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INTRODUCTORY DATA ON MEIOTIC STRUCTURES IN *ALLIUM SENESCENS* L. SUBSP. *MONTANUM* (POHL) HOLUB

Romuald Kosina * & Agnieszka Małecka

Abstract. Examples of zygotene chromosomal configurations in *Allium senescens* subsp. *montanum* are presented. Bivalents do not dominate in a zygotene stadium. Some bivalent segments show deletions. Also, tri- and tetravalents segments are observed. The conjugation of SAT-chromosome shows that a translocation has occurred in the SAT segment. In addition, univalents indicate that meiotic behaviour of the species is unstable.

Key words: *Allium senescens*, meiosis, conjugation, translocations

*Plant Speciation Group, Institute of Environmental Biology, University of Wroclaw, Przybyszewskiego 63-77, 51-148 Wroclaw, Poland; * kosina@biol.uni.wroc.pl*

Introduction

Allium senescens L. subsp. *montanum* (Pohl) Holub is a perennial plant distributed mainly in Central Europe, with extreme points in Northern Germany, Central Ukraine, Northern Spain and Southern Italy (Friesen & Herrmann 1998). Zhang *et al.* (2012) have provided data on its vegetation also in China. Its synonyms are *A. montanum* Lam. and *A. lusitanicum* F.W. Schmit. The species has been recognized as allotetraploid (Gillies 1989) with chromosome numbers ranged from 16 to 32 (Pastor 1982; Speta 1984). Breeding strategy is the factor that decides the survival of the species in the environment, and hence research of meiotic behaviour is important in assessing the evolutionary fate of the species. A detailed study by Małecka (2008) showed that the process of microsporogenesis in the species is highly unstable. Laggards, bridges, micronuclei of different size and extrusion of nucleolar RNA into cytoplasm are common. Zhang *et al.* (2012) observed similar anomalies in plants of *A. senescens* growing in China. Therefore, presence of B chromosomes has to be considered when meiotic behaviour is evaluated in alliums. One B chromosome was noted in *A. senescens* (Shopova 1966). However, Loidl (1988) did not find too many anomalies during pachytene synapsis in allotetraploid *A. montanum*. In this polyploid species one can at least expect some meiotic configurations related to multivalents formation.

Material and methods

Stamens of *A. senescens* subsp. *montanum* were collected during two years, 1993 and 2006, from plants cultivated in the Botanic Garden, University of Wroclaw, Wroclaw, SW Poland. The stamens were fixed in a Carnoy's solution and stored in a freezer at -20 °C. Before slide preparation, stamens were washed in distilled water and further three times for 5 min in a 0.01 M citrate buffer. Material was enzymatically digested in a mixture of pectinase and cellulase in a hybridization oven at 37 °C. The digested material was centrifuged three times for 3 min at 800 g, each time in a fresh

citrate buffer and the supernatant was discarded. The material was prepared by squash or dropping methods according to Schwarzacher *et al.* (1980) and Ambros *et al.* (1986). For better dispersion of chromosomes, a hot plate method according to Henegariu *et al.* (2001) was applied. For chromosome staining, 100 µl/slide of 0.5 µg/ml DAPI and that of 0.025 µg/ml propidium iodide were used, respectively. Slides were washed in PBS buffer and mounted in a medium that prevented the fading of fluorescence. Slides were stored in a refrigerator at 5 °C. Slides were documented under an epifluorescence microscope Olympus BX60 with a DAPI filter and pictures were taken with Zenith TTL camera and Fuji 400 film.

Results and discussion

The size of an *Allium* L. chromosome can be observed in a specimen shown in the left upper part of Fig. 1 A. This is a univalent chromosome, not conjugated. A univalent segment is also shown in the lower part of the picture. Another chromosome, in which chromatids are indicated by black arrows (Fig. 1 A), shows probably two crossing-overs with two other chromosomes not distinguished in a chromosomal mass. These chromosomes form a trivalent segment. Trivalent association is also indicated by green arrows in the lower right part of the zygotene group. The arrows point to the conjugation of heteromorphic arms of different length. This trivalent group is associated by a short terminal segment with another chromosome, and a tetravalent group is formed as a result of several translocations. In Fig. 1 B, a univalent segment (see white arrow in the upper left part) proves that in this site some deletion is present. Several long bivalent segments are shown (red arrows); however, in the lower right part, two bivalent segments are involved to form a tetravalent segment. A short translocation forms a trivalent segment between the bivalent and SAT-chromosome. A NOR-constriction of the SAT-chromosome is well documented. Both zygotene pictures show that some uni-, bi- and multivalents are formed during meiosis. Translocations are responsible for such configurations. SAT-chromosomes also undergo these changes.

Małecka (2008) showed that the studied *Allium* is an allotetraploid, therefore it could expect a high percent of bivalents. In fact, meiosis in this accession was highly irregular. Multiple translocations, multivalents, rings, heteromorphic bivalents, laggards, bridges, dicentric chromosomes and a spectrum of micronuclei were noted. The frequency of these chromosomal aberrations reached 15%.

