

The occurrence of synaptic and asynaptic plants in one accession of *Allium rubellum* M. Bieb.

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Summary: We found asynaptic individuals within the studied accession of *Allium rubellum* in addition to normal plants. The male meiosis cytology of both normal and asynaptic plants are described in details here. Though in mutants, we could not detect any true cytological exchanges between homologous chromosomes, we counted nearly two chiasmata per each bivalent in the normal plants. Consequently, in the asynaptic mutants, the lack of chiasmata upsets entirely the course of meiosis and results in a complete ‘meiotic chaos’, while regular meiosis occurs frequently in the normal plants and more than 95% of their pollen grains are fertile. Though a nearly complete male sterility was found in the asynaptic plants, they can produce viable seeds.

Keywords: Amaryllidaceae, asynapsis, cytology, Iran, lagged chromosomes, meiosis

In the evolution of living organisms, sexual reproduction has proven to be a successful strategy (BELL 1982). Meiosis is the key event of eukaryotic sexual cycle. It properly reduces a cell’s chromosome number by the half, while also creates new gene combinations through independent chromosome assortments and crossovers (LENORMAND et al. 2016). For correct segregation of homologous chromosomes at anaphase I, they must pair, recombine and synapse (CNUDDÉ & GERATS 2005), but ‘synaptic mutants’ fail to pair chromosomes during prophase I (RILEY & LAW 1965). In these mutants, either no synapsis takes place (asynaptic mutants) or normal pachytene pairing occurs, but homologues are immediately separated (desynaptic mutants). High frequency of univalents at diakinesis and metaphase I is the common feature of the two meiotic mutants. Furthermore, in synaptic mutants, more or less similar types of irregularities occur at later meiotic stages. But depending on the mutant type, the percentage of male sterility could be varied (BIONE et al. 2002). PRAKKEN (1943) classified desynaptic mutants into weak, medium strong and strong types on the basis of the frequency of univalents and bivalents in microsporocytes. It seems that synaptic mutants are more common in plants than in animals. KODURU & RAO (1981) reported these mutants in 20 plant families. Synaptic mutants have also been described in several species of the genus *Allium* (GOHIL & KOUL 1971; and references cited in the chapter Discussion).

Working on cytology of Iranian *Allium* species (Dolatyari et al., unpublished data), we found not only normal plants but also asynaptic individuals in one accession of *Allium rubellum* M. Bieb. This species has a rather widespread distribution in Iran and belongs to section *Scorodon* K. Koch of subgenus *Allium* (WENDELBO 1971). This paper deals with the detailed cytology of male meiosis in the normal and asynaptic plants.

Materials and methods

Bulbs, herbarium vouchers and material for the meiotic studies were collected from NW of Iran (Ardebil province: 45 km before Ardebil from Meshginshahr, Arbab Kandi village, N 38°29’14.6”

Table 1. Meiotic characterization of normal and asynaptic plants.

Plants	Chromosome configurations at MI	Chiasma frequency per cell	% Irregularities in anaphase I – telophase I	% Irregularities in anaphase II	% Pollen sterility
Normal	8II, 7II + 2I	14.6	5.9	2.4	3.7
Asynaptic	16I	0	96.2	rarely seen	more than 95

E 48°02'3.1", 1192 m). The bulbs were planted in a trial field and the vouchers were deposited at Iranian Biological Resource Center Herbarium [IBRC].

Inflorescences of ten individuals were prefixed in Carnoy's I solution (1:3 v/v of glacial acetic acid and absolute ethanol) for 24 h, then preserved in alcohol 70% in refrigerator till laboratory works. The inflorescences enclosed by spathes are usually young and suitable for meiotic studies. The spathes of young inflorescences were removed for better penetration of the fixative. White-colored anthers were squashed in a drop of 2% aceto-orcein on microscope slides applying the commonly used technique. Fresh preparations were immediately investigated by Olympus BX50 light microscope equipped with DP21 digital camera. The preparations were made permanent using absolute alcohol for dehydrating and Euparal as mounting medium. Normal pollen mother cells (PMCs) and irregularities were photographed at magnifications of 600x or 1200x. At least 100 cells were scored to calculate the percentage of irregularities at anaphase I – telophase I and also at anaphase II – telophase II stages. Also, 2% aceto-orcein was used for staining pollen grains and estimating the percentage of pollen sterility. We counted pollen grains with unstained nuclei as well as pollen grains with apparent abnormalities (Fig. 1h) as sterile pollen.

