

## Structural and physiological relationships between embryo and endosperm at the early stages of development

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### Abstract

In *Zea mays* different factors cause the considerable aberrations both in the ratio of the dividing cells of proembryo to the endosperm nuclei and in their interaction. These factors are: age of female flower, type of pollination, high and low temperatures, high humidity and drought, shortening of maize silk or its treatment with saturated vapours of naphthalene or poison gases.

Investigations by light and electron microscopy revealed that the egg cytoplasm contains the developed mitochondria, Golgi apparatus, endoplasmic reticulum, ribosomes and plastids with starch, lipids, proteins and probably some carotenoids; the sperm cell contains only promitochondria, proplastids and undeveloped Golgi bodies (Fig. 1 a-b).

As can be seen in Fig. 1c, the sperm cytoplasm, while going out from the pollen tube as the hyaloplasm, remains between the egg cell and the central cell. If the cell organelle desintegration of sperm cytoplasm during the pollen tube growth is absolute, the question arises what is the origin of zygote neoplasm. This question raised by Muntzing (1967) was not dealt with by the embryologists though it is without doubt of great interest (Chebotaru, 1972).

The study on the influences of different factors has shown that besides the changes of the fertilization rate and embryogenesis pattern, some special changes may arise is synergids, central cell, polar nuclei, antipods.

Different factors cause the considerable aberrations in ratio of number of the dividing cells of proembryo to the endosperm nuclei as well as their interaction. These factors are: age of female flower, type of pollination, high and low temperatures, high humidity and drought, shortening of maize silk or its treatment with saturated vapours of naphthalene or poison gases.

In most crop plants the primary endosperm nucleus begins to divide the first. The division of zygote begins 5-8 h after interstrain pollina-

tion in maize and 10-12 h after selfpollination. The temporal fixation at the early embryogenesis (Table 1) in the case of free pollination showed that the zygote divides 24 h after fertilization in the vicinity of Kishinev, 27 h near Cuban (Korobova, 1962) and 42 h near Moscow (Ustinova, 1953).

Table 1

Formation rate of endosperm nuclei and proembryo cells after interstrain and free pollination

Kind of pollination	Number of	Time after pollination								
		15	24	25	29	33	2	3-4	5	10-12
		hours					days			
Interstrain pollination	endosperm nuclei	—	4-8	8-14	16-18	16-24	40-50	60-70	—	—
	proembryo cells	—	—	2	2-4	2-4	2-4+1	4+1	10-14+2	mp
	cellular endosperm	—	—	—	—	—	—	ce	ce	ce
	endosperm nuclei	2	6-8	12-16	18-20	30-40	59-62	70-80	—	—
Free pollination	proembryo cells	—	2	2-3	3-4	4-8	8+2	8-10+2	13-17+2	mp
	cellular endosperm	—	—	—	—	—	—	ce	ce	ce
	endosperm nuclei	2	6-8	12-16	18-20	30-40	59-62	70-80	—	—
	proembryo cells	—	2	2-3	3-4	4-8	8+2	8-10+2	13-17+2	mp

ce—cellular endosperm; mp—maceshaped proembryo.

Such an extending of the fertilization process was also observed in wheat, barley, rye and sunflower — when the pollination and fertilization took place at different ecological conditions.

The type of pollination has a special effect on the fertilization rate and early embryogenesis in the first 3-4 days. In maize, pollen of other strains influences the division rate of proembryo cells and nuclear endosperm. If the plants are pollinated with the pollen of the same strain then on the second day after pollination 80 endosperm nuclei are corresponding to the 8-celled proembryo, while if pollinated with pollen of the other strain the two-celled proembryo and the 60 endosperm nuclei are developing.

After artificially delayed pollination the embryo sacs and their elements are of very large size. This concerns mainly the central cell of maize embryo-sac (Fig. 1d). It is highly vacuolated and contains little cytoplasm, the polar nuclei are pressed to the micropylar part of embryo sac. The number of antipods reaches 48-80. The embryogenesis in case of fertilization of female gametophyte of different age is accompanied by considerable morphophysiological aberrations.

