Probl Cryobiol Cryomed 2020; 30(3):287
https://doi.org/10.15407/cryo30.03.287

Прояв морфогенетичних реакцій калусної культури пшениці озимої за тривалого впливу низької температури

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Morphogenetic Responses of Winter Wheat Callus Culture Under Prolonged Period of Low-Temperature Exposure

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Temperature as one of the most important exogenous factors determines the plant morphogenesis program [Ivanova, 2014]. For winter wheat, a prolonged period of a low-temperature exposure, *i. e.* vernalization is required to implement the morphogenetic development and to go into the generative stage of ontogenesis [Dennis, 2009]. Currently, *in vitro* culture methods are widely used to study the plant development, in particular that of soft wheat [Khlebova, Nikitina, 2016; Dubrovnaya *et al.*, 2014]. The effect of duration of the low temperature exposure on the plant *in vivo* and culture *in vitro* may play a key role in the morphogenetic responses. In this regard, the purpose of the study was to investigate the effect of different periods of the low-temperature exposure (15, 30, and 45 days) on the winter wheat morphogenesis *in vitro*. The research object was the callus culture of the second passage of three wheat varieties, *i. e.* Doridna, Statna and Astet. The low-temperature exposure was performed during 15, 30 and 45 days at (4 ± 1)°C in a refrigerator in the dark, and the control variant was accomplished at 26°C in the thermostat in the dark. After 15, 30, 45-day exposure the calluses were cultured with Murashige and Skoog's (MS) medium with adding the growth stimulators, namely the synthetic phytohormones 6-BAP and 1-NAA in a concentration of 3 and 0.5 mg/l, respectively. Cultivation was carried out under 2 klx intensity of illumination and temperature of (22 ± 1)°C during one month. On day 28, the direction of the morphogenetic reactions was analyzed. There are the processes of hemogenesis, rhizogenesis, chlorophyllogenesis. According to the results of experiments, it was shown that after 15 days of low-temperature exposure in all the control plants, except Doridna, and experimental variants, greening of the callus tissue was found, a phenomenon of chlorophyllogenesis. The latter was appeared as the formation of green roots, as well as the general greening of callus tissue due to an active biosynthesis of the main photosynthesis pigment, chlorophyll and the formation of chloroplasts. The maximum index was a feature of Astet and the minimum was found for Doridna. The efficiency of hemogenesis in the calluses of the experimental variants was higher than in the control ones, except Astet. There was an active rhizogenesis, especially in all variants of Statna and Astet varieties, regardless of the experimental conditions. After 30 days of low-temperature exposure, the frequency of chlorophyllogenesis and hemogenesis increased in Statna and Astet varieties. After 45 days, the callus of Statna formed the above-ground organs, *i. e.* coleoptiles and primary leaves. In general, the prolonged low-temperature exposure stimulated the formation of meristematic cells and roots, that is, the morphogenetic development of callus culture of the studied varieties of winter soft wheat.

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https://doi.org/10.15407/cryo30.03a.287

Вплив розчинів диметилсульфоксиду і температури інкубації на функціональну активність окремих популяцій клітин наднирників щурів

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Influence of Dimethyl Sulfoxide Solutions and Incubation Temperature on Functional Activity of Individual Adrenal Cell Populations of Rats

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Mammalian adrenal glands are represented by different cell types, synthesizing and secreting the vital hormones. The whole cell composition of the adrenal glands and their isolated populations are used to solve various problems of cell biology [Saito *et al.*, 2016] and transplantation [Balyura *et al.*, 2018]. Therefore, the establishing of low temperature banks of adrenocytes as accessible sources of cells is relevant. To cryopreserve the whole suspension of cells or the ones of adrenal cortex, the dimethyl sulfoxide (DMSO) is used [Ustichenko, 2008; Dudetskaya *et al.*, 2010; Yurchuk, 2016]. However, DMSO in its various concentrations has not only a protective but also a toxic effect on biological objects. The temperature of the incubation medium influences the transport rate of substance molecules through the cell membrane.

The aim of this research was to investigate the influence of the solutions with different concentrations of DMSO and incubation temperature on functional activity of individual adrenal cell populations of rats.

Adrenal cell populations were obtained in a density gradient of ficoll by the method [Dudetskaya *et al.*, 2016]. Three main populations were obtained: the fasciculata-reticularis zone cells, the ones of glomerulosa zone and medulla zone. DMSO was used as a cryoprotectant; final concentrations of 5, 7 and 10% were achieved by adding to the cell suspension a twofold concentration of a cryoprotectant solution prepared with DMEM supplemented by 20% calf serum. The saturation/removal of the cryoprotectant was carried out at temperatures of 0–4, 22 and 37°C. The samples were incubated for 20 min at the appropriate temperature. The percentage of cells with an intact membrane was determined by supravital staining using a 0.4% aqueous trypan blue solution [Strober, 2001]. The cell functional activity was estimated using the MTT assay [Ferrari, 1990]. The highest number of cells with intact membrane and high functional activity was detected for the zona medulla cells using the solutions of 7 and 10% DMSO at 37ºC. For the zona glomerulosa cells that was noted with 5% DMSO at 22°C. For the zona fasciculata-reticularis cells this was 7% DMSO at 0–4 and 22°C. The medulla cells at physiological temperature were found to be less sensitive to an increase in the concentration of DMSO in a cryoprotective medium. For the cells of the fasciculata-reticularis and glomerulosa zones, the cytotoxic effect of cryoprotectant reduces with a decrease in temperature and the DMSO concentration in the cryoprotective medium. The data on the permeability of adrenal cell membranes, which were obtained earlier [Dudetskaya, 2011] support our assumptions.