Megasporogenesis and development of the embryo sac in the Palestine variety of *Trifolium alexandrinum* L.

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Abstract

The structure and development of the embryo sac in *Trifolium alexandrinum* (Palestine variety), are very similar to that in *Trifolium pratense*. The embryo sac of *Trifolium alexandrinum* is monosporal, has eight nuclei and is of the *Polygonum* type. The Palestine variety of *Trifolium alexandrinum* can be autogamous.

MATERIAL AND METHODS

The material was fixed from plants raised by doc. Dr. T. Kazimierski in the greenhouses of the Institute of Genetics of the Polish Academy of Sciences in Piątków, Poznań. The plants were strictly isolated from the access of bees, bumble-bees and other insects. The material was fixed in 1970 and 1971 during the absence of Professor S. Krupko by Mrs. Laskowska, M.Sc., under the supervision of doc. Dr. T. Kazimierski.

The following fixatives were used: F.A.A., Navashin's fixative (normal and diluted), Carnegie Laboratory XIV fixative. The results of fixing were not very good. The material embedded in paraffin was sliced by a microtome. The preparations were 8, 10, 12 and 15 μ thick.

The preparations were stained with the following stains: 1) Heidenhein's ferrous hematoxylin with counter-staining by fast green, 2) Gentian Violet according to Newton with counter staining with Orange, 3) Feulgen's method.

RESULTS

In *Trifolium alexandrinum* there is no correlation between the stage of development of pollen and of the embryo sac. Often, when the embryo sac is in the tetrad stage, the pollen on the same flower is already surrounded by a membrane, or even has two nuclei. In other plants the megaspore division or tetrad stage occurs when in the anther microspores are separating, or tetrads are present.

In the material of *Trifolium alexandrinum* which I examined the degeneration of the nucellus in ovules was very rare, but degeneration caused by fungi was rather common.

Fig. 1 and Fig. 1a

Fig. 1 — Campylotropous ovule. Fig. 1a — Megaspore mother cell
 oi — outer integuments; ii — inter integuments; f — funiculus; mmc — megaspore mother cell;
 pc — parietal cells

In the embryo sac there are two campylotropous ovules (Fig. 1) similarly as in *Trifolium pratense* (Hindmarsh, 1964). In general there was only one archesporial cell (Fig. 1a) which initially formed parietal cells and then the megaspore mother cell. Meiosis in the megaspore mother cell in general started in a very early stage of ovule development, when the outer integument had just started to grow (Fig. 2). In the course of meiosis a normal megaspore tetrad is formed (Figs 3, 4). Deviations from this normal course of development were also found. Sometimes two archesporial cells were found (Fig. 5). This phenomenon has been observed in other species of clover (Krupko, 1971/72; Hindmarsh, 1964; Grala, 1964). Irregular tetrads (Fig. 6) or triads of megaspores are also found. Two embryo sacs were never observed. When the embryo sac is in the stage of one nucleus its pressure on the cells of the nucellus is so great that most of these cells are destroyed

Figs 2 — 5

Fig. 2 — Prophase of first meiotic division in megaspore mother cell. Fig. 3 — One nucleate embryo sac. Fig. 4 — One nucleate embryo sac. Fig. 5 — Two megaspore mother cells and mitotic division in parietal cell

mmc — mother megaspore cell; mc — megaspores; es — embryo sac; pc — parietal cells

(Fig. 4). The embryo sac at the stage of two nuclei is much larger and has a large central vacuole which separates the two nuclei (Fig. 7). Shortly after this stage mitosis in the embryo sac nuclei takes place and a sac with four nuclei is formed (Fig. 8).

A young embryo sac can be seen in Fig. 9. The egg cell is covered by synergids. Such a young embryo sac still has antipodes which are young nucleated cells. These antipodes disappear very quickly in *T. alexandrinum*.

The polar nuclei are very close to the egg cell in this early stage, and remain unfused for a very long time (Fig. 10), even in sacs in which traces of pollen tubes can be seen and even in the presence of the pro-embryo. A striking but rare phenomenon was the occurrence of remains

Figs 6 — 9

Fig 6 — Tetrad. Fig. 7 — Two nucleate embryo sac. Fig. 8 — Four nucleate embryo sac. Fig. 9 — Embryo sac

es — embryo sac; eap — egg apparatus; pn — polar nucleus; ac — antipods

of the pollen tube or the two-cell proembryo in the examined material (Fig. 11).