Since distinction between strong desynapsis and asynapsis is not possible at late prophase I and also at later meiotic stages, we considered 'asynapsis' as an extremely decreased number of paired chromosomes during prophase I, nearly equivalent to 'strong desynapsis' in the sense of PRAKKEN (1943).

Results

The inflorescence structure of *Allium* presents some advantages in cytological studies. One can record data for each individual separately and also can simply compare meiosis between different flowers of a plant. Our results showed that the studied accession is a mix of synaptic and asynaptic individuals. More precisely, two of eight examined plants were asynaptic, and meiosis was regular in the rest. The gametic chromosome number of all examined plants was $n = 8$.

We arranged meiotic images illustrating chromosome number and behaviour of both normal and abnormal plants in Fig. 1. Also, meiotic data of the normal and asynaptic plants are given in Table 1.

Cytology of meiosis in normal plants

All meiotic stages could be studied with a considerable clearness. In the studied individuals, we found predominantly eight bivalents in metaphase I (Fig. 1a). Also in some cells, seven bivalents and two univalents were observed (Fig. 1b, arrowheads indicate univalents). The mean chiasma frequency per cell was 14.6. Thus, on average, slightly more than two chiasmata per bivalent were estimated (Table 1). The majority of the meiocytes were normal, though we detected also

