Cryogenic freezing of apple, carrot, and tobacco suspension cultures and their cryopreservation peculiarities

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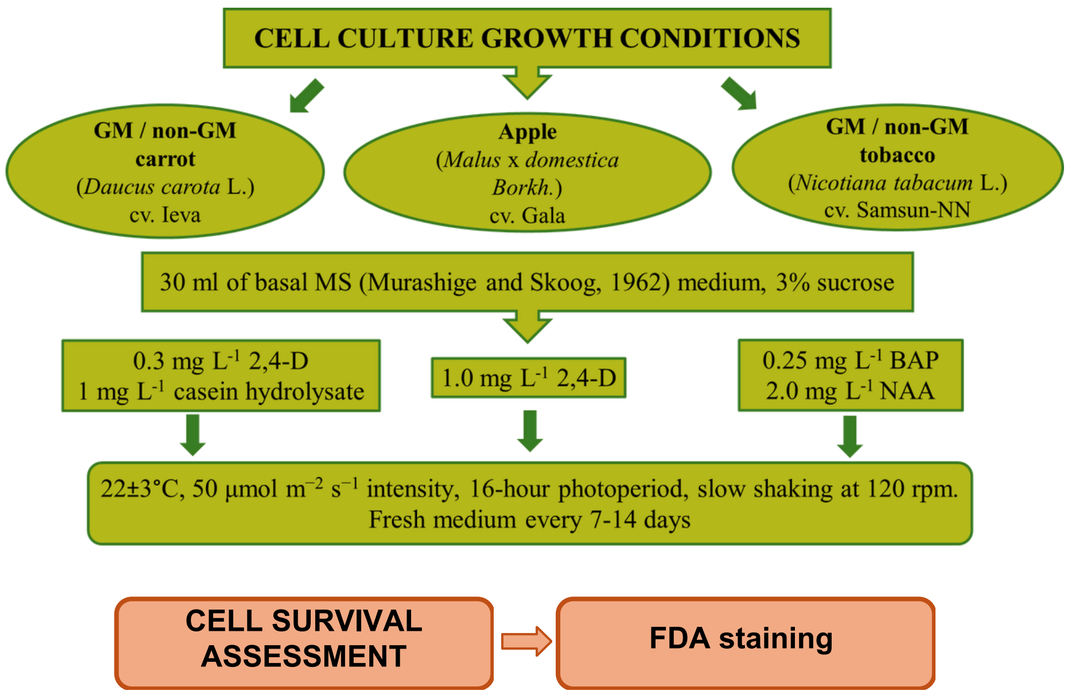
***In vitro* plant cell suspension culture** is considered an effective alternative to traditional recombinant protein production methods, the induction of somatic embryogenesis, and other biotechnological purposes. The performance and transferability of CP protocols for plant cell cultures are currently limited by the diverse properties of plant cells from different species or even different tissues of the same species. Therefore, unique protocols may be required in order to achieve effective preservation of plant cells.

- **The aim of the study** was to determine the most suitable CP conditions for long-term storage of **apple, tobacco and carrot (GM / non-GM) cell suspensions** in liquid nitrogen using different cryoprotectors and their mixtures (sucrose, glycerol, vitrification solution, sorbitol, and DMSO), dehydration and freezing time regimes ensuring the viability of cells after CF treatment.

