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Experimental study of embryo differentiation in angiosperms

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Abstract

By comparison of peony and lotus embryos in *in vitro* culture we established differences in their morphogenetic potential. We traced in peony embryo culture: embryogenesis, embryoidogenesis, organogenesis and histogenesis, whereas the lotus embryo enhibited only one pathway of development — embryogenesis. At the critical stage the embryo becomes independent of the maternal organism, i.e. self-sufficient. Self-sufficiency of the lotus embryo on the 10-11th day after pollination correlates with development and greening of the first leaf in the plumule, with a sharp increase in embryo dry weight and content of carbohydrates, decrease in the size of the endosperm, degeneration of the inner integument, appearance of starch in the embryo and ovary walls an increase of its content in the outer integument.

There exists considerable factual evidence for spore-, gamete- and embryogenesis in angiosperms which led to a number of valuable conclusions concerning the evolution of plants. However, descriptive evidence cannot reveal the mechanics of single processes which constitute the complex picture of embryo development. Hence, emphasis is being shifted to experimental embryology, and to an isolated-embryo culture technique, in particular.

The discoveries of recent years, such as experimental induction of embryo (embryoid) and plant development from the somatic cell, formation of haploids and parasexual hybridization drew attention to some problems of classical embryology and raised new questions.

Of interest are the problems of: cell differentiation in embryo development, both *in vivo* and *in vitro*; research into tissue and organ determination and establishment of the morphogenetic potential of embryonal structures; investigation of morphophysiological correlations between an asexual or sexual embryo and the surrounding tissues of the maternal organism; study of initial stages of shoot formation in relation to structural and functional characteristics of the seed components; inquiry into formation of embryo structures from ecological and evolutional points of view. Solution of these problems requires different strategies of investigation as well as development of new approaches and techniques.

We consider promising the following system of approach based on description and experiment study of: 1) embryogenesis in relation to the surrounding maternal tissues; 2) embryogenesis as a dynamic process and comparison of morphogenetic kinetics with that of physiological and biochemical processes in the embryo; 3) embryo growth and differentiation regularities *in vivo* and *in vitro*; 4) study of plant species contrasting in embryogenesis type and in relationship between embryo and maternal tissues (e.g. in the number of cotyledons, cellular or coenocytic proembryo structure, presence or absence of endosperm and perisperm, etc.); 5) reproduction of conditions for each stage of embryo and shoot development, based on the evidence provided by complex morphophysiological study.

On the basis of research of the embryo morphogenetic potential in various angiosperms and morphophysiological correlations in embryo development we attempt to show the advisability of such an approach.

The objects of this research were the embryos of contrasting species: *Nelumbo nucifera* Gaertn., *Vicia faba* (L.) H. House, *Triticum aestivum* L., *Paeonia anomala* L., *P. suffruticosa* Andr., *P. lactiflora* Pall., *Dactylorhiza maculata* (L.) Soo.

Collection of our and literature evidence suggests that the principal pathways of morphogenesis for entire plant reproduction are similar both in natural and in *in vitro* conditions (B a t y g i n a et al., 1978). The