The analysis of early embryogenesis in the isolated pistils revealed a specific relationship between the endosperm and the developing em-

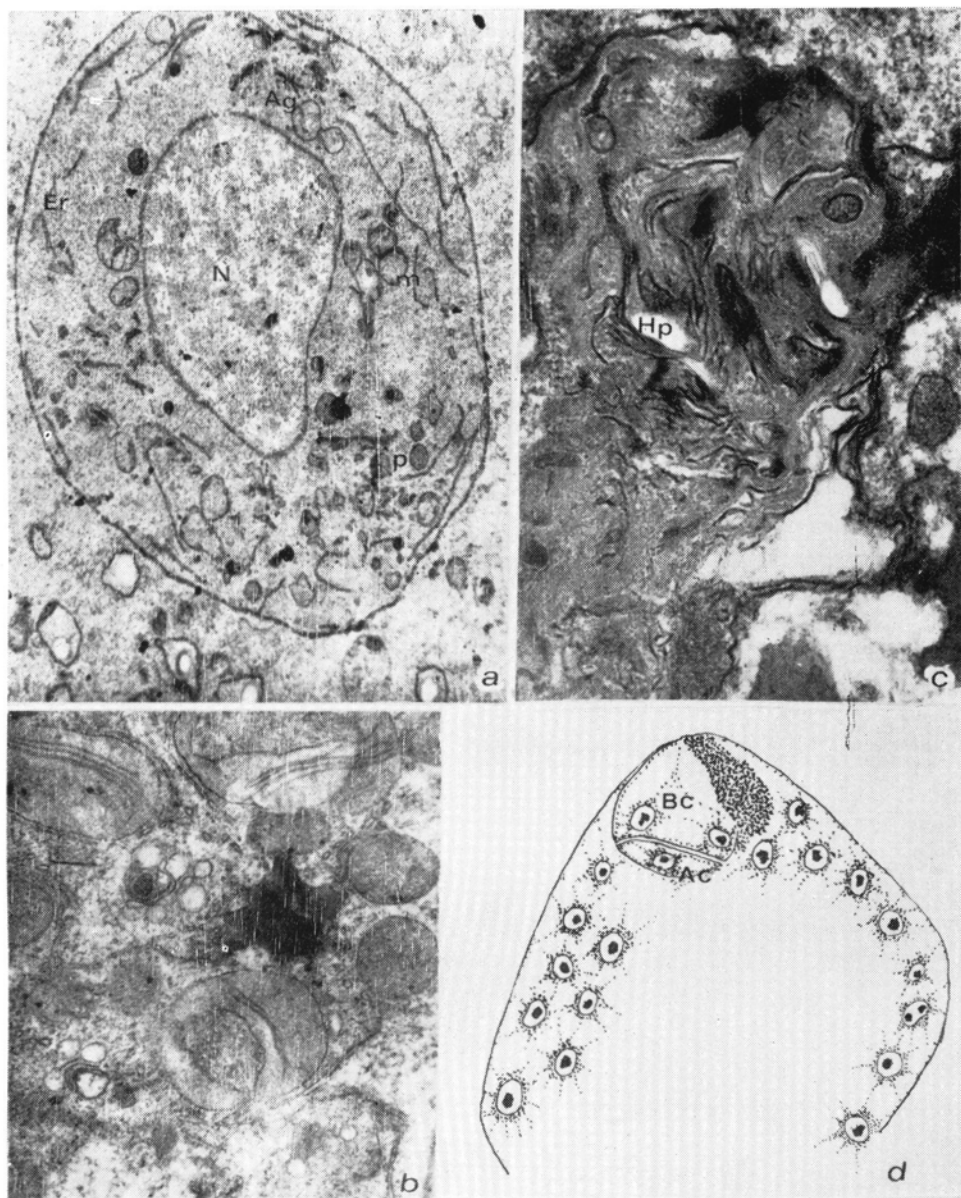


Fig. 1a. *Zea mays* sperm-cell (SC)

