

## CHROMOSOMAL STUDIES ON FOUR IRANIAN CROCUS SPECIES (IRIDACEAE)

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Four species of Iranian *Crocus* were chromosomally investigated and their karyotypes were determined: *C. sativus* ( $2n=24$ ), *C. cancellatus* subsp. *damascenus* ( $2n=8A$ ), *C. gilanicus* ( $2n=24$ ) and *C. speciosus* subsp. *speciosus* ( $2n=12$ ).

On the meiotic studies of pollen mother cells in *Crocus sativus* unbalanced distribution of chromosomes in two poles of cells and chromosomal lagging were frequently observed. Karyological studies on the meristematic cells of neoformed root tips in explant of saffron corm showed cells with  $2n=16$  chromosomes.

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## مطالعات کروموزومی چهار گونه زعفران

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چهار گونه زعفران ایران از نظر کروموزومی مورد بررسی قرار گرفت و کاریوتایپ آنها تعیین شد.  $2n$  در این گونه‌ها برابر با ۲۴ در زعفران مزروعی (*Corcus sativus*)، 8A در زعفران مشبک زیرگونه دمشق (*Crocus cancellatus subsp. damascenus*)، ۲۴ در زعفران گیلان (*Crocus gilanicus*) و ۱۲ در زعفران زیبا زیرگونه زیبا، (*Crocus speciosus subsp. speciosus*) بود. در بررسی تقسیم میوز سلولهای مادر دانه گرده در زعفران مزروعی، توزیع نامتعادل کروموزومها در قطبین سلول و تأخیر کروموزومی به فراوانی مشاهده شد. بررسی کاریولژیکی ریشه‌های نوپدید در قطعه جداگشت بنه‌های زعفران مزروعی وجود سلولهایی با  $2n=16$  را نشان داد.

## INTRODUCTION

*Crocus* species are perennial, grassy and ornamental plants from *Iridaceae* family, flowering in early spring or early autumn, according to the species. The number of species are reported 80 to 100 (Mathew, 1982). Their distribution centre is mainly Turkey and Greece. Mobayyen (1974), Wendelbo and Mathew (1975) and Wendelbo (1977) have reported eight wild and cultivated *Crocus* species in Iran. Brighton (1977a, b), and Brighton, Mathew and Marchant (1973) showed that *Crocus* is a highly complex genus with a wide range of chromosome numbers ( $2n=6-64$ ) and a considerable variation in karyotypic morphology (Rudall Paula, Owens and Kenton 1984). On the other hand, some species reveal distinct constancy in characteristics of their karyotype.

This report examines chromosomal particularities in *Crocus sativus* and three wild species (*C. gilanicus*, *C. cancellatus* and *C. speciosus*) from Iran.

Among the above mentioned species, *C. gilanicus* is endemic of Iran. It is a small white flowered species, flowering in October in grazed pastures or at the edge of beech forests, at an altitude 1500-2400

meters above s. l. Distribution of *C. gilanicus* is in N. and NW. Iran, in the provinces of Azerbayejan and Gilan. Mathew and Brighton (1976) reported  $2n=24$  for the species.

*C. cancellatus* subsp. *damascenus* distributes to the west of Iran and  $2n=8, 10, 12$  were reported for it (Mathew, 1982). *C. speciosus* is one of the most beautiful ornamental species in the garden and it includes three subspecies, two of which are endemic to small areas of Turkey, and the third, namely subsp. *speciosus* occurs in a much wider area of Iran (Caspian region extending to just East of *Gorgan*). Its chromosome number was reported  $2n=12$  (Brighton, 1983; Mathew, 1982).

