Probl Cryobiol Cryomed 2020; 30(3):289
https://doi.org/10.15407/cryo30.03.289

Особливості впливу кріоконсервування на структурні та функціональні характеристики мононуклеарів кісткового мозку з метою одержання з них *ex vivo* дендритних клітин

Г.Г. Кісєльова, А.М. Гольцев,
Т.Г. Дубрава, К.Є. Ямпольська
Інститут проблем кріобіології і кріомедицини НАН України, м. Харків, Україна

Peculiarities of Cryopreservation Influence on Structural and Functional Characteristics of Bone Marrow *Ex Vivo* Derived Dendritic Cells

G.G. Kisielova, A.M. Goltsev,
T.G. Dubrava, K.E. Yampolska
Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine

Tolerogenic properties of dendritic cells (DCs) are of a particular interest worldwide when searching the novel approaches to treat the autoimmune diseases (AIDS). Taking into account that cryopreservation of DC is a mandatory step during biotechnological process to use them in clinical practice the search for adequate freezing methods are still in progress. Cryopreservation either peripheral blood or bone marrow (BM) of mononuclear cells (MNCs) to obtain *in vitro* the tolerogenic DCs (tolDCs) has been shown to be more effective if compared with freezing of only the DCs. Therefore, there is a need to optimize the methods of MNCs cryopreservation to ensure the tolerogenic potential of the DCs generated from them, namely, the ability to stimulate T-regulatory cells (T_{regs}) with a suppressive activity against autoimmune processes in the recipient body.

The aim was to investigate the peculiarities of the influence of different cryopreservation regimens on structural and functional characteristics of DCs.

Experiments were performed in CBA/H mice. Two regimens of cryopreservation of BM MNCs were analyzed: 1 deg/min down to –80ºC (R1); 1 deg/ min to –40ºC (R2) followed in each case by an immersion into liquid nitrogen (–196ºC) under the protection of 10% DMSO. TolDCs were obtained in culture from the cryopreserved according to different regimens MNCs (cryoR1MNC, cryoR2MNC) according to the conventional protocol. The tolerogenic potential of DC was studied *in vitro* for its ability to stimulate T_{reg} ($FOXP3^+$) cells.

The most effective mode of cryopreservation of MNCs (R2) was determined, which provided their maximum number, safety, metabolic activity and content of $CD14^+$ monocytes, *i. e.* the DC precursors. In DCs obtained from cryoMNCs, a decrease compared to the native control of the expression of co-stimulatory molecules CD80, CD86 and an increase in the number of cells on their background, with the integrin marker CD11b, indicating the support of their immature phenotype. DCs derived from cryoR2MNCs had the most pronounced ability to stimulate T_{regs}, which increased their content in 5 times in comparison with DCs obtained from native MNCs and in 1.5 times in comparison with those obtained from cryoR1MNCs.

Probl Cryobiol Cryomed 2020; 30(3):289
https://doi.org/10.15407/cryo30.03a.289

Посівна якість насіння гібридів спаржі лікарської (*Asparagus officinalis* L.) після низькотемпературної та гідротермічної обробки

О.В. Лялюк[1], Т.В. Івченко[1],
Н.О. Шевченко[2], Т.Ф. Стрибуль[2]
[1]Інститут овочівництва і баштанництва НААН України, сел. Селекційне, Харківська обл., Україна
[2]Інститут проблем кріобіології і кріомедицини НАН України, м. Харків, Україна

Sowing Quality of *Asparagus Officinalis L.* Hybrid Seeds After Low-Temperature and Hydrothermal Treatment

O.V. Lialiuk[1], T.V. Ivchenko[1],
N. O. Shevchenko[2], T. F. Stribul[2]
[1] Institute of Vegetable and Melon Growing of the National Academy of Agrarian Sciences of Ukraine, Selektsiine village, Kharkiv region, Ukraine
[2] Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine

Stimulating and increasing the field germination of seeds, activating the growth of the root system and enhancing the productivity are the actual tasks, which can be solved by priming the seeds of Asparagus officinalis L.

The aim of this research was to determine the influence of low-temperature and hydrothermal treatment, solutions with succinic acid and microelements on seed germination and yield of asparagus hybrids.

Samples of Atlas-15 and WB 210-15 hybrids were cooled in 1.8 and 50 ml polypropylene centrifuge containers down to –70 or –196°C and then heated on air at 22ºC, or soaked for 24 hours in water, in solutions of microelements according to Murashige&Skoog (1–3 mg/l) or succinic acid (1–3 mg/l). The treated seeds were germinated in a thermostat at a temperature of 26...30ºC and a humidity of 90%. The non-treated seeds were used as the control.

Various low-temperature treatments did not affect the germination. Yield study after 120 days of growing the Atlas-15 seedlings obtained from the seeds cooled to –70°C in 1.8 ml containers showed a bigger number of shoots (10.5), their weight (17.5g) and roots (45.0g) compared with the control (7.4 units, 10.5 and 29.5g, respectively), other variants did not differ significantly by certain indices from the control values. Seeds of the WB 210-15 hybrid responded with increasing all the seedling yields in the freezing version to –70°C in both types of containers, while freezing down to –196°C inhibited the development of asparagus seedlings in the 50 ml containers and did not change in the 1.8 ml ones.

When treating the seeds of Atlas-15 and WB 210-15 hybrids with microelements and succinic acid no significant increase in plant viability was observed. The hydrothermal treatment of seeds and its further cultivation at optimal temperature to promote significantly higher germination of asparagus seeds compared to the control and other options with soaking the seeds. If this variant is used, the germination increased in the Atlas hybrid from 28.1 to 34.0%, and for WB 210-15 it did from 28.2 to 52.3%, respectively.

We can conclude that to improve the qualities of hybrid asparagus seeds the pretreatment at low temperatures (–70°C) or hydropriming for the day before sowing into soil should be used.