Proc. Indian natn. Sci. Acad. B **44**, 30-42 (1978)
Embryology

EMBRYOLOGICAL PROCESSES AT DISTANT HYBRIDIZATION IN GRAMINEAE FAMILY*

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

(*Received* 2 *August,* 1977)

Distant hybridization of plants is of great theoretical and practical importance (Karpechenko, 1934; Vavilov, 1935; Kostov, 1938; Zhukovsky, 1971 and others.) Application of the distant hybridization method to plant breeding involves a number of difficulties. The principal ones are the non-crossing and sterility of hybrids. The non-crossing problem has for long attracted attention of many scientists. However, this problem still remains to be solved. The knowledge of embryological processes occurring normally and at distant hybridization is of great help when attempting to discover the reasons for failures in crossing as well as for the incompatibility of the components that are being crossed.

Cereals, and particularly wheat, were demonstrated (Batygina, 1962 b, 1974) to have morphogenetic correlations between the development of the egg cell and that of the endosperm expressed in certain ratios between their development rates and the state of their sexual nuclei. When wheat is pollinated normally, this correlation in development is, as we shall demonstrate below, not violated and the system 'zygote→embryo→endosperm' shows normal development. At distant crossings the mobile equilibrium (homeostasis) in the development of zygote, embryo and endosperm can also be occasionally maintained, though we observe some changes in the development rates of individual elements of the correlative system. However, in most cases of distant crossings the correlative system is so much violated that it breaks down. The comparative analysis of embryological processes occurring normally and at distant hybridization makes it possible to divide them into three stages: (i) progamic phase — interaction of pollen and pollen tubes with pistil tissues, (ii) fertilization process (syngamy and triple fusion), and (iii) development of embryo and endosperm.

The study of fertilization and embryogenesis processes at distant crossings in Gramineae family is dealt with in relatively few publications (Poddubnaya-Arnoldi, 1939, 1964, 1970; Fedorova, 1964; Kandelaki, 1968, 1969; Khudiak & Bannikova, 1968; Fursov & Shkurenko, 1968; Frumkina, 1968; Khvedynich, 1972,

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* **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)**

and others). Most of these works discuss the reasons for disturbances occurring at the middle and late stages of embryo and endosperm development.

The persistent attempts to obtain hybrids involving *T. monococcum* are being undertaken by the plant specialists as this species is highly resistant to many diseases. However, the attempts to cross the einkorn wheat with other wheat species seldom end successfully. We have studied the fertilization and embryogenesis processes mainly in reciprocal crossings of *T. monococcum* with *T. aestivum* (Diamant variety).

At reciprocal crossings of these species the normal interactions of the male and female sexual elements may be violated starting from the moment the pollen gets on the stigma, this violation being expressed in degeneration of ungerminated pollen grains. During germination of the pollen tubes various abnormalities in their growth were observed (Fig. 1) (Batygina, 1959, 1962 *a, b*, 1966, 1974; Batygina, Dolgova & Korobova, 1960, 1961).

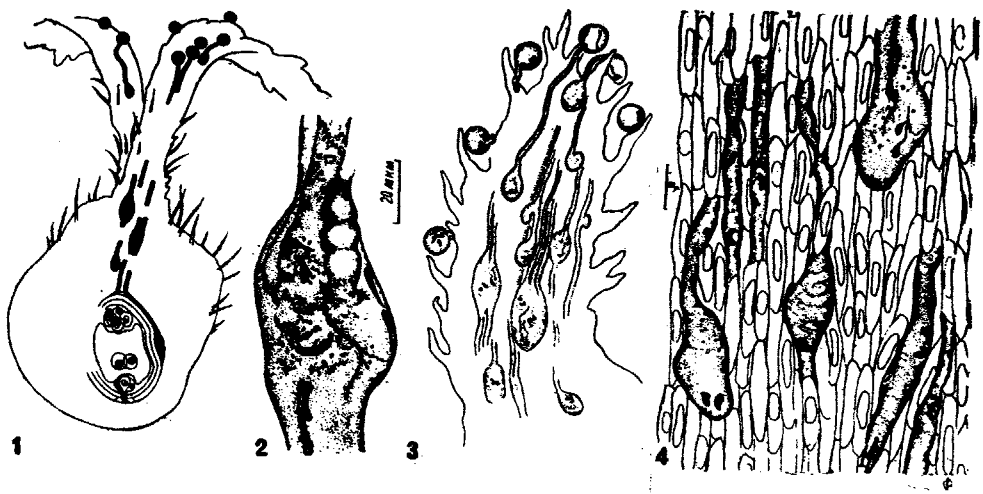


FIG. 1. Growth of pollen tubes in wheat ovary at distant hybridization. 1, general view of wheat ovary with growing pollen tubes; 2, 'synthetic' swelling formed by several pollen tubes; 3, pollen swellings formed as a result of fusion of several pollen tubes; 4, pollen tubes in pistil tissues.