In first cytological studies on *C. sativus* that were taken up in 1926 by Himmerbaur the chromosome number was determined  $2n=24$ . Later on, other researchers confirmed the first report (Brighton, 1977 b; Karasawa, 1933, 1943; Morinaga and Fukushima, 1931; Pathak, 1940; Pogliana and Grosso, 1971). Karasawa (1933, 1940, 1943) reported that the cultivated *C. sativus* was an autotriploid with  $2n=3x=24$  chromosomes. He also,

Table 1. Chromosome numbers and origins of the *Crocus* collections studied.

| Taxon  | Cytology<br>no. | Collector      | Locality                    | 2n |
|--|-----------------|----------------|-----------------------------|----|
| <i>C. cancellatus</i> Herb. subsp. <i>damascenus</i><br>(Herb.) Mathew | 301             | Saboora et al. | Iran: Arak                  | 8  |
| - -  | 303             | Saboora et al. | Iran: Golpayegan            | 8  |
| <i>C. speciosus</i> M. B.  | 801             | Saboora et al. | Iran: Golestan forest       | 12 |
| <i>C. gilanicus</i> Mathew   | 601             | Saboora et al. | Iran: Syah-Bisheh           | 24 |
| <i>C. sativus</i> L.   | 901             | Saboora et al. | Iran: Cultivated (in vivo)  | 24 |
| -  | 902             | Saboora et al. | Iran: Cultivated (in vitro) | 16 |

reported  $2n=2x=16$  and  $2n=5x=40$  for *C. sativus*. Noori-Dalooi (1975), Aghamohammadi (1976) and Estilai and Aghamohammadi (1997) have reported that Iranian saffron has  $2n=24$  chromosomes. Ghaffari (1986, 1991) observed that *C. sativus* plant is an autotriploid which formed up to eight trivalent groups of chromosomes at both diakinesis and metaphase stages during meiosis. Furthermore, presence of anomaly that occurring in segregation of chromosomes during meiosis produces a variety of microspore with gametic chromosome numbers ranging from 8 to 16.

## MATERIALS AND METHODS

The plant materials were investigated in autumn when root formation is active.

Corms of *Crocus sativus* were collected from a farm situated in Gonabad and, three wild species, including *C. cancellatus* subsp. *damascenus* from two populations in Arak and Golpayegan, *C. speciosus* subsp. *speciosus* from Golestan forest on the road toward Bojnourd and *C. gilanicus* from Siah-Bisheh (Table 1).

Root tips were collected and pre-treated in 0.002 M 8-hydroxyquinoline for seven hours at a temperature of 25-30°C. They were then fixed in Carnoy and Piennar's fixator for 24 hours (Philips, 1981) and stored in 70% ethanol at 4°C. After staining and squash by 1% Fe-acetocarmine (Philips, 1981), suitable slides were prepared. Average of length and diameter, and arm ratio (long: short) were computed for each chromosome and they were categorized in tables 2-5.

Table 2. Morphometric characteristics of *C. cancellatus* subsp. *cancellatus* chromosomes.

| Number of chromosome pair | Total length ( $\mu$ ) | Arm ratio (long: short) | Diameter ( $\mu$ ) |
|---------------------------|------------------------|-------------------------|--------------------|
| 1                         | 8.50                   | 3.97                    | 2.37               |
| 2                         | 7.82                   | 4.99                    | 2.42               |
| 3                         | 6.75                   | 4.06                    | 2.26               |
| 4                         | 5.35                   | 2.33                    | 2.18               |
| average                   | 7.1                    | 3.84                    | 2.31               |

Table 4. Morphometric characteristics of chromosomes of *C. gilanicus*.

| Number of chromosome pair | Total length ( $\mu$ ) | Arm ratio (long: short) | Diameter ( $\mu$ ) |
|---------------------------|------------------------|-------------------------|--------------------|
| 1                         | 19                     | 1.11                    | 1.75               |
| 2                         | 10                     | 1.33                    | 2                  |
| 3                         | 9.5                    | 5.3                     | 2                  |
| 4                         | 9                      | 2                       | 2                  |
| 5                         | 8.5                    | 3.66                    | 2                  |
| 6                         | 7.5                    | 2.8                     | 2                  |
| 7                         | 6.5                    | 5                       | 2                  |
| 8                         | 6                      | 3                       | 2                  |
| 9                         | 5.5                    | 1.2                     | 2                  |
| 10                        | 5                      | 2.33                    | 2                  |
| 11                        | 5                      | 4                       | 1.5                |
| 12                        | 4.5                    | 2.5                     | 2                  |
| average                   | 8                      | 2.84                    | 1.94               |

In order to study the changes occurring in cells of corm explants of *C. sativus* with regard to chromosome number, segments of callus and neofomed roots were stained with the same method.

