AS-120 TTC TEST FUNCTIONALITY IN EVALUATION OF SURVIVING OF GENTIANA PEM AFTER CRYOPRESERVATION

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Introduction: The cryopreservation method developed numerous tests helping to describe the survival of low temperature treated plant material. The best test concerns the ability of plant material undergo cell division after freezing when cultures are implanted on agar regeneration medium. There are biochemical tests based on colour reaction of the formozan obtained from the TTC and fluorescence of FDA for estimation of freezing injury. The aim of the paper is the evaluation of usefulness of TTC test for estimation of freezing injury of the gentian cell suspension.

Material and Methods: Experiments were carried out on the cell suspension of Gentiana tibetica King. and G. kurroo Royle. Before freezing the cell suspensions were cultured on standard medium. A few combinations of tissue pre-treatment before freezing were employed in these experiments: a) with vitrification solution, b) with sorbitol, c) with sorbitol + DMSO, d) with sorbitol + proline and e) control - without treatment

Results and Conclusions: The viability of cell suspensions after freezing was strongly correlated with the time of TTC testing and the level of cryoprotection efficiency. The highest level of TTC testing always time connected with direct defreezing of the tissue. Both programmed and direct freezing of tissue in LN2 without cryoprotectant treatment resulted in a large destruction of organelles of the cell which was proved by the TEM analysis. In those cells the dehydrogenases were active in the following times: 1h (3% sucrose pre-treatment), 5 hrs (6% sucrose) and 24 hrs (9% sucrose). Plasmolyzed cultures presented high level of cryoprotection efficiency directly after freezing, however the hours followed it decreased according to the passing time of the experiments. Two-day long sorbitol pre-treatment with DMSO or proline resulted in the restoration of the whole culture from the cells which were able to survive the freezing procedure. The dehydrogenase activity in the cells protected by DMSO decreased directly after thawing for 50% of the control culture and 77% after 24 hrs. Finally its level reached only 3%. The highest level of dehydrogenase activity was expressed by cells treated with vitrification solution, which resulted in quick restoration of cell viability and after 48 hrs the enzyme activity get the level of 100%.