CRYOPRESERVATION OF *MALUS* CV. GALA MICROSHOOTS USING ENDOPHYTIC BACTERIA

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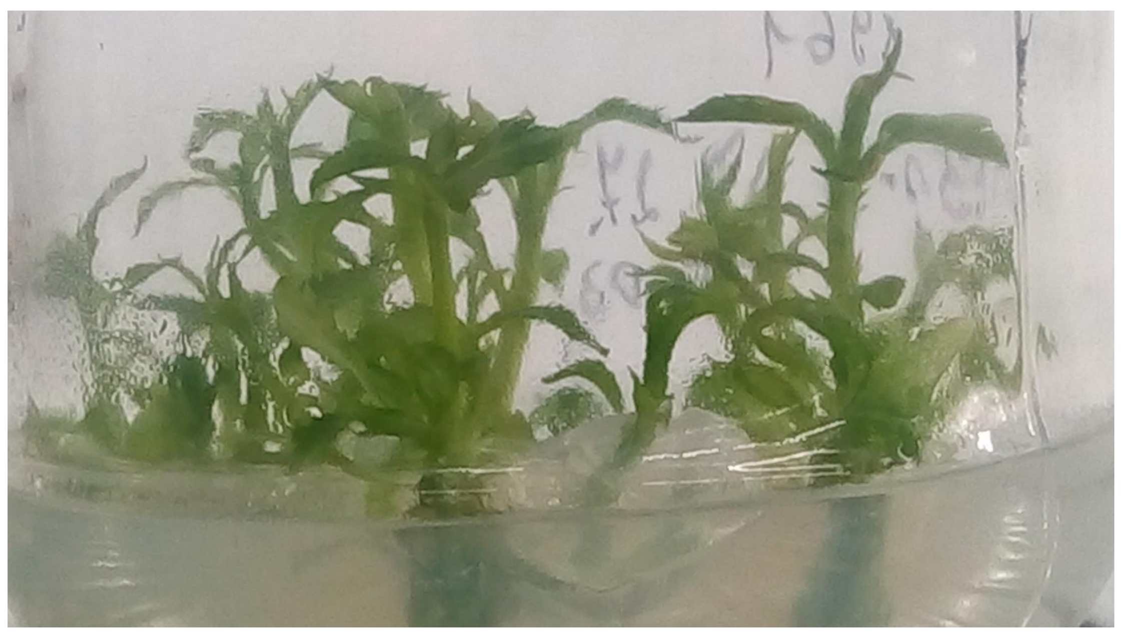
ABSTRACT

Apple (*Malus* spp.) is one of the most economically important temperate fruit crops. In vitro cultivation and cryopreservation techniques are essential for ex situ preservation of genetic diversity and the production of pathogen-free plant propagation material (Wang et al, 2018). Cryogenic freezing (CF) is the only ex situ conservation method for the long-term preservation of the biodiversity of clonal crops (Nagel et al., 2024). Nevertheless only a part of the frozen microshoots survives and regenerates plants after cryopreservation. Successful survival after cryopreservation depends on many factors, among which is cold acclimation (Bilavčík et al, 2012). It has been reported that complementation of axenic culture with endophytic bacteria could mitigate stress, improve growth or rooting, and facilitate acclimation under in vitro conditions (Tamosiune et al., 2018; Andriunaite et al., 2021). However, a potential for the inoculum of plant growth-promoting bacteria in plant recovery and adaptation after cryopreservation has not been directly addressed until now. The aim of the present study was to assess a potential of the inoculum of bacterial isolate to improve the survival and regeneration of apple cv. Gala microshoots after cryopreservation. The research results show a reliable increase in percentage of survived microshoots from 37,1 to 48,3% and increase in the regeneration rate from 18.2 to 27,1% when they were inoculated with a selected strain of bacterial endophytes before 4 week cold acclimation and freezing. No reliable positive effect of endophytes on cryopreservation results was observed for microshoots that were not cold acclimated. The obtained results show the perspective of using endophytic microorganisms to improve apple and other plant cryopreservation and the need for further research by selecting new endophytes and their combinations, evaluating their influence on cold acclimation and recovery after cryopreservation.

