Proc. Indian natn. Sci. Acad. B **44**, 13-29 (1978)
Plant Embryology

EMBRYOLOGY OF WHEAT*

by T. B. BATYGINA, *Embryology Laboratory, Plant Morphology Department, Komarov Botanical Institute, The USSR Academy of Sciences, 2, Professor Popov Street, Leningrad* 197022, *USSR*

(Received 2 August, 1977*)*

Fertilization and embryogenesis in cereals was studied by many researchers (Sax, 1918; Yakovlev, 1946, 1950; Lvova, 1950; Oksiyk & Khudiak, 1955; Morrison, 1955;

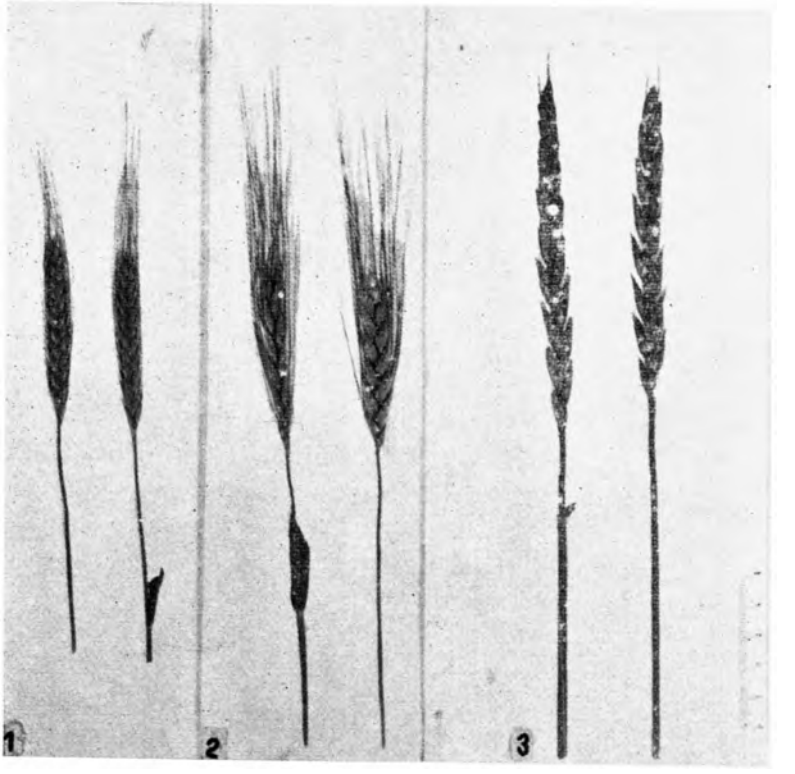

FIG. 1. Some representatives of the polyploid wheat series : 1-*T. monococcum* (2n = 14); 2, *T. dicoccum* (2n = 28); 3, *T. aestivum* (2n = 42).

*Paper presented at the Indo-Soviet Symposium on Embryology of Crop Plants, held at New Delhi on 2-6 March, 1976 (Convener : Professor B. M. Johri, FNA)

Tchebotar, 1972 ; Chandra, 1976 ; Johri, 1976, and others.). However, only certain aspects of fertilization have been studied while correlations between syngamy and triple fusion, and between development of zygote and endosperm remain unconsidered.

In order to achieve a better understanding of fertilization in wheat and embryogenesis, the author (Batygina, 1957; 1961 a, b; 1962 a, b; 1974 a, b) undertook a detailed study of embryogenesis in various wheat species, like *T. monococcum*, *T. dicoccum* and *T. aestivum*.

It should be noted that wheat has proved to be a very convenient object for embryological research. Its fertilization process is prolonged which makes it possible to follow all the stages, to observe, for instance, the sperms in synergids, in egg-cell cytoplasm, etc. Apart from that, the prolonged fertilization permits to make clear a correlation between syngamy and triple fusion at all stages. In addition, all sexual elements of wheat are large enough. At R. H. 80-90% and temperature 14-16°C the pollen tubes reach the embryo sac after 15-20 min of pollination (morning, afternoon or evening being unimportant for the growth rate). The wheat ovule is apotropous by its position in the ovary, and pollen tubes have to traverse quite a long distance from the stigma

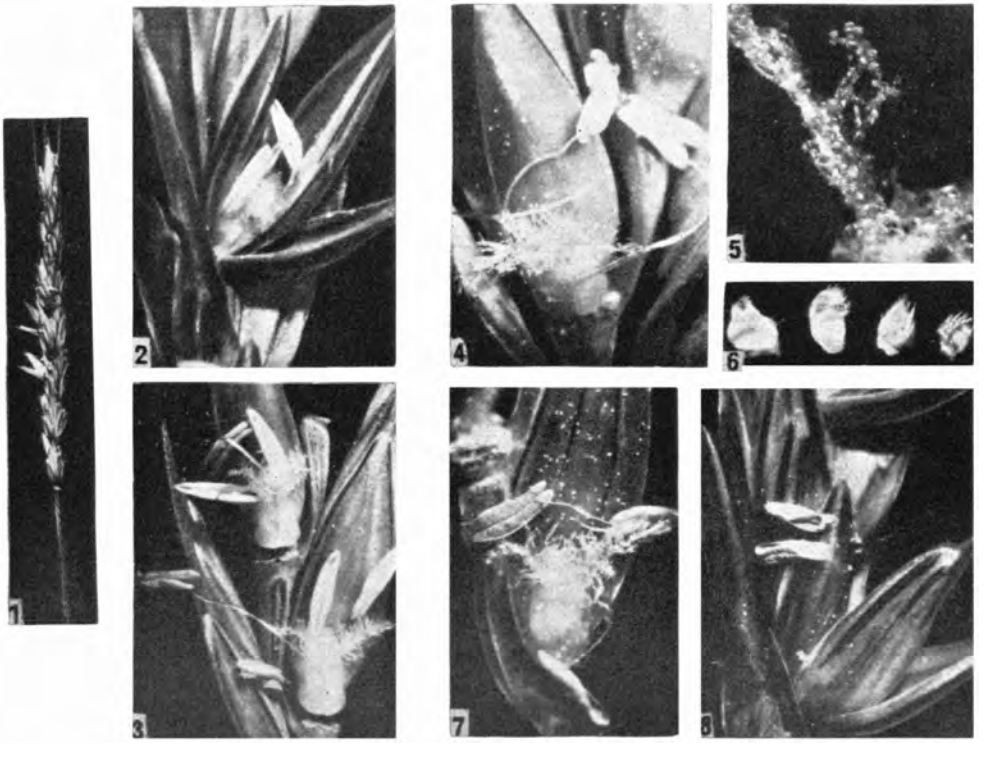

FIG. 2. Flowering and pollination (1-8) of *T. aestivum* (Diamant variety), lodicle (6) and its alterations in the course of flower development.

to the micropyle (Fig. 3A). One or more pollen tubes can enter the ovary and embryo sac. Pollen tubes pass through the lobes of the plumose stigma into conducting stylar tissue moving apart the tightly closed cells which result in deformation of the latter, or destruction of many cells when a massive growth occurs (Fig. 3 B, C).