Comparing distant hybridization processes with normal development, one is able to separate two main types of disturbances — reversible and irreversible ones (Fig. 2). The disturbances affecting correlations in development of these or other structures are referred to as irreversible disturbances. They are beyond the limits of the possible self-regulation.

The incompatibility of the pollen tubes and pistil at crossing *T. monococcum* with *T. aestivum* becomes evident just 15-30 min after pollination (Fig. 1).

At the backcrossing of *T. aestivum* with *T. monococcum* a slightly different situation is observed. At this crossing, most of the pollen grains die off already

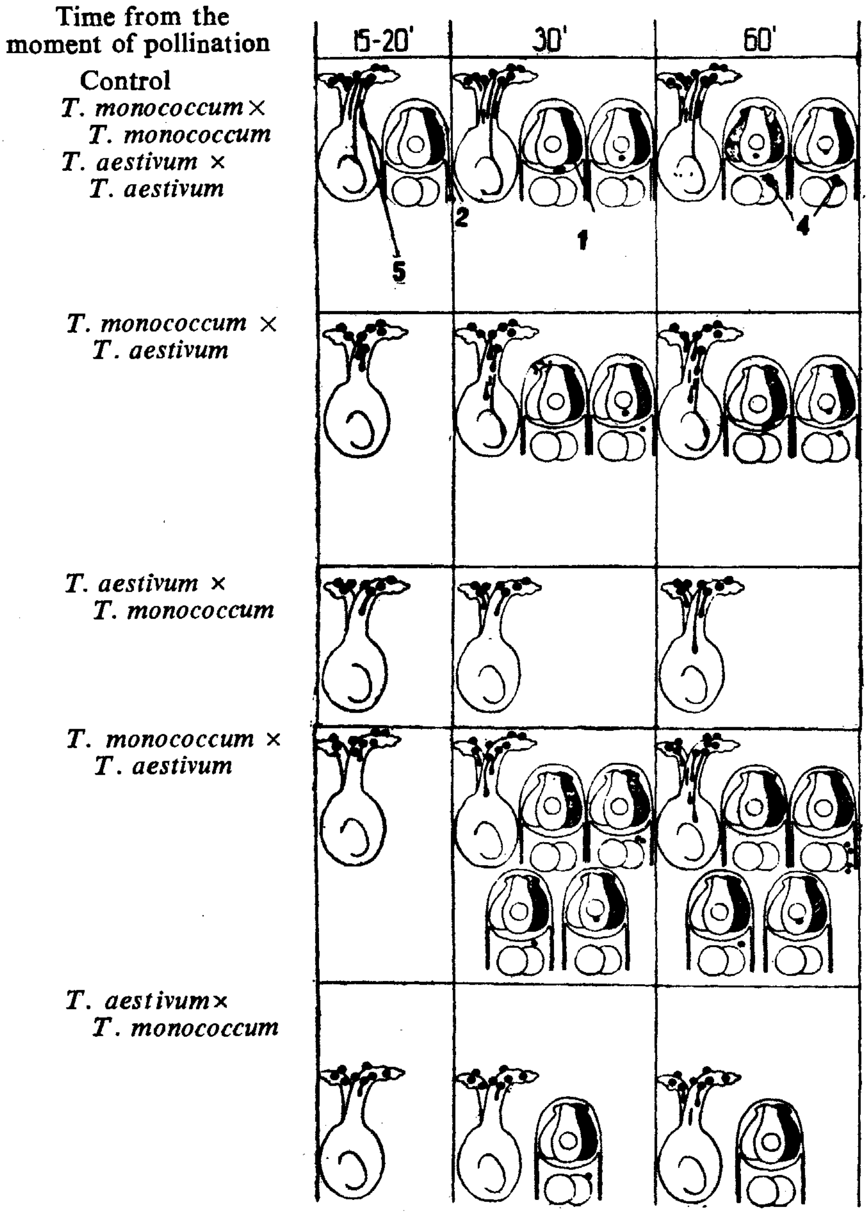
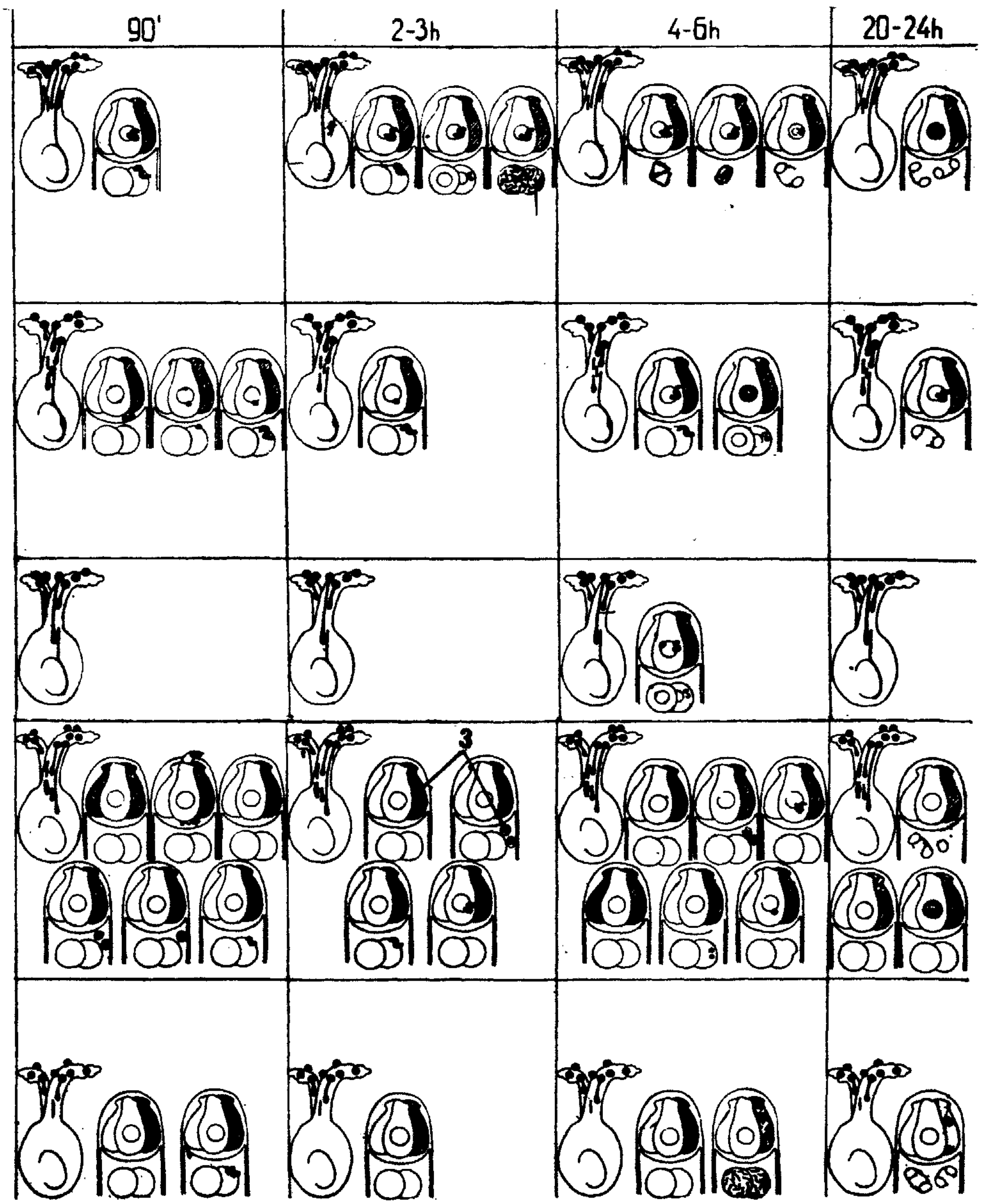


FIG. 2. Reversible (A), and irreversible (B) disturbances of fertilization process at distant hybridization in *Triticum*. 1, destroyed synergid; 2, sperm; 3, degenerated sperm; 4, 'dissolution' of sperm; 5, pollen grains and pollen tubes.


90'
2-3h
4-6h
20-24h
3

on the stigma; formation of the pollen tubes can be seen only 60-90 min after pollination; most of them end in swelling.