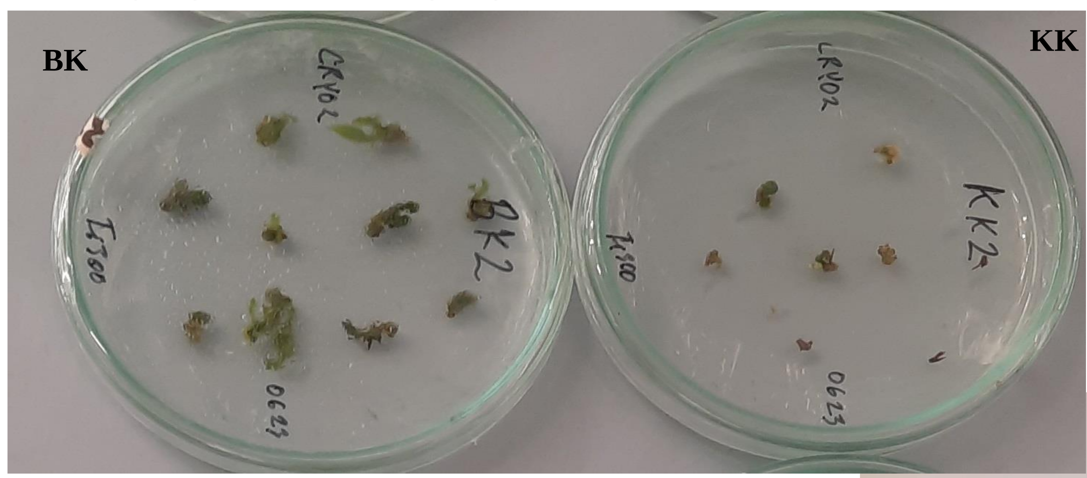
Fig.1 *Malus* microshoot cultivation *in vitro* materials and methods.

Shoot culture *in vitro* of cv. Gala were cultured on MS medium (Murashige and Skoog, 1962) supplemented with vitamins thiamine, pyridoxine, and nicotinic acid) at 0.5 mg l−1 each, 0.5 mg l−1 BAP, 0.01 mg l-1 indole-3-butyric acid, 0.1 mg l-1 gibberellin, 1 mg l−1 ascorbic acid, 3% sucrose, and 0.7% plant agar (pH 5.8), at 22±3 °C under the illumination of 60–70 μmol m−2 s−1 intensity and a 16-hour photoperiod. The established shoot culture was transferred to fresh media every 4 weeks.

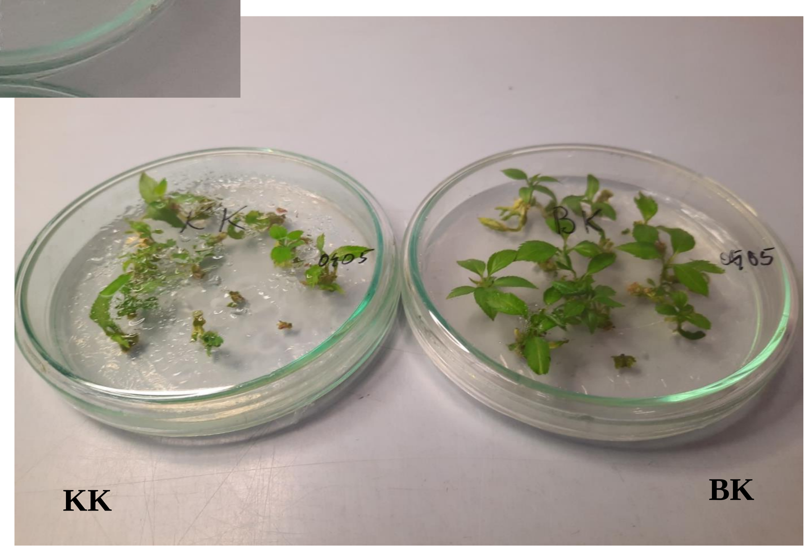


Apical shoot tips pre-treatment with CP and loading solution was performed using the modified vitrification technique (Shatnawi et al., 2007; Sakai et al., 2008; Barraco et al., 2012). Shoot tips were cryoprotected by PVS2 solution (Sakai et al., 1991).

Fig. 2. Meristem survival and regrowth after cryopreservation after inoculation with endophytic bacteria (BK) and control (KK).