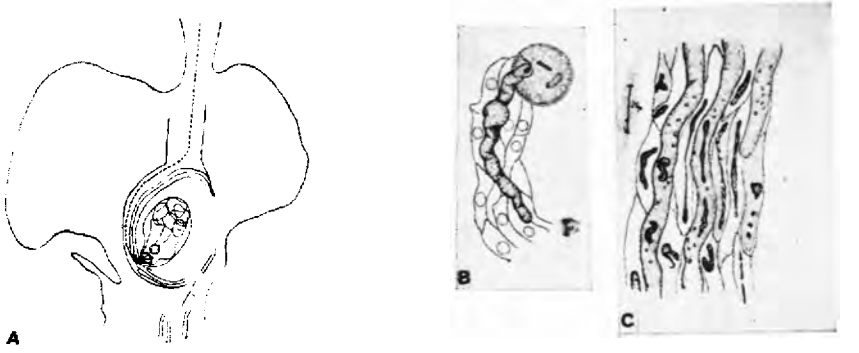

FIG. 3. Route of pollen tubes (indicated by dotted line) traversing in wheat ovary (*A*), and their growth in pistil tissues (*B*, *C*).

The sperms in the pollen tubes of wheat differ slightly from those in the pollen grains. With the growth of the pollen tubes the sperms undergo some changes in shape and structure. They may also differ within each pair in their external appearance.

The structure of sperms in pollen tubes varies in various species as it does in pollen grains (Fig. 3).

On entering the embryo sac the pollen tube meets the synergids and discharges its content into them (Fig. 4). Destruction of one or both synergids has been observed in all wheat species studied. The discharged content of the pollen tube is delivered, together with both sperms, into the space between the egg cell and the central cell of embryo sac. From there the sperms move in the opposite directions; one gets into the central cell and another into the egg cell (Fig. 5). Despiralization of the sperm, its "dissolution" in the egg cell nucleus lasts 4-5 hr. (Figs. 5, 1-17). In the polar nuclei this process lasts 1·5-2 hr and the primary endosperm nucleus enters prophase in 3-4 hr (Figs. 5, 26). The duration of dormancy period is also varied: in zygote it lasts about 18 hr, while in endosperm only 0·5-1 hr (Figs. 5, 25). If the male and female chromatins are indistinguishable in zygote during prophase, it is only during metaphase that they fuse in polar nuclei which might be explained by varying duration of dormancy period in these cells.

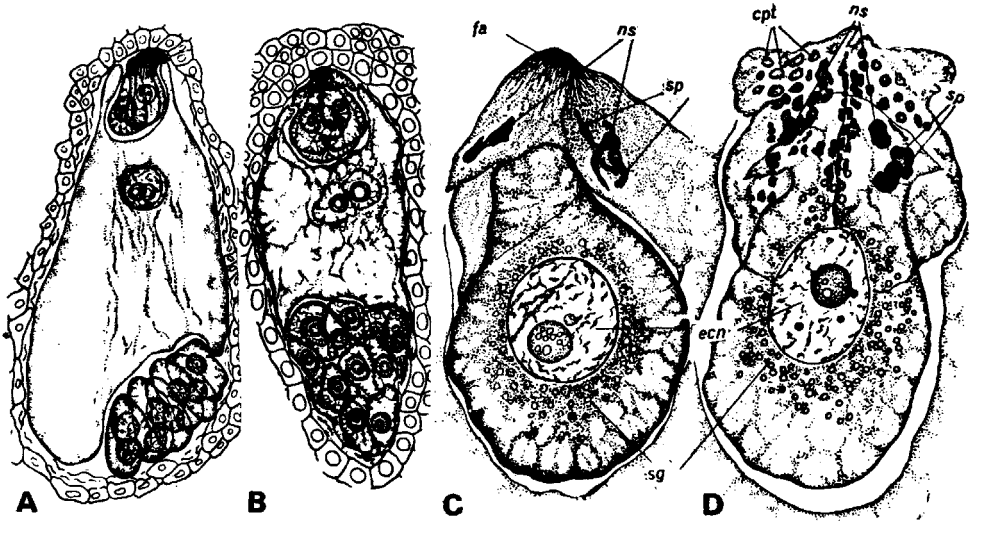

FIG. 4. Embryo sac of wheat. *A*, profile view; *B*, face view; *C*, *D*, egg apparatus of *T. aestivum* 20 min after pollination. *sp*, sperms; *ns*, nuclei of synergids; *fa*, filiform apparatus; *cpt*, content of pollen tube; *ecn*, egg cell nucleus; *sg*, starch grains. (*C*—by Feulgen with light green colouring; the filiform apparatus can be observed; *D* — by Modilevsky colouring; the pollen tube content in the form of drops in both synergids can be clearly seen).

Thus, as a result of different rates with which each of the sperms of a pair goes through every development phase, there appears a false impression of great difference between them. These differences might be explained by different conditions which affect the sperms of the same pollen tube.

Sperms fuse with the embryo sac nuclei at different rates. However, in spite of their difference there exists quite a definite rate in the course of each process and their correlation. Certain states of polar nuclei, sperm, and endosperm always

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FIG. 5. Successive stages of shape and structural changes in wheat sperm from the moment of its entering the embryo sac till its complete "dissolution" in the egg cell nucleus (1-17). 1, *T. dicoccum*, the sperms from synergids; 2, *T. aestivum*, the sperms from the "slot"; 3-6, *T. aestivum*, the sperm has entered the egg cell; 7-15: "dissolution" of the sperm in the egg cell nucleus; 7-9 & 15, *T. monococcum*; 10, 11 & 14, *T. aestivum*; 12, *T. dicoccum*; 16, the nucleus of *T. dicoccum* zygote in the state of dormancy; 17, the prophase of *T. monococcum* zygote. By Feulgen with light green colouring.