At distant hybridization the example of reversible disturbance may be the case when having pollinated *T. aestivum* with *T. zhukovskyi* pollen, we observed the final phase of sperm sinking into egg cell nucleus and anaphase of division in the endosperm only 10 hr after the pollen had got on the stigma. At normal pollination it takes place after 5-6 hr. Here we have a reduction in the rate of the entire fertilization process expressed in the slowdown of the pollen tube's growth and in the lower rate of sperms passing toward the female cells as well as in the lower rate of sexual nuclear fusion. However, certain rhythm of development peculiar to the fertilized egg cell and zygote and, correspondingly, to endosperm is maintained. During further development of embryo and endosperm such disturbances are likely to be levelled though the general slowing of the processes may be observed for a long time.

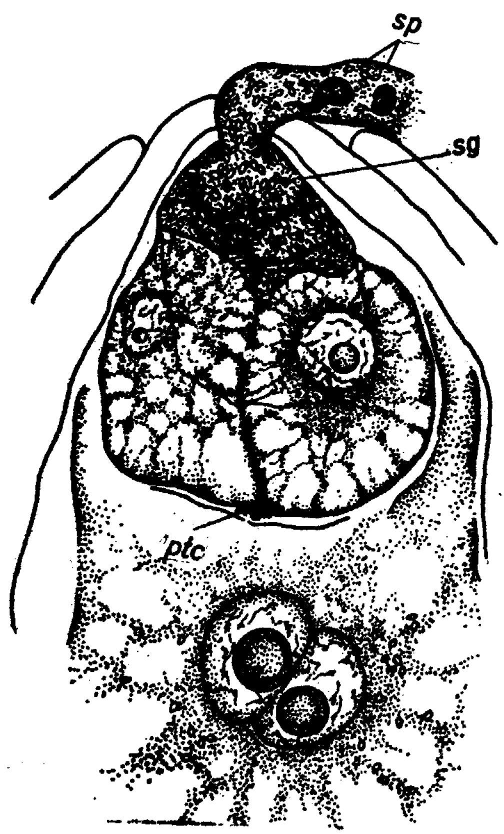


FIG. 3. Disturbance related to anomalous discharge of the pollen tube content. *sp*, the sperms ; *sg*, starch grains ; *ptc*, pollen tube content.

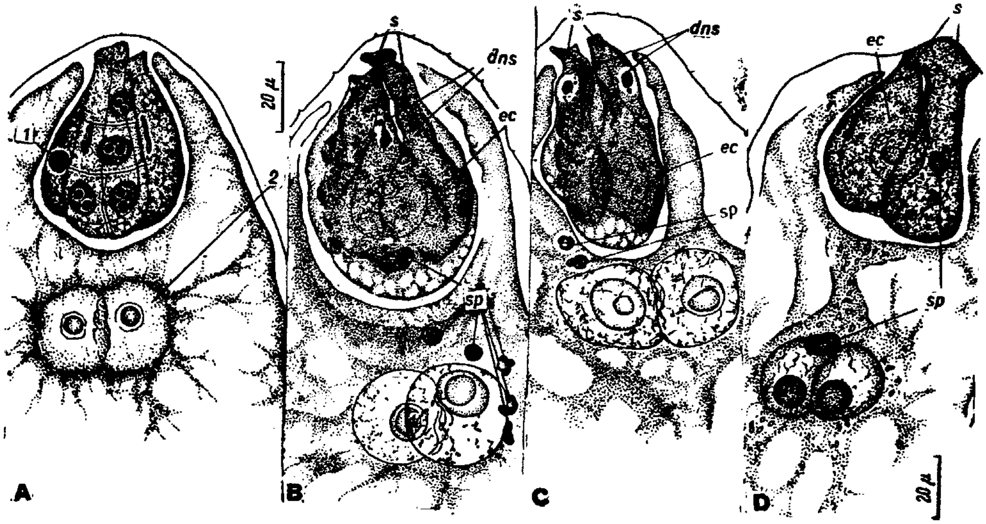


FIG. 4. A part of *T. monococcum* embryo sac pollinated with the *T. aestivum* pollen (A). 1, degenerated sperm in synergids ; 2, degenerated polar nuclei.

The apical part of *T. monococcum* embryo sac 30 min (B), 2 hr (C), 4 hr (D) after pollination with the *T. aestivum* pollen. *ec*, egg cell ; *s*, synergids; *dns*, destroyed nuclei of synergids *sp*, the sperms.