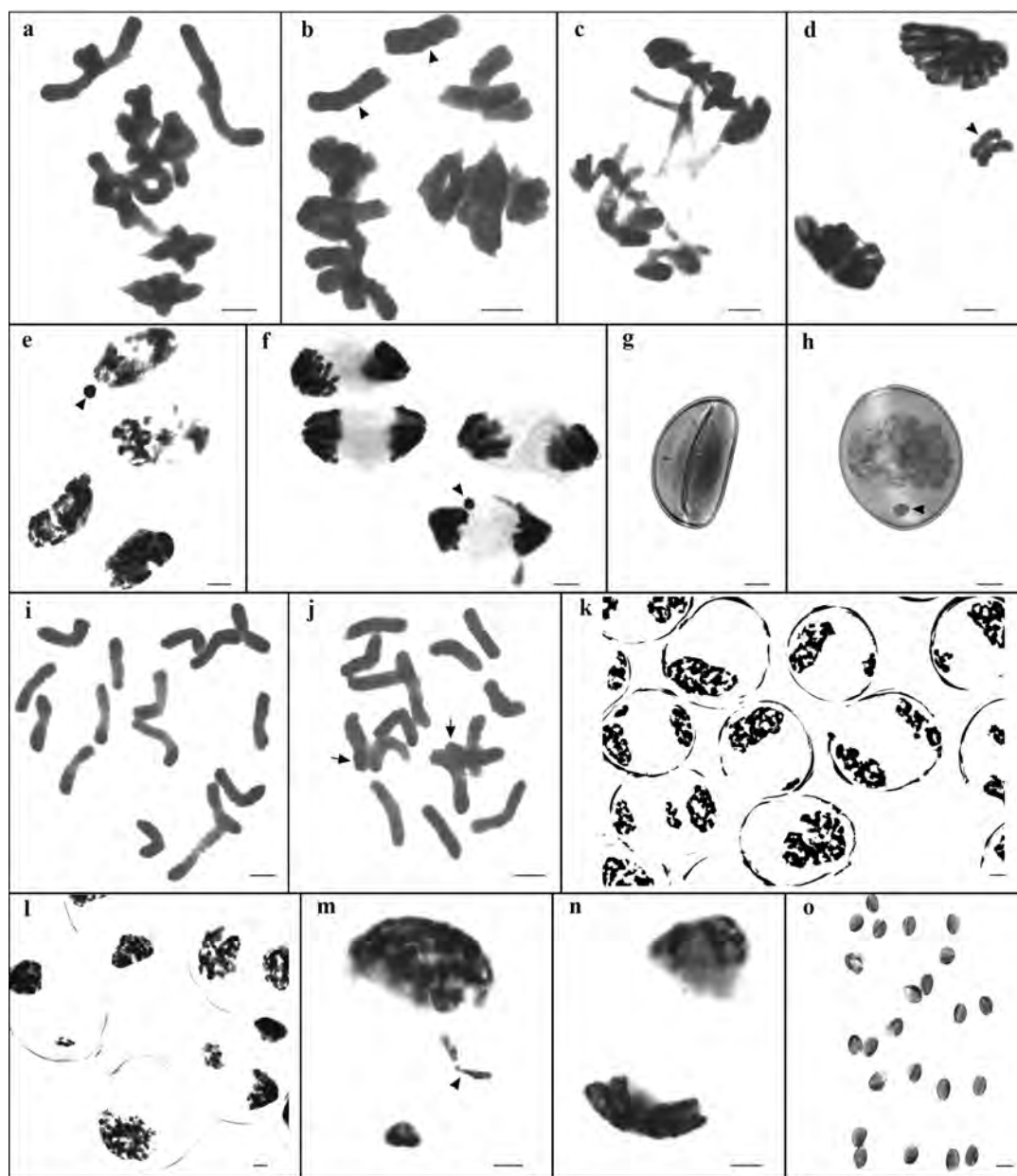


Figure 1. a–h male meiosis in normal plants. a – late diakinesis, 8II; b – late diakinesis, 7II + 2I; c – anaphase I, chromatin bridges; d – telophase I, lagging chromosome; e – telophase I, micronucleus; f – telophase II, normal and abnormal meiocytes; g – unstained sterile pollen; h – pollen grain, micronucleus. i–o male meiosis in asynaptic plants. i – metaphase I, 16I; j – metaphase I, two nonchiasmatic associations (arrows); k – telophase I, complete aberration cells; l – telophase I, in the lower cell all chromosomes remained in one pole; m – telophase I, lagging chromosome; n – telophase I, probably normal; o – sterile pollen grains. Scale bars = 5 µm. Arrowheads indicate irregularities.

abnormal cells at anaphase I – telophase I, e.g. chromatin bridges, lagged chromosomes and micronuclei (Fig. 1c–e). Altogether 487 cells at anaphase I – telophase I were investigated to count the percentage of abnormalities in which on average 5.9% of the scored cells (29 cells) showed one or two of the above mentioned aberrations. This value considerably varied from 1% to 11.3% between investigated individuals. The same types of abnormalities were found in

anaphase II – telophase II (e.g. Fig. 1f), but the counted percentage of abnormalities in meiosis II was much lower than those of meiosis I (Table 1). At least 500 pollen grains were counted to test pollen fertility. Only 3.7% of pollen grains were sterile (Fig. 1g). Often, 1–2 nucleoli were seen during development of the fertile pollen grains.

Cytology of meiosis in the asynaptic plants

All microsporocytes of the two mutant plants showed 16 univalents at diakinesis and metaphase I (Fig. 1i). In some cells, 0–6 nonchiasmatic associations were formed between univalents (Fig. 1j, arrows indicate two nonchiasmatic associations). However, we could not detect true cytological exchanges between homologous chromosomes, i.e. chiasmata were not formed. Yet, during meiosis I, sister chromatids were held together completely so that in all examined cells no splitting between daughter chromatids was visible. These univalents resembled somatic metaphase chromosomes in appearance and the less stained median or submedian regions may be clearly discerned as primary constrictions (Fig. 1i,j). At metaphase I, all univalents were randomly scattered at surface of cells with little tendency to assemble at equator. In fact, the significant role of chiasmata in the orientation and proper segregation of homologous chromosomes at meiosis I could obviously be inferred in the asynaptic plants.

In the absence of chiasmata, homologues experience unclear destinations: all chromosomes may remain in one pole or they may divide unequally to two or three poles (Fig. 1l,k). Also, in some cells, lagged chromosomes arranged between two unbalanced poles of chromosomes (Fig. 1k,m). About 160 cells were counted at late anaphase I – telophase I stages and only in six cells the chromosomes were doubtfully divided equally to two poles at telophase I (Fig. 1n); all other cells were apparently abnormal. The second nuclear division was rarely seen. More than 95% pollen grains were sterile (Fig. 1o).

Discussion

Many somatic chromosome counts were reported earlier for *A. rubellum* based on material from different countries (index to plant chromosome numbers of Missouri Botanical Garden, <http://www.tropicos.org/Project/PCN>). HOSSEINI & GO (2010) studied three Iranian accessions and presented $2n = 16$ as the somatic chromosome count for this species. ZAKIROVA & VAKHTINA (1974) studied meiosis in *A. rubellum* and reported diploid and tetraploid levels, though KHOSHOO & SHARMA (1959) reported a triploid level in material from India. The latter authors reported high frequency of multivalents, and their results were completely different from our results in normal plants since they studied a triploid accession. In accordance to the previous study on material from Iran by HOSSEINI & GO (2010), we confirm $2n = 16$ and diploid level for this species.