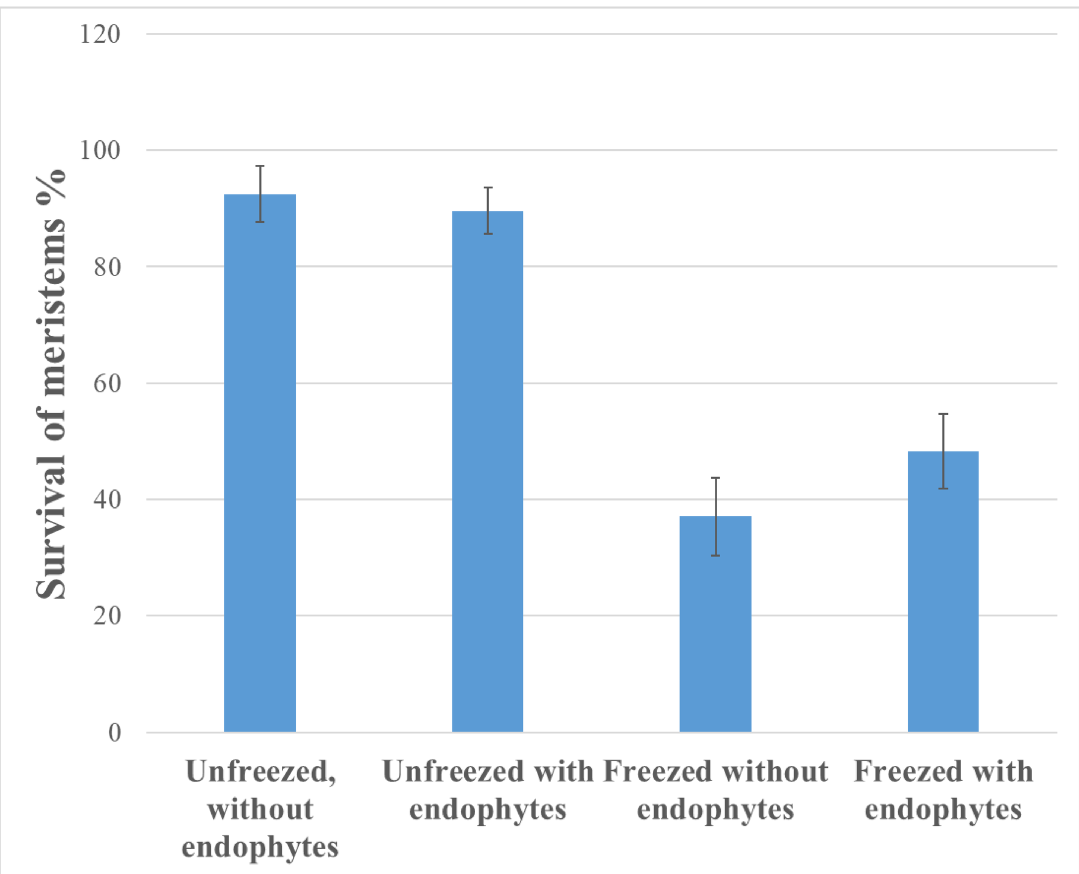


Slow freezing to -40°C at a rate of 0.2-0.3°C/min in a freezer was used before transferring tubes to liquid nitrogen for at least half an hour.