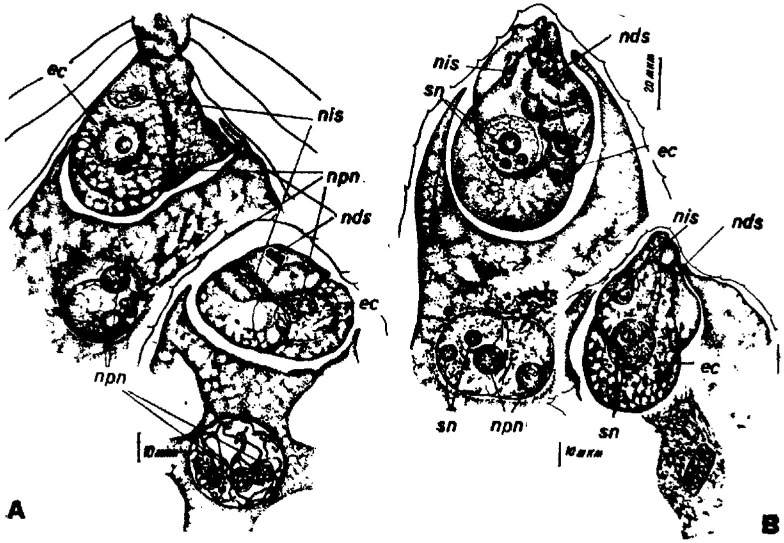


FIG. 5. The apical part of *T. aestivum* embryo sac 3 hr (A) and 7 hr (B) after being pollinated with *T. monococcum* pollen (above) and with *T. aestivum* pollen (below). *sp*, the sperms ; *nds*, nucleus of destroyed synergids; *nis*, nucleus of intact synergids ; *npn*, nucleoli of polar nuclei ; *sn*, sperm nucleolus ; *ec*, egg cell.

All irreversible disturbances can be divided into three following groups:

(i) the disturbances related to abnormal discharge of the pollen tube content into the embryo sac; e.g. part of the pollen tube content is discharged into the embryo sac and the sperms remain lying in the pollen tube at the micropyle (Fig. 3).

(ii) the anomalies related to the disturbance in the sperm-passing mechanism (Fig. 4).

(iii) the changes affecting both general course of syngamy and triple fusion and their correlation, e.g. when crossing *T. monococcum* with *T. aestivum*, the sperms on both female nuclei can be seen 3 hr after pollination, whereas at normal pollination, after 3 hr we can see only the process of the first division (Fig. 5A). A similar picture of disturbance can be seen 7 hr after pollination (Fig. 5B).

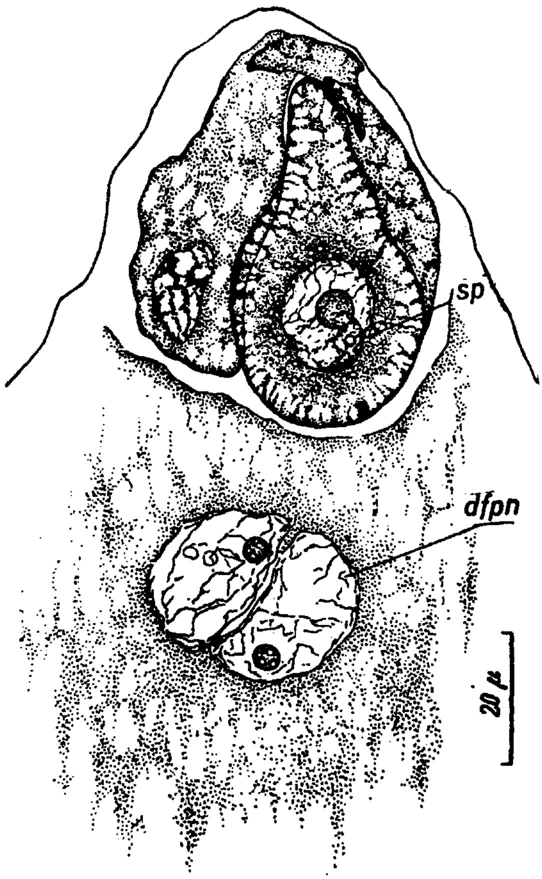


FIG. 6. A part of *T. aestivum* embryo sac 4 days after being pollinated with *T. monococcum* pollen. *sp*, the sperm on the egg cell nucleus; *dfpn*, degenerated fertilized polar nuclei.