Successive stages of shape and structural changes of the sperm from the moment of approaching central nuclei till its complete 'dissolution' in polar nuclei before primary division of endosperm nucleus (18-28). 18, 19, the sperms on the border of two polar nuclei; 20, 21, 23, 24, the sperm is fusing with both polar nuclei simultaneously; 25, the dormant nucleus of endosperm; 26, 27, endosperm prophase; 28, endosperm metaphase; 18-22, 25-27, *T. aestivum*; 23, 24, 28, *T. monococcum*.

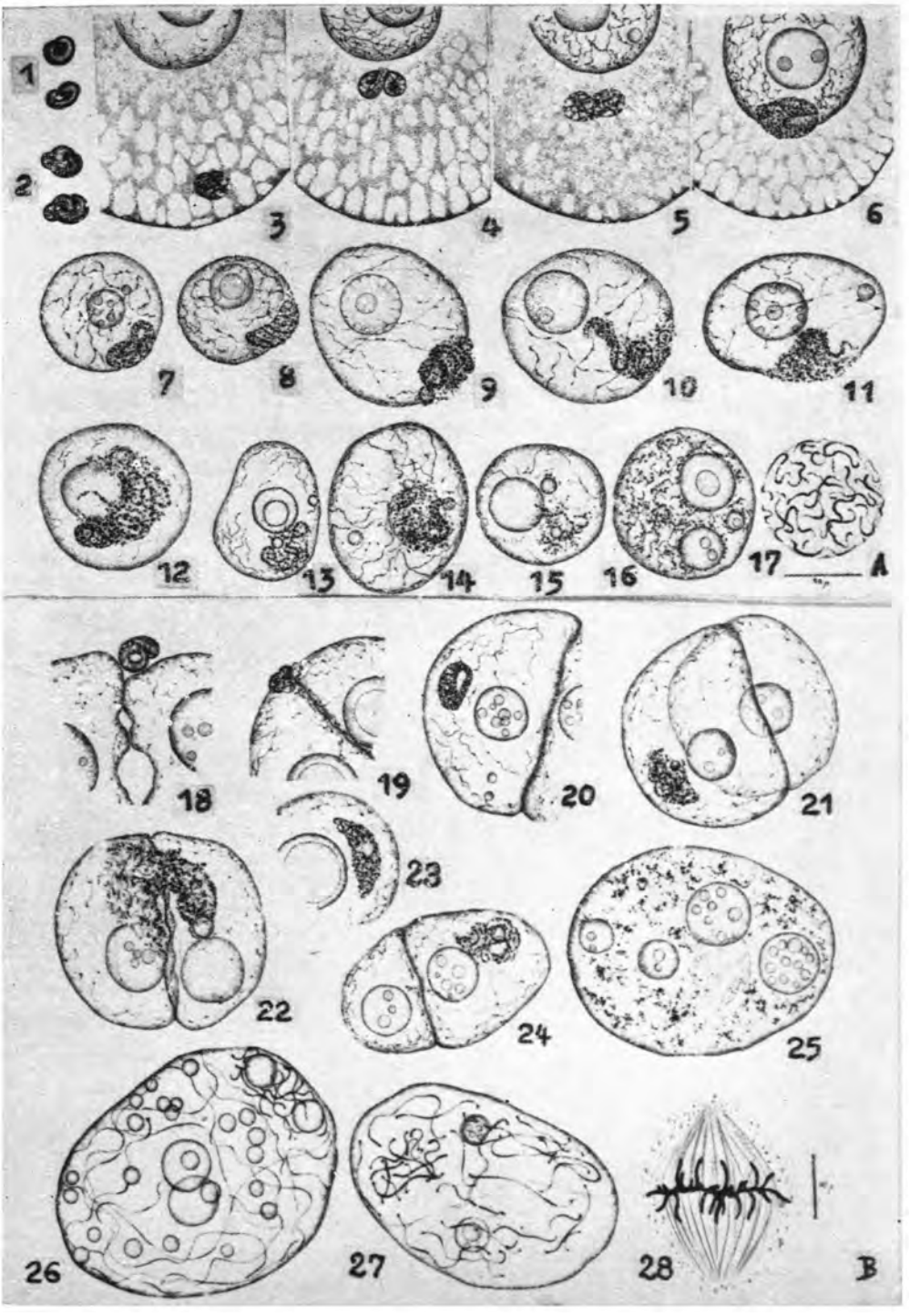


FIG. 5.