MATERIAL AND METHODS



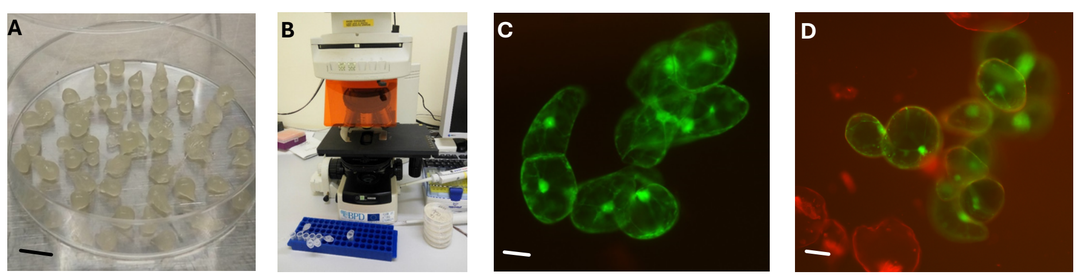
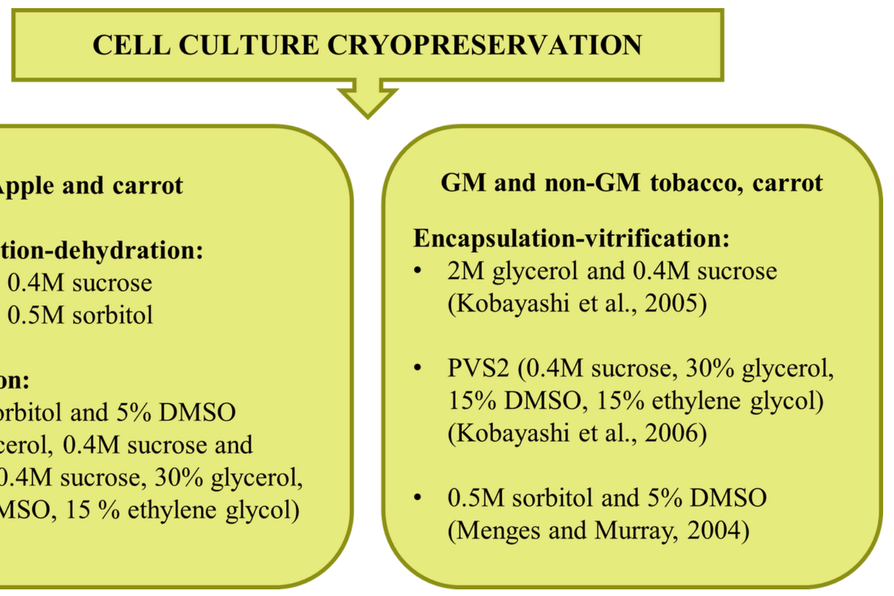


Figure 1. (**A**) CS cells of apple encapsulated in sodium alginate (SA). Scale bar 1 cm. (**B**) Nikon Eclipse 80i (Nikon, Tokyo, Japan) fluorescent microscope. Viable (**C**) apple and (**D**) tobacco CS cells in 0.02 FDA dye solution after CF treatment. Scale bar 1 mm = 10µm, magnification 100x.



RESULTS

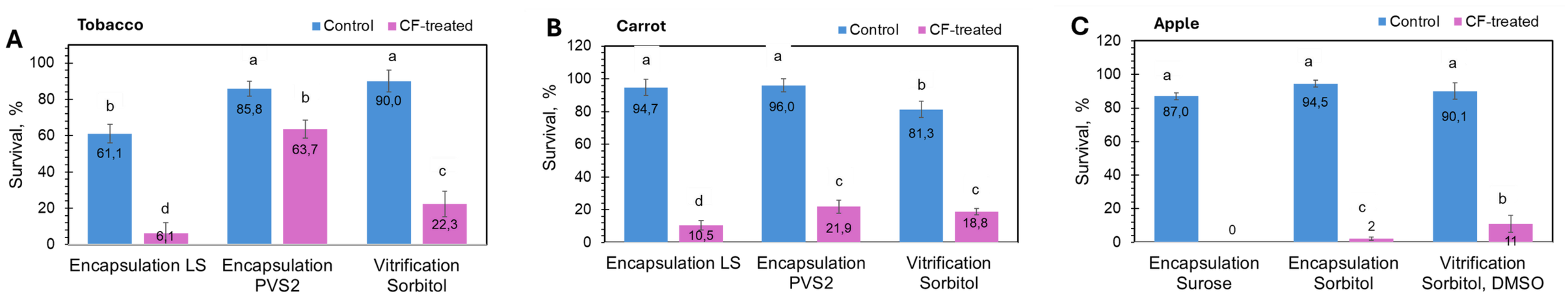


Figure 2. Survival rate of tobacco cv. Samsun-NN (**A**), carrot cv. Ieva (**B**) and apple cv. Gala (**C**) CSCs depending on different cryoprotectants and pretreatment technology. Different letters denote significant differences between experimental groups, ANOVA ($p \leq 0.05$).

