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МОРФОГЕННАЯ МИКРОСПОРА КАК ИНИЦИАЛЬНАЯ КЛЕТКА АНДРОГЕНЕЗА *IN VITRO*: ОБЗОР ПРОБЛЕМЫ

Аннотация
Явление андрогенеза *in vitro* активно используется в биотехнологических исследованиях коммерчески ценных растений. При решении конкретных задач важно вводить в культуру *in vitro* пыльники, содержащие спорогенные клетки, морфогенетически компетентные к смене программы развития с гаметофитной на спорофитную. Анализ собственных экспериментальных и литературных данных свидетельствует, что такими клетками являются сильновакуолизированные микроспоры, обладающие признаками физиологически тотипотентных, меристематических и стволовых клеток и находящиеся в критической стадии развития. Сильновакуолизированные микроспоры следует расценивать как инициальные клетки андрогенеза *in vitro* в культуре пыльников. С позиции системного подхода дается анализ гистологического статуса стенки пыльников, содержащих инициальные клетки андрогенеза *in vitro*.
Ключевые слова: андрогенез *in vitro*; морфогенетическая компетентность клетки; сильновакуолизированная микроспора.

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MORPHOGENIC MICROSPORE AS AN INITIAL CELL OF ANDROGENESIS *IN VITRO:* REVIEW OF THE PROBLEM

Abstract
The phenomenon of androgenesis *in vitro* is widely used in biotechnological investigations of commercially valuable plants. When solving the specific tasks it is important to enter into the culture *in vitro* anthers containing sporogenous cells which are morphogenetic competent to change the development program from gametophytic to sporophytic one. Analysis of own experimental and literature data shows that such cells are strongly-vacuolated microspores which have the characteristics of physiologically totipotent, meristematic and stem cells and which are at the critical stage of development. Strongly-vacuolated microspores should be regarded as the initial cells of androgenesis *in vitro* in anther culture. By the system approach the analyses of the histological status of wall anthers containing the initial cells of androgenesis *in vitro* was given.
Keywords: androgenesis *in vitro*; morphogenetic competency of cell; microspore.

Biotechnological method of anther culture *in vitro* is widely used in modern breeding programs [8, 12, 25]. This method is based on the phenomenon androgenesis *in vitro* (or androclinia, in other term) – the formation of haploid plant-regenerant from initial sporogenous cell, which development switches from normal gametophytic morphogenetic pathway to a fundamentally different one – sporophytic under the action of external stress factors [2, 9, 23, 26, 34]. Two pathways of morphogeneis *in vitro* in anther culture are distinguished: the embryoidogenesis (plant-regenerant arises from initial cell through formation of the embryoid – embryo-like structure) and the callusogenesis (initial cell at first forms undifferentiated callus, giving the origin to plant-regenerant after the its transfer to the organogenesis induction medium) [4, 9, 17, 18, 20, 21, 28, 29, 30, 31, 33, 38, 43-45].

Despite the great strides made in the field of theoretical and applied study androgenesis *in vitro* in recent years [1, 6, 7, 8, 9, 12, 13, 14, 32, 39] continues to be an unresolved question of what anther sporogenous cell is the initial cell of this process.

Numerous studies have shown the importance of the stage of development initial sporogenous anther cell in the induction of androgenesis *in vitro.* Such observations indicate the presence of a certain critical stage in the genesis of sporogenous cell, during which it is morphogenetic competent to change the development program. In other words, as the initial cell of androgenesis *in vitro* should be considered the normal sporogenous cell in a critical stage of development. The main symptom of a critical stage

of development of such sporogenous cell should be considered to increase its sensitivity to the action of external stress factors.

Morphogenetic competence of initial cell of androgenesis *in vitro* must be determined by its toti- and pluripotency. This sign of initial sporogenous cell leads to the continuity of morphogenesis in anther culture, the multiplicity of ways and forms of reproduction and pathways of morphogenesis *in vitro*. In addition, this initial cell must possess the characteristics of a meristematic cell. The initial cell can be regarded as a stem cell, in understanding of T.B. Batygina [3].

What stage of development of anther sporogenous cell is the critical? What anther cell at this stage has signs of totipotential, meristematic and stem cell?