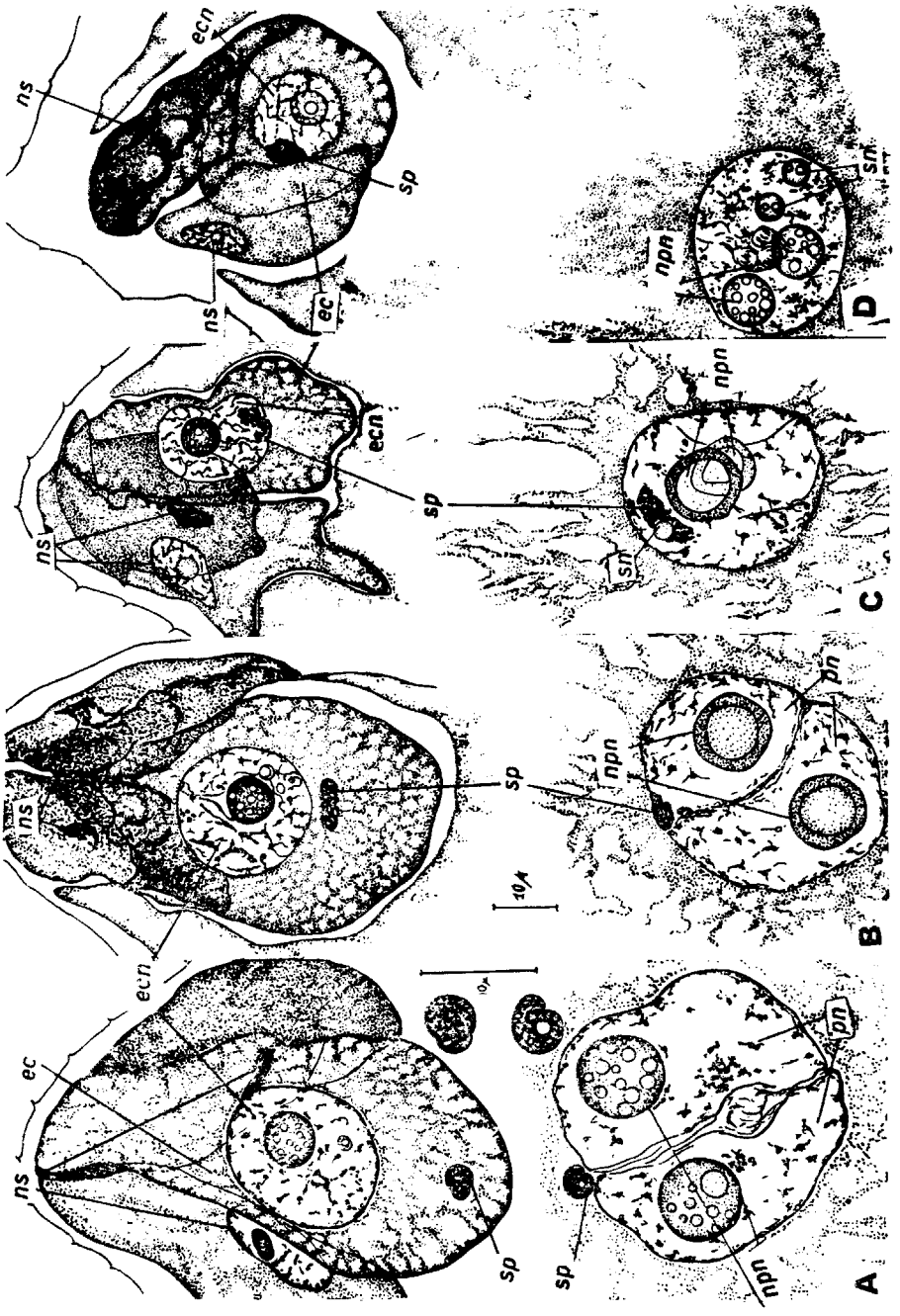


FIG. 6. Portions of embryo sacs. *T. aestivum* (*A*), 30 min after pollination: *T. dicoccum* (*B*), 1 hr after pollination: *T. aestivum*, 1·5 hr. (*C*), and 2·5 hr. (*D*), after pollination. *ec*, egg cell; *ecn*, egg cell nucleus; *pn*, polar nuclei; *ns*, nuclei of synergids; *npn*, nucleoli of polar nuclei; *sp*, sperms; *sn*, sperm nuclei. By Feulgen with light green colouring.

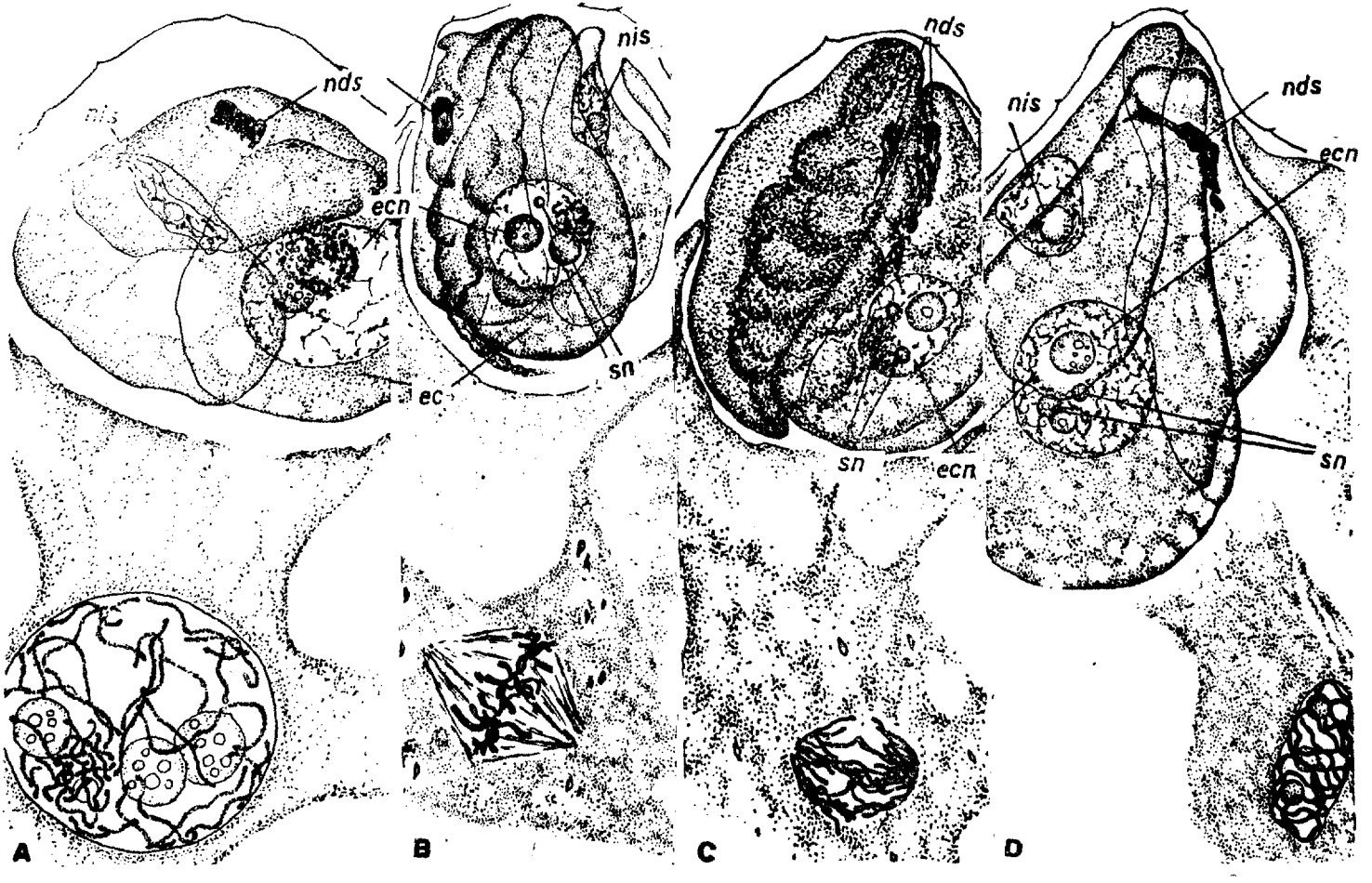


FIG. 7. Portions of embryo sacs. *T. aestivum* (*A*), 3 hr after pollination; and *T. monococcum*, 5 hr (*B*); 6 hr (*C*); and 7 hr (*D*) after pollination. *nis*, nucleus of intact synergid; *nds*, nucleus of destroyed synergid. Other indications same as in Fig. 6.

strictly correspond to certain states of egg cell, sperm and zygote (Figs. 6, 7, 8, 9, 10). Such correlation is least affected by external factors and does not practically depend on them as can be confirmed by the comparative study of these processes under varying conditions. It should, however, be noted that the rate of the embryological processes on the whole is dependent on the changes in environmental conditions. The comparative temporal study of fertilization process in various wheat species makes it possible to believe that the correlation between development rates of zygote and endosperm is similar in all the species studied, and does not depend on their ploidy.