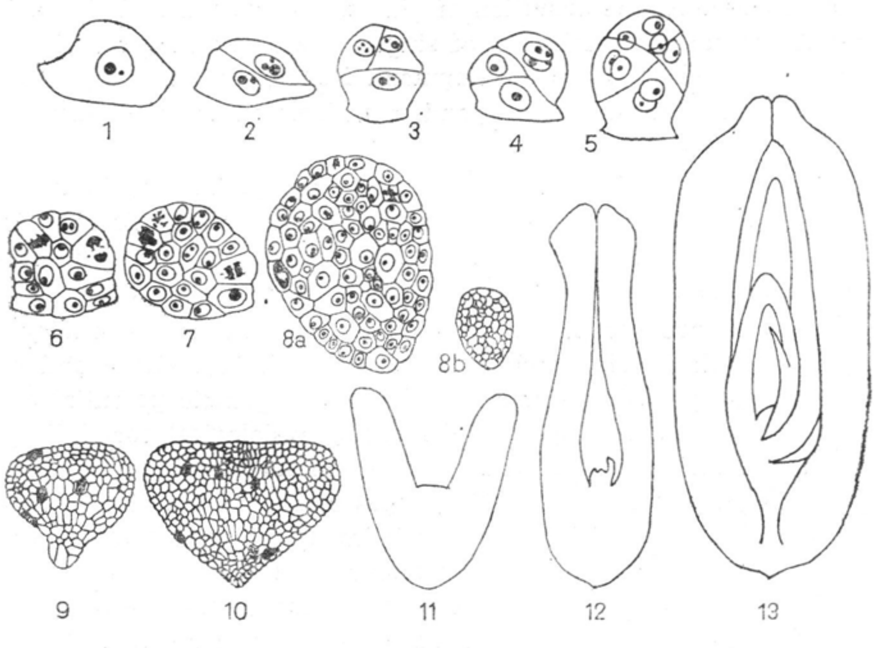

Fig. 1. Lotus embryogenesis (*Nelumbo nucifera*) *in vivo*, schematic 1-13

particular patway of morphogenesis *in vitro* depends on the plant species, embryo isolation stage and conditions of culture.

The study of peony and lotus embryos cultured *in vitro* on a wide range of nutritive media demonstrated differences in their morphogenetic potential, manifested in different pathways of morphogenesis. In peony embryos, four pathways of morphogenesis were traced, beside anomalous ones: embryogenesis, organogenesis, embryoidogenesis and histogenesis (Batygina, Butenko in press) while lotus embryos, irrespective of media composition, exhibit only one development pathway embryogenesis (Batygina, Vasilyeva, 1979), similar to that *in vivo* (Fig. 1).

During embryo development "breaking points" or "critical stages" can be observed. They are featured for each taxon by specific morphological changes: definite size and differentiation stage of embryo, definite relation between embryo and surrounding structures inside the ovule, etc. One of the important critical stages of embryogenesis is that at which the embryo becomes independent (i.e. self-sufficient) of the material organism and capable of producing a normal plant *in vitro*.

This stage represents also the specific physiological state of the embryo, one of its traits being hormonal self-sufficiency, manifested by normal development in simple medium containing mineral salts, vitamins and saccharose (Vaisilyeva, Batygina, 1981). The mecha-

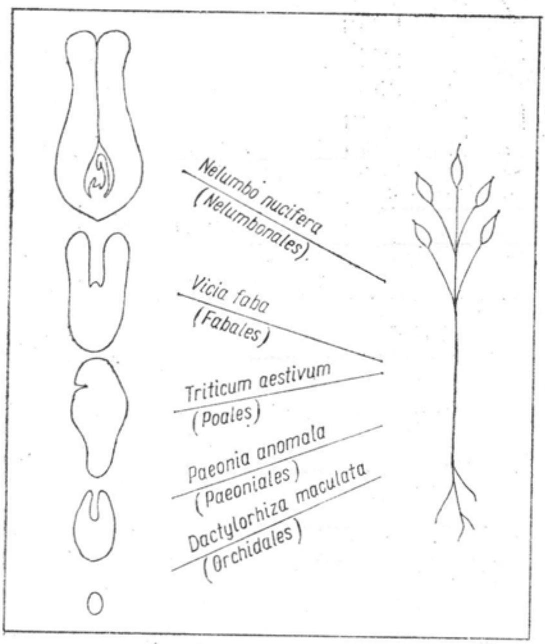

Fig. 2. Development of angiosperm embryos *in vivo* and *in vitro* — stage of embryo self-sufficiency

nisms of embryo self-sufficiency and its different, as regards time, manifestation in different angiosperms have not been so far explained.