According to our experimental data for wheat [9, 12] and literature data for representatives of different plant families [5, 10, 11, 16, 22, 24, 27, 34, 36, 40-45 and many others] the strongly-vacuolated microspore (the term according to [19]), or late microspore (fig.) gives the origin to the morphogenic structures (embryoid or callus) in the cultural conditions *in vitro*. This developmental stage (and in this respect the period of anther development on the whole) is favourable for anther or isolated microspores inoculation with the aim to successful culture *in vitro*.

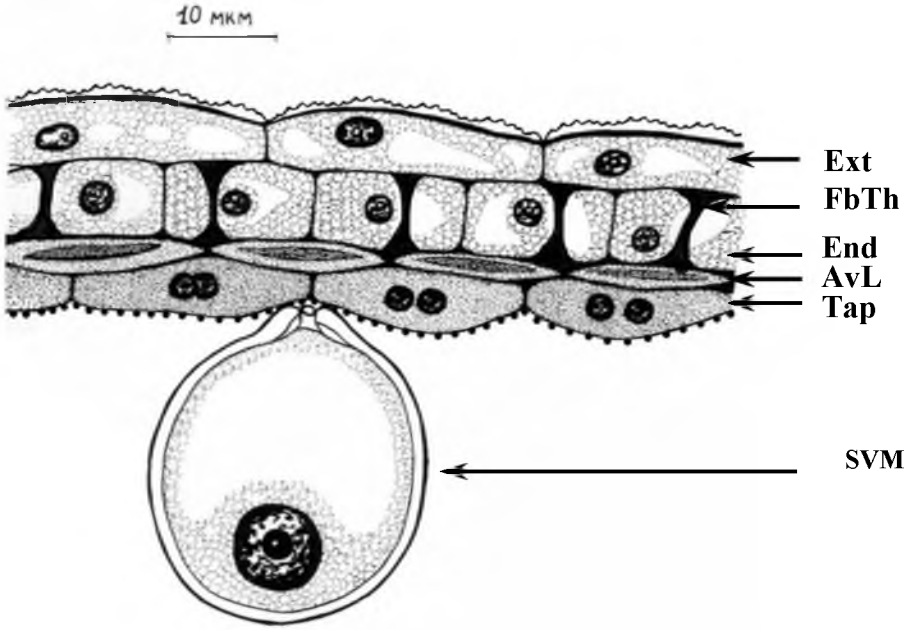

Fig. Longitudinal section of wheat anther during a critical period of development (scheme). Legend: SVM – strongly-vacuolated microspore, AvL – average layer, Tap – tapetum, FbTh – fibrous thickening, Ext – exothecium, End – endothecium (according [9])

In this connection it is necessary to discuss the terms used for the characteristic of initial microspore. So, the morphogenically competent microspores are considered so-called S-microspores from English "small" (the term was proposed by N. Sunderland [35]) and the so-called P-microspores from English "premeiotic" (the term was proposed by E. Heberle-Bors [15]). Such terms as «androgenic microspore» and «embryogenic microspore» are used also. Apparently, these terms, except the latter, have the right to existence. The term «embryogenic microspore» causes serious objections. It is commonly known, embryogenesis is the process of embryo development formed by a way of amphimixis. The embryogenic microspore in comprehension of the authors of the term gives origin to either embryoid or callus, therefore would be more correct to call it either embryoidogenic or callusogenic. However, at the stage of microspore it is impossible to determine the pathway of morphogenesis *in vitro* – embryoidogenesis or callusogenesis – of this cell (if it will enter on the pathway of morphogenesis *in vitro*). Therefore, we offer to use the term «morphogenic microspore».

The term «late microspore» is used by researchers. It is necessary to explain this term. The concept «late» is rather subjective and in such periodicity there is no precise criterion. On the basis of presence or absence of vacuoles, the degree of their expressiveness and also location of nucleus concerning the germ pore at the stage of «microspore» we offer to distinguish the following phases: an unvacuolated microspore (nucleus occupies the central part of cell, vacuoles are absent),

a feebly-vacuolated microspore (the nucleus occupies various positions concerning of germ pore, in the cytoplasm there are small number of fine vacuoles), a strongly-vacuolated microspore, or the microspore with a central vacuole (fine vacuoles are merged in a single large vacuole, the nucleus is posed on the party opposite to germ pore).

