Micro- and megagametogenesis in *Cichorium intybus* L.: successive stages and some symptoms of apospory

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Abstract

In *Cichorium intybus* L., the mode of reproduction could be at the same time sexual and apomictic. This could explain the contradictions existing in the interpretation of the heritability of the characters in this species. In this particular case, the only way of describing facultative apospory, functional or not, is to proceed to a cytomorphological study of the megagametogenesis. In the present study, symptoms of apospory have been observed and described.

INTRODUCTION

The genetic interpretation of the heritability of the characters in *Cichorium intybus* L. is difficult and contradictive (Plumier, 1960; Bannerot, Fouilloux, 1970; Louant et al., 1978). The theories of auto-incompatibility and facultative autocompatibility are no longer sufficient for the geneticist (Stout, 1916; Pecaut, 1962; Bannerot, Fouilloux, op. cit.).

From a cytomorphological point of view, we can distinguish two great lines of evolution during the megagametogenesis of *C. intybus*: the normal or sexual process on the one hand, and the aposporic process which expresses itself through a serie of particular features or "abnormalities" on the other.

The description of the "normal" megagametogenesis allows us to say that the sexual development in *C. intybus* gives rise to a monosporic octonucleate embryo sac. It is, thus, of the "Polygonum type" according to Strasburger (1879). This description is necessary to establish a relationship between the development of the megagametogenesis and of the microgametogenesis, relation which shall serve for a subsequent study of the symptoms of apospory. Indeed, the observation of the microgametogenesis enables a good estimation of the degree of global development of the flowers.

MATERIAL AND METHODS

Buds were collected at all stages of development from diploid plants of various origin. They were fixed 24 hours at 4°C in FAA (formalin-acetic acid-alcohol) or in Navashin's fluid. The material was then rinsed and stored in 70 per cent ethyl alcohol. The buds, embedded in paraffin, were sectioned at 12 μ m and stained with Haematoxylin and Fast Green. A few sections were stained by the Feulgen method for the observation of the second mitosis in the pollen grains.

Only stages before anthesis were observed. The development of the flowers of a single capitulum was generally synchronous.

OBSERVATIONS AND DISCUSSION

I. The sexual process

We divided microgametogenesis into ten stages and related to them the corresponding stages of the megagametogenesis. This description shall constitute a reference calendar.

First stage

The first stage begins when the anthers are filled with rows of microspore mother cells. In the corresponding ovarian cavity (the ovary is inferior, unilocular and contains only one ovule), there is an undifferentiated and straight ovule. Afterwards, the ovule curves and one can distinguish a nucellus and one integument. In the hypodermal layer of the nucellus appears one archesporial cell, which functions here directly as a megaspore mother cell.

Second stage

The microspore mother cells enter into meiosis. The meiosis progresses very quickly and after the first prophase, several phases are often coexisting in the same capitulum (for instance from metaphase I to telophase I, sometimes to metaphase II, and from prophase II to cytokinesis or the stage of young tetrads). But in the anthers of a some flower, all the microspore mother cells are at the same stage. The cytokinesis is of the simultaneous type. The ovule curves on while the male meiosis occurs and everything is growing.

Third stage

When there are tetrads in the anthers, or sometimes when the microspores are already released, the ovule is definitively anatropous. The integuments is at the same level or exceeds the nucellus in length. The archesporium grows on.

Fourth stage

The microspores are surrounded by the first deposit of sporopollenin. The cells of the tapetum are still intact. The ovule grows on and fills the whole ovarian cavity. The integument covers almost completely the nucellus. The cells of the nucellus gradually elongate. The archesporium enters into meiotic prophase.

Fifth stage

There is a large vacuole in the microspores and the exine gets thicker. The cells of the tapetum begin to disintegrate. The ovary and the ovule develop further. The integument elongates parallel to the funiculus and comes in contact with it. The cells of the inner layer of the integument become differentiated, prefiguring in this way an endothelium. The cytoplasm in the cells of a few layers lying against the endothelium becomes denser. The heterotypic division of the megaspore mother cell has ended and given rise to a dyad.

Sixth stage

The first mitosis occurs in the microspores. It is not synchronous in all the microspores of the same anther but is spread out over almost all the period during which the vacuole is present. Three cytoplasmic protuberances are seen at the place of the pores. The exine is now fully developed. The cells of the tapetum further disintegrate. The homeotypic division has given rise to a tetrad in the ovule. In *Cichorium*, the tetrads are, generally linear, rarely T-shaped. The three micropylar megaspores degenerate and only the chalazal megaspore becomes functional. There are anticlinal divisions in the endothelium. The cells at the base of the funiculus at the micropylar side start to weaken.

Seventh stage

The vacuole in the microspores and young pollen grains decreases in size and the first mitosis is almost finished. The cytoplasmic pro-

tuberances are more developed. The intine is clearly distinguishable. The cells of the tapetum are completely broken up and their multinucleate scraps persist among the pollen grains.

The enlarged chalazal megaspore divides and gives rise to a binucleate embryo sac. At the micropylar end, there are remnants of the three disappearing megaspores and also of the first degenerated nucellar cells. The nucellus degenerates in an acropetal way. Furthermore, the cells of the endothelium still widen. The cells of the funiculus on the micropylar side weaken on and the same happens to the cells of the dense area against the endothelium (see the fifth stage).