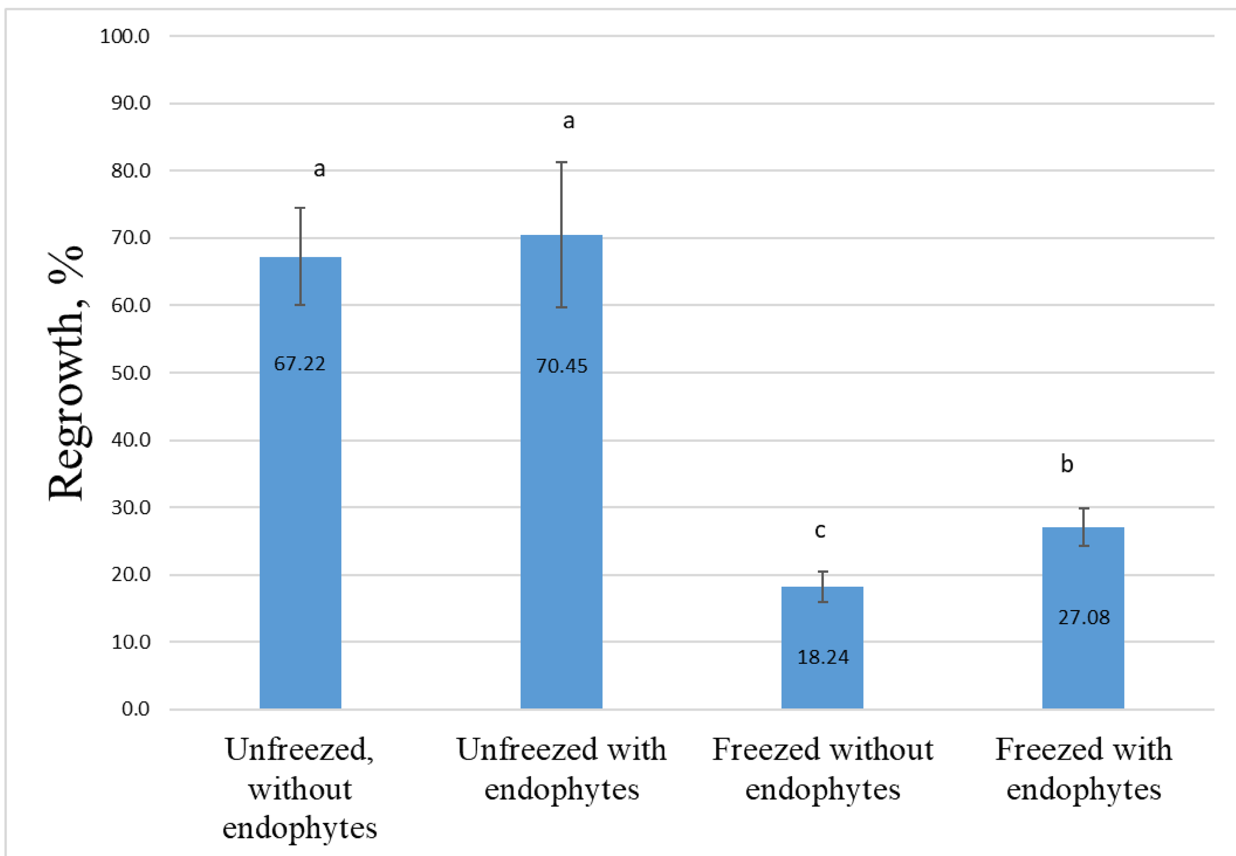
CF samples were fast thawing in a 40°C water bath for 2 min. the shoot tips were transferred to a Petri dish with MS medium supplemented with 1 g l−1 polyvinylpyrrolidone and 1% plant agar and incubated at 22 °C in the dark for 1 week.

Fig. 3. Survival of meristems after pre-conditional treatment at 4°C for four weeks with and without endophytic bacteria in control and CF treated meristems.



