Preculture medium optimization for *in vitro* microcuttings in the cryopreservation process of grapevine cultivar 'Graševina'

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Abstract

Grapevine (*Vitis vinifera* L.) is one of the oldest agricultural culture. Wide application of this culture in the economy makes it one of the most important agricultural fruit culture in the world. Republic of Croatia is important gene center for native, as well as for introduced cultivars of grapevine, so the viticulture's aim is to conserve and revitalize its cultivation. Crypreservation is the most efficient procedure of the conservation of the plant material. In the procedure different preculture of microcuttings, cryoprotectants and steps during freezing can be toxic and make the stress within cultivars of grapevine.

The aim of this study is the optimization of preculture medium for preculture of microcuttings with addition of antioksidans (salicylic acid) with the purpose of the successful growth of shoot tips of the cultivar 'Graševina'. The study was made on microcuttings of cultivar 'Graševina', planted on ½ MS medium, with or without cytokinins benzylaminopurine and different concentrations of salicylic acids (0, 0.1, 0.5 and 1 mMol). The highest percentage of regenerated plants was achieved on the medium without the salicylic acid and BAP (68, 38%), and the lowest on the medium with addition of salicylic acid in concentration 0.1 mMol and supplement of 1µmol BAP (35, 00%). After cryopreservation, of microcuttings of the cultivar 'Graševina', the higher results of regeneration was achieved in controled explants in comparioson on the freezing ones. Given results are implying that some additional studies should be done for successful crzopreservation of this cultivar.

Introduction

´'Graševina' is the most cultivated cultivar (4 524,85 ha) and the most popular white wine in Croatia

Cryopreservation is an efficient technique for long-term storage of plant material

´'Graševina can present a model-cultivar for in vitro conditions

Efficient protocol should be tested for each cultivar of interest

Materials and methods

Regeneration *in vitro* of microcuttings

-microcuttings were planted on half-strenght MS medium (Murashige and Skoog, 1962.) with or without BAP (6-benzylaminopurine; 1µMol) four replications with 25 explants on four concentrations of salicylic acids (0-control; 0,1 ; 0,5 and 1 mMol)

-8 weeks after inoculation, regeneration of plants was evaluated (plants with 2-3 leaves)

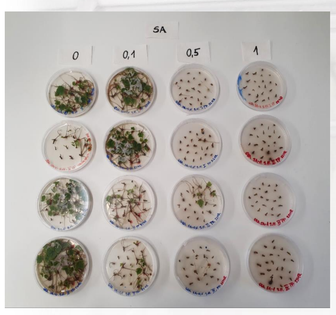
Cryopreservation

-cryopreservation protocol 'droplet-vitrification' for grapevine was performed on regenerated plants

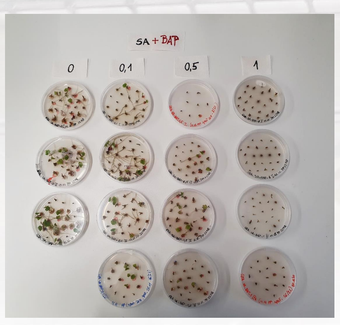
Results

Preculture of microcuttings (MC):

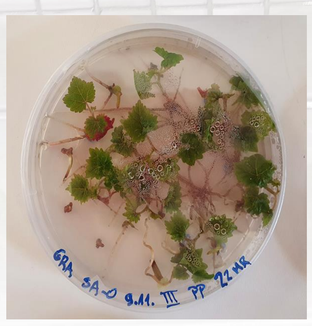
- 0 & 0,1 Mmol SA_improved growth of MC
- BAP+SA_inhibited growth of MC
- the highest regeneration of MC without SA and BAP



Preculture with SA



