Pollen and sperm nuclei development in rye

AGNIESZKA MOSTOWSKA, MARIA CHARZYŃSKA

Institute of Botany, Warsaw University, Warsaw

(Received: February 23, 1977)

Abstract

The development of rye (*Secale cereale* L. *Gramineae*) pollen grains was followed in the light microscope from the differentiating division in the microspore to pollen shedding. Particular attention was given to the localization of the generative cell before and during mitosis and to the change in the structure of sperm nuclei during pollen grain maturation.

INTRODUCTION

The development of pollen grains in Angiosperms has been recently the object of intensive research. Pollen grains of Angiosperms are an interesting model for studies on the mechanism of differentiation, owing to the asymmetric division of the microspore and the distinct differentiation of the two cells of the male gametophyte.

Investigations of the ultrastructural and biochemical aspects of microspores and male gametophytes differentiations were performed mainly on plants with binucleate pollen grains (Mascarenhas, 1975).

The development of trinucleate pollen grains has not been analysed in such detail.

Ultrastructural investigations of several species of Angiosperms demonstrated that the male gametes formed in the pollen tube and in grain are cells (Sassen, 1964; Jensen and Fischer, 1968; Hoefert, 1969 and Cass, 1973). The latter two papers concerned species containing sperm cells in mature pollen grains (Hoefert, 1969 in beet and Cass, 1973 in barley). Surrounded by plasmalemma sperm cells besides typical organelles with the exception of plastids contain a microtubule system to which some authors attribute the role of motor (Hoefert, 1969) or cytoskeletal (Cass, 1973) elements. Both authors found small nucleoli in mature sperm nuclei.

Early stages of trinucleate pollen grains development were analysed in the light microscope mainly in cereals (Modilevski et al., 1958; Carniel, 1961; Romanov, 1966, 1971; Oryol, 1969, 1972). These papers concern the microsporogenesis, microspore division and early stages of the vegetative and generative cells development. Cytological differentiation of the pollen grains of *Gramineae* during mitosis of the generative cell has not been so far analysed in the light microscope. Korobova (1959, 1961) and Oryol (1972) claim that the mitosis occurs in the generative cell of maize pollen grains after the translocation of this cell into the grain centre and the vegetative cell contains no more vacuoles. Similar information is reported by Modilevski et al. (1958) in their description of cereal pollen grain development. It results, however, from the paper by Christensen and Horner (1974) on *Sorghum bicolor* that during mitosis the generative cell lies in the vegetative cytoplasm layer near the cell wall and that at this time the vegetative cell contains a large vacuole.

The aim of the present work was to study in the light microscope the cytomorphological transformations of *Secale cereale* (*Gramineae*) pollen grains during their development. Special attention was paid to the mitosis in the generative cell and to changes in the structure of sperm nuclei during their maturation up to pollen shedding.

MATERIAL AND METHODS

Spikes of *Secale cereale* L., variety Dańkowskie Złote were collected in May 1975 from experimental fields of the Institute of Plant Breeding and Acclimatization in Radzików.

Spikes were always chosen from the main shoots. Anthers for analysis were separated from the central earliest flowering part of the spike. The lengths of spikes and anthers were measured and a close correlation between these values and the developmental stages of pollen grains was found only in early stages of development. Similarly the colour of the anthers in early stages changes according to the anther length. The differences in the above mentioned parameters in further stages of development are large and depend on individual variations.

The rye anthers were fixed in chrome-aceto-formalin (CrAF) — 0,4 : 0,5 : 20 and in aceto-alcohol (AA) — 1 : 3.

The material was dehydrated in ethanol series with increasing concentrations and embedded in paraffin. Longitudinal and cross sections, 5 and 7 μ thick, were prepared and stained in the Feulgen reagent with fast green and also by the PAS reaction.

Squash preparations stained with acetocarmine were prepared from material fixed in AA and fresh (unfixed) material.

RESULTS

1. Development of microspores and binucleate pollen grains

Microspores in the anther locule of *Secale cereale* are radially distributed and constitute a unicellular layer adjacent to the tapetum. At the stage of high vacuolization the microspores are elongated with one circular aperture localized close to the external pole (adjacent to the tapetum). The opposite pole is referred to as the internal pole (Fig. 1).