Eighth stage

The pollen grains are filled with reserves. These are very refringent and hide the nuclei. The cytoplasmic protuberances and the intine are fully developed. There are now but traces of the tapetum. The embryo sac is enlarged. Its center is occupied by a large vacuole and the two daughter nuclei have moved apart to opposite poles. The nucellus degenerates further.

Ninth stage

A Feulgen staining reveals two sperm nuclei resulting from the division of the generative nucleus. In the beginning, they remain together. Afterwards, they move away from each other and elongate. The vegetative nucleus is no more visible. There are now two nuclei at each pole of the tetranucleate embryo sac. The first degenerated cells of the nucellus have disappeared and the process of degeneration goes on at the chalazal end.

Tenth stage

The sperm nuclei are now threadlike and well straight. They make their way towards the pores. The tapetum has completely disappeared. At this moment, the embryo sac is octonucleate and the cytoplasm acquires their own identity. Soon after, the two polar nuclei will fuse together and give rise to the secondary nucleus. The three antipodal cells degenerate quickly. The embryo sac is highly vacuolated. There is no more trace of the nucellus. The cells of the endothelium are now elongated in accordance with the vertical axis of the embryo sac and undergo still anticlinal divisions. The cells of the hypostase are very compressed by the highly swollen cells of the integument.

II. Abnormalities during megagametogenesis

The abnormalities encountered during the megasporo- and megagametogenesis are numerous and most of them constitute signs of apospory (Esa u, 1946; Maheshwari, 1950). In *Cichorium* the apospory would be occasional but it can not be said as yet if it was functional or not because only young stages before anthesis were observed. The symptoms are the following:

1) At young stages, there are sometimes several differentiated cells, or several potential archesporia, under the epidermis of the nucellus. Sometimes they can also be seen in the epidermis.

2) Later, there are archesporia which do not divide immediately but increase a lot. In most cases, their nucleus is highly hydrated. Often, simultaneously, the cells of the nucellus are very large, especially at the micropylar end. There is often also one big cell at the chalazal end, just beneath the archesporium. There is already a well formed endothelium.

3) Other archesporia swell and become highly vacuolated. Their nucleus shows a low chromaticity. In these cases, there is often one or more highly vacuolated and sometimes very enlarged cells at the chalazal end, just beneath the archesporium. The cells of the nucellus are also swollen. At this time two or three rows of endothelial cells can generally be seen. The first row on the archesporium side is normally developed while the others finish halfway.

4) There are still archesporia with a large and somewhat elongated nucleus with two identical nucleoli. Such nuclei could be restitution nuclei but it cannot be stated with certainty because division stages have not been observed. The cells of the nucellus are very elongated and vacuolated as at the telophase I stage in the sexual process. Sometimes, there are nuclei of the nucellus which are very large and enter into prophase.

In the last three cases described above, the corresponding pollen stage is more advanced than at the stage of archesporium in interphase or in meiosis of the sexual development. Moreover, the ovule is larger and more developed. A well formed endothelium is present while we have an abnormally enlarged archesporium.

5) In some cases, we found rows of cells. These rows were of different kinds. Their cells were of the same size or of different sizes with an alternation of little and big ones. Sometimes, they seemed to be tetrads which were prolonged by one or several large vacuolated cells at the chalazal side.

6) There are cases where there is a constriction between an enlarged archesporium or a tetrad and large cells at the chalaza. This constriction is due to the presence of one or two narrow cells between them.

7) In other cases, there are supernumerary cells between the nucellus and the future "gametophyte". A development of cells of the nucellus, at one side, giving rise to a second "gametophyte" next to the first one was also noted.

How all these cases evolve is unknown but at later stages there are many embryo sacs which degenerate. There are also abnormalities during the microgametogenesis. They are less numerous than in the megagametogenesis and not necessarily correlated with these ones.

III. Abnormalities during microsporogenesis

The only abnormality noted during the microsporogenesis is the formation of rows of very flat microspore mother cells. Their cytoplasm is denser than in normal microspore mother cells and they secrete callose very quickly. There is a partitioning of the cytoplasm as in a normal cytokinesis but it cannot be stated whether meiosis took place or not. The resulting "tetrads" remain together in a row and the same happens in the microspores and the pollen grains.

At later stages, there is sometimes only a slight deposit of sporopollenin around the microspores. In other cases, there is also a deposit of sporopollenin but the exine is not completely structured or it gets loose from the microspores.

In some cases, the cytoplasm and the nuclei dissolve and the contents of the pollen grains disappear almost completely.

Finally, we also found many pollen grains which were collapsed.

The observation of mega- and microgametogenesis in *C. intybus* revealed a series of abnormalities which are characteristic of a tendency to apospory. In fact, those abnormalities correspond to the aposporic symptoms as described in the literature. These observations would explain the contradictions and the difficulties encountered in the genetical and cytogenetical interpretation of the heritability of the characters. The cytomorphological study undertaken may allow to identify in a more accurate way the existence of a tendency to facultative apospory in *C. intybus*. This apospory could be functional or not according to ecological conditions.

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