Karyological and palynological studies on *Astragalus hamosus* and *A. glycyphyllos* in Turkey

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Abstract. *Astragalus hamosus* and *A. glycyphyllos* species have been investigated karyologically (the somatic chromosome numbers) and palynologically in this study. Chromosome numbers were found as $2n = 44$ for *A. hamosus* and $2n = 16$ for *A. glycyphyllos*. The chromosome bridges were observed in the mitotic cells of adventive root tips of *A. hamosus*. In both species the mature pollen grains were 2-celled; the pollen type is tricolporate, and the ornamentation was reticulate. They all were fertile.

Key words: *Astragalus*, chromosome numbers, European Turkey, *Fabaceae*, pollen

Introduction

In terms of species number, *Astragalus (Fabaceae*) is the richest genus of the vascular plants on Earth, represented by a total of ca. 2500 taxa (Maasoumi 1998). In Turkey, *Astragalus* has ca. 445 species in 62 sections and 210 of these taxa are endemic (Chamberlain & Matthews 1969; Chater 1968; Aytaç 2000; Akan & Civelek 2001; Ekici & Aytaç 2001; Podlech 2001; Hamzaoğlu & Kurt 2002; Duman & Akan 2003).

Although there are many systematic, anatomic, karyological and palynological studies on the *Astragalus* species, some taxonomic problems concerning this genus have not been resolved yet (Wojciechowski & al. 1999; Karamali & al. 2007; Khodaei & al. 2007). Thus, in the present study *A. hamosus* and *A. glycyphyllos* species have been investigated karyologically (the somatic chromosome numbers) and palynologically. This is the first report on these species based on Turkish material for these properties.

Material and methods

The specimens of *A. hamosus* and *A. glycyphyllos* were collected from a natural population in Edirne in European Turkey. Voucher specimens were deposited in the Herbarium of Trakya University (EDTU). These specimens were determined according to the *Flora of Turkey* (Chamberlain & Matthews 1969).

Pollen slides were prepared according to the methods described by Wodehouse (1935), Erdtman (1952) and Reille (1992). An Olympus photomicroscope with an apochromatic oil immersion objective (×100) and a periplan eyepiece (×10) were used for the measurements. Thus, the polar axis, equatorial diameter, exine thickness, and colpi length were measured. Generally, 100 pollen grains of specimens were measured (Sokal & Rohlf 1960). The terminology used is in accordance with Erdtman (1952) and Punt & al. (1994). Pollen grains of both species have been ascribed to one pollen type by Moore & al. (1991) and Beug (2004). Size and fertility of the pollen grains were examined by

staining them with cotton blue in lactophenol (Stanley & Linskens 1974).

Chromosome numbers were counted on root tips from seeds germinated in Petri dishes and bulbs. The chromosome preparations were made by using a standard root-tip squash technique. Seeds were germinated in darkness, at 25 °C, on moist filter paper in Petri dishes. Actively growing root tips 1 cm long were excised from the germinating seeds. Root tips for karyotype analyses were pretreated with ABN for 24 hours, at +4 °C, then fixed in Carnoy (3:1 = ethyl alcohol:acetic acid) for 24 hours. The root tips were hydrolyzed with 1N HCl for 15 min, at 60 °C, in an oven. They were stained with Feulgen reagent for 2 hours, in darkness, at 25 °C. Dissected meristems were squashed and counterstained with acetic orcein**.** Several chromosome atlases were also consulted to check on the chromosome numbers of the studied taxa (Darlington & Janaki Ammal 1945; Goldblatt & Johnson 1991).

The slides were examined under an Olympus photomicroscope and photographs were taken with the same microscope.

Results

***Astragalus hamosus* L.** (Table 1, Figs 1, 3)

Examined specimens: A1 (E) Edirne : Center, around Gullapoğlu Campus, 20.05.2002, coll. *F. Dane*, EDTU (8515).

Karyology

Astragalus hamosus was found to be pentaploid. However, the examined specimens were aneuploid. The chromosome number was determined as $2n = 2x = 44$ (Fig. 1a), with a basic chromosome number $x = 8$**.** But its chromosomes are relatively small (ca. 1 µm) and their morphology cannot be examined precisely. According to an earlier report, *A. hamosus* has very different chromosome numbers. So we examined the mitotic divisions and some chromosome abnormalities, such as chromosome bridges, and lagging chromosomes were seen in the somatic cells of the root tip of *A. hamosus* (Figs 1b, 1c).

***Astragalus glycyphyllos* L.** (Table 1, Figs 2, 4)

Examined specimens: A1 (E) Edirne: Center, Söğütlük forest, 10.08.1989, coll. *F. Dane*, EDTU (3826)!; Center, Izzet Arseven Forest, 20.07.2002, coll. *O. Dalgic*, EDTU (8516)!

Karyology

Astragalus glycyphyllos was found to be diploid. The chromosome number was determined as $2n = 2x = 16$ (Fig. 2).

Palynology

Palynological properties of the investigated specimens are given in Table 1 and Figs 3, 4.