Meiotic chromosomes of *Crocus sativus*

Table 3. Morphometric characteristics of *C. speciosus* subsp. *speciosus* chromosomes.

| Number of chromosome pair | Total length ( $\mu$ ) | Arm ratio (long: short) | Diameter ( $\mu$ ) |
|---------------------------|------------------------|-------------------------|--------------------|
| 1                         | 12                     | 1.37                    | 2.5                |
| 2                         | 12                     | 1.22                    | 2.5                |
| 3                         | 11.87                  | 1.43                    | 2.5                |
| 4                         | 10.75                  | 1.53                    | 2.5                |
| 5                         | 9                      | 1.52                    | 2.5                |
| 6                         | 7                      | 1.08                    | 2.5                |
| average                   | 10.44                  | 1.36                    | 2.5                |

Table 5. Morphometric characteristics of *C. sativus* chromosomes.

| Number of chromosome pair | Total length ( $\mu$ ) | Arm ratio (long: short) | Diameter ( $\mu$ ) |
|---------------------------|------------------------|-------------------------|--------------------|
| 1                         | 6.41                   | 3.40                    | 0.61               |
| 2                         | 5.80                   | 2.22                    | 0.61               |
| 3                         | 4.58                   | 1.07                    | 0.5                |
| 4                         | 3.66                   | 1.10                    | 0.61               |
| 5                         | 3.62                   | 1.2                     | 0.61               |
| 6                         | 3.58                   | 2.56                    | 0.61               |
| 7                         | 3.05                   | 1.89                    | 0.61               |
| 8                         | 2.14                   | 1.12                    | 0.61               |
| average                   | 4.11                   | 1.82                    | 0.6                |

were observed in pollen mother cells. Immature flower buds taken from the underground corms, and then fixed in Piennar's solution for 24-48 hours at 4°C. After washing with distilled water, they were stored in 70% ethanol at 4°C. The

anthers were then stained and squashed in 2% Fe-acetocarmine.

## RESULTS

The results obtained from karyological studies in *Crocus* species are summarized as follows:

### ***Crocus cancellatus* subsp. *damascenus***

Mitotic studies on meristematic cells of root tips in collected specimens from Sefidkhany plain of Arak and Golpaygan had no distinct difference in chromosome number,  $2n=2x=8A$ . Usual karyotype of these populations consisted three pairs of acrocentric and one pair of smaller acrocentric chromosomes. There are extremely small satellites on the long arms of the fourth pair which were faintly stained and observable in some specimens with difficulty (Fig. 1a, 2a). Morphometric characteristics of *C. cancellatus*, cytotype  $2n=8A$ , are summarized in Table 2.

### ***C. speciosus* subsp. *speciosus***

The cytological studies of this subspecies collected from the margin of Golestan

forest, reveals  $2n=12$ . This species shows a distinctive karyotype of two pairs of metacentric chromosomes with small satellites on the one pair, three pairs of submetacentric and one pair of smaller metacentric chromosomes (Fig. 1b, 2b). Morphometric characteristics of *C. speciosus* are presented in Table 3.

### ***C. gilanicus***

The collections of *C. gilanicus* from Syah-Bisheh in Mazandaran province have  $2n=24$ , the karyotype consisting of two pairs of metacentric, one pair of smaller metacentric, one pair of submetacentric, one pair of acrocentric, four pairs of middle-sized acrocentric and three pairs of smaller acrocentric chromosomes. There are satellites on the short arms of one pair of acrocentric chromosomes (Fig. 1c, 2c). Morphometric characteristics of chromosomes of *C. gilanicus* are presented in Table 4.