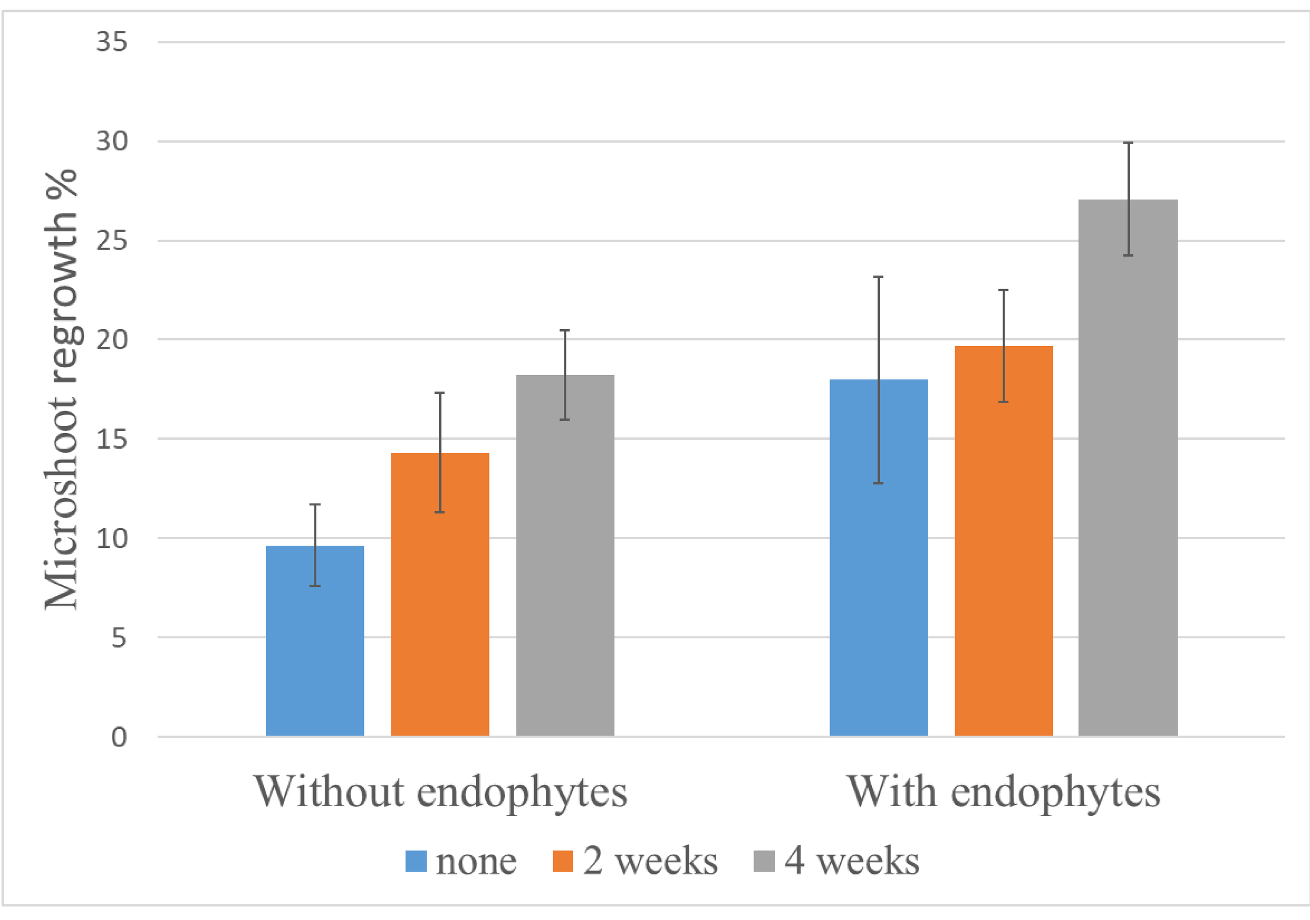
The survival rate of apical shoot tips was evaluated after 2 weeks weeks of incubation on MS medium supplemented with PVP (1g l-1) under standard illumination.

Fig. 4. Regrowth of microshoots after pre-conditional treatment at 4°C for four weeks with and without endophytic bacteria in control and CF treated meristems



The regrowth was evaluated after 7-8 weeks on survived explants transferred to the shoot propagation medium..

Fig. 5. Effect of pre-conditional treatment at 4°C on regrowth of meristems with or without endophytic bacteria inoculation.



For pre-conditioning treatment, *in vitro* shoots were hardened at 4°C for 2 and 4 weeks before the preparation of apical shoot tips. Unhardened shoots were grown at standard cultivation conditions *in vitro*.

CONCLUSIONS

- Pre-conditioning treatment at 4°C improved tolerance to cryopreservation of 'Gala' shoot tips. The longest pre-treatment (4 weeks) caused higher survival and regrowth rate.
- Apical shoot tips pre-treatment with CP and loading solution decreased both survival and regrowth of control meristems that were not cryo-freezing treated. The effect on regrowth being more pronounced.
- *In vitro* cultivation after inoculation with endophytic bacteria lead to better survival and regrowth of CF treatment.
- Inoculation with endophytic bacteria had smaller effect on survival of microshoots without pre-conditional treatment at 4°C.
- Regrowth of microshoots inoculated with endophytic bacteria and treated at 4°C for four weeks had the highest percentage, however the regrowth did not reach 30%.

Abbreviations. BAP: benzylaminopurine; CF: cryo-freezing; CP: cryoprotector; MS: Murashige and Skoog; PVP: polyvinylpyrrolidone; PVS2: plant vitrification solution.

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