B chromosomes which present in alliums during meiosis are mostly univalents and are preferentially transmitted. In polyploids, like the allotetraploid here, they are lost. In *A. senescens* one B chromosome has been detected (Shopova 1966). No multivalents have been observed from diplotene to metaphase stage in allotetraploid *A. montanum* (Loidl 1988). But Fig. 1 shows that some partial multivalent associations occur. Also, multivalents were noted in diakinesis (Małecka 2008). In addition, the presence of heteromorphic bivalents and some deletions contradict the possibility of pure bivalents occurrence. Here, chromosomal configurations cannot be 'true multivalents' but multivalent association caused by translocations. Configurations presented in the upper part (Fig. 1 A, black arrows) and in the lower part (Fig. 1 B, blue and red lines) can be interpreted as tetravalent formation, and such figures have been documented in *A. porrum* L. by Khazanehdari *et al.* (1995).

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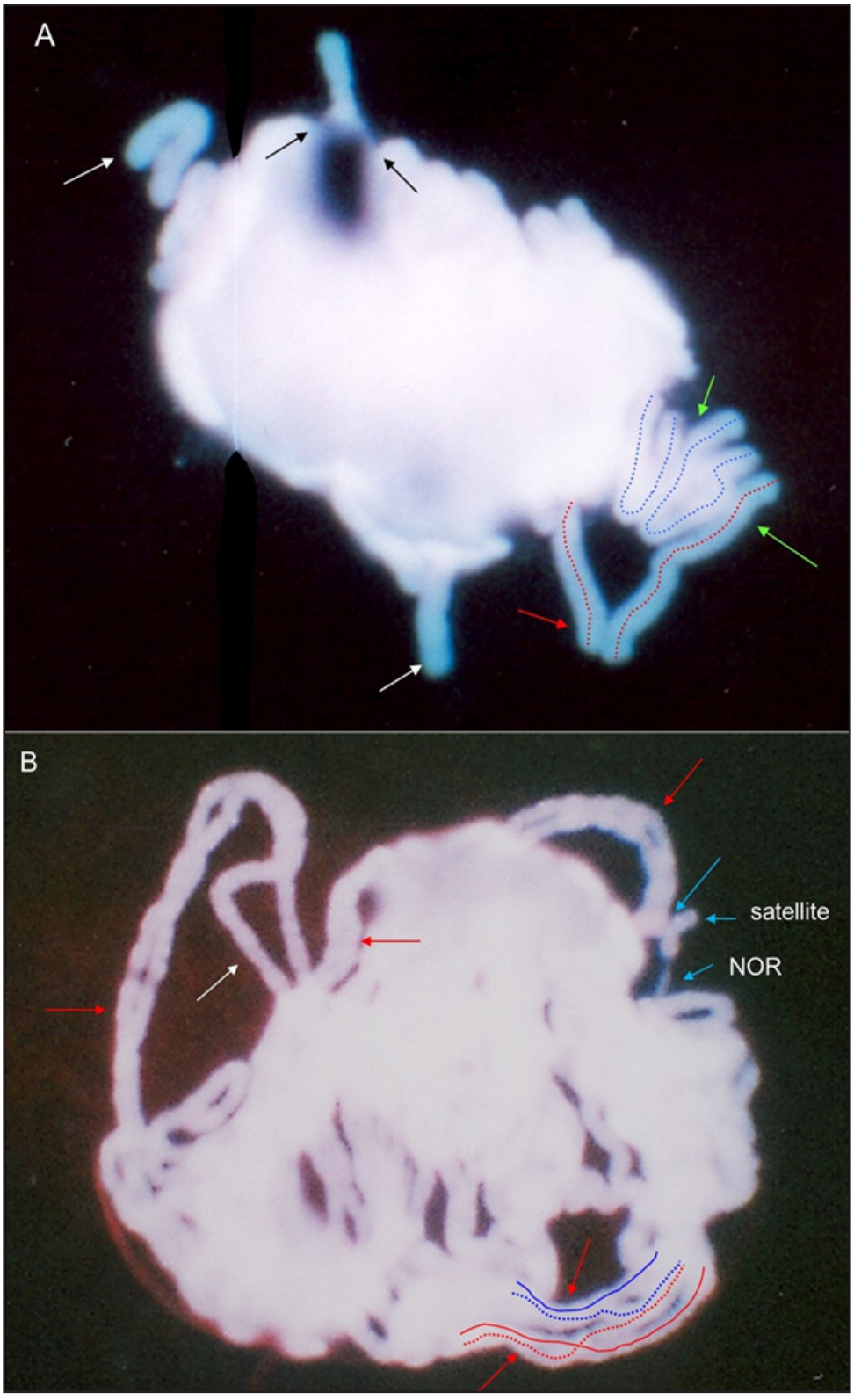
Fig. 1. Zygotene chromosomal configurations in *Allium senescens*. Univalents (**white arrows**), bivalents (**red arrows**), ▸ and trivalent formation (**black** and **green arrows**), SAT-chromosome (**blue arrows**).


A
B
satellite
NOR

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