Besides the above-mentioned reversible and irreversible disturbances, we have also come across the disturbances which are hard to refer to any of these groups. For instance, wheat has been found to have a pre-mitotic type of fertilization. However, at occasional crossings one could see the fusion of sexual nuclei which corresponds to the transitional stage of pre- to post-mitotic type of fertilization, this phenomenon occurring at various intervals after pollination (Fig. 6). The penetration of two and more pollen tubes is shown in Figs. 7A and 7B, respectively.

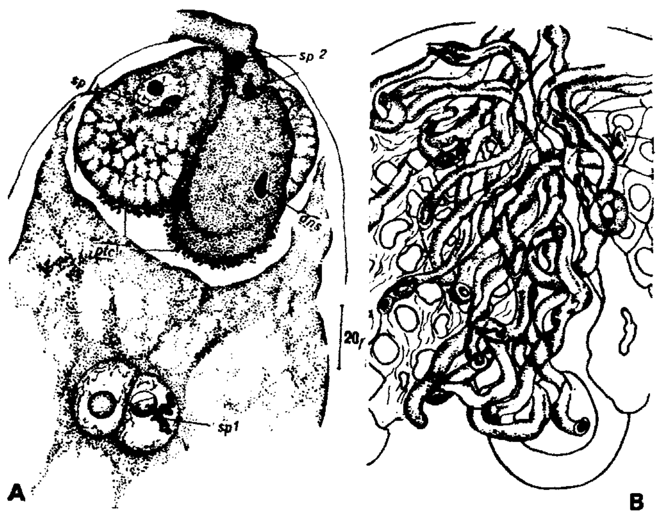


FIG. 7. The apical part of *T. monococcum* embryo sac 60 min (A) and 3 hr (B) after being pollinated with the *T. aestivum* pollen. sp^1, the sperms of the first pollen tube ; sp^2, the sperms of the second pollen tube ; *dns*, destroyed nucleus of synergids ; *ptc*, pollen tube content.

In the development of hybrid embryo and endosperm also there are two types of disturbances. The following disturbances can be referred to the reversible ones: e.g. at normal development 5-6 days after pollination there appear multicellular embryo and multicellular endosperm; whereas at distant crossings one can observe at the same moment also proembryo and nuclear endosperm; the development rate of both structures is noticeably lower but correlation is maintained. In spite of general lower rate of embryo and endosperm development, these crossings can produce seeds.

One can also refer to self-regulating reversible processes those rare instances when formation of embryo and endosperm at the first stages goes on with increased rate though certain synchronism in their development is maintained. There are data demonstrating the increased rate of embryo and endosperm development (as compared with the parental forms) at distant hybridization of other cereal species (Khukhan, 1961; Bannikova, 1963; Kandelaki, 1969, and others).

The disturbances of correlations during embryo and endosperm development are referred to the irreversible disturbances. Abnormalities of this kind are quite frequent at distant hybridization. Thus, for instance, 6 days after pollination a multicellular embryo can be observed while endosperm still remains nuclear.

When crossing *T. monococcum* with *T. aestivum* (Batygina, 1959, 1966, 1968, 1974) irreversible disturbances at which only embryo or only endosperm developed were observed (Fig. 4A).

In some cases (especially when studying early stages of hybrid embryo and endosperm development) it is hard to determine which type of disturbance is present. Besides, one type of disturbance (reversible) can often readily transform into another, irreversible one, when violation of correlation in embryo and endosperm development is very strong.

The character of anomalies found in the hybrid embryo and endosperm development should be given a detailed consideration.

In embryogenesis of various wheat species there are, as we mentioned above, two phases: blastomerization and organogenesis (Batygina, 1969, 1975). At reciprocal crossing of *T. monococcum* with *T. aestivum* disturbances in the development of hybrid embryos are possible in each of these phases. In the first phase they are comparatively rare and the process of blastomerization proceeds as usual. Changes affect here mainly the succession of blastomere divisions; there are single cases of mitosis disturbances expressed in chromatin discharges and formation of strands. More essential anomalies are found at the middle stages of embryogenesis. They are displayed in the massive violation of mitoses as well as in the