All data obtained on the structure of sperms in pollen grain and pollen tube, as well as in embryo sac, confirm once again the concept of Gerassimova-Navashina (1947-1959) that sperms approach the female cells in a state of telophase, and accomplish their development cycle affected by the dormant female cell.

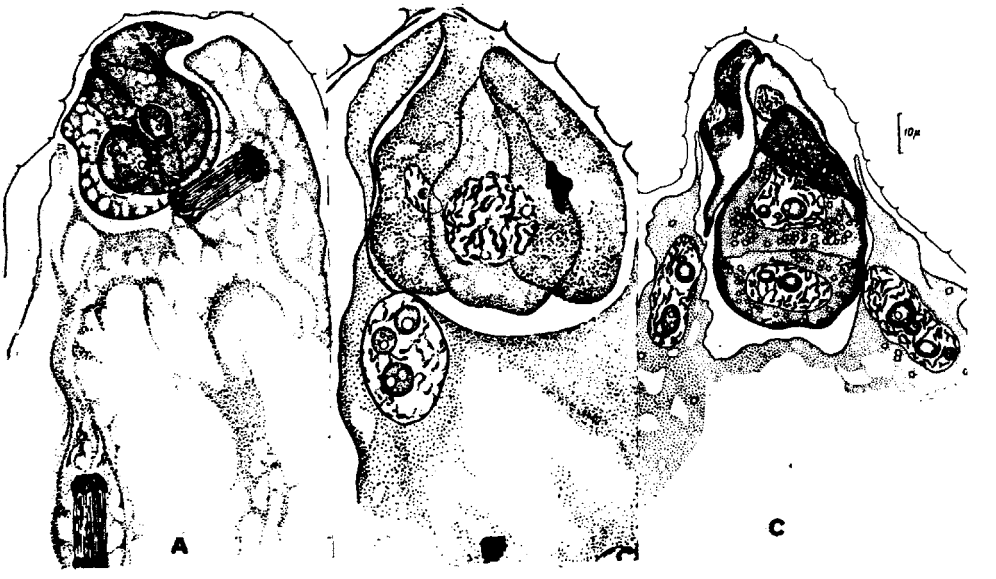


FIG. 8. Micropylar part of *T. monococcum* embryo sac 8 hr after pollination *(A)*; zygote in the state of dormancy, second division of endosperm, one of the synergids destroyed. The same 24 hr after pollination *(B)*; prophase in zygote, two endosperm nuclei can be observed. Bicellular proembryo of *T. aestivum (C)*.

As a result of a detailed study of fertilization process in some wheat species, and also of analysis of the data given by Sax, Morrison & Vazart, the author, together with Gerassimova-Navashina (Batygina, 1957, 1961 a, b; 1962 a, b; Gerassimova-Navashina & Batygina, 1959; Batygina, 1974 a, b), found that the premitotic type of fertilization is characteristic of cereals, including wheat.

Our data on embryo of various wheat species (*T. monococcum*, *T. dicoccum*, and *T. aestivum)* showed that the two stages can be separated in its development: initial differentiation (blastomerization) and organogenesis (Fig. 11), the latter consisting of initial morphological isolation of organs of the embryos and their successive tissue differentiation (Batygina, 1968 a, b, 1974 a, b).

What is characteristic for wheat as well as for other cereals is that both first as well as successive divisions are unequal. The peculiar position of proembryo cells shows that the wheat embryo has a specific type of blastomere tetrad structure (all walls are at right angles to each other) (Figs. 11, 12). Related to this are the following works: Tchebotar (1960, 1972) and Korobova (1961) on maize embryogenesis; Paulson (1969) and Khazova (1970) on *Sorghum* embryogenesis; Norstog (1972) on barley proembryo structure; and Batygina (1971) on embryogenesis of representatives of the polyploid series of meadow grass in which the authors considered the initial stages of embryogenesis.

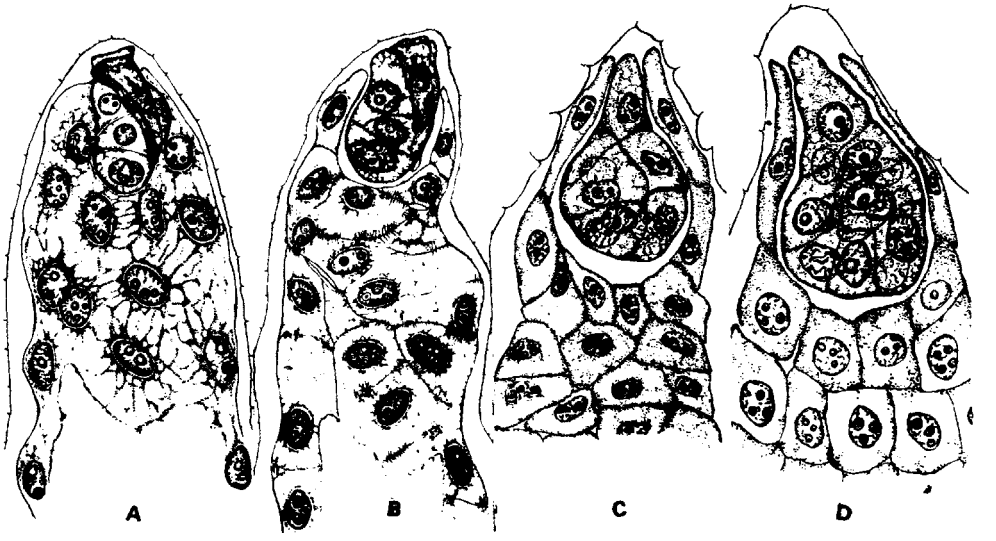


FIG. 9. Micropylar part of embryo sacs. *T. monococcum* 42 hr *(A)*, 52 hr *(B)*, 72 hr *(C)*, and 96 hr *(D)* after pollination. Successive stages of embryo development are shown.