### ***C. sativus***

Karyological studies on cultivated *C. sativus* (saffron) revealed an autotriploidy with  $2n=24$  chromosomes. The karyotype of which consists two

triplets of acrocentric, three triplets of metacentric, two triplets of smaller acrocentric and one triplet of smaller metacentric chromosomes. There are satellites on the large arms of the second groups of acrocentric chromosomes (Fig. 1d, 2d). Characteristics of chromosomes of *C. sativus* are presented in Table 5.

Meiotic studies on pollen mother cells in cultivated *C. sativus* showed special anomalies of autotriploid plants. The segregation of chromosomes and their migration to two poles of cell in anaphase I is occurred at random and has caused the derangements. In addition, retardation of some chromosomes and chromosome elimination are also observed in some cells. The result of this process is unbalanced distribution of chromosomes in two poles of cell (Fig. 3).

Staining and counting of chromosomes in callus cells and neoformed root tips of explant shows that in the basic medium LS (Linsmaier and Skoog, 1965) which contains 1 mg/lit each of both hormone NAA and BAP, there are cells with different chromosome numbers in the meristematic zone of neoformed roots. Chromosome counting of these cells in

mitotic metaphase shows  $2n=16$  (Fig. 2e).

## DISCUSSION

*C. cancellatus* subsp. *damascenus* ( $2n=8$ ) as compared with other species which are investigated in this paper has enjoyed less antiquity for smaller basic chromosomal number ( $x$ ) and chromosome number (Bidault, 1971), existence of acrocentric chromosomes and small satellites (Pathak, 1940).

Constant difference in size of the same or different pairs in a species may be due to the stage of evolution of the particular species in the genus, the most primitive ones possessing large, and the advanced ones possessing minute satellites [Pathak, 1940].

Specimen of *C. speciosus* subsp. *speciosus*, collected from the margin of Golestan forest showed a chromosome number  $2n=12$ . Brighton (1983) reviewed this subspecies and introduced seven cytotypes. In these studies, the collections of North of Iran had  $2n=12$  with a distinctive karyotype of three pairs of large metacentric chromosomes with small satellites on one pair, and three pairs of

submetacentric with small satellites on the long arms of one pair Karyotype of the above specimen and those investigated in this study are fundamentally similar. Only in the latter case one pair metacentric chromosomes is replaced by one pair of submetacentric that could be caused by exchange of chromosome pieces.

Karyotype of *C. gilanicus* shows  $2n=24$  which consists of two distinctive groups of chromosomes i. e., large and small. These two types of chromosomes are derived probably from similar karyotypes with the origin of polyploid, smaller resulting from differential loss of the large chromosomal pieces. Translocation of chromosomal pieces is also another probability that caused decrease of length of donor chromosomes and increase of length of receptors. An example is karyotype of *Aloinae* that consists of approximately equal number of small chromosomes and large chromosomes [Stebbins, 1989].

The results obtained from mitotic and meiotic studies in *C. sativus* show that it is a autotriploid plant ( $2n=3x=24$ ) conforming with previous reports [Aghamohammadi, 1976; Brighton, 1977b; Chichiricco, 1984; Chichiricco and Grilli

Cariola, 1982; Ghaffari, 1986, 1991; Karassawa, 1933, 1943; Noori-Daloi, 1975; Pathak, 1940; Pogliana and Grosso, 1971]. Reported karyotype was similar to karyogram of *C. sativus* that was reported from central region of Iran by Brighton (1977 b).

Investigation of the stage of microspore formation from pollen mother cells reveals cytologically abundant abnormal states. During the meiosis, disorderly distribution of chromosomes, occurrence of lagged chromosomes and, in some cases, chromosomal deletion is observed. Therefore, after the completion of meiosis, the microspores are with different chromosome number that their germination power are incomplete and a few number of them remain alive. Ghaffari (1986, 1991) reported existence of gametes that have 8-16 chromosomes. This state caused that gametes with respect to presence of genes have been unbalanced and consequently sterile. One of the reasons for the sterility of *C. sativus* were mentioned its autotriploidy and appearance of anomalies during formation of microspores (Chichiricco and Grilli Cariola, 1982, 1986; Chichiricco, 1984) and megaspores



(chichiricco, 1987).

