Embryological studies on *Anthemis tinctoria* L.

K. BIJOK, T. PAWLAK, B. KREŃSKA

Institute of Biology, University of Gdańsk, Gdynia

Abstract

The chromosome number of *Anthemis tinctoria* from natural habitats in Poland is $n=9$.

The development of pollen grains is normal. The embryo-sac is of tetrasporic type and the number of antipodal cells is seven, all derived from one-cellular archesporium. The embryo development is of *Aster* type and the endosperm is of nuclear type.

INTRODUCTION

The purpose of present work was to perform embryological investigations of the species *Anthemis tinctoria* from natural habitats in Poland.

Karyologically this species was studied by Lundegård (1909), Holmgren (1915), Tischler (1934), Harling (1950), Löve and Löve (1956), Koul (1967), Hundakowa (1967), and Skalińska (1971). The number of chromosomes $n=9$ or $2n=18$ was established by all these authors.

In the family *Compositae*, which includes species *Anthemis tinctoria*, like in *Graminae* the apomictic reproduction occurs very often (Bergman, 1935; Gustafsson, 1947). Good examples are genera *Antennaria*, *Hieracium* and *Taraxacum* where the reproduction by diplosporic parthenogenesis is often coupled with apospory (Schnarf, 1929; Gustafsson, 1947).

Gustafsson (1947) has established that in the genus *Crepis* the species with high polyploidy levels propagate apomictically whereas the low polyploids propagate sexually.

Bergman (1935) in his studies on *Leontodon hispidus* and *Picris hieracioides* has shown, that apomictic reproduction may also occur in diploid species. Wcisło (1952) studying the genus *Doronicum* has

found, that apomixis is not entirely connected with polyploidy and may be found, although rarely, also in diploid species.

For this reason was decided on to investigate embryologically the species *Anthemis tinctoria*.

MATERIAL AND METHODS

The plant material for present studies has been collected in 7 natural habitats in Poland (Table 1). The flower heads in various stages of development, from very young ones, were fixed in the field with

Table 1

List of habitats of *Anthemis tinctoria* L.

Place of origin	
Koniaków	district Cieszyn
Ustroń	„ „
Czechowice-Dziedzice	„ Bielsko
Częstochowa	„ Częstochowa
Działdowo	„ Działdowo
Olsztyn-Kortowo	„ Olsztyn
Puszcza Piska	„ Pisz

50% Navashin fixative. The material embedded in paraffin wax was cut into 15 μm thick sections. The part of material for study of pollen grains and embryo-sac development was stained with 1% gentian violet and differentiated using a phenol solution in xylene (1:3). The second part of microtome sections for study the older stages of embryo-sac and embryo development were stained with Meyer's haematoxylin.

The drawings were done from Amplival microscope with 100 $\times$ objective and K 20 $\times$ and 15 $\times$ Zeiss ocular.

RESULTS

Number of chromosomes

The chromosome number in *Anthemis tinctoria* $n=9$ was established in various stages of the meiotic division of pollen and egg mother cells (Figs: 1, 3, 16, 18).

The somatic number of chromosome $2n=18$ for this species from natural habitats in north-western Poland was established by Skalińska (1971).

Microsporogenesis

In flowers from one flower head all stages of meiotic division were found, from early prophase up to microspore tetrads and in the most peripheral flowers mature pollen grains. Within one pollen sac the pollen mother cells were approximately at the same stage of division.

Meiosis was studied in detail from diakinesis stage. In this stage 9 bivalents were distinguished (Fig. 1). The paired chromosomes showed always the same characteristic configurations (Fig. 1).