Analysis of the above mentioned and a number of other works (Kihara & Nishiyema, 1932; Modilevsky *et al.*, 1958, etc.) decisively shows that the first three divisions of proembryo result in formation of a peculiar tetrad of blastomeres (similar to that of wheat) but not the distinctive 3-layer (T-shaped) tetrad described earlier by Soueges (1924) for meadow-grass and later generally accepted for proembryo of all cereals (Fig. 12).

Organogenesis of embryos of a large number of cereals studied by Guignard (1961) was found to be similar to that of wheat. To which type of development may be referred the development of cereal embryos has been a matter of controversy for long.

According to Yakovlev (1946), a cereal embryo developed as Onagrad type, whereas Poddubnaya-Arnoldi (1964) opines that its first developmental stages are closer to Aterad type. Soueges (1938) refers development of cereal embryo to Megarchi type 1, while Guignard to Megarchi type 2. Maheshwari (1950) referred development of cereal embryo to anomalous type which is not included in existing classifications, arguing that cereal proembryo does not have any regular pattern in orientation of cell divisions.

Dorsiventral structure of proembryo is characteristic for all studied *Triticum* species. It is expressed already at the first division of zygote, and is maintained later on throughout the whole course of embryo development, being increasingly expressed morphologically at its successive stages. However, if in the process of development of zygote and early proembryo, dorsiventrality is established mainly due to the differences in the degree of cell wall elongation on the dorsal and ventral sides of zygote and proembryo, at the next development stages dorsiventrality of the embryonal structure is expressed in the specific orientation and frequency of cell divisions as well as in the elongation of cell walls.

In the process of growth and development the wheat embryo changes its shape significantly. In these changes as well as in all intricate structural transformations at the stage of organogenesis a decisive role is played by successive

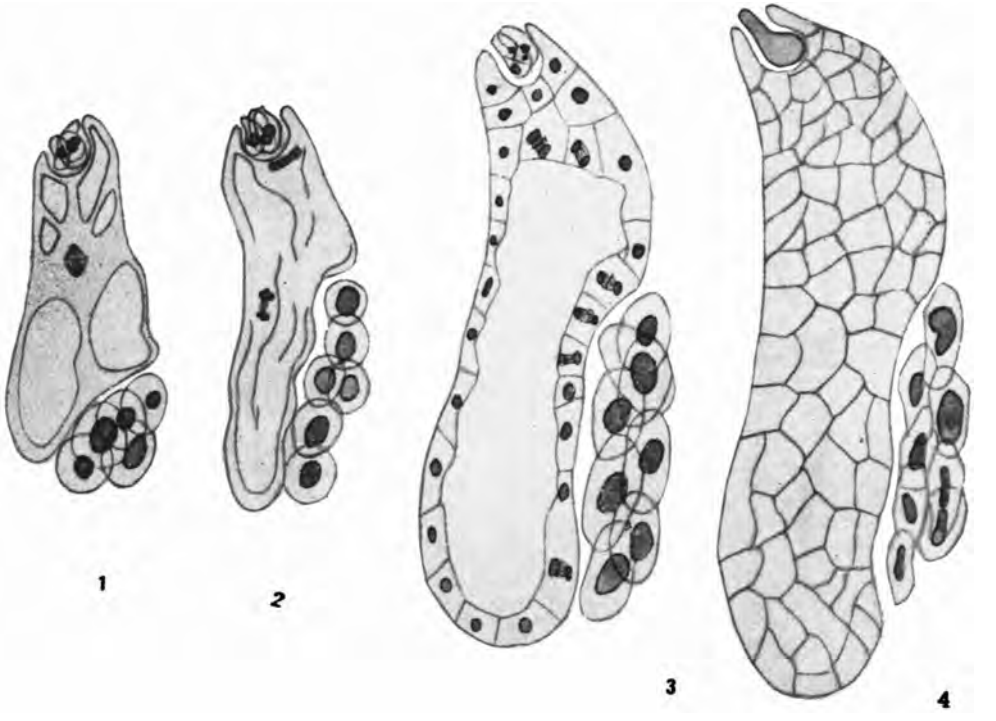


FIG. 10. Successive stages of embryo and endosperm development (schematic): 1, 5 hr after pollination; 2, 10 hr; 3, 48 hr; 4, 6-8 days after pollination.

changes in the rate of mitotic activity in certain embryonal parts (Yakovlev, 1946, 1951 a, b; Batygina, 1968 a, 1969, 1975). Changes in the rate of mitotic activity can be easily observed in preparations and are expressed mainly in the staining degree of separate embryo parts. Meristematic centres in developing embryo can be estimated by staining capacity.

In the process of further development of embryo the complicated transformations occur resulting in scutellum taking the shape similar to that observed in mature embryo. Organogenesis of wheat embryo starts with the formation of scutellum rather than coleoptile, as many scientists believe. Further development of embryo results, firstly, in formation of the constriction dividing cotyledon and epicotyle and, secondly, in isolation of coleoptile and shoot apex. Beginning of main root formation is simultaneous with the formation of coleoptile and differentiation of procambial strand in scutellum. The root formation is endogenous and takes place in the basal part of embryo. The presence of independent plerome hystogens, periblem and root cap in wheat embryo makes it possible to refer development of wheat root to the closed type (Guttenberg, 1960 ; Batygina, 1968 b ; Sokolovskaya 1968). The entire wheat embryogenesis shows that coleorhiza and root cap appear and develop together like a single formation, and that it is only at the end of embryogenesis when the embryo is mature that coleorhiza detaches itself from the