The cytoplasm surrounded by a thin double membrane with pores. The nucleus (N), promitochondria (M), proplastids (P), Golgi structures (AG) and poorly developed endoplasmic reticulum (ER).  $\text{KMnO}_4$ , Epon,  $\times 28\ 000$

b. Fragment of egg cell cytoplasm at fertilization

Desintegrating plastids. Mitochondria are round shaped, cristae small and poorly developed; cisternae of Golgi apparatus form swellings; smooth ER cisternae.  $\text{OsO}_4$  Epon,  $\times 67\ 000$ .

c. Hyaloplasm of a sperm cell (Hp) seen between central cell and egg cell. Numerous tubules and remnants of plastids and mitochondria.  $\text{OsO}_4$ , Epon,  $\times 4000$ .  
d. The apical (AC) and binucleate basal cells (BC), proembryo nuclear endosperm (E)

bryo (Table 2). It can be seen that in pistils pollinated two days before the anthesis, the division of primary endosperm nucleus and that of zygote proceeds slowly, while in pistils pollinated on the 5-th day of anthesis there is a more active division. The embryogenesis in the young embryo sacs is more balanced than in the aged ones. The ratio of number of endosperm nuclei to the embryo cells on the 3rd day after pollination is approximately 1 : 20.

Table 2

The formation rate of endosperm nuclei and proembryo cells in embryo sacs of different age

The pistils age at the moment of pollination	Time after pollination (h)	Number		Diameter (μm)	
		endosperm nuclei	proembryo cells	endosperm nuclei	nucleoli of endosperm nuclei
2 days before anthesis	24	10-18	0-2	—	—
	48	37-38	2-4	—	—
Anthesis (control)	48	59	7	9.69	5.6
	72	213	13	—	—
5-th day after anthesis	72	390	19	—	—
10-th day after anthesis	72	260-336	4-25	5.6	3.48
15-th day after anthesis	72	—	—	—	—

Our data suggest also the influence of a number of other factors on the pattern and the rate of embryogenesis. In the experiments when anthesis occurred at the end of September or the beginning of October (maximal temp. 8-10°C and minimal — at night — 4-5°C) the rate of fertilization and embryogenesis was decreased 4-5 times. At normal conditions fertilization is observed 16-18 h after the pollination, while in autumn fertilization occurred on the 4-th day and the transformation of the nuclear endosperm into the cellular was observed on the 10-12th day. At normal conditions this process takes 48-50 h. Disturbances of division succession of proembryo were also observed. The apical cell often appears to be excluded from the "embryological framework" and then the basal cell of the two-celled proembryo begins to divide first. It is not known if it gives to the embryo.

High temperature (43-45°C) during 2 h before and after pollination, different poison vapours and gases, physiologically active substances, cause nonspecific structural changes. They concern mainly development of embryo sac elements and course of early embryogenesis.

The cells of the embryo sac, as well as the apical and basal cells of proembryo, represent genetically unequal structures. Moreover, the inequality can be traced in all taxons of the angiosperms (Maheshwary, 1954). Nevertheless, it is necessary to outline that the racemical combination of the terminal and basal cells is not a combination of the inactive bodies. Genetic inequality of the terminal and basal cells which can be well traced structurally and physiologically is masked by the diploid nature which makes then separately active physiologically.

In conclusion, interactions of the proembryo and the endosperm at early stages of the embryogenesis must be considered as a necessary event providing the aromorphose condition of the proembryonal structures. Moreover, the morphogenetic embryonal initials may serve as example, both of growing of the levels of organization and integration, and their interrelationships. This process can be easily traced at different stages of embryo development. Undoubtedly the two-celled proembryo represents a bipolar formation with strictly determines aromorphose.

We can say that pattern of the apical and basal cells and their interaction with the primary endosperm are transformed into a complicated system of reactions on the external factors. This, finally, will determine the rate of adaptivity of the future organism.

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