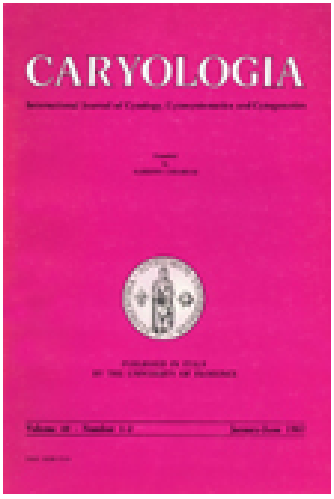

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MEGASPOROGENESIS AND DEVELOPMENT OF EMBRYO SAC IN *CROCUS SATIVUS* L.

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SUMMARY — Cytological observations during megasporogenesis and embryo sac development of *Crocus sativus* L. are reported. During meiotic divisions irregular assortment of chromosomes was observed; consequently, megaspores resulted numerically variable (4-6) and they were genetically unbalanced. The megaspore tetrads-hexads showed chalazal heteropolarity, however, the microphylar pole resulted more active than the chalazal one in promoting megaspore development. This was attributed to the nutritional factors liberated from degenerated micropylar spores. Independently on the number of produced megaspores the micropylar ones (M_1, M_2, M_3), generally, degenerated, the chalazal spores (M_4 - M_6) persisting; therefore, besides single embryo sacs, megaspores close to embryo sac or development of two embryo sacs could be observed. Frequently, the megasporogenesis or development of the persistent megaspore were not completed; consequently, ovules lacking embryo sac or showing immature embryo sac occurred. The mature embryo sac was 7-nucleate. Sometimes, ovules were fertilized with subsequent development of embryo and endosperm.

INTRODUCTION

Previous studies (CHICHIRICCÒ 1984; CHICHIRICCÒ and GRILLI CAIOLA 1982, 1984, 1986), aimed at investigating on the sterility of *Crocus sativus* L. (saffron), an infertile autotriploid ($2n = 3x = 24$) (KARASAWA 1933, 1943; BRIGHTON 1977; MATHEW 1977; CHICHIRICCÒ 1984), revealed that several cytological abnormalities occur during microsporogenesis; consequently, the saffron pollen results partially viable and it shows a defective germination and pollen tube growth both *in vitro* and *in vivo*. *In vivo*, a low percentage of pollen tubes reaches the ovary and a few of them penetrate the ovules.

For the same purpose embryological studies, which are still in progress, were undertaken. The present paper reports some cytological abnormalities observed during megasporogenesis and embryo sac development, as well as some data concerning the ovule fertilization.

MATERIALS AND METHODS

Bulbs of *Crocus sativus* L. from Navelli Highlands, L'Aquila (Italy), where this plant is in cultivation, were cultivated at the Botanical Garden of the University of L'Aquila. Ovaries, detached from flower buds at different developmental stages and from flowers which in part had been pollinated as described previously (CHICHIRICCÒ and GRILLI CAIOLA 1984), were fixed in 1:3 acetic ethanol; then they were dehydrated in a graded ethanol series and were embedded in LKB 2218-500 historesin. Serial longitudinal sections thro of the ovules were cut at 4-8 μm and stained with toluidine blue.

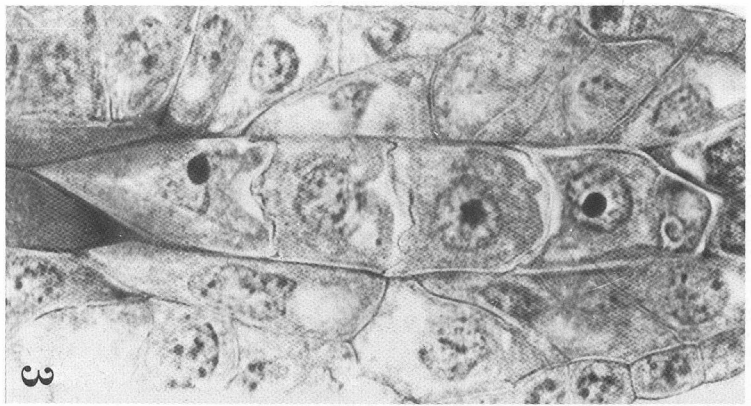
The nomenclature used for the arrangement of megaspores in the tetrads-hexads is the following: M_1 - M_2 - M_3, micropylar megaspores; M_4, chalazal megaspore; M_5 - M_6, supernumerary chalazal megaspores.