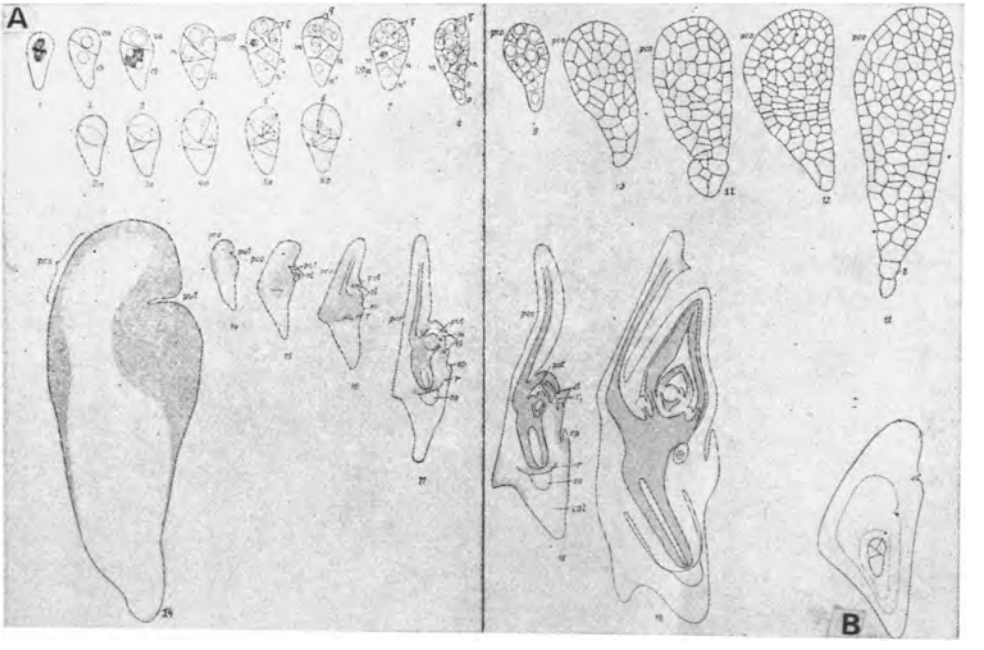


FIG. 11. Embryo development in *Triticum* (schematic). A, 1-19 — *T. aestivum* embryo in dorsiventral section; 2a-6a — its stereoscopic representation. B, scheme of development of wheat embryo *pco*, scutellum; *cl*, coleoptile; *pvt*, shoot apex; *ep*, epiblast; v^1, first leaf of gemmule; *r*, root; *co*, root cap; *s*, suspensor.

root cap as was shown by Sokolovskaya (1968) for other cereals as well. The root dermatogen appears from the outer layer of periblem. The epiblast gets isolated after the root has been formed. Then formation of other embrynal structures takes place—further differentiation of vegetative cone, formation of the ligule, and basal scutellum excrescence.

In the mature wheat embryo the following structures can be found : scutellum, ligule, coleoptile, mesocotyle, epiblast, coleorhiza which consist of several primordia, several roots and auxillary buds.

The analysis of the intricate morphogenetic transformations in the wheat embryo organogenesis shows that seemingly lateral position of shoot apex and seemingly terminal position of cotyledon are the result of specific features of embryogenesis (Fig. 11).

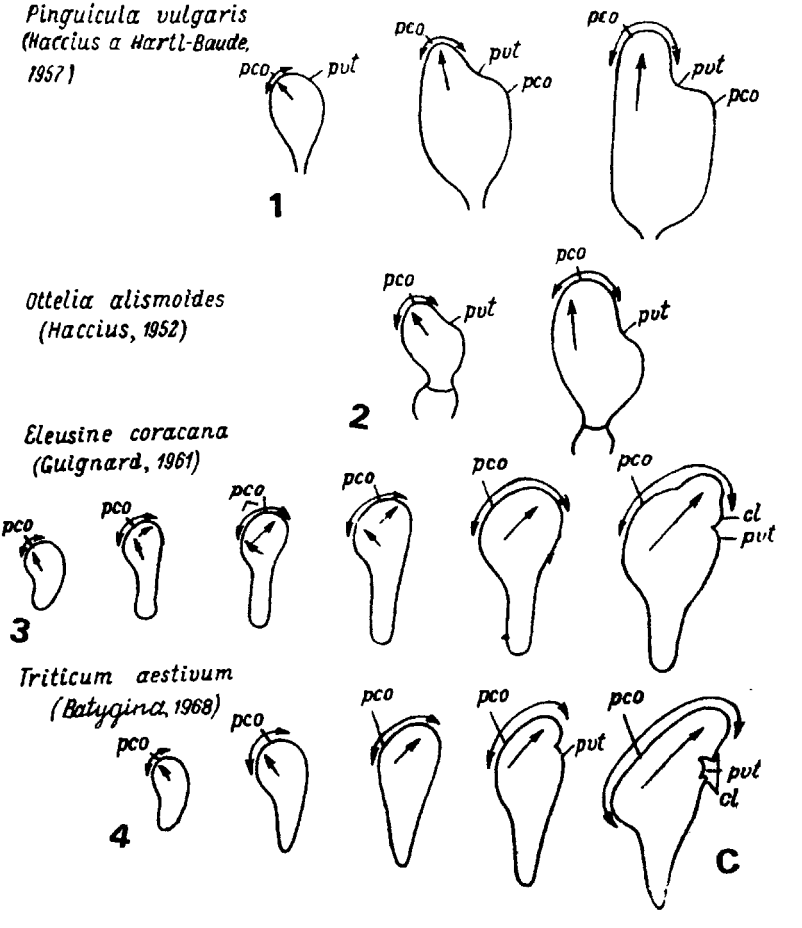
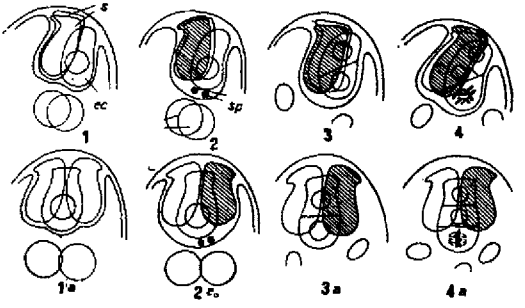


FIG. 11. C, evolutionary formation of embryo, dorsoventral organization. 1, predominant development of one of the seed lobes in pseudocotyledonous *Pinguicula vulgaris*; 2-4, evolutionary formation of monocotyledonous features (character of seed-lobe growth, and shift of shoot apex in abaxial position).

The analysis of literature and the results of our study showed that in one group of plants the relatively slow formation of shoot apex makes it possible to observe its shift in the ontogenesis of embryo, whereas in another group to which we refer also cereals, it happens so rapidly that one can get a false impression of initial lateral formation of shoot apex (Fig. 11 C).

Thus, as regards the origin of shoot apex and morphologic significance of cotyledon there does not appear to be great difference between mono- and dicotyledons. These differences are the evolutionary secondary result of specialization.