It seems that embryo self-sufficiency becomes apparent at different stages of embryogenesis in lotus and *Vicia* at the stages of differentiated embryo with well-developed plumule: in *Triticum* at the stage of scutellum differentiation and shoot apex formation; in peony at the stage of initial differentiation of cotyledons, but without shoot apex; in orchid

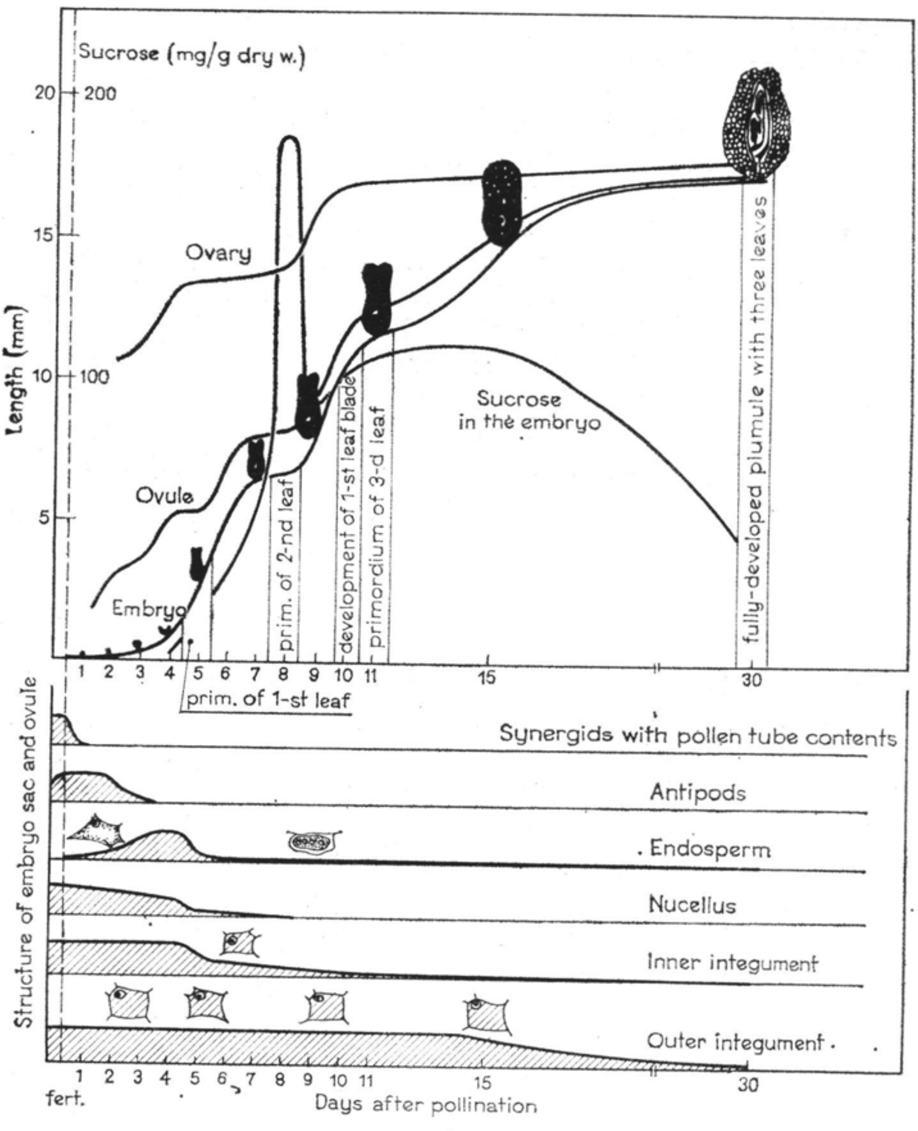

Fig. 3. Development of angiosperm embryos *in vivo* and *in vitro* — correlations of growth and development of *Nelumbo nucifera* embryo and surrounding structures *in vivo*

at the stage of proembryo (Fig. 2). Thus, the widely-held opinion that all angiosperm embryos, isolated at the stage of cotyledon and shoot apex differentiation, should exhibit normal embryogenesis and produce normal shoots, has not been confirmed.

In order to study the regularities in the development of embryo self-sufficiency, we investigated the morphophysiological aspects of lotus embryogenesis. It was found that self-sufficiency of the lotus embryo (10-11th day after pollination) correlated with the development of the first plumule leaf plate, with reduction in endosperm size and degeneration of inner integument in the ovule (Fig. 3).