As the specimens of *T. alexandrinum* from which the preparations were made were very strictly isolated from insects the assumption that we were dealing with cases of selfpollination could be made. This assumption is rendered more probable by comparison with the works of such experts on clover as doc. Dr. T. Kazimierski and Dr. Ewa Kazimierska (T. Kazimierski, E. M. Kazimierska, Cz

Fig. 10. Mature embryo sac
ec — egg; syn — synergids; pn — polar nucleus; ac — antipods

Fig. 11. Proembryo
pe — proembryo; pn — polar nucleus; ac — antipods

Photo 1. Fertilization

ec — egg; sn — secondary nucleus; pt — pollen tube; sp. n. — sperm nucleus

Strzyżewska, 1972; T. Kazimierski, E. M. Kazimierska, 1972; T. Kazimierski and E. M. Kazimierska, 1969).

These authors have found that *Trifolium alexandrinum* from Israel (i.e. the Palestine variety) is autogamous, while *T. alexandrinum* from Egypt (Egyptian variety) is heterogamous and self-fertile.

I had further evidence of autogamy in the material I examined. I found a clear picture of fertilization (Photo 1). In this preparation a secondary nucleus with the sperm nucleus can be seen under the egg cell.

It must be remembered that in many *Trifolium* species the polar nuclei fuse some time after fertilization of the egg cell has taken place. In other preparations (not very numerous) pollen tubes, or their remains, could be found in the embryo sac (Photo 2).

There is thus evidence that *T. alexandrinum* (Palestine variety) is autogamous. This species has been described by Kazimierski and Kazimierska (1972) and Kazimierski et al. (1972). These authors reported that Bobrov (1947) created a new subgenus *Lagopus* in the genus *Trifolium*. In this subgenus and its section *Hiatia* Bobrov these authors place *T. alexandrinum*. They have investigated the Egyptian variety of *T. alexandrinum* and have found that it is genetically self-fertile but only after the carina of the flower has been torn. The authors proposed new term for this form of self-fertility crypto-self-fertility. The crosses of *T. alexandrinum* with *T. apertum* and *T. Vavilovii* gave seeds, and the hybrids were fertile.

The Egyptian variety of *T. alexandrinum* after self-fertilization gave

Photo 2. Egg apparatus
ec — egg; syn — synergids

only 20% of seeds. After mechanical self-fertilization it gave 42.9% of seeds, and after cross-fertilization 47.7%.

In the *T. alexandrinum* material I examined there were no proembryos older than the two cell stage and there were no endosperm nuclei.; This is indubitably not a characteristic trait of the examined variety but the result of not collecting older ovules.

CONCLUSIONS

The Palestine variety of *Trifolium alexandrinum* L. has an embryo sac with eight nuclei of the *Polygonum* type. It is an autogamous species. There is no synchronization between the development of the embryo sac and microsporogenesis.

EXPLANATION OF FIGURES AND PHOTOS:

Fig. 1 Campylotropous ovule. Drawing from preparation 39, slice thickness, 10 μ , Navashin's fixative.

Fig. 1a. Megaspore mother cell. Drawing from preparation 371, slice thickness 10 μ , diluted Navashin's fixative.

Fig. 2. Meiotic prophase I in the megaspore mother cell. Drawing from preparation 336, slice thickness 7.5 μ , diluted Navashin's fixative.

Fig. 3. One-nucleate embryo sac. Drawing from preparation 118, slice thickness 10 μ , Carnagie fixative.

Fig. 4. One-nucleate embryo sac. Drawing from preparation 357, slice thickness 10 μ , FAA fixative.

Fig. 5. Megaspore mother cell. Drawing from preparation 411, slice thickness 7.5 μ , dilute Navashin's fixative

Fig. 6. Tetrad. Drawing from preparation 471, slice thickness 10 μ , dilute Navashin's fixative.

Fig. 7. Embryo sac with two nuclei. Synthetic drawing from preparation 16, FAA fixative.

Fig. 8. Embryo sac with four nuclei. Drawing from preparation 354, slice thickness 10 μ , Navashin's fixative.

Fig. 9. Mature embryo sac. Synthetic drawing from preparation 4, slice thickness 10 μ , FAA fixative.

Fig. 10. Embryo sac with disappearing antipodes. Drawing from preparation 348, slice thickness 10 μ , FAA fixative.

Fig. 11. Proembryo. Drawing from preparation 66, slice thickness 10 μ , Carnegie fixative.

Photo 1. Fertilization. Photograph from preparation 45, slice thickness 10 μ , Navashin's fixative. Magnified 1200 times.

Photo 2. Egg apparatus. Photograph from preparation 162, slice thickness 12 μ , Carnegie fixative. Magnified 1200 times.

All preparations were stained with ferrous hematoxylin and fast green.

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Megasporogeneza, rozwój woreczka zalążkowego Trifolium alexandrinum
L., odmiana palestyńska

Streszczenie

Trifolium alexandrinum L., odmiana palestyńska ma woreczek 8-jądrowy typu *Polygonum*. Cały rozwój zalążka, megasporogeneza i rozwój woreczka zalążkowego jest podobny do *Trifolium pratense*.

Koniczyna ta jest rośliną samopylną.