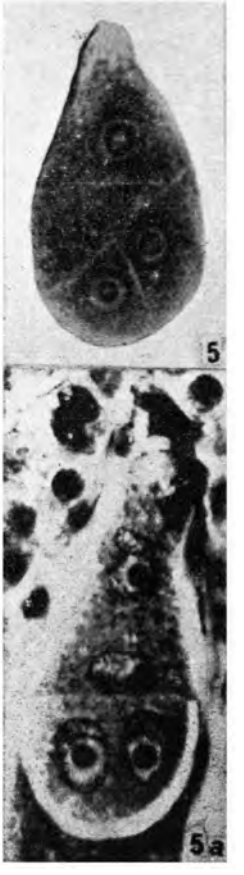
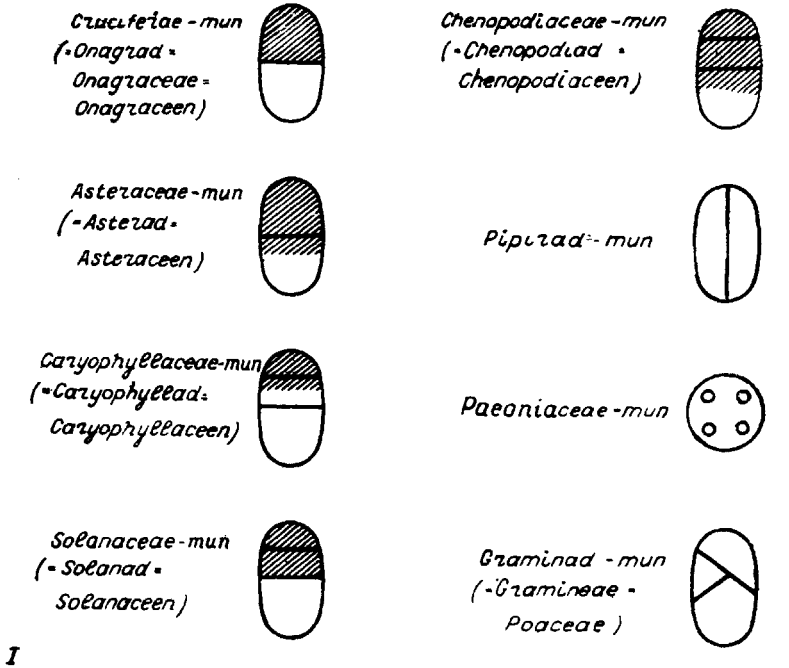


FIG. 12. Apical part of *Triticum* embryo sac in dorsiventral (1-4) and bilateral (1a- 4a) sections : 1 & 1a, before fertilization; 2 & 2a, at the time of fertilization; 3 & 3a, bicellular embryo; 4 & 4a, tricellular embryo; the destroycd synergid is hatched. Microphotographs of 4-nucleate embryo in dorsiventral (5) and bilateral (5a) sections. *ec*, egg cell; *s*, synergids; *pn*, polar nuclei; *sp*, sperm.

Our study of embryogenesis in *T. monococcum*, *T. dicoccum* and *T. aestivum* shows that the original dorsiventral structure is acquired by wheat embryo as a result of the first two divisons. Their stable reproduction of specific 4-celled proembryo in every embryo, and preservation of dorsiventrality at all successive stages allow us to believe that the character of first proembryonal divisions is directly related to dorsiventrality of embryo at its successive developmental stages.

One should not, of course, think that orientation of the first division determines orientation of divisions in embryonal cells during the middle and late stages of embryogenesis. It is rather the character of the primary blastomerization that produces the most rational correlation of cells in proembryo for further develop-



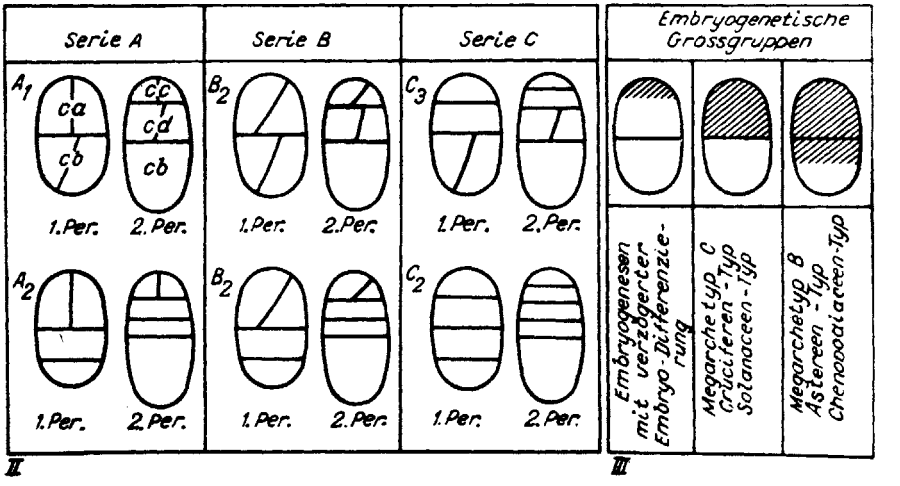


FIG. 13. Main types of angiosperm embryo development. I, *BY* Schnarf, Jahansen, M. S. Yakovlev, T. B. Batygina and Haccius; II, *BY* Soueges (Haccius, 1971); and III *BY* Schnarf, Meestre and Haccius (Haccius, 1971). The portion of the proembryo, derivatives of which will later produce the main body of embryo is hatched.

ment of, dorsiventrality. Beginning from organogenesis (scutellum formation), the mutual position of separate blastomeres in the embryo becomes less important; other morphogenetic regulations come into force being effective at the level of the entire cell complexes. The analysis of initial accrescences taking place both as a result of differences in mitotic activity and different rates of cell elongation in different embryonal parts shows that usually the "redistribution" of cell masses in embryo results in formation of scutellum on the dorsal side of embryo facing placenta-chalaza. Thus, the specific position of organs in the mature wheat embryo is determined by the specific complicated redistribution of mitotic activity zones during organogenesis.

The characteristic dorsiventral structure of wheat proembryo, its method of development and specific participation of blastomeres in embryo formation in accordance with criteria of existing classifications (Schnarf, 1927, 1929; Soueges, 1938; Johansen, 1950, *et. al.*) allowed us to recognize a new type of embryogenesis in Gramineae (Poaceae) (Fig. 13) (Batygina, 1968 a, b).

Formerly, when defining any embryogenetic type, the structure of mature embryo and peculiarities of its embryogenesis were not taken into account. For better understanding of Poaceae type, (Graminad type) in addition to the characters used by other authors defining embryogenetic types, we decided to take into account also the character of embryogenesis as a whole, and the structure of mature wheat embryo. Introduction of additional criteria enabled us to define more correctly the essence of embryogenetic type of cereals.

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