# CALLUSOGENESIS AND MORPHOGENESIS IN THE CULTURE OF ISOLATED ORGANS AND TISSUES OF *MELISSA OFFICINALIS* L. *IN VITRO*

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*Melissa officinalis* L. – is a perspective essential oil, medicinal and spice-taste plant, widespread in many countries of the world. To improve the efficiency of breeding it is appropriate to use biotechnological methods for obtaining a new initial material, as well as accelerate its propagation. Such cell technologies are based on obtaining of callus cultures

and induction of morphogenesis. In this work we investigated the influence of some factors (donor plant origin, the time of introduction to the culture, the hormonal composition of the nutrient medium, seedling age and type of explant) on the induction of callus formation and morphogenesis in melissa. The materials for the study were *M. officinalis* L. plants varieties 'Citronella'. Explants were isolated from seedlings, developing from the seeds *in vitro* ("plants *in vitro"*), and from plants of glass-covered ground ("plants *in situ"*). Sterilization of plant material was performed using 70% ethanol (1 min) and 50% antiseptic solution "Bradofen" (4 min). As a result of researches the regimes of obtaining callus culture from different types of explants (leaf, stem, petiole and hypocotyl and cotyledonary leaves of seedlings) were chosen. Callus obtained from the different types of explants of melissa had morphological differences. The maximum frequency (up to 70-95%, depending on the type of explant) and intensity of callus formation were on MS medium supplemented with 1.0 mg/l NAA (or 2,4-D) and 0.5 mg/l BAP. Better ability for proliferation in isolated culture on the majority of tested nutrient media showed explants of leaf and stem of the plants or seedling hypocotyl. The frequency of callus formation for explants of leaf and petiole was up to 1,5-2,9 times higher than that of the stem. The influence on the callusogenesis the origin of the donor plants, which were used as a plants, were grown in a glass-covered ground, and the test-tube plants (30-35 days old), obtained from the seeds *in vitro*, was investigated. For explants from "plants *in vitro*" in many variants of experiment the indicators of callus formation were 2-2.5 times higher than that of the explants from "plants *in situ*". On the optimal MS medium the frequency of callus formation from segments of leaves and stems of "plants *in vitro*" was respectively 80.0 and 85.0%, whereas for these explants, isolated from "plants *in situ*", this indicator was respectively 46.4 and 25.0%. It was found that in the winter (January - February) the frequency of callus formation on the majority of nutrient media from explants of leaf, stem and petiole were 1.5-2 times higher than in the summer (July - August). The possibility of using for the callus induction the explants of cotyledonary leaves and hypocotyls, isolated from seedlings, was investigated. In this experiment the seedlings of different ages (4-, 8- and 12-days-old), obtained from the seeds *in vitro*, were used. It has been found that the formation of callus occurred only from organs, excised from a young seedlings (4- and 8-days- old). Callusogenesis was observed with the highest frequency (up to 78.5 – 95.0%, depending on the explants) on the same modifications of MS medium (with BAP and NAA or 2,4-D), which we previously chosen for the cultivation of other explants of melissa. When cultured cotyledonary leaves of 4-day-old seedlings on the mediums with cytokinins the direct morphogenesis was induced. However when cultured hypocotyl the morphogenesis on any of medium was not observed. The maximum frequency of the direct morphogenesis ( 23,1%) was observed upon addition in the medium 1.0 mg/l kinetin. With small frequency this process was also marked from cotyledons of 8-day-old seedlings, but on a medium with 2,4-D and BAP. During the induction of morphogenesis at the surface of explants the buds and shoot development were found. For explants of more age seedlings the direct shoot formation was not revealed. With the aim of developing a method of clonal micropropagation for melissa the peculiarities of morphogenesis when cultured stem nodal segments (isolated from *in vitro* seedlings) on nutrient media of different composition were investigated. It

was shown the advantage of introduction in the nutrient medium kinetin, which provided a high frequency of multiple shoot formation (up to 86,9 ± 7,1%) and development of shoots with a length up to 41,1 ± 0,4 mm. This allows to use for micropropagation of melissa two methods – induction of multiple shoot formation and micro cuttings of shoots.

***Keywords****: Melissa officinalis* L., callusogenesis, morphogenesis, micropropagation, *in vitro*.

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