**RESULTS**

**Without cryoprotectants**

The viability of cell suspensions after freezing was strongly correlated with the value of TTC testing and the level of cryoprotection efficiency. The highest values of TTC test were always observed immediately after sample rewarming. In those cells the dehydrogenases were active for direct freezing for 1 h for all sucrose concentration pretreatments and only for 5 hrs for 9% sucrose. The programmed freezing extended time of TTC test expression up to 24 hrs for 9% sucrose pretreatment both, direct freezing of tissue in LN, (Tab. 1A) and programmed freezing (Tab. 1B) without cryoprotectant treatment resulted in a large destruction of organelles of the cells which was proved by the TEM analysis (Fig. 1A, B).

![Fig. 2A. General view of destruction of cell protoplasm of PEM after freezing Fig. 2B. Total disorder of membranes](image)

**With cryoprotectants**

1M sucrose treatment presented high level of cryoprotection efficiency at the presence of 6 and 9% sucrose in standard medium directly after freezing, however it decreased according to the passing time from the rewarming - up to 24 hours (Tab. 2).

![Fig. 3A. Cells with little bit shrunkenoplast and 3B. dilatation of nuclear envelope](image)

**Vitrification**

Vitrification solution PV52 protected PEM cells against freezing injury. TTC test confirmed high viability of aggregates directly after thawing and 24 hours (Tab. 4). After 48 hrs frozen samples reached level of control. High production of formazan after 48 hrs may suggest high metabolic activity of studied cell suspension. Fig 4 A & B presents ultrastructure of cells which successfully survived LN2 treatment. 5 hrs after thawing cells of PEMs started to divide (Fig. 4 C - arrow). Cell ultrastructure was likely to control after 48 hrs of culture (Fig. 4 D).

![Fig. 4. Ultrastructural and cytological evidences of cell viability after vitrification. A & B - directly after thawing; C- after 5 hrs; D & E - after 48 hrs in agar culture](image)

**Conclusions**

1. TTC test might be a good indicator of culture viability
2. For reliable results, TTC test should be used at least 24 after thawing

![Fig. 5. Consecutive stages of development of vitrificated PEMs A – directly after thawing; B – 5 weeks later and C – 8-week-old culture with newly formed somatic embryos (SE)](image)

High performance in TTC test reflects viability of vitrificated aggregates (Fig. 5)