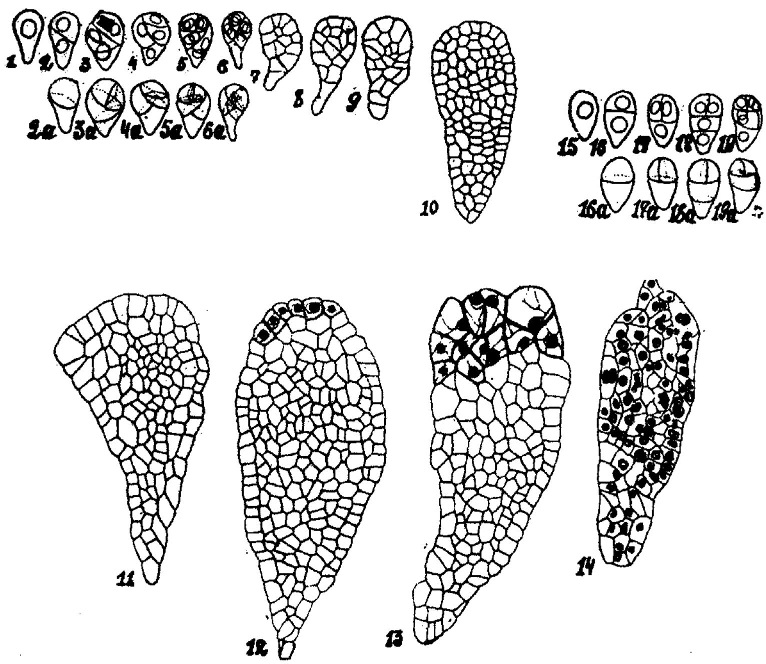


FIG. 8. Development of the *T. monococcum* x *T. aestivum* embryo : 1-14, embryo in dorsiventral section ; 2a-6a, its stereoscopic representation; 15-19, embryo in bilateral section; 16a-19a, its stereoscopic representation.

violation of division rate of cells in various embryo parts. All this results in distortion of the embryo shape and the shape of its organs, i.e. in disturbance of differentiation (Figs. 8, 9).

These anomalies are first localized in the tissue of developing scutellum and then they involve other embryo structures. When crossing *T. monococcum* with *T. aestivum*, the hybrid embryo ceases in most cases its development at the stage of transition from blastomerization to organogenesis (Fig. 8). At the back crossing, most embryos are observed to have the formation of all organs but both formation and development proceed abnormally (Fig. 9). Among most frequently found are the embryos with strongly altered scutellum which may be of various shapes and sizes. Such embryos have a violated differentiation of the conducting system. Even if the system is developing, this development is not very pronounced, only small proportions of procambial strand being observed. Formation of the vegetative point is also disturbed. It is interesting to note that abnormal embryos always have a developed root (Fig. 9).

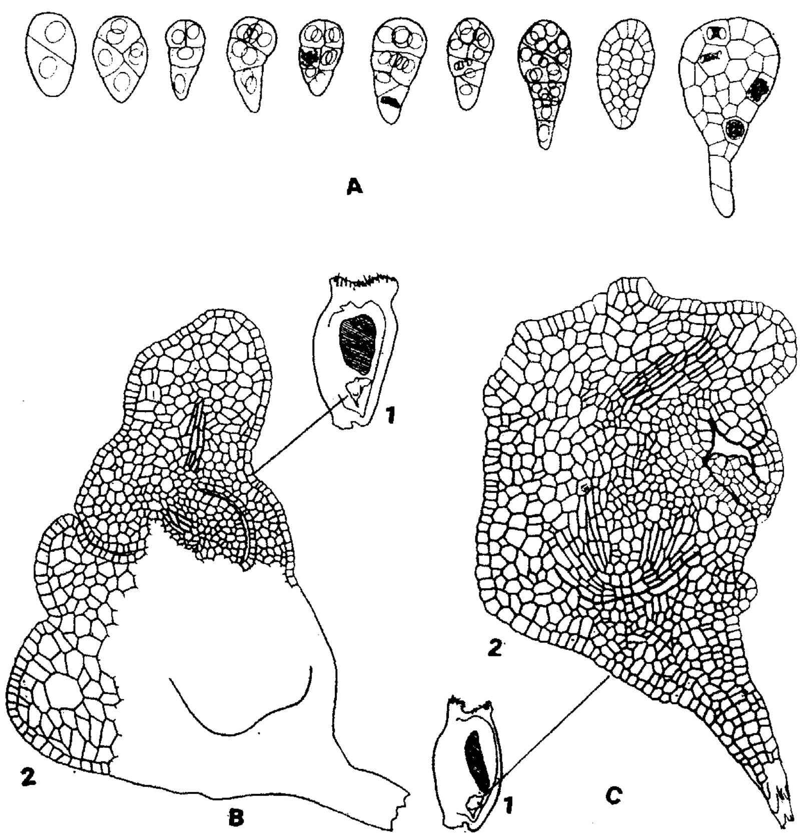


FIG. 9. Development of *T. aestivum* × *T. monococcum* embryo (A), sections in different planes : B and C, anomalous hybrid embryos produced 20 days after pollination : 1, position of embryo in caryopsis; 2, the same at higher resolution.

