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MORPHOGENESIS PATHWAYS AND REGENERATION OF PLANTS FROM FLOWER ORGANS *IN VITRO* IN SOME REPRESENTATIVES OF *ASTERACEAE*, *CAMPANULACEAE* AND *ORCHIDACEAE* FAMILIES

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Universality of morphogenesis pathways in flowering plants, both *in vitro*, and *in situ*, allows us to use the reproductive structures of plants cultivated *in vitro*, as the models for experimental study of the morphogenetic potential of cells in various parts of a flower.

A special role is given to the micropropagation techniques that are most effective in renewal of the population of natural species and especially for accelerated breeding valuable genetic combinations and varieties (Batygina et al., 2007, 2010).

Objects of study - representatives of three families of flowering plants, growing in the Botanical Garden BIN RAS: *Helianthus tuberosus* L. (Asteraceae), *Campanula bononiensis* L., *C. mirabilis* Albov (Campanulaceae) and *Cynorkis seychellarum* Aver., *Microterangis hariotiana* (Kraenzl.) Senghas (Orchidaceae). We used separate flower organs - stamens, ovary, petals and sepals as explants, as well as intact flowers at different stages of development. They were cultivated on Murashige-Skoog and Fast mediums with the addition of BAP and NAA (Teplitskaya, 2007; Pimenova, Andronova, 2008).

These species revealed different ability to callus formation. Most actively callus was formed in *Campanula* and *Helianthus.* After a short cultivation of separate stamens at Campanula bononiensis we observed endogenous thickening in the sporogenic part of anther lobes. One month later, under the influence of rapidly dividing cells of the callus, the anther wall was torn in areas of thickening. In contact with the nutrient medium this callus (first type callus) formed friable aggregates of large (up to 130-230 microns in diameter) round cells. These cells had the thin transparent plasmolemma, large central vacuole and well distinguishing nucleus. Some peripheral cells of the callus conglomerate after prolonged cultivation on the nutrition medium elongated and formed short chains of cells. In this case the cells were 50-65 microns in diameter and 110-230 mm in length. In the most of these chains a regular branching were observed, in some cases dichotomous one.

Cytological studies have revealed that the first type of callus was formed from immature highly vacuolated microspores. This process was similar to the obtaining androclinous callus from immature microspores *in vitro* as a result of switching over morphogenesis program from sporophyte to gametophyte in wheat (Batygina et al., 2010). However this type of callus in *C. bononiensis* had another pathway of morphogenesis: vacuolated microspore divided after destruction of its wall, whereas in wheat the morphogenic callus formed from microspores divided inside the wall (Batygina et al., 2010). In *C. bononiensis*, on the contrary, this type of callus was not of morphogenic type and displayed no ability even to histogenesis during more than 10 months of cultivation *in vitro*.

The second type of callus was obtained from cambial tissues of the stamen filaments, the base of the style, sepals and stems of *C. bononiensis*, which were cultivated in isolation from other parts of the flower. This callus was consisted of smaller cells with thick walls. The chlorophyll and anthocyanin pigments were presented in these cells. One month later, the second type callus manifested distinct ability to histogenesis. The cells of the peripheral parts of callus formed a looser tissue from relatively larger parenchymal cells, but in the central zone they formed tissue with smaller cells, and the numerous vascular tissues elements were differentiated from them. Similar features were founded in other flowering plants (Teplitskaya et al., 2010).

During cultivation of isolated flower buds of *C. mirabilis* their complete opening and prolonged flowering *in vitro* (in a Petri dish) were observed. Herewith self-pollination did not occur, probably because in nature *C. mirabilis* is a strongly cross-pollinated. After flowering, on the 18-20th day of cultivation, the callus formation was observed in the base of sepals and on the top of the ovary. Like the first type of callus in *C. bononiensis*, the callus of *C. mirabilis* was also consisted of conglomerates of large parenchyma cells. However, its cells had not transparent cell wall. After reaching this callus a certain critical mass (5-7 mm in diameter) an active gemmogenesis began in its peripheral parts. In the course of 2 next weeks from 20 to 45 vegetative shoots were formed on the each conglomerate. Rhizogenesis began much later - on 2-3rd month after the formation of callus. Multiplication coefficient was 103 for the 10 months.

During the cultivation of *H. tuberosus* intact flowers callus formation from the microspores, from tissue of stamen filaments near the connective, from the base of style in the area of nectaries and from the sepals and petals of corolla was observed. As in *C. bononiensis*, the first type of callus from immature microspores in *H. tuberosus* formed

conglomerates of large parenchymal cells with transparent plasmolemma. In most cases, they were surrounded by the smaller and rapidly dividing cells of callus second type which was formed from anther wall. Callus of the same type in *H. tuberosus* and *C. bononiensis* showed identical ability to histogenesis.

In the orchid species studied, despite the prolonged cultivation, we failed to obtain callus from anthers and perianth petals of blossoming flowers and buds, before opening. Most likely, it was due to extreme protandry of flowers and deep specialization of perianth elements in orchids. In *C. seychellarum*, for example, the anthers opened even in buds and massula reached the stigma a week before flowers blooming. It is known that for orchids of temperate zone the mononuclear microspore stage is also critical for introducing anther in culture *in vitro*, but it occurs long before flowering (Teplitskaya et al., 2010). High degree of aggregation of the pollen in the pollinium can be another factor preventing to the successful cultivation of anthers.

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