Table 1. Pollen grain characteristics of the examined specimens from three different natural populations.

	A. hamosus	*A. glycyphyllos*
Origin of the specimen	A1E Edirne, Güllapoğlu Yerleşkesi.	A1E Edirne, Karaağaç
Date of collection	03. 05. 2002	03.05.2002
Pollen type	Tricolporate	Tricolporate
Pollen shape	oblate, P/E=0.69	oblate-sphaeroidal, P/E= 0.89
Exine	avarage thickness 1.50 ± 0.98 µm	avarage thickness 1.20± 0.23 µm
Apertures	colpus: long and narrow; porus: long and wide; plg/plt= 0.88	colpus; long and narrow; porus: long and wide; plg/plt= 1.00
Sculpture (exine ornamentation)	reticulate	reticulate
Intine	thin	thin
P (polar axis)	24.11 ± 1.85 µm	24.13 ± 1.13 µm
E (equatorial axis)	34.57 ± 0.43 µm	26.92 ± 0.05 µm
Clg (colpus length)	30.50 ± 0.09 µm	22.40 ± 0.33 µm
Plg (pore length)	7.14 ± 0.36 µm	4.64 ± 1.17 µm
Plt (pore width)	8.07 ± 1.20 µm	4.60 ± 0.44 µm

Discussion

According to the chromosome number studies, all New World *Astragalus* species have aneuploid chromosome numbers (n = 11–15), while the Old World *Astragalus* species are generally euploid (n = 8, 16) groups including both aneuploid and euploid species (Ledingham & Rever 1963; Ledingham & Fahselt 1964; Spellenberg 1976; Goldblatt & Johnson 1991; Wojciechowski & al. 1999).

In the present study, the chromosome number was determined as $2n = 2x = 44$ for *A. hamosus*. According to earlier records, the chromosome number was found as $2n = 14$ by Prete & Miceli (1994), who concluded that the plants examined by them represented a prim-

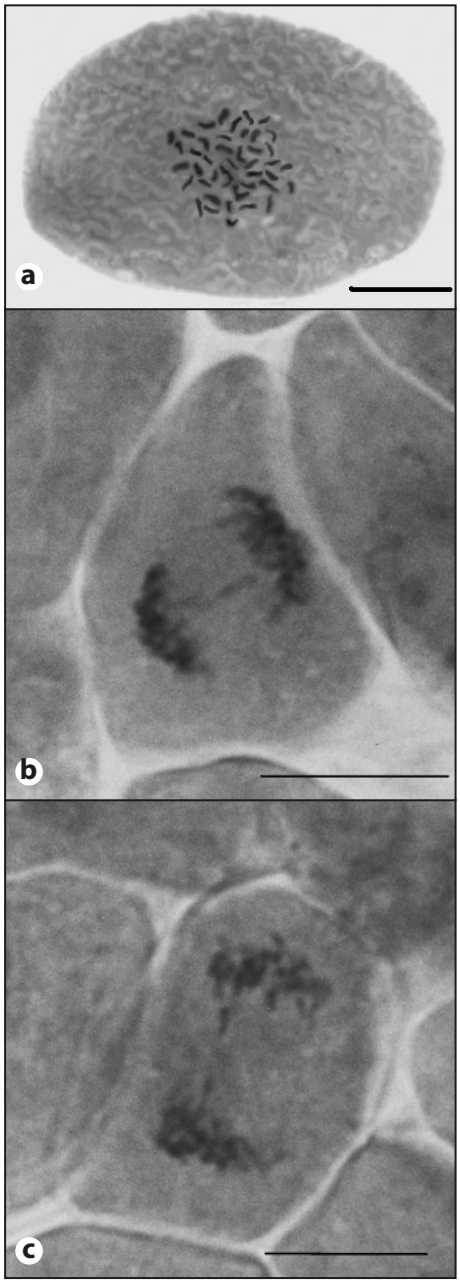

Fig. 1. Microphotographs of *A. hamosus* chromosomes: $2n = 44$. **a** – somatic metaphase; **b**, **c** – chromosome bridges of telophase. Scale bar = 10 µm.

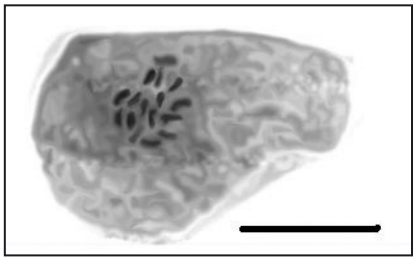

Fig. 2. Microphotographs of somatic metaphase chromosomes of *A. glycyphyllos*: $2n = 16$. Scale bar = 10 µm.