In our view, the frequently used terms «uninuclear microspore» (it is a tautology) and «uninuclear pollen grain» (such phase in pollen grain development is not present) are incorrect.

The problem of the morphogenic structure formation in cultured anthers is connected to the decision of a problem of «switching» of microspore development from its usual gametophytic way to essentially other that is a sporophytic way of development. Already at early stages of work on the study of androgenesis *in vitro* the idea about existence of the special fraction of morphogenically competent microspores which are capable to develop on sporophytic way has arisen [35]. The question of whether the competence to sporophytic development appears only under the conditions *in vitro* or morphological equivalents of competent microspores are various anomalous microspores already presented in anthers *in vivo* up to culture is not unequivocally yet decided, though there are many data for the benefit just of last assumption [12].

The ability of the morphogenic strongly-vacuolated microspore to change the development program under the influence of the inducing stress factor (in other words, the morphogenetic competence of such microspore) is determined, in our opinion, by several circumstances.

The first of these is the premitotic state of a cell that indicates an unstable status of strongly-vacuolated microspore.

The second of these is the structural similarity (namely the presence of a large nucleus and well-developed vacuole as well as the polarity of cell) of strongly-vacuolated microspore and plant egg cell giving the zygotic embryo in a case of amphimixis and cells of embryo sac, nucellus and integument forming the adventive embryo in a case of apomixes. It is possible to think that the structure of all initial cell giving origin to new plant organism is universal despite the specificity of reproduction systems. Thus there is a homology of the initial cells in various systems of plant reproduction [3].

The second of these is the high level of transcriptional activity of the nucleus of strongly-vacuolated microspore (e.g. [37]).

All these circumstances characterize the strongly-vacuolated microspore as a very active and «intense» system. The action of an external stress factor able to break the «dynamic equilibrium» of such a system and to induce sporophytic development of cell.

Unfortunately, the researches including the data on wall status of anther which contains the strongly-vacuolated microspores are rare. Meanwhile, in our view, taking into account the approach to anther as a complex integrated system, such data is absolutely necessary. It is important to take into account the particularities of separate tissues of the anther loculus wall, their interaction with each other and with strongly-vacuolated microspores during the inoculation of anthers into nutrient media. Besides, the processes of embryoid and callus development depend on the status of anther loculus wall tissues in the course of culture. According to our data (fig.) on wheat, the beginning of degeneration of tapetum cells and average layer cells, the beginning of formation of fibrous thickenings in endothecium cell walls and eginning of cutinization of exothecium cell walls are characteristic at the moment of the inoculation of anther in wheat [9, 12].

In general, the role of somatic tissues in the induction or in inhibition of androgenesis *in vitro* is obvious. However this major problem is rather far from the final decision. In this connection it is necessary to develop a prospective approach to cultured anthers as to the complex integrated systems. All these questions discussed above need a detailed knowledge of the object investigated from the viewpoint of the system approach. Perhaps, the optimal stage (phase) for obtaining haploids will be determined by a complex of factors and in a certain sense is species-specific. One of the reasons for species-specification is a different type of anther wall formation and because of this its various structures at one and the same stage of the microspore and pollen formation, a different type of tetrad formation (successive or simultaneous) and so on.

Morphogenic microspore can serve as a convenient model system to study some discussion problems of plant development. One of such problems is the presence of plant stem cells. As suggested by T.B. Batygina [3], microspore capable of transition from normal gametophytic way (the formation of pollen grains with sperms) into sporophytic one (the formation of plant), in other words, capable to switch of reproductive pattern from sex into asex, is the stem cell. Besides, morphogenic microspore has such properties of the stem cell as the toti- and pluripotency, namely the ability to form different types of tissues and organs, but also new individual organism due to different pathways of

morphogenesis *in vitro*. We subscribe to this view and believe that the property of the stem microspore is most convenient to study in controlled conditions of culture *in vitro*.

Conclusion

Thus the analysis of our experimental and literature data shows that the strongly-vacuolated microspore is the initial cell of androgenesis *in vitro* in plants of different families. This cell has the characteristics of physiologically totipotent, meristematic and stem cell and is at the critical stage of anther development. By the system approach the analyses of the histological status of wall anthers containing the initial cells of androgenesis *in vitro* must be given.

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