In the mitotic studies on the corm explant of *C. sativus* (in vitro), we observed reduction of chromosome number ( $2n=16$ ). Unfortunately, karyotyping of these cells were difficult and existence of two series of chromosomes (diploidy) were not confirmed. Whereas various examinations for obtainment of plants that can produce the germinative seeds were not successful [Aghamohammadi, 1976; Chichiricco, 1986; Ghaffari, 1991].

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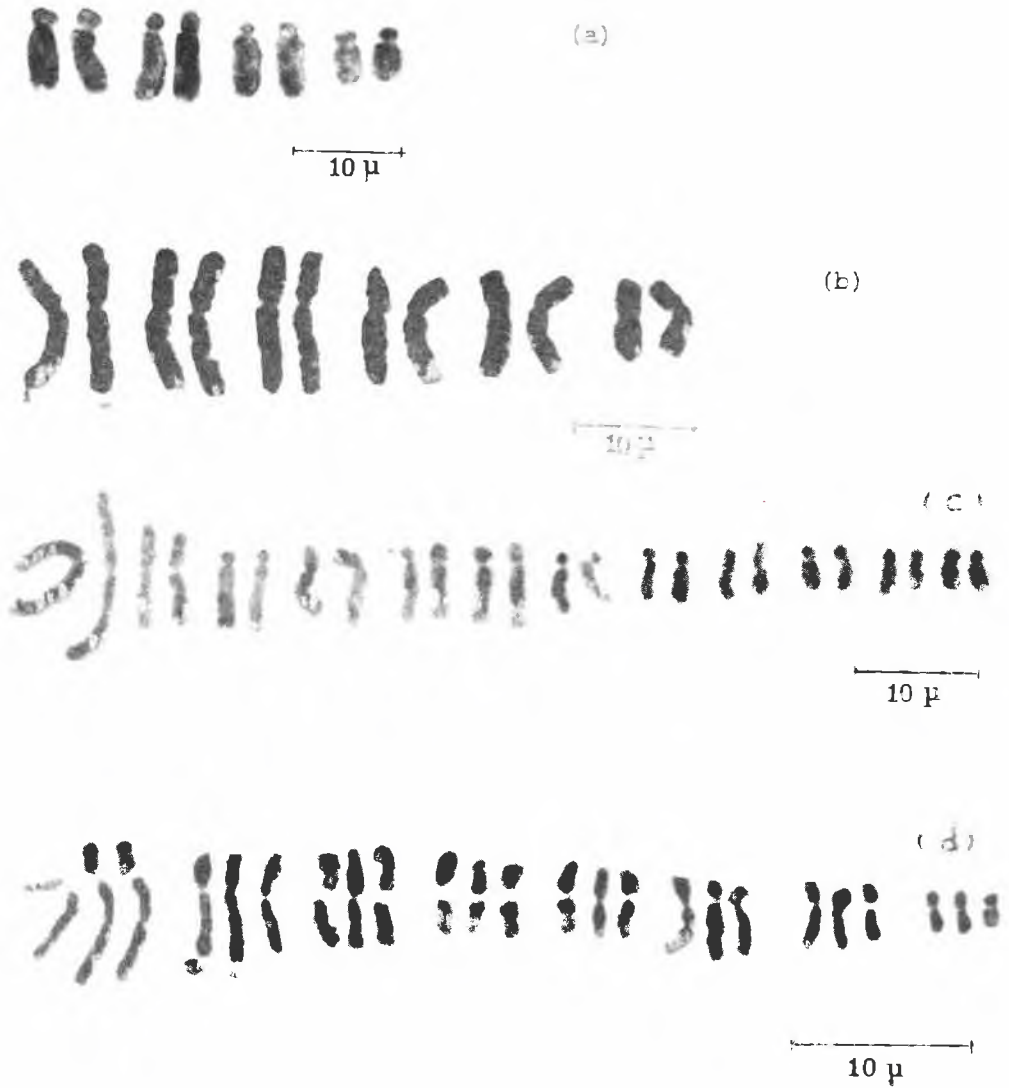


Fig. 1. Karyotype of (a) *Crocus cancellatus* subsp. *damascenus* ( $2n=8A$ ); (b) *Crocus speciosus* subsp. *speciosus* ( $2n=12$ ); (c) *Crocus gilanicus* ( $2n=24$ ); (d) *Crocus sativus* ( $2n=3x=24$ ).