The manner of variation in embryo size and dry weight during development *in vivo* indicates alternation of intensive and slow growth periods, conditioned presumably to the course of embryo differentiation. The embryo growth (mm) is expressed by the three-apex curve with maximum growth points on the 7th, 10th and 30th days after pollination (Fig. 3). Here discontinuation of embryo elongation and decrease in dry weight accumulation occurred in the embryo on the 7-8th and 10-11th days after pollination. The ovule and ovary repeated, largely, the embryo growth curve.

Biochemical analysis of carbohydrates in the lotus embryo, ovule integuments, ovary and receptacle tissues, combined with histochemical identification of starch suggest certain critical points of carbohydrate metabolism, presumably related to the differentiation stages of the embryo. Thus, the sharp increase in the content of mono- and disaccharides in the embryo and starch accumulation in the inner integument on the 7-8th day after pollination correlated with discontinuation of embryo growth, formation of the second plumule leaf primordium as well as with changes in the endosperm cell structure (Fig. 3).

At the moment of acquiring self-sufficiency (10th day after pollination), the second upsurge in the content of soluble carbohydrates was observed in the embryo, as well as the appearance of starch in the ovary walls and embryo, and an increase of starch content in the outer integuments. This was accompanied by a sharp increase in embryo dry weight and plumule greening. The latter agrees with the evidence of Yakovlev and Zhukova (1973) on formation of prolamellar bodies and initiation of chlorophyll synthesis in the lotus embryo plastids on the 10-12th day after pollination.

Changes in carbohydrate metabolism correlated with development of the first leaf plate in the embryo plumule, reduction in endosperm size, and complete degeneration of the inner integument in the micropylar part of the ovule. It should be emphasized that it is at that stage that the lotus embryo becomes independent of exogenous hormones (Fig. 4).

There are indications that the appearance of the moment of self-sufficiency depends on the plant species (Raghavan, 1976; Vasilye-

Days after pollination	3	4	5-7	8	9	10	11-30
Stage of development / Medium							
BM	no	no	no	no	no	shoot	plant
BM + IIA + A + K	no	no	no	no	shoot	shoot	plant

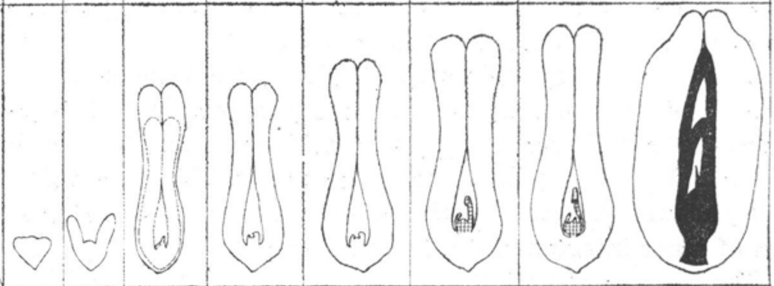

Fig. 4. Development of angiosperm embryos *in vivo* and *in vitro* — germination of *Nelumbo nucifera* embryos, isolated at various stages of development *in vitro* (the relationship between stage of development and germination)

BM — basal medium (modified Murashige and Skoog's medium); IAA — indole-3-acetic acid; A — adenin; K — kinetin

va et al., 1978; Batygina, Vasilyeva, 1979, etc.) and is presumably conditioned by differences in the nature of structural and functional interrelations between the embryo and the surrounding tissues.

The dependence of the embryo on the maternal organism has been discussed in relation to phylogeny and systematics and the type of embryo with less-pronounced development and self-sufficiency, i.e. stronger dependence on endosperm is considered as primitive (Zinger, 1958; Poddubnaya-Arnoldi, 1976). This criterion does not seem particularly clear and distinctive.

We came to the conclusion that there exists a definite genetic characteristic, i.e. the "self-sufficiency" (independence) of the embryo. This characteristic deserves special attention as it is of major theoretical and practical importance.

One cannot possibly doubt the fact that descriptive and experimental methods are combined in dialectical study of nature and lead to a more detailed knowledge of individual development processes. Thourough investigation of the regularities in embryo differentiation will help to control embryogenesis, this being a major task of experimental embryology.

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