Before and during mitosis the nucleus in the rye microspore occupies a definite position, namely on the internal pole of the highly vacuolized microspore opposite to the aperture (Plate I, photos 1, 2). The mitotic spindle and fragmoplast lie slantingly to the microspore long axis (Plate I, photos 2, 3).

Fig. 1. Cross section through one of the anther locules in rye at the stage of vacuolized microspores

in — internal pole; ex — external pole; a — aperture; t — tapetum; v — vacuole; n — microspore nucleus

After the end of mitosis a two-celled gametophyte with initially identical nuclei lying next to one another is formed (Plate I, photo 4). The generative lens-shaped cell with a distinctly visible cytoplasm borders on the pollen grain wall at the internal pole.

The structure of both nuclei changes during the further development. The vegetative nucleus gradually increases in volume, its structure is loose and the nucleus relatively large (Plate I, photo 5). The generative nucleus is more compact and has a small nucleolus.

The vegetative cell nucleus and the generative cell are initially located next to one another but later change their mutual position. The vegetative nucleus displaces along the pollen grain wall to a position close to the grain aperture and opposite to the generative cell (Plate I, photos 5, 6). The generative cell, on the other hand, detaches itself at

this time from the pollen grain wall and becomes completely surrounded by the cytoplasm of the vegetative cell (Plate I, photo 6). Next the generative cell moves to the external pole where it finally remains close to the vegetative nucleus (Plate I, photo 8). Numerous small rod-like starch grains appear in the vegetative cytoplasm during the translocation of the generative cell, their number greatly increases as the pollen grain ripe. They remain in the pollen grain until the pollen sheds.

2. Mitosis in the generative cell

The internal pole of the pollen grain is occupied by a large vacuole before the beginning of the mitosis in the generative cell (Plate I, photo 8, Plate II, photo 9). The vegetative nucleus is always located on the external pole (the one which borders the tapetum) on which a great part of the vegetative cytoplasm is present. Before and during mitosis the generative cell is located in the vegetative cytoplasm layer parallel to the pollen grain wall at various distances from the external pole (Plate II, photos 9—12). The position of the generative cell relatively to the pore and to the long axis of the grain is not constant (Plate II, photos 11, 12).

Mitosis in the generative cell proceeds similarly as in somatic cells (Plate II, photos 9-12). Prophase chromosomes are created in the elongated nucleus (Plate II, photos 9, 10), they form in metaphase the typical plate (Plate II, photo 11). The mitotic spindle is tangent to the pollen grain wall but its orientation relatively to the long pollen axis is not constant (Plate II, photos 11, 12).

The formation of fragmoplast and the cell plate indicate that the separation of the cytoplasm around the sperm nuclei takes place (Plate II, photo 13), that is the generative cell is divided into two sperm cells. However, the cytoplasm of the sperm cells cannot be made visible with the help of our staining methods.

Young sperm cells lie not far from one another and have spherical nuclei with reticular structure (Plate II, photo 14).

3. Maturation of sperm nuclei

Nuclei of young sperm cells of rye, initially spherical gradually elongate during pollen grains maturation. They become ovoid, more elongated and with blunt ends, fusiform and finally they become shorter prior to shedding. This shortening is preceded by splitting of one end of the nucleus (Plate II, photos 14-16, and Plate III, photos 17, 19, 22).

The changes in shape of sperm nuclei are accompanied by a modification of their structure both as regards the position of chromosomes within the nucleus and the degree of their condensation. It is difficult to analyse these structural changes. Fixation in CrAF causes a high

Photos 1—3. Differentiating division in *Secale cereale* microspore

Photo 4. Young binucleate male gametophyte

Photo 5, 6. Opposite position of vegetative nucleus (on the external pole) relatively to the generative cell (on the internal pole)

Photo 7. Vegetative nucleus after translocation to the external pole, generative cell on its way to this pole

Photo 8. Older binucleate pollen grain with generative cell and vegetative nucleus situated close to the external pole

Photos 1—4, 7 — stained with acetocarmine

Photos 5, 6, 8 — Feulgen reaction $\times 1000$

Photos 9—13. Mitosis in the generative cell of *Secale cereale*

Photos 14—16. Changes of shape and structure of sperm nuclei during pollen maturation, stained with acetocarmine $\times 1000$

Photos 17, 18a and b. Fusiform sperm nuclei at the same stage of pollen grain development. Differences in size and structure are due to fixation and preparation methods. Photo 17 — fixed with CrAF, microtome section, Feulgen reaction. Photo 18a — fixed with AA, squash in acetocarmine. Photo 18b — squash in acetocarmine without previous fixation. Nucleus mechanically drawn apart, parallel chromosome arrangement relatively to each other and to the long nuclear axis is visible