Fig. 1—15. *Anthemis tinctoria*. Pollen development

1 — diakinesis, 2 — first metaphase in P.M.C's., 3 — first metaphase in P.M.C's. — polar view, 4, 5 — first anaphase and telophase in P.M.C's., 6, 7, 8 — second metaphase, anaphase and telophase in P.M.C's., 9, 10 — formation of microspore tetrads, 11, 12, 13 — original pollen grain, 14 — pollen grain with vegetative and generative cell, 15 — pollen grain with vegetative cell and two sperm nuclei ($\times 2400$)

In the metaphase of first meiotic division also only 9 bivalents occurred with similar number of chiasmata and their distribution (Figs 2, 3, 16).

In the anaphase of the first meiotic division the movement of chromosomes to the poles was synchronized (Fig. 4). The phragmoplast formed at this stage was visible up to formation of the two daughter nuclei and then disappeared (Figs 4, 5).

At the metaphase of the second meiotic division the axis of both karyokinetic spindles were as a rule perpendicular (Fig. 6) and only rarely parallel.

At the anaphase of the second meiotic division as in anaphase of the first meiotic division the movement of chromosomes to the poles was synchronized. Between separating chromosomes the phragmoplasts were well visible (Fig. 7).

In the telophase of the second meiotic division all PMC's contained four nuclei. Between them six phragmoplasts were formed (Fig. 8). In further developmental stages as a result of simultaneous division of PMC's in each of them a microspore tetrad was formed (Figs 9, 10).

The primary pollen grains soon after the separation from one another had only single wall — intine (Fig. 11). During their growth the second wall — exine was formed (Figs 12—15). Three pores were observed in exine (Figs 12—15).

Within the primary pollen grains a large and centrally located vacuole pushed the nucleus towards the cell wall (Fig. 13).

Fig. 16. *Anthemis tinctoria*. 9 — bivalents from first metaphase in PMC's ($\times 2400$)

After the first mitotic division of nucleus the pollen grains contained bigger vegetative cell and smaller generative one (Fig. 14).

After the second mitotic division of generative nucleus the pollen grains were three nuclear (Fig. 15).

The diameter of pollen grains ranged from 12.9 to 13.9 μm . The average diameter of 100 pollen grains was 13.36 μm .

Development of ovule

Topography of ovule

The ovule of *Anthemis tinctoria* was typical of the *Compositae*. It was anatropous, tenuinucellate, with a single integument. The archesporium consisted of a single cell (Figs 17—20). The nucellar cells on longitu-

dinal sections in relation to ovule axis were rectangular, had one big nucleus and strongly granular cytoplasm.

In the first stages of embryo-sac development the beginning of nucellus degeneration was observed. Its nuclei decreased in their stainabi-

Fig. 17—18. *Anthemis tinctoria*. Early stages of embryo sac development
17 — megaspore mother cell, 18 — diakinesis in megaspore mother cell ($\times 2400$)

Fig. 19—20. *Anthemis tinctoria*. Early stages of embryo sac development
19 — diad, 20 — four coenomegaspore nuclei ($\times 2400$)

lity and the cells were more and more flattened. In eightnucleate stage of embryo-sac the nucellar cells were hardly visible (Fig. 21).

The integument at early stages of ovule development was composed of cylindrical cells with strongly stainable nuclei. The cells of internal,

integumental epidermis were similar to the rest of the cells. Beginning from four-nucleate stage of embryo-sac these cells were strongly elongated in perpendicular direction to the longitudinal axis of the ovule and formed so called "nutritive jacket" (endothelium).

Archespore

The results of investigations of 44 species from the tribe *Anthemidae-
-Chrysanthemidae* have shown that in 6 species the archespore was unicellular, in 11 — two- or many-cellular and in 27 only multicellular

Fig. 21—23. *Anthemis tinctoria*. Development of embryo-sac
21 — embryo sac with six nuclei and seventh nucleus in anaphase stage, 22 — embryo sac
with 12 nuclei, 23 — mature embryo sac with 7 antipodal cells ($\times 2400$)

(Hoffman, 1894; Harling 1951; Bijok, 1964; Piotrowicz, ms). In *Anthemis tinctoria* from natural habitats in Poland archespore was always unicellular (Fig. 17).