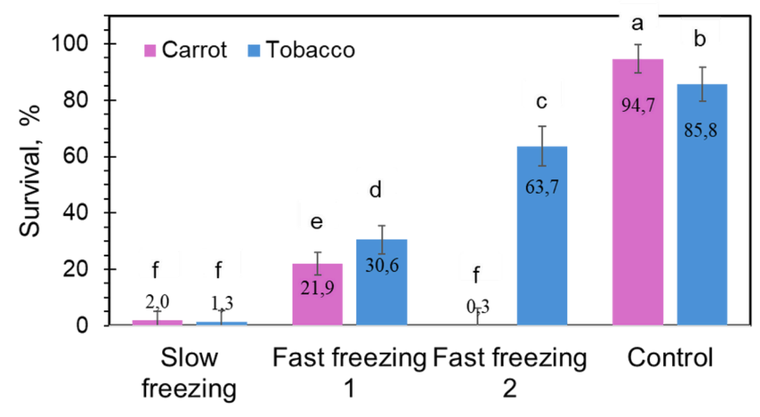


Figure 3. Survival rate of carrot cv. Ieva and tobacco cv. Samsun-NN CSCs depending on freezing regime and incubation in PVS2 solution. Different letters denote significant differences between experimental groups, ANOVA ($p \leq 0.05$).

Table 1. Survival rate of GM tobacco cv. Samsun-NN and carrot cv. Ieva CSCs.

	Survival rate,
	C1 (CF-untreated)	**C2 (5 M sorbitol + 5 % DMSO)**	**C3 (C2 + slow freezing -40°C 2 h)**	**V1 C2+C3+CP treatment (LN 30 min.)**
Tobacco				
CT (non-GM cells)	90.0	71.8	33.3	22.3
GM cells_N4	80.0	65.6	31.5	16.1
LSD0.5 = 4.8				
Carrot				
CC (non-GM cells)	81.3	51.0	28.4	18.8
GM cells_M1	76.7	47.6	25.0	13.7
LSD0.5 = 5.1				

Abbreviations. 2,4-D: 2,4-dichlorophenoxyacetic acid; BAP: 6-benzylaminopurine; CF: cryogenic freezing; CP: cryopreservation; CS: cell suspension culture; DMSO: dimethyl sulfoxide; FDA: fluorescein diacetate; GM: genetically modified; LN: liquid nitrogen; LS: loading solution; MS: Murashige and Skoog; NAA: naftil acetic acid; PVS2: plant vitrification solution 2; SA: sodium alginate.

CONCLUSIONS

- **63.7%** and **21.9%** viability of tobacco cv. Samsun-NN and carrot cv. Ieva CS cells were obtained using the vitrification–encapsulation method with **PVS2**.
- **The vitrification method** using **0.5M sorbitol** and **5% DMSO** cryoprotectants for CP of apple cells cv. Gala was better compared to 2M glycerol and 0.4M sucrose or PVS2, but a low viability (11 %) was obtained, and it should be improved.
- For CP of tobacco and carrot CS cells, fast freezing was more reliable compared to slow freezing. **63.7%** of tobacco CS cells survived after 40 min. exposure in 100% PVS2 solution, and **21.9%** of carrot CS cells survived after two steps exposure in PVS2 solution.
- Pre-treatment with **5M sorbitol** and **5% DMSO** following slow freezing was successful for CP of GM tobacco and carrot CS cells. Survival after CP was lower compared to non-GM cells, and this difference was similar to that of the CF-untreated control CSCs.

References. Kobayashi T. et al. 2005. Plant Biotech, 22.2: 105-112; Kobayashi T. et al. 2006. Plant Biotech, 23.3: 333-337; Menges M. and Murray J.A.H. 2004. Plant J, 37(4): 635-644; Murashige T. and Skoog F. 1962. Physiol Plant, 15(3):473-497.

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