Photos 19, 20. Sperm nuclei with one end "forked", photo 19 — fixed in CrAF, Feulgen reaction, photo 20 — squash in acetocarmine

Photo 21. Sperm nucleus just before pollen shedding, squash in acetocarmine

Photos 22 and 23. Sperm nuclei in shed pollen grains, photo 22 — fixed in CrAF, Feulgen reaction, photo 23 — squash in acetocarmine $\times 1000$

condensation of the chromosome material and the Feulgen reaction reveal the changes in the shape of the nucleus only. On the other hand the nuclear structure on squash is hardly seen in maturing pollen grains because of the large number of starch grains in the vegetative cytoplasm.

Spherical and ovoid sperm nuclei have a reticular structure (Plate II, photos 14, 15) and a clearly visible nucleolus (Plate II, photos 15, 16). The structure of more elongated nuclei resembles an advanced prophase (Plate II, photo 16). At this time the chromosomes probably change their position within the nucleus reaching parallel arrangement relatively to each other and to the long nuclear axis, characteristic for sperm nuclei of elongated shape.

The gradual elongation of nuclei is caused not only by a change in position of chromosomes within the nucleus but also by their despiralization and a more compact arrangement. A parallel arrangement of chromosomes was demonstrated in fusiform nuclei. Photos 17 and 18 (Plate III) show spindle-like sperm nuclei at the same stage. The different size of sperm nuclei on photos 17 and 18 (a and b) is the result of different fixation and preparation methods. Photo 18 b shows a rye sperm nucleus isolated from a pollen grain and mechanically drawn apart to visualize the chromosomes. Within the nucleus the chromosomes are arranged parallelly to each other and to the long nuclear axis so that the arms of the neighbouring chromosomes overlap.

The changes occurring in the nuclei at the final stage of development are difficult to interpret and require further observations. One end of the nucleus splits (Plate III, photos 19, 20) and the other one remains fusiform and sharp.

The sperm nuclei become much shorter just before pollen shedding. This seems to be due to the spiralization of the nucleus as a whole. This state persists in mature pollen grains (Plate III, photos 21, 22, 23).

The sperm nuclei stain much weaker in mature pollen grains in the Feulgen reagent than at earlier stages (Plate III, photo 22). The nucleolus is not noticeable in the light microscope in the fusiform stage of the nucleus and in further stages.

Young sperm cells are located close to each other and to the vegetative nucleus immediately after the division of the generative cell. At the stage of fusiform nuclei they are still localized on the external pole of the pollen grain (adjacent to the tapetum), while the opposite pole is occupied by a vacuole which diminishes in size as the pollen mature (Plate III, photo 17).

After disappearance of the vacuole, the sperm cells and the vegetative nucleus displace to the center of the pollen grain. The vegetative nucleus is irregular in shape and its structure is hardly visible. At this stage the nucleolus in the vegetative nucleus is not noticeable.

DISCUSSION

A single layer of microspores in the anther locule and the formation of circular aperture close to the external pole of the microspore (adjacent to the tapetum) is characteristic for the family *Gramineae* (Romanov, 1966, 1971 in *Secale cereale* and *Zea mays*; Huynh, 1972, 1975 in *Agropyron* and *Avena*; Christensen and Horner, 1974 in *Sorghum bicolor*). Asymmetrically situated aperture and constant relatively to the tapetum position of pollen grains during their development facilitate the observation of polar differentiation and changes in the position of the generative cell in the pollen grain.

The present observations have demonstrated that, like in other representatives of *Gramineae* examined to date, polar differentiation of the microspore of rye is manifested, among other processes, by the translocation of the nucleus to the internal pole before the asymmetrical division. The position before division is decisive for the localization of the young generative cell and vegetative nucleus on the internal pole.

In the further development of binucleate pollen of rye, the vegetative nucleus and later the generative cell displace to the external pole of the pollen grain, close to the aperture. The same process was demonstrated by Oryol (1969, 1972) in maize pollen grain and by Romanov (1971) in wheat. It seems that this process is characteristic for all *Gramineae*.