Megasporogenesis

The archesporium cell of *Anthemis tinctoria* was transformed directly into a megaspore mother cell without cutting off the "cover cell" (Figs 17, 18). The megaspore mother cell before meiosis was slightly elongated in direction of longitudinal axis of the ovule. After the first and second meiotic divisions no transverse walls formation between longitudinally arranged nuclei were observed (Figs 19, 20). Thus four-nucleate coenomegaspore was formed. Between the nuclei in cytoplasm of coenomegaspore small vacuoles were observed (Fig. 20).

Fig. 24—25. *Anthemis tinctoria*

24 — micropylar part of embryo sac, fertilization of egg cell and secondary nucleus, 25 — chalazal part of embryo sac with degenerating 7 antipodal cells ($\times 2400$)

Fig. 26—28. *Anthemis tinctoria*. Young and older embryos in nuclear endosperm
 $\times 2400$ fig. 26, 27, $\times 1200$ fig. 28

Development of embryo-sac

The embryo-sac derived from elongating coenomegaspore. The eight-nucleate embryo-sac originated from coenomegaspore by one mitotic division of each of the four nuclei (Fig. 21). From the fig. 21 it is visible that the nuclei on micropylar side of coenomegaspore were divid-

ing earlier than nuclei on the chalazal side. In further developmental stages 12-nucleate embryo-sacs were observed (Fig. 22). From the fig. 22 it could be assumed, that the three chalazal nuclei of embryo-sac underwent further mitotic division and from 6 resulting nuclei one was additionally divided. Thus a 12-nucleate embryo-sac probably was formed.

The structure of mature embryo-sac

The structure of egg apparatus of *Anthemis tinctoria* was typical, it consisted of one egg-cell and two synergids (Fig. 23). The egg-cell, strongly elongated, in the enlarged basal part oriented to the centre of embryo-sac had big nucleus with disperse structure. Above nucleus in egg-cell on micropylar side a big vacuole was visible in the cytoplasm.

The synergids also strongly elongated, but shorter than the egg-cell, had also big nuclei. Vacuoles were present in their enlarged parts turned towards the chalazal pole.

The polar nuclei were near to egg-apparatus (Fig. 22). Their fusion into the secondary nucleus of embryo-sac took place just before fertilization. The secondary nucleus with very loose structure had two nucleoli (Fig. 23).

The antipodes of *Anthemis tinctoria* embryo-sac in the number of seven uninucleate cells occupied the chalazal, narrow part of embryo-sac (Fig. 23). At the time of fertilization they were already degenerated (Fig. 25).

Development of embryo

The embryo of *A. tinctoria* developed from the fertilized egg-cell (Figs 26—28) according to *Aster* type (Schnarf, 1929, 1931; Maheshwari, 1950). In investigated material any case of apomictic development of the embryo was observed.

Development of endosperm

The development of endosperm in embryo-sac was of nuclear type (Figs 24—28). At the beginning the free nuclei of endosperm surrounded the young embryo, and later they formed the layers along the embryo-sac. At the time of the formation of cotyledones in the embryo the nuclear endosperm transformed to cellular endosperm, the cell wall formation in the endosperm was observed. At that time the embryo-sac

was folded. Following further development the cellular endosperm was resorbed by growing embryo.

Simultaneously with the embryo development was observed the formation of seed coat from integument, and its fusion with wall of ovary which transformed in the pericarp. Many histological changes lead to the formation of achene which is characteristic for *Compositae* family.

DISCUSSION

The somatic number of chromosomes $2n=18$ established by Skalińska (1971) for *Anthemis tinctoria* from natural habitats in Poland was confirmed in meiotic division ($n=9$) of pollen mother cells and coenomegaspore mother cells.

Observations of the course of meiotic division in PMC's demonstrated that it is normal. No degenerated pollen grains were found.