A specific feature of hybrid embryos both at the early and middle stages of development is the presence of large quantities of starch in their cells. In some embryos the cell nuclei are almost invisible due to the abundance of starch (this is never the case with normal embryos).

When crossing *T. monococcum* with *T. aestivum*, the disturbances are observed also in the endosperm. Hybrid endosperm is formed abnormally and appears either in the central part of the embryo sac or at its periphery or in the form of disconnected fragments. All three types of endosperm development are likely to be the result of disturbances in mitoses, synchronism of divisions and formation of cell walls which take place at the early and middle stages of endosperm development. Anomalies of mitoses in endosperm sometimes lead to formation of giant restitutional nuclei of irregular shape with a large number of nucleoli.

All described anomalies are observed more often in the combination of *T. monococcum* × *T. aestivum* (almost in every hybrid caryopsis) than in combination of *T. monococcum* × *T. zhukovskyi.*

The process of aleurone layer formation at distant crossings is worth attention. At the reciprocal crossings of *T. monococcum* with *T. aestivum*, various anomalies which take place during differentiation of aleurone layer can be observed. They are expressed in violation of all divisions, irregular formation of aleurone layer (1 to 5 rows of cells) or in its complete absence. All these disturbances are found when endosperm is both well and underdeveloped. Correlations between differentiation of aleurone layer and development of embryo at hybridization have not been found. There are cases when normally differentiated embryo is formed at the endosperm which is developed quite sufficiently but with disturbance in normal differentiation of aleurone layer; there are also cases when clearly abnormal embryo is formed in the presence of the well-developed endosperm and the well-differentiated aleurone layer. The phenomenon of formation of several aleurone layer cell rows in the area of embryo scutellum is of considerable interest. This phenomenon is significant due to the fact that in these cases it is the differentiated embryos that develop while endosperm in the area of embryo scutellum remains 'untouched'. Aleurone layer might be said to form a barrier between embryo and endosperm. Similar observations were made when crossing wheat with elimus (Ivanovskaya, 1964, 1965), and wheat with rye (Khudiak, 1963).

Thus, incompatibility at distant crossings in *Triticum* may occur at three stages : (i) interaction of pollen and pollen tubes with pistil tissues ; (ii) fertilization process (syngamy and triple fusion); and (iii) embryo and endosperm development. Incompatibility at every next stage is not always related to the character of processes at a previous stage, e.g. the good growth of pollen tubes does not always guarantee successful double fertilization.

Different character of developmental disturbances in hybrid embryo and endosperm as well as different degree of manifestation of these disturbances (e.g. in the cases of direct and back crossing) provide evidence in favour of the fact that at each crossing and in each individual plant used as a maternal form, there is formed its own specific type of interrelations between hybrid and maternal tissues. When working out techniques for obtaining viable seeds in the incompatible crossings, it is important to take into account not only the character of the disturbances observed but also the time when they take place.

The analysis of literature and the study of embryological processes in *T. monococcum* L. (n=7), *T. dicoccum* Schrank (n=14), and *T. aestivum* L. (n=21) showed high stability of principal embryological characters, and their higher degree of similarity in the representatives of this polyploid series of wheat as well as morphological similarity of distant hybridization processes in Gramineae.

Disturbances occurring during pollen germination, growth of pollen tubes, fertilization and embryogenesis process that are observed at distant hybridization of various angiosperm species and, in particular, cereals are studied insufficiently. However, facts have been found, explanations given, and hypotheses that deserve attention proposed.

Among non-crossing problems the incompatibility in size and structure of reproductive organs of the components crossed is the principal problem. Among others are differences in their genetic and physiological nature, and in the conditions under which crossings take place.

All facts presented in this paper on the causes of non-crossing of various wheat species, as well as the analysis of the works on distant hybridization in Gramineae family, indicate that this problem is a very complicated one and is far from its final solution.

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