As we have demonstrated, the generative cell in rye, before detaching from the pollen grain wall, is surrounded by the cytoplasm of the vegetative cell. Oryol and Romanov demonstrated that the callose wall which has separated the generative cell lying next to the wall disappears at this time. The present observations as well as those of the above cited authors do not agree with the results of Karas and Cass (1974) who reported that during mitosis the generative cell of barley still adheres to the pollen grain wall and is separated by a wall from the vegetative cytoplasm.

The biological meaning of the vegetative nucleus and the generative cell translocation within the pollen grain and the mechanism of this process are not known. Oryol (1969) claims that it is a passive movement which reflects the polar differentiation of the pollen grain.

The generative cell and vegetative nucleus again move away from the pollen grain aperture before mitosis. The present observations prove, contrary to the results of Korobova (1961), that the pollen grain protoplast is polarly differentiated during the mitosis in the generative cell. A large vacuole occupies the internal pole and the generative cell lies in the cytoplasm layer next to the wall close to the external pole. The vegetative nucleus lies at a certain distance from the aperture.

The sperm cell move towards the interior of the pollen grain as the

vacuole, still persisting at the stage of fusiform sperm nuclei, is gradually reduced. As in rye the high vacuolization of pollen grains and the same localization of the generative cell before mitosis have been also demonstrated by Christensen and Horner (1974) in *Sorghum bicolor*.

It is known that sperm nuclei in mature pollen grains of cereals have a more or less fusiform shape, however, the changes in their structure from the moment of their formation to maturity have not been analysed in detail.

Korobova (1961) suggests after Gerasimova-Navashina (1951) that, in maize, the mitosis of the generative cell is not definitively ended within the pollen grain, that is, the sperm nuclei remain in the telophase up to the moment of fertilization. The present observations indicate, however, that during maturation of the sperm nuclei the changes in the arrangement of the chromosomes within the nucleus are so large that the final state can hardly be considered as telophase. A distinct regrouping of chromosomes from the typical telophase arrangement through uniform distribution in the spherical nucleus to parallel arrangement in the fusiform nucleus is observed. It is interesting that the chromosomes ordering in the fusiform sperm nuclei in maturing pollen grains of rye resembles chromosome ordering in the fusiform nucleus of the generative cell in maturing binucleate pollen grains of, for instance, *Tradescantia* or *Eremurus* (parallel arrangement of chromosomes relatively to each other and to the nuclear long axis). The question arises whether such a state of the nucleus is not conditioned in both cases by the physiological state of maturing pollen grains and the nuclei preparation to displace to the pollen tube. In mature binucleate pollen grains of *Tradescantia* the generative nucleus is in the G₂ stage (Charzyńska, Maleszka — in preparation). So far opinions are not common as to the phase of the life cycle in which the sperm nuclei are present in mature pollen grains. Gerasimova-Navashina (1970) on the basis of studies on *Crepis* sets up the hypothesis that the sperm nuclei in all Angiosperms are in phase G₁ at this moment. Controversial results were obtained by d'Amato et al. (1965) in barley. According to these authors, sperm nuclei in mature trinucleate pollen grains are in phase G₂, and DNA synthesis occurs just before pollen shedding.

The authors wish to express their thanks to professor Henryk Teleżyński of the Warsaw University for his helpful discussion of the paper and to dr Lucjan Madej of the Institute of Plant Breeding and Acclimatization in Radzikow for the plants from his collection which were investigated in this work.