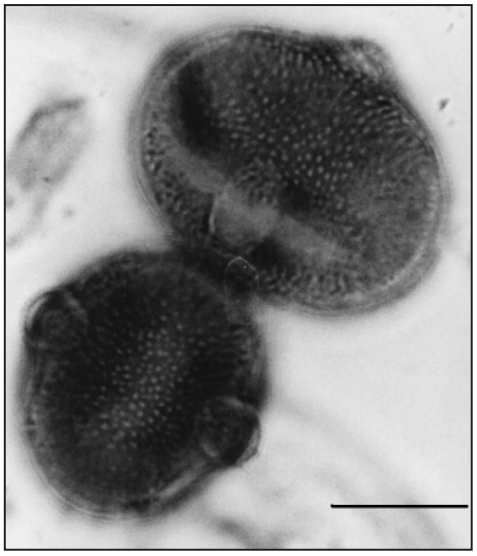

Fig. 3. Pollen morphology of *A. hamosus*. Scale bar = 10 µm.

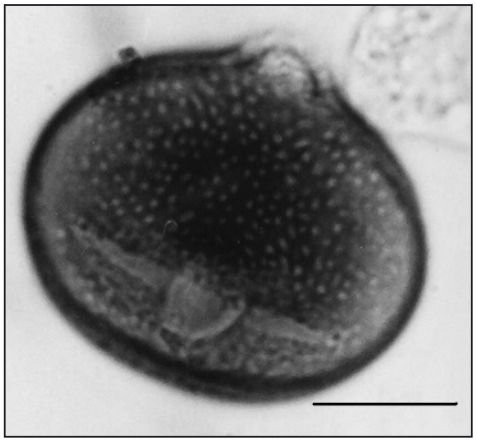

Fig. 4. Pollen morphology of *A. glycyphyllos*. Scale bar = 10 µm.

itive and relic Sardinian cytotype, so it was possible to conclude that *A. hamosus* evolved elsewhere into different chromosomic races with a higher ploidy level.

Our karyological investigations showed that the chromosome number of $2n = 44$ agrees with the earlier counts (Federov 1969; Pretel Martinez 1974; Pretel & Sanudo 1978; Gohil & al. 1981 – $n = 22$; Maassoumi 1987). Other data are also known: $2n = 32+2b$ (Chuxanova 1967), $2n = 48$ (Fernandes & Santos 1971; Löve & Kjellquist 1974), $2n = 32$ (Borgen 1974; Kuzmanov & Georgieva 1976; Dalgaard 1987; Colombo & al. 1983); $2n = 24, 32, 40, 44, 46, 48$ (Horjales 1976). The chromosome numbers $2n = 40, 42, 44, 46, 48$ were reported from Bulgarian populations and for the first time for the same population Pavlova also reported it as $2n = 88$ by (1995). The results of the present study clearly indicate that *A. hamosus* is an aneuploid species and most likely mitotic irregularities accounted for this.

Astragalus glycyphyllos was found to be diploid. The chromosome number was determined as $2n = 2x = 16$ (Fig. 1), with a basic chromosome number $x = 8$, which agrees with that reported by Albers & Pröbsting (1998) for material from Slovakia, and also $2n = 16$ for material collected from Europe (Chater 1968).

As a result of our karyological studies, we have found that *A. glycyphyllos* in the Turkish material was euploid, while *A. hamosus* was aneuploid. The results of earlier, similar chromosome studies on these taxa confirm these results. For instance, Ledingham & Rever (1963) and Ledingham & Fahselt (1964) found the chromosome number of the Old World *Astragalus* species as $x = 8$ and showed that they expressed ploidy.

Pollen morphology of some *Astragalus* species has also been studied by various authors. Evren & Çobanoğlu (1992) and Çelik & al. (1995) studied some endemic *Astragalus* from Turkish populations. Aytaç (1997) studied the section *Dasyphyllium* of *Astragalus* from Turkey. Pavlova & al. (1994, 1995) conducted similar studies on the subgenera *Hypoglottis, Phaca*, *Astragalus*, *Cercidothrix*, *Calycocystis, Trimeniaeus, Epiglottis,* and *Calycophysa*. In this study, *A. hamosus* of section *Bucerus* and *A. glycyophyllos* of section *Glycyphyllos* (Chamberlain & Matthews 1969) were investigated. Our results provided the first palynological data on these species based on Turkish material.

In the present study, pollen grains of *A. hamosus* and *A. glycyphyllos* were two-celled, tricolporate, with reticulate ornamentations. Similar pollen characteristics have been also known in other *Astragalus* species (Evren & Çobanoğlu 1992; Pavlova & al. 1994, 1995; Çelik & al. 1995; Evren & Aytaç 1997). There are some differences between the pollen grains of both investigated species. They differed morphologically from each other in terms of their equatorial axis size, pore length and pore width. Pore width and pore length of the pollen grains of *A. hamosus* exceeded nearly twice those in *A. glycyphyllos.* Similarly, equatorial axis and colpus lengths were greater in *A. hamosus* than in *A. glycyphyllos.* There were also differences in the shape of pollen grains. While in *A. hamosus* pollen shape was oblate, it was oblate-spheroidal in *A. glycyphyllos.* The pollen of both species was also fertile, a fact which shows that in both species meiosis regularly took place. Only mitotic irregularities were observed to occur and further studies will be needed for meiotic investigations of *A. hamosus.*

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