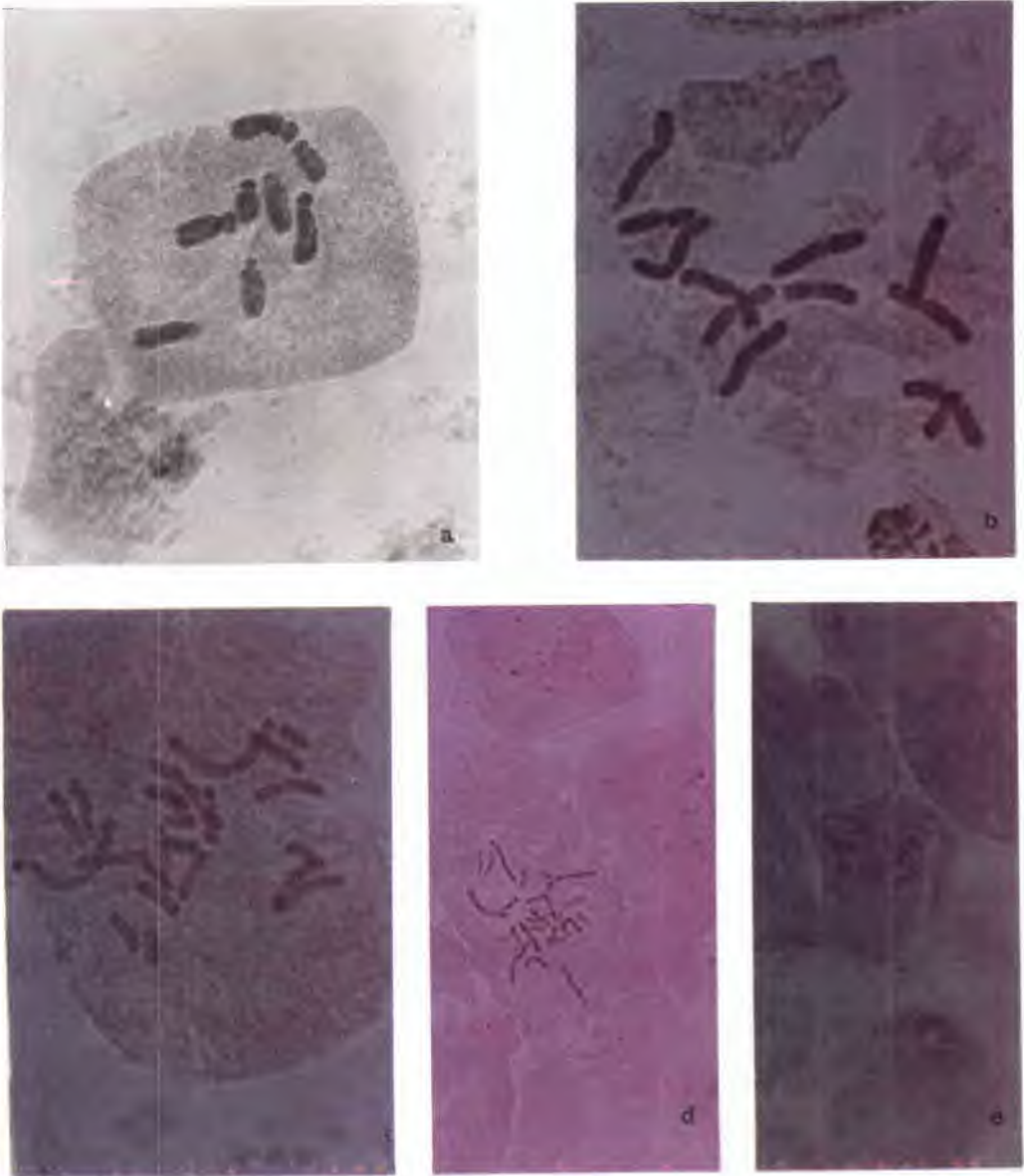


Fig. 2. Mitotic chromosomes of *Crocus* species: (a) *C. cancellatus* subsp. *damascenus*; (b) *C. speciosus* subsp. *speciosus*; (c) *C. gilanicus*; (d) *C. sativus* (e) *C. sativus*, metaphase in meristematic cells of neoformed root tips of term explant,  $2n=16$ .

