DATE:
NAME:
THE TOPIC: **Cryopresevation**

Cryopreservation is characterized as a viable storage of tissues at ultra low temperatures. It is used to minimize growth and development of plants *in vitro*, to preserve viability and genetic stability of breeding materials, to preserve developmental and functional potential of material and for labour and cost saving. Cryopreservation methods are often genotype specific and require considerable modification. We demonstrate cryopreservation method with encapsulation / dehydratation sequence. Shoot tips are encapsulated in an alginate gel, cultured in sucrose solutions, dehydrated in airflow, and than usually rapidly cooled (Benson 1993). The method uses shoot tips or buds, protoplasts, cells, tissues and somatic embryos.

Generalized flow chart for encapsulation / dehydratation (Benson 1993)

1. Shoot tip culture
↓
2. Conditioned plant
↓
3. Isolated shoot tips
↓
4. Transfer to 3% alginate medium
↓
5. Draw alginate with meristems into the tip of Pasteur pipette
↓
6. Expel the meristem and medium as a drop into Ca^{2+} solution
↓
7. Polymerisation of alginate (encapsulation)
↓
8. Transfer the gelled drops (beads) to 0,5M sucrose solution (dehydratation 1-5 days)
↓
9. Partial desiccation in sterile air flow (laminar flow hood) for 1-4 hours period)
↓
10. Rapid freezing in liquid nitrogen (-196 °C)
↓
11. Thawing
↓
12. Test of viability

THE THEME: **The Effect of Isolation and Encapsulation on Viability of *Solanum tuberosum* Meristems.**

Each treatment of cryopreservation method should be examined for chosen plant material to identified variables of species and improve survival.

MATERIAL AND EQUIPMENT:

Shoot tips culture of Solanum *tuberosum*, 3% natrium alginate in modified medium without Ca^{2+} ions, 0,1M $CaCl_2$, Petri dishes containing moistened filter paper, tools (scalpels, forceps), Bunsen burners, sterile pipettes, sterile blue adapted tips, laminar flow hood.

PROCEDURES:

(See above the steps no. 3. – 7. of the flow chart)

1. Excise apical and axillary meristems from sterile shoot tips culture of *Solanum tuberosum* in a sterile Petri dish with water-moistened tissue under a stereomicroscope.
2. Draw meristems into the adapted blue tip of the pipette along with some alginate medium.
3. Keep the pipette perpendicularly and slowly expel the meristem and medium as a drop with a central localization of the meristem into the 0,1M solution of $CaCl_2$.
4. Allow the gelled drops (beads) to sit (polymerisation) about 30 min.
5. Place the beads on the surface of solidified M-S medium.

EVALUATION:

Make weekly observations and evaluate the growth and development of meristems.

LITERATURE:

- Benson E. E. Cryopreservation. – In: Dixon R.A. *et* Gonzales R.A. (Eds.): Plant Cell Culture: A Practical Approach. - ILR Press, Oxford University Press, 1993.
- Kováč J. (1995): THE USE OF *IN VITRO* METHODS FOR PLANT GENETIC CONSERVATION (využití kultur *in vitro* k uchování genových zdrojů rostlin). – Zahradnictví, 22: 143 – 148.