LITERATURA

- Carniel K., 1961. Beiträge zur Entwicklungsgeschichte des sporogenen Gewebes der Gramineen und Cyperaceen L. *Zea mays*. *Osterr. bot. Zeitschr.* 108: 228-237.
- Cass Dd., 1973. An Ultrastructural and Nomarski Interference study of the sperms of barley. *Canad. J. Bot.* 51: 601-605.
- Charzyńska M., Maleszka J. Inkorporacja ^3H tymidyny do mikrospor i ziarzen pyłku *Tradescantia bracteata in vitro*, (in press).
- Christensen J. E., Horner T. J. R., 1974. Pollen pore development and its spatial orientation during microsporogenesis in the grass *Sorghum bicolor*. *Amer. J. Bot.* 61: 604-623.
- D'Amato F., Derreux M., Scarascia G. T., 1965. The DNA content of the nuclei of the pollen grains in tobacco and barley. *Caryologia* 18: 377-382.
- Gerasimova-Navashina E. H., 1951. Pylcevoie zerno gamety i polowoi proces u pokrytosemennykh. *Morfologia i anatomia rasteni* II. Moscow: 294-363.
- Gerasimova-Navashina E. H., 1970. O pieriodakh mitoticheskogo cykla gamet pokrytosemennykh. *Vestnik Mosc. Univ. Biol.* VI: 93-94.
- Hoefert L., 1969. Fine structure of sperm cells in pollen grains of Beta. *Protoplasma* 68: 237-240.
- Huynh K. L., 1972. The original position of the generative nucleus in the pollen tetrads of *Agropyron*, *Itea*, *Limnanthes*, *Onosma* and its phylogenetic significance in the Angiosperms. *Grana* 12: 105-112.
- Huynh K. L., 1975. Un phénomène de polarité peu connu dans l'anthere des Graminées et des Cypéracées: la position constante du pore germinatif du pollen. *Bull. Soc. Bot. Suisse* 85: 18-24.
- Jensen W. A., Fischer D. B., 1968. Cotton embryogenesis. The Sperm. *Protoplasma* 65: 277-286.
- Karas J., Cass Dd., 1974. Generative cell division in barley. *Amer. J. Bot.* 61 suppl. 58.
- Korobova C. H., 1959. O khodie oplodotvorennya u kukuruzy (*Zea mays* L.). *Dokl. Akad. Nauk SSSR* 127: 921-923.
- Korobova C. H., 1961. Mikrosporogenes i rozvitie pylcevo zerna u kukuruzy. *Dokl. Akad. Nauk SSSR* 136: 233-236.
- Mascarenhas J. P., 1975. The biochemistry of Angiosperm pollen development. *Bot. Rev.* 41: 259-314.
- Modilewski J., 1958. Cytoembriologia osnovnykh khlebnnykh zlakov. *Kiev*: 10-23.
- Oryol L. J., 1969. Polarity of microspores and movement of nuclei and of generative cell in *Zea mays*. *Rev. Cytol. et Biol. Veg.* 32: 37-42.
- Oryol L. J., 1972. Cytologia muzhskoi cytoplazmatischekoi sterilnosti kukuruzy i drugikh kulturnykh rastenii. *Akad. Nauk SSSR. Leningrad*: 14-18.
- Romanov J. D., 1966. Specyficheskiye osobennosti rozvitiya pylcy zlakov. *Dokl. Akad. Nauk SSSR* 169: 456-459.
- Romanov J. D., 1971. Développement du gametophyte mâle chez le froment (*Triticum aestivum*) d'après les observations in vivo. *Ann. Univ. et A.R.E.S.* 9: 188-194.
- Sassen M. M. A., 1964. Fine structure of *Petunia* pollen grain and pollen tube. *Acta Bot. Neerl.* 13: 175-181.

Author's address:

*Mgr Agnieszka Mostowska and dr Maria Charzyńska
Institute of Botany, University of Warsaw,
Krakowskie Przedmieście 26/28; 00-927 Warsaw; Poland
Department of Plant Metabolism*

Rozwój ziaren pyłku i jąder plemnikowych u żyta

Streszczenie

Zanalizowano w mikroskopie świetlnym rozwój ziaren pyłku żyta — *Secale cereale* (Gramineae) od podziału różnicującego w mikrosporze do wypylenia. Szczególną uwagę zwrócono na lokalizację komórki generatywnej przed i w czasie mitozy oraz na zmianę struktury jąder plemnikowych w czasie dojrzewania ziarna pyłku.

Wykazano, że w czasie mitozy w komórce generatywnej ziarno pyłku zróżnicowane jest biegunowo; biegun wewnętrzny (zwrócony do komory pyłkowej) zajmuje dużą wakuolę, jądro wegetatywne leży na biegunie zewnętrznym (przylegającym do tapetum) w pobliżu apertury a komórka generatywna odsunięta od ściany ziarna pyłku położona jest w przyściennym pokładzie cytoplazmy w pewnej odległości od bieguna zewnętrznego.

Młode jądra plemnikowe są kuliste o równomiernie rozmieszczonych chromosomach. W miarę wydłużania się jąder dochodzi do przegrupowania chromosomów. We wrzecionowatych jądrach plemnikowych chromosomy są ułożone równolegle do siebie i długiej osi jądra, ale nieco przesunięte względem siebie. Skrócenie jąder plemnikowych w ostatnim etapie dojrzewania wydaje się być rezultatem ich spiralizacji.