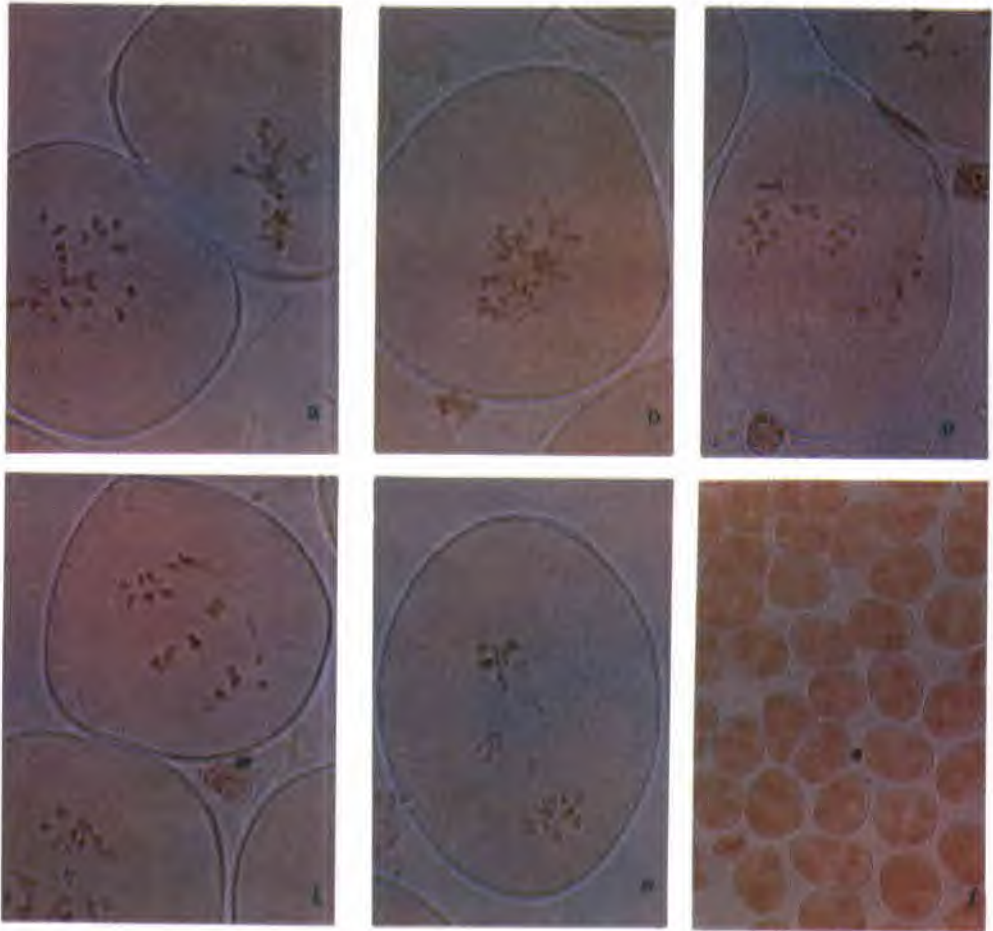


Fig. 3. Meiosis in pollen mother cells of *Crocus sativus*: (a) metaphase I,  $2n=24$ ; (b) early anaphase I, migration of chromosomes to two poles of cell; (c) segregation in the form of 11-3-10; (d) 9-7-8 segregation; (e) 8-4-12 segregation; (f) stage of tetrad, formation of four cells with one or two nuclei; separated microspores with two nuclei, is also observed.

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