As it could be expected, there were substantial differences between the normal and asynaptic meiosis. In the asynaptic plants, a nearly complete absence of chiasmata upsets entirely the course of meiosis. We assumed some observed connections between univalents as ‘nonchiasmatic associations’ based on comparison of morphology of these connections with chiasmata in the normal plants and also considering the behavior of such connected chromosomes at later stages. Nearly a complete irregular segregation of chromosomes was found in the mutants. However, immunostaining with class I crossover markers, e.g. MLH1 or HEI10, is necessary to confidently judge, if they are not chiasmatic (JAHNS et al. 2014). Furthermore, the centromeres remained

undivided during meiosis I and the univalents distributed randomly to poles or occupied unclear positions at anaphase I. As a consequence, bi- or multinuclear interkinesis stage was initiated, while nuclei contain unequal chromosome sets. A near complete failure of the first meiotic division was found in asynaptic triploid *Allium amplexans* Torr. by LEVAN (1940). In this case, chromosomes were not divided to poles, and only a uninuclear interkinesis stage was formed. Since second meiosis in the asynaptic triploid occurred regularly, pollen grains with balanced somatic chromosome sets were formed eventually.

Meiosis in a low percentage of PMCs of the normal plants was also irregular. These abnormal cells may develop to unbalanced pollen grains, though they unexpectedly could compete with normal pollen grains and could transfer successfully their genetic material to the next generations (GOHIL & KAUL 1981). The same percentage of irregularities was found in several studied accessions of subg. *Allium* which possessed solely normal individuals (Dolatyari et al., unpublished data).

Both genetic and environmental factors can influence chromosome synapsis (LEVAN 1940; KODURU & RAO 1981). Temperature, age and chemical substances, as non-genetic factors, may cause asynapsis in plants. BARBER (1942) in *Fritillaria* L. and SOOST (1951) in *Lycopersicum esculentum* Mill. noticed negative relationships between temperature and synapsis; conversely, IWAMASA & IWASAKI (1963) reported positive relations between temperature and synapsis in *Citrus* L. We assume asynapsis in our examined material as a genetically caused mutation, because the asynaptic mutants could be traced again among plants of the cultivated accession in the second year examinations. Also, the material studied in the first year were collected from nature and all were grown under the same environmental conditions. Additionally, both normal and asynaptic forms were handled with an identical method.

In nearly all earlier cases of synaptic mutants reported in the genus *Allium*, the suppression of chiasmata was never complete (MATHUR & TANDON 1965 in *A. tuberosum* Roxb.; GOHIL & KOUL 1971 in *A. consanguineum* Kunth; KAUL 1975 in *A. cepa* L.). However, GOHIL & KOUL (1971) in a diploid clone of *A. sativum* L., and as mentioned above, LEVAN (1940) in a triploid accession of *A. amplexans* reported strong desynapsis and complete asynapsis, respectively. Our studied mutants were in all aspects different from the *Allium* species already studied. Firstly, no chiasma was formed between homologous chromosomes; secondly, in contrast to the last two mentioned works, the undivided univalents moved erratically to one or several poles at anaphase I; thirdly, meiosis II rarely occurred. Nevertheless, meiotic behavior similar to our data was earlier reported by SJODIN (1970) and PALMER (1974) in asynaptic plants of *Vicia faba* L. and soybean, respectively.

Female meiosis in the male asynaptic plants was earlier studied in different species and different results were reported. NELSON & CLARY (1952) observed a normal female fertility in spite of male asynapsis in corn. DAVIES & JONES (1974) and JONSSON (1973) found the same types of abnormalities in both sexes in rye and *Picea* A. Dietr., respectively, while in *Brassica* L. the effect of asynapsis on megasporogenesis was reported to be more than its effect on the male side (GOTTSCHALK & KONVIČKA 1971). Since our asynaptic plants were able to produce seeds and also considering the cross pollination by insects in *A. rubellum* (personal observations) which ensures allogamy, it seems that meiosis probably takes place normally in the female side of our

studied mutants. But we avoided a certain conclusion before detailed research of female meiosis in a separate study. Also, the normal and asynaptic plants must be accurately studied, if there are marked morphological dissimilarities.

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