Degeneration of pollen grains often connected with apomictic way of reproduction were observed by Schnarf (1929) and Gustafsson (1935) in the genera *Hieracium* and *Crepis*. Bergman (1935) connected the meiotic disturbances in *Leontodon hispidus* with the occurrence in this species of additional aposporic embryo-sac.

The reason for embryological study of *A. tinctoria* was to establish if its reproduction is only strictly sexual or if aposporic reproduction may also occur.

In one celled archesporium of *A. tinctoria* the course of meiosis was normal and led to formation of one coenomegaspore which later formed the embryo-sac of tetrasporic type.

Holmgren (1915) and Harling (1950) in *A. tinctoria* from Botanical Gardens observed one-, two-, and even three-celled archesporium. The normal meiotic divisions in plants studied by these authors occurred only in one archesporial cell, in the remaining archesporial cells many meiotic disturbances leading to their degeneration occurred. From the archesporial cell in which meiotic division was normal a four-nucleate coenomegaspore was formed. In later investigations these authors have established that in the further development of embryo-sac one or two nuclei of coenomegaspore were involved. These were the nuclei from micropylar pole of coenomegaspore, while the nuclei from chalazal pole were degenerating.

In *Anthemis tinctoria* from natural habitats in Poland the mature embryo-sac had enlarged number of nuclei to twelve, the egg-apparatus consisted of egg-cell and two synergids, the secondary nucleus originated from the fusion of two polar nuclei, while the antipodals consisted of seven one-nucleate cells.

Holmgren and Harling also have observed in *A. tinctoria* normal egg-apparatus and secondary nucleus of embryo-sac but three

antipodals one- or four-nucleate could divide during further developmental stages.

Although, it is known that in family *Compositae* in some genera — for instance in genera *Hieracium*, *Taraxacum* — some diploid species propagate apomictically, in the species *Anthemis tinctoria* from natural habitats in Poland only sexual reproduction was observed.

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Author's address:

Prof. dr. Karol Bijok,
Mgr. T. Pawlak, and Mgr. B. Kreńska
Institute of Biology,
Czołgistów Str. 46; 81-378 Gdynia; Poland

Studia embriologiczne nad Anthemis tinctoria L.

Streszczenie

Materiałem do badań embriologicznych u *Anthemis tinctoria* były rośliny ze stanowisk naturalnych w Polsce.

Liczba $n=9$ chromosomów u *A. tinctoria* została ustalona w podziałach mejoetycznych komórek macierzystych pyłku i komórek macierzystych coenomegaspor.

Komórki tapetum *A. tinctoria* w stadium premejoetycznym komórek macierzystych pyłku były jednojądrowe o jądrach diploidalnych. Od stadium zygotenu do diakinezy komórek macierzystych pyłku, jądra komórek tapetum dzieliły się dwukrotnie podziałem mitotycznym. Powstawały wtedy dwu- i czterojądrowe komórki tapetum. W czasie pierwszej i drugiej mitozy występowały zaburzenia, które prowadziły do powstania komórek o jądrach o różnym stopniu poliploidalności. Szczegółów różnicowania się warstwy komórek tapetum u *A. tinctoria* zostały omówione w pracy poprzedniej (Bijok, Kreńska, Pawlak 1977).

U *A. tinctoria* mejoza miała przebieg normalny i stąd nie obserwowano zdegenerowanego pyłku.

W zalążkach *A. tinctoria* występował archespor jednokomórkowy. Po podziałach mejoetycznych, w zalążkach powstawały pojedynczo coenomegaspory.

Woreczek zalążkowy u *A. tinctoria* rozwijał się według typu tetrasporowego. W dojrzałym woreczku zalążkowym występowała podwyższona liczba antypod do siedmiu komórek.

Zarodek u *A. tinctoria* rozwijał się według typu Aster, a bielmo według typu nuclearego.