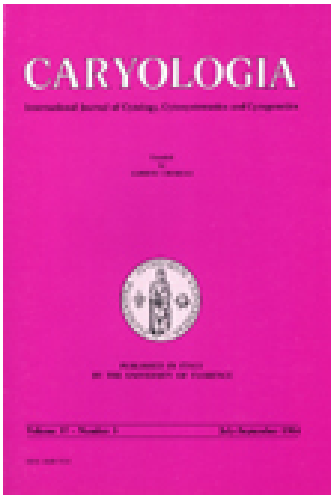

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KARYOTYPE AND MEIOTIC BEHAVIOUR OF THE TRIPLOID *CROCUS SATIVUS* L.

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SUMMARY — The karyotype and microsporogenesis of triploid *Crocus sativus* were studied. The high percentage of trivalents observed at metaphase I (on average, 7.3 trivalents per cell) together with the karyotype configuration confirm autotriploidy. Some considerations about the presence of one unique chromosome in the karyotype and about the origin of triploidy are presented.

INTRODUCTION

Crocus sativus is a sterile triploid ($2n = 24$) that rapidly reproduces itself by means of bulbs; it is cultivated to obtain the saffron drug while it is unknown as a wild plant (MATHEW 1977). Several karyological studies are reported for this species (BRIGHTON *et al.* 1973; GATES and PATHAK 1938; HIMMERBAUR 1926; KARASAWA 1932; MATHER 1932; MORINAGA and FUKUSHIMA 1931; PATHAK 1940; POGLIANI and DEL GROSSO 1971), while little is known on its microsporogenesis (KARASAWA 1933, 1943). This Author reported that at metaphase I eight trivalents were usually observed in *C. sativus*, indicating its autotriploidy, and that tetrads, triads and supernumerary microspores were formed as a result of an irregular division. The autotriploidy of saffron has been subsequently supported by BRIGHTON (1977) on the basis of her karyotipical study on *C. sativus* from different countries (France, Iran, Turkey, England, and Majorca).

In the present paper the chromosome behaviour during meiosis in *C. sativus* from L'Aquila (Italy) and the description of its karyotype are reported in order to further contribute to the cytology of this species that is economically important particularly in Abruzzo (Italy). In another work the germination and viability of the pollen were studied (CHICHIRICCÒ and GRILLI CAIOLA 1982).

MATERIALS AND METHODS

Bulbs of *Crocus sativus* L. were collected at Navelli (L'Aquila, Abruzzo, Italy) where the saffron is cultivated from ancient times.

For the study of mitotic chromosomes, the basal part of young leaves, pretreated with 0.2% colchicine in 1,4-dichlorobenzene (satured solution), fixed in 1:3 acetic ethanol and stained in acetic orcein, was used.

For the study of meiotic chromosomes in PMCs, young anthers, fixed in the above mentioned fixative and stored in 70% ethanol at 4° C till the staining in acetic orcein, were used. In both cases, squash preparations were temporary.

RESULTS

Karyology.

Twenty-four chromosomes were counted in the cells observed (Fig. 1). On the basis of overall length and centromeric position, the somatic chromosomes could be assembled in seven triplets, one pair, and one single chromosome (Fig. 2). Among the triplets, three consisted of metacentric, two of submetacentric, and two of subtelocentric chromosomes of which one showed a small satellite on the long arm. The chromosome pair was submetacentric, and the single chromosome was metacentric (Table 1).

TABLE 1 — *Karyotype of triploid «Crocus sativus».*

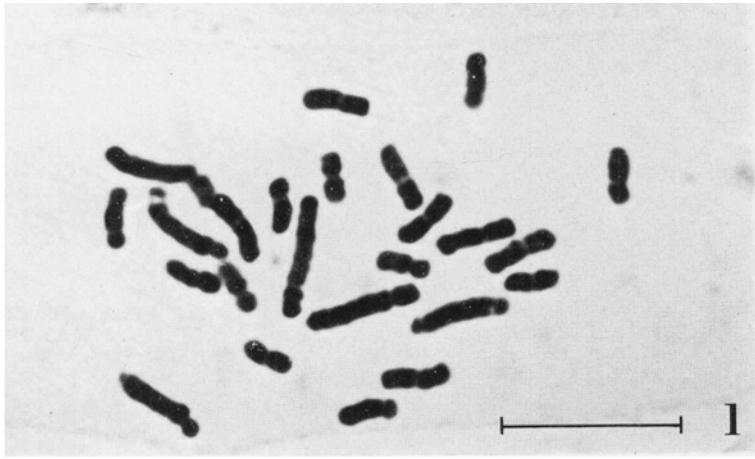
Chromosome type	Number of chromosomes examined	Average length of long and short arms in μm	Long arm / short arm	Position of the centromere
a	33	5.05 – 1.60	3.15	st
b	22	3.79 – 0.78	4.85	st
c	30	2.34 – 1.59	1.47	m
d	23	2.16 – 0.74	2.91	sm
e	25	1.93 – 0.93	2.07	sm
f	21	1.84 – 1.05	1.75	sm
g	12	2.46 – 1.76	1.39	m
h	26	1.79 – 1.65	1.08	m
i	26	1.33 – 1.22	1.09	m

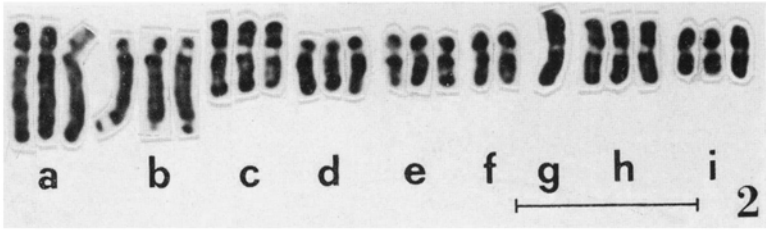
Pollen mother cell meiosis.

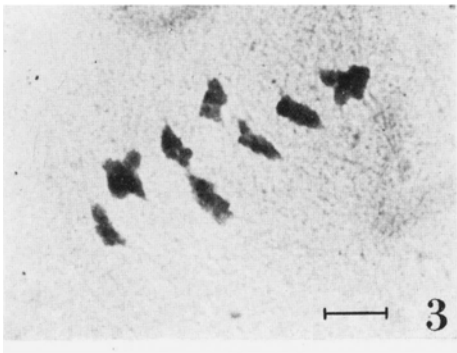
The earliest stage studied in the PMCs was metaphase I. The chromosomes at this stage were much condensed and tightly joined in the associations

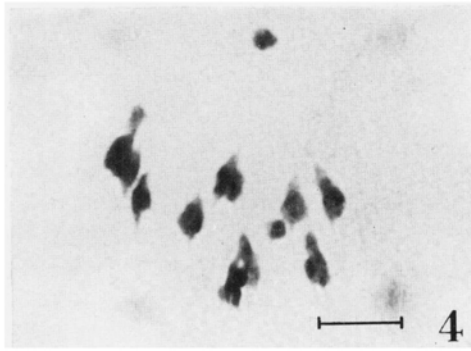
Figs. 1-13. — The bar marker represents 10 μm in Figs. 1-12 and 100 μm in Fig. 13.

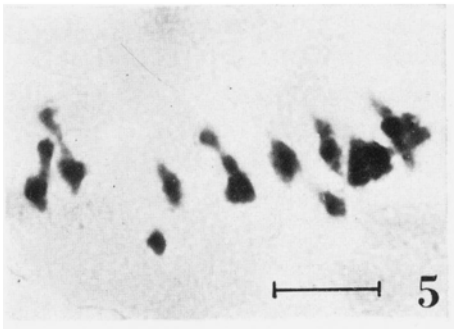
Fig. 1 — A somatic plate of *C. sativus* showing 24 chromosomes. Fig. 2 — Karyogram of the cell in Fig. 1. Figs. 3, 4, 5 — Pollen mother cells at metaphase I showing: 8III – 7III + 1II + 1I – 6III + 2II + 2I. Figs. 6, 7 — Anaphase I. Figs. 8, 9 — Telophase I. Fig. 10 — A pollen mother cell at metaphase II. Fig. 11 — Two pollen mother cells at telophase II showing laggards and a multipolar distribution of chromatids. Fig. 12 — Polyad and triad. Fig. 13 — Unripe pollen grains. Some of them are collapsed.

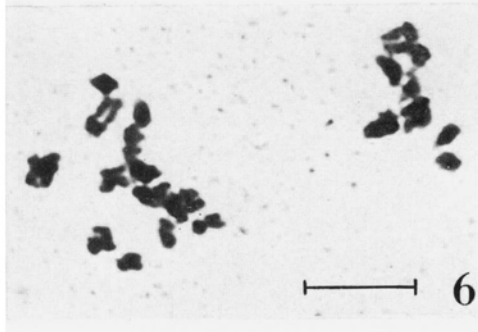


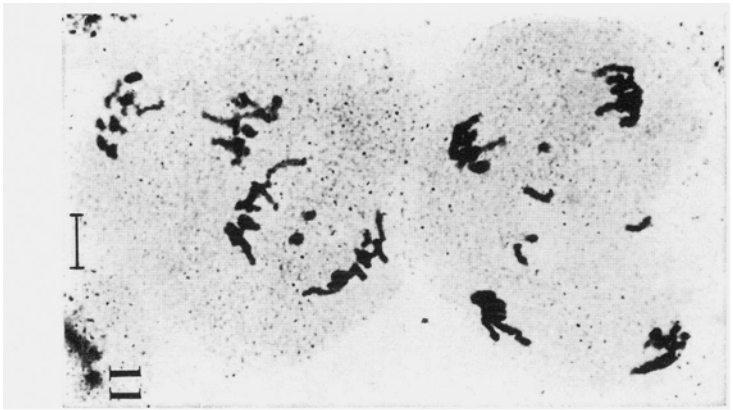


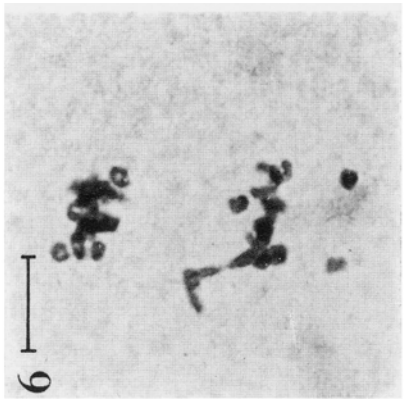


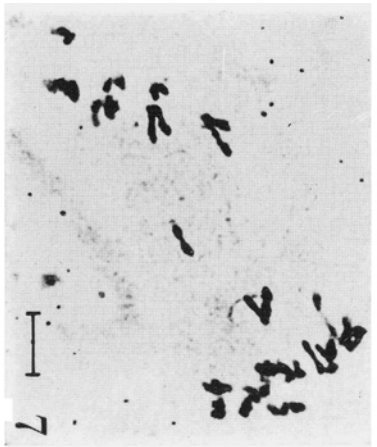


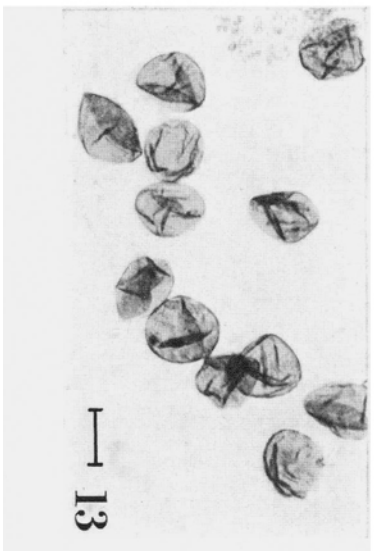


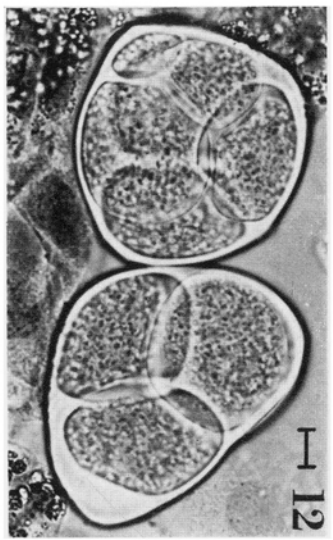


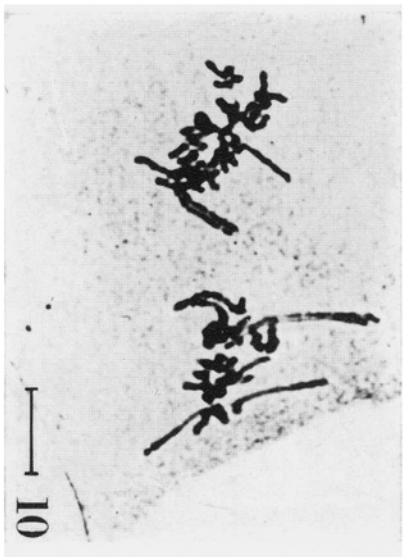












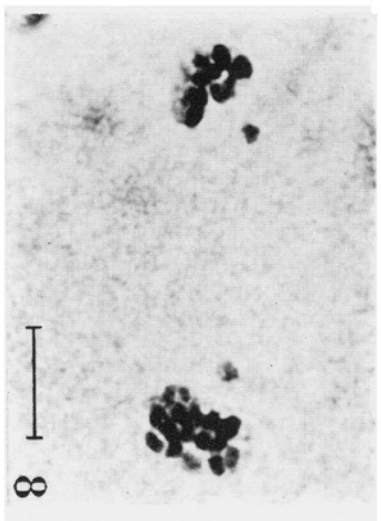


TABLE 2 — *Chromosome configurations at metaphase I.*

Configurations	8III	7III + 1II + 1I	6III + 2II + 2I	5III + 3II + 3I	1IV + 6III + 1II	Total
No. of cells	75	59	19	2	2	157
% of cells	47.7	37.5	12.1	1.27	1.27	
No. of trivalents	600	413	114	10	12	1149
No. of bivalents	/	59	38	6	2	105
No. of univalents	/	59	38	6	/	103
No. of quadrivalents	/	/	/	/	2	2

TABLE 3 — *Chromosome segregation at anaphase I.*

Distribution of chromosomes										
	13	12	14	15	12	13	11	12	13	9
					1	1	2	2	2	7
	11	12	10	9	11	10	11	10	9	8
No. of cells	13	5	5	1	2	1	1	1	1	1

TABLE 4 — *Pollen-tetrad, triad, monad, and supernumerary microspores.*

Tetrads	Triads	Monads	Polyads							Total
			supernumerary microspores							
			1	2	3	4	5	6	7	
205	8	8	27	23	9	8	4	/	1	293

so that it was difficult to distinguish them. As shown in Table 2 trivalents occurred in all cells studied, varying from 5 to 8 per cell (Figs. 3, 4 and 5) with a mean of 7.3. Bivalents and univalents, when present, were numerically corrispondent, suggesting a previous association. Quadrivalents, caused by the occasional pairing between trivalents and univalents, were seldom observed.

At anaphase I irregular distribution of chromosomes at the poles was observed (Fig. 6). Moreover, laggards generally consisting of univalents occurred (Fig. 7). The chromosome number at the poles varied from 8 to 15 (Table 3), only 5 cells showed equal disjunction. Bridges were rarely seen.

Laggards persisted till telophase I (Figs. 8 and 9), being present in 4 of the 33 cells studied.

Metaphase II was of normal appearance in the 5 PMCs studied (Fig. 10).

Laggards and multipolar distribution of chromatids were observed during telophase II (Fig. 11).

Cytokinesis, as expected, was abnormal and resulted in monads (2.7%), triads (2.7%), tetrads (70%), and polyads (24.6%) (Table 4, Fig. 12).

Pollen grains, genetically unbalanced due to erratic segregation of the chromosomes, were variable in size ranging from about 50 μm to about 100 μm. About 20% resulted collapsed and empty (Fig. 13).

DISCUSSION

The results of the present study confirm the autotriploidy of *Crocus sativus*, already reported by KARASAWA (1933, 1943) and BRIGHTON (1977).

The karyotype consists of a series of chromosome triplets with the exception of one group of three chromosomes of which one is different from the others; the presence of a unique chromosome in the karyotype was reported also by BRIGHTON (1977) in *C. sativus* coming from Majorca. Although this karyotype configuration seems to support autoploidy, it does not allow to definitely exclude alloploidy. However, the chromosome pairing during meiosis speaks undoubtedly in favour of the autotriploidy. At metaphase I a high frequency of trivalent formation (on average 7.3 trivalents per cell) is observed, indicating a very high degree of homology between chromosomes. The percentage of trivalents observed is higher or similar to that generally reported for autotriploids (CARROL 1966; ERICHSEN and ROSS 1963; KHOSHOO and SHARMA 1959; LIN and ROSS 1969; MADHUSUDANA and REDDI 1970; PANTULU 1968; WAFAI and KAUL 1981). The presence of some quadrivalents indicates that incidental pairing between non homologous or partly homologous chromosomes occurs.

Some considerations about the origin of this autotriploid can be formulated. The cross between tetraploids and diploids seems to be hardly probable because tetraploids do not occur among allies of *C. sativus* (BRIGHTON 1977; MATHEW 1977). Therefore, a cross between diploids can be hypothesized, by assuming that fertilization of a functional *2n* egg by haploid sperm or of the haploid egg by a *2n* sperm nucleus occurred; the selfing can be excluded because of the presence of the unique chromosome. Since *C. sativus* is sterile, the presence of this chromosome might be explained by assuming the existence of chromosomal polymorphism at diploid level in the progenitor species, most probably *C. thomasii* or *C. cartwrightianus* (BRIGHTON 1977).

Whatever the mechanism involved in the triploidy of *C. sativus*, it is puzzling that this species does not spontaneously occur as its suggested progenitors do (BRIGHTON 1977); this fact cannot be attributed to the drawback of sterility because several vegetatively reproducing autotriploids spontaneously occur in nature (KHOSHOO and SHARMA 1959; KOLLMANN 1972; GUPTA and SRIVASTAVA 1970).

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