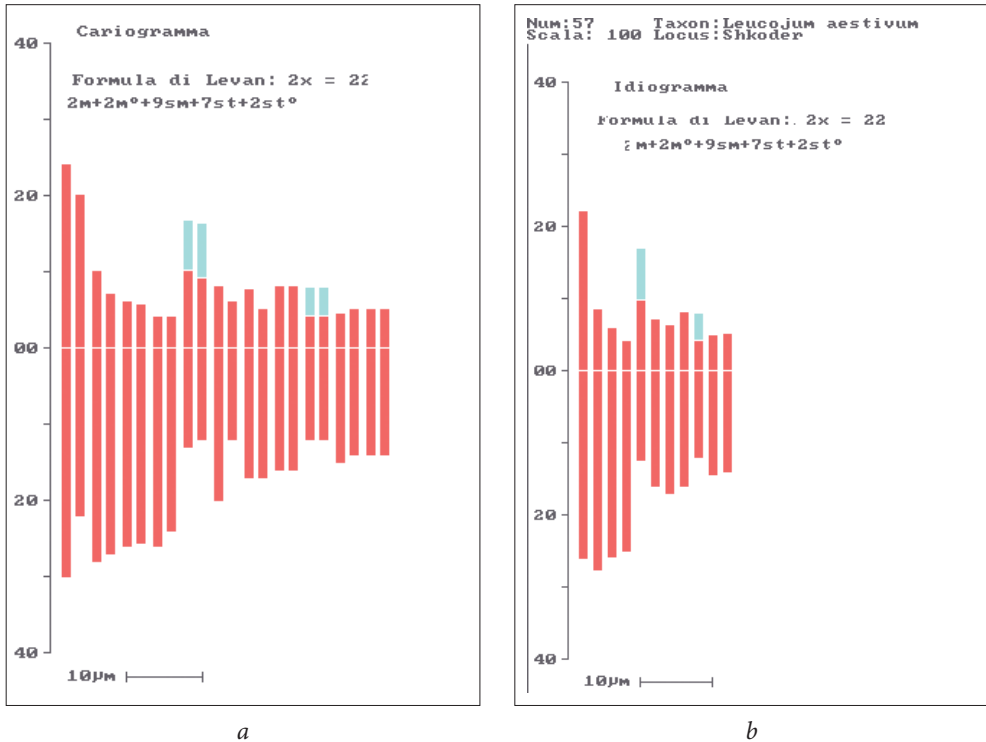


Figure 7. *Leucojum aestivum* L. (a) – Karyogram and (b) – Idiogram, for cytotype 22 n=22

chromosome pair shows very often minor structural heterozygosity. No differences in the number of SAT-pairs were registered in contrast of the other authors (Bareka *et al.*, 1998; Ignatova *et al.*, 2006). The karyotype is highly asymmetrical in shape.

CONCLUSIONS

According the data of the National Herbarium, botanical literature and authors' survey, *Leucojum aestivum* L. result to have a restricted distribution in threatened habitats in Albania, and therefore we propose to engage in the „Red List” as a threatened species.

Only the cytotype 2 n=22 at habitats of this species is studied for chromosomes number. Karyomorphological typology of chromosomes for this cytotype results:

$$2n = 2x = 2m + 2m^{\circ} + 9sm + 7st + 2st^{\circ} = 22$$

However, that study does not give information about the others known localities of this species.

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