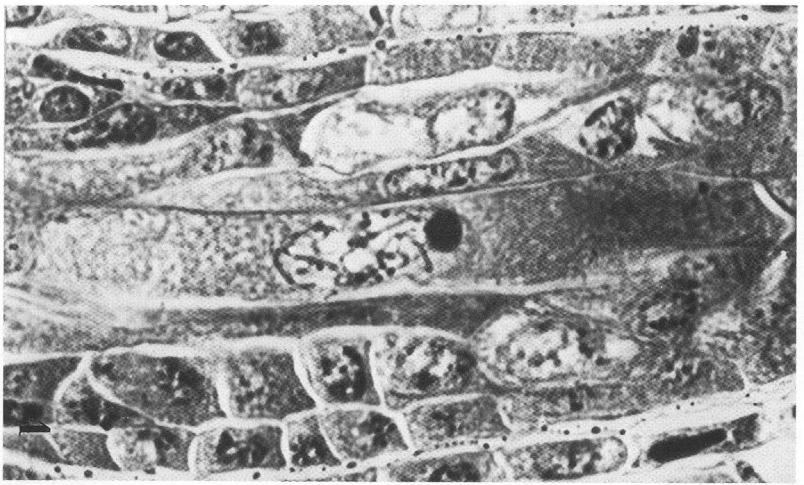
RESULTS

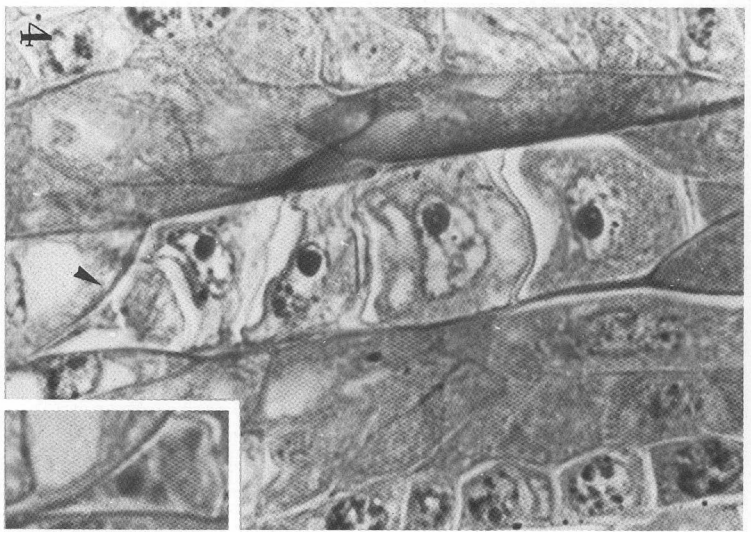
The ovary of *C. sativus* is inferior and 3-chambered, each chamber containing, on average, 9-10 ovules arranged on two rows on an axile placenta. Ovules are anatropous and comprise abundant nucellar tissue with a large hypostase.

Meiosis starts during early developmental stages of flower buds, as has been reported for other *Crocus* species (RUDALL *et al.* 1984), and goes on slowly. During the first stages of ovule development the nucellus comprises some layers of cells surrounding a single enlarged cell showing dense cytoplasm. This acts as the megaspore mother cell and undergoes meiosis (Fig. 1), forming firstly a large dyad (Fig. 2) and later a linear tetrad of megaspores (Fig. 3); in addition, pentads (Fig. 4) or, sometimes, six megaspores are produced. The meiotic divisions were characterized by both irregular assortment of chromosomes and the occurrence of lagging chromosomes, as it was reported for microsporogenesis (CHICHIRICCÒ 1984). After meiosis the micropylar megaspores degenerate and the M_4 spore, generally, increases in size assuming first a

Figs. 1-15. — Abbreviations: a = antipodal; e = embryo; ec = egg cell; en = egg nucleus; h = hypostase; pn = polar nuclei; pt = pollen tube; s = synergid; sm = persistent supernumerary megaspore; sn = sperm nucleus.
Fig. 1. — Megaspore mother cell at leptotene stage. × 910.
Fig. 2. — Dyad. × 640.
Fig. 3. — Tetrad of megaspores. × 700.
Fig. 4. — Pentad of megaspores. Megaspore with arrow more evident in the insert. × 920.







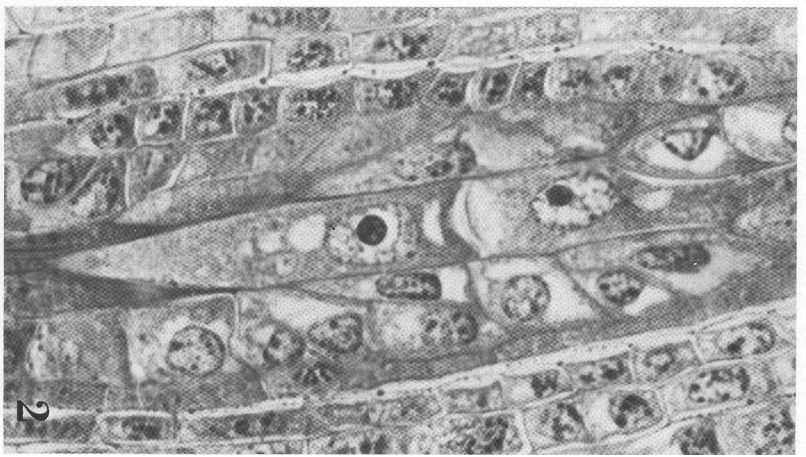
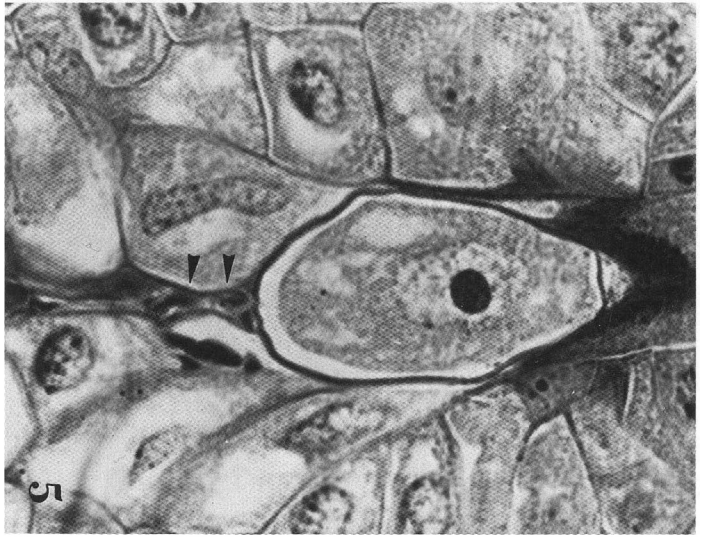


Fig. 5. — Persistent megaspore after meiosis. Note degenerated micropylar spores (arrows). × 890.
Fig. 6. — M_4 megaspore at pro-metaphase of the first mitosis, showing 11 chromosomes. × 1370.
Fig. 7. — Two megaspores (M_4, M_5) persisting after meiosis, the M_4 spore being at the two nucleate stage. × 570.
Fig. 8. — Persistent megaspore (M_5) close to the hypostase after embryo sac maturation. × 340.



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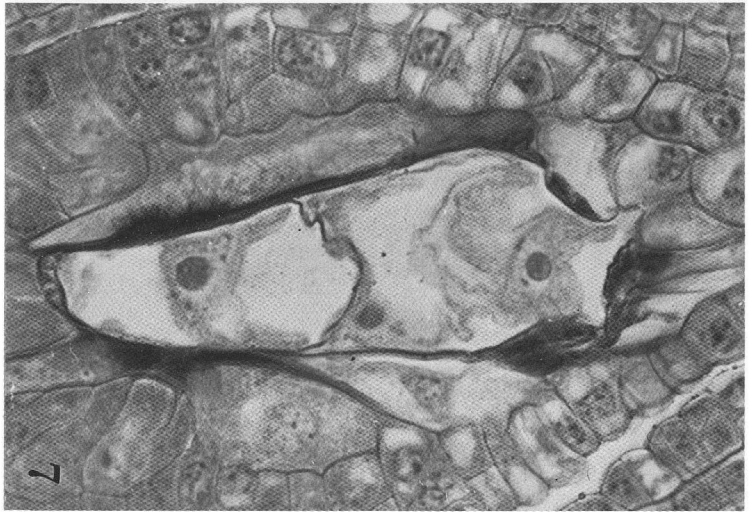

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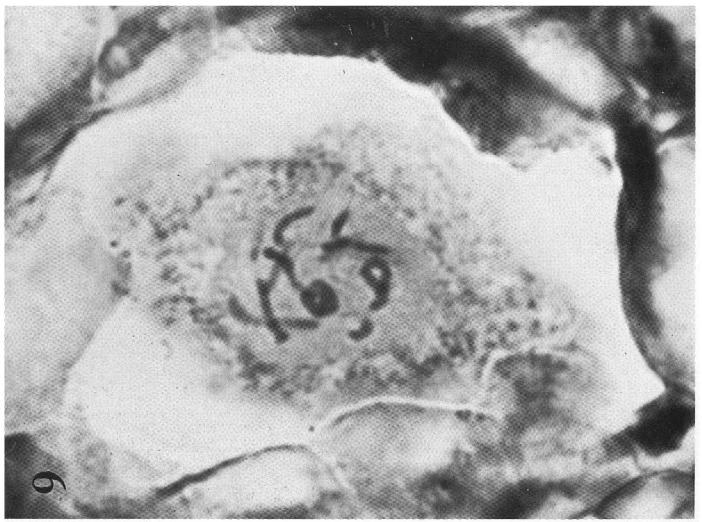

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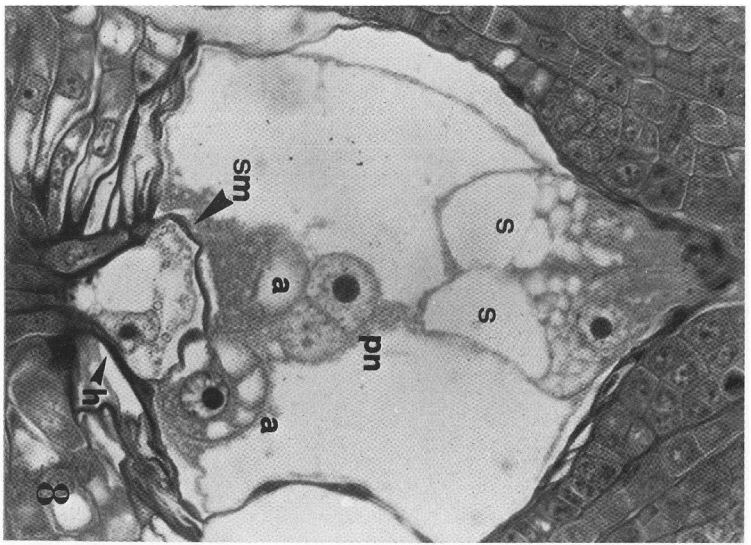

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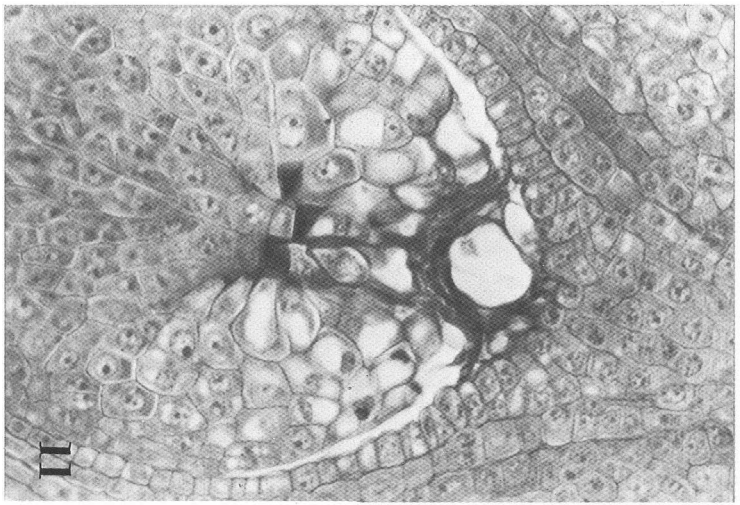
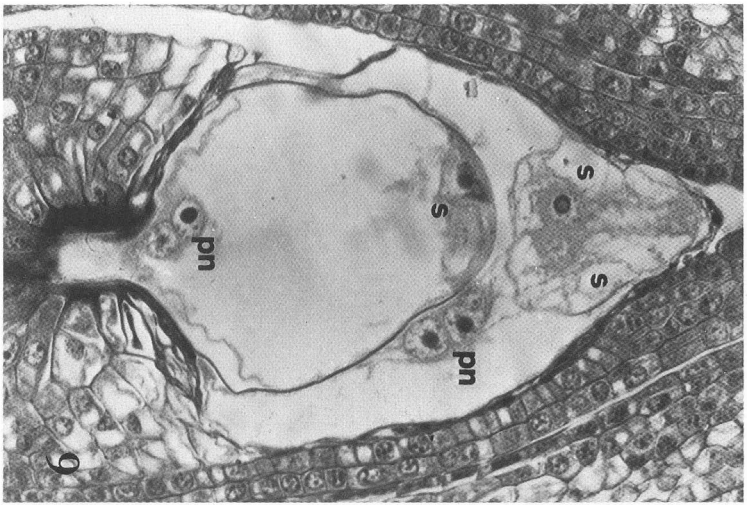
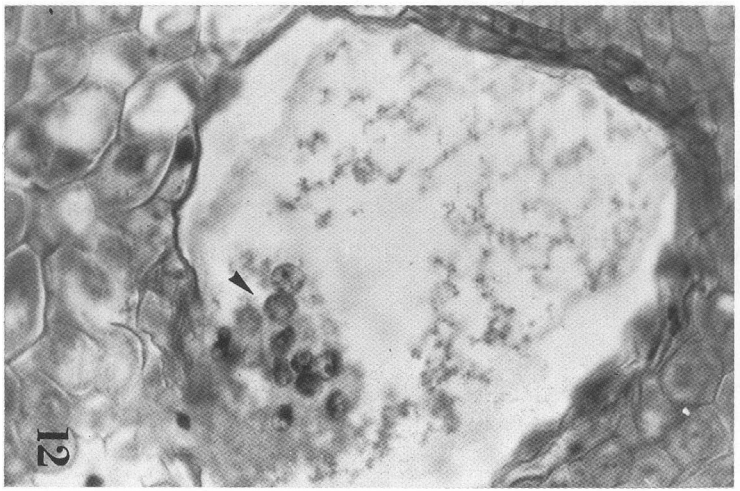
Fig. 9. — Two embryo sac in one ovule. × 285.
Fig. 10. — Mature embryo sac. × 285.
Fig. 11. — Ovule lacking embryo sac with abundant nucellar tissue. × 285.
Fig. 12. — Embryo sac containing both granular material and micronuclei (arrow). × 430.







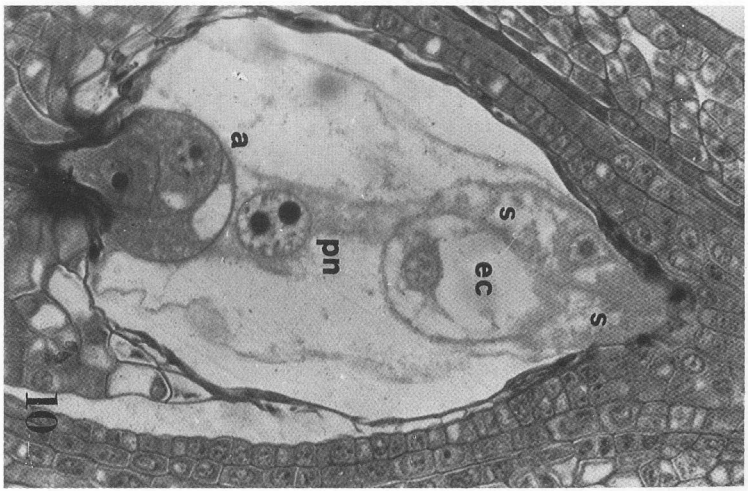
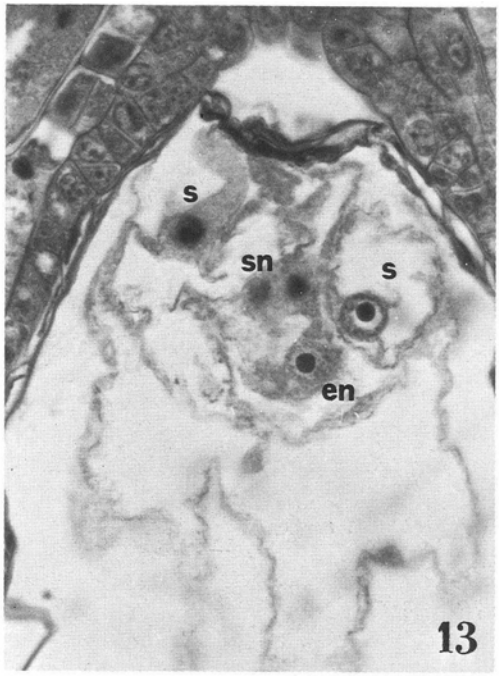
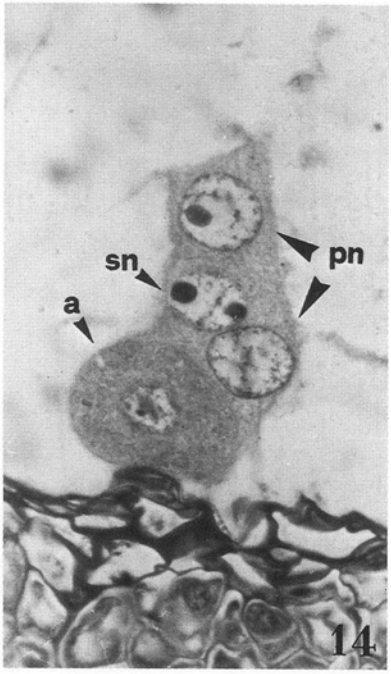
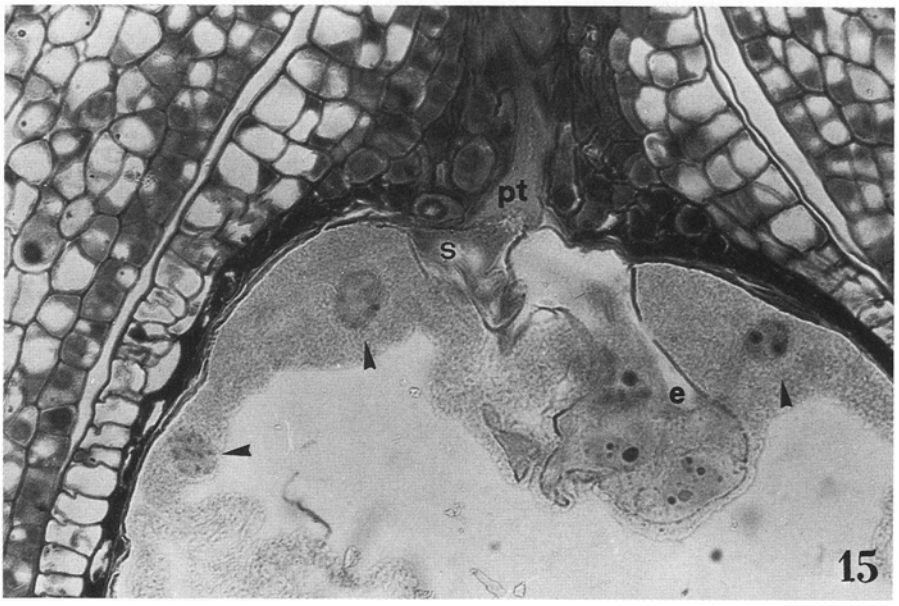


Fig. 13. — Ovule 4 days after pollination, showing one sperm nucleus with two nucleoli, close to the egg nucleus. × 440.
Fig. 14. — Ovule of Fig. 13 showing the other sperm nucleus close to the polar nuclei. × 440.
Fig. 15. — Ovule 12 days after pollination, showing developing embryo and endosperm (arrows). × 340.







pear-shape (Fig. 5) and then, after undergoing three mitotic divisions, it develops into an embryo sac. A variable chromosome number is observed during mitosis of the megaspores, frequently $n = 11$ (Fig. 6) or $n = 13$. Supernumerary megaspores do not seem to degenerate, they are observed during mitotic divisions of the M_4 spore (Fig. 7), and also after embryo sac maturation borne on the hypostase (Fig. 8). About 7% of the ovules show one (M_5) or, sometimes, two megaspores (M_5, M_6) close to embryo sac. Sometimes, two persistent megaspores undergo nuclear divisions, consequently, two adjacent embryo sacs develop into one ovule (Fig. 9).

The mature embryo sac is broad and 7-nucleate (Polygonum-type) (Fig. 10); the organization of the cells in the embryo sac will be described in a further paper.

Frequently, ovules do not reach a fertilizable stage due to unsuccessfulness of either megasporogenesis or megaspore development. About 12% of them develop a small embryo sac generally comprising acellular nuclei which may be numerically variable, often no more than four nuclei are observerd. A higher percentage of ovules (18%) are quite lacking embryo sac; these show proliferation of nucellar tissue which increases up to micropyle (Fig. 11). Degeneration at embryo sac stage occurs infrequently; in this case, the embryo sac contains abundant granular material and, sometimes, also micronuclei (Fig. 12).

Ovules are fertilized infrequently. Four days after pollination, one sperm nucleus was observed close to the egg nucleus (Fig. 13), the other being close to the polar nuclei (Fig. 14); developing embryo and endosperm were observed 12 days after pollination (Fig. 15).

DISCUSSION

Megasporogenesis in *C. sativus* is accompanied by some cytological abnormalities previously reported also in microsporogenesis (CHICHIRICCÒ 1984) and due to the triploidy of this plant. Consequently, the megaspores result numerically variable and they are genetically unbalanced so that a part of them is not able to develop successfully into embryo sac. On the whole, non-functional ovules are more than 30%; generally, they show defective embryo sac or they quite lack embryo sac. The remaining ones display mature embryo sac that is 7-nucleate as in other *Crocus* species (RUDALL *et al.* 1984). It is interesting to note that, although the tetrads-hexads of megaspores show heteropolarity in the chalazal direction (Polygonum-type), the micropylar pole results more active than the chalazal one in promoting megaspore development. In fact, independently on the number of produced megaspores (4-6), the micropylar ones (M_1, M_2, M_3), generally, degenerate and the M_4 spore develops into embryo sac. The growth is most likely stimulated, at least initially, by nutrients and or

other factors coming from the degenerated megaspores (NOHER DE HALAC and HARTE 1977).

Although the high male sterility of *C. sativus* (CHICHIRICCÒ and GRILLI CAIOLA 1986) and the partial female sterility, some times, ovules are fertilized after pollination, with subsequent embryo and endosperm development. However, capsule and seeds have never been observed; probably, rare fertilized ovules are not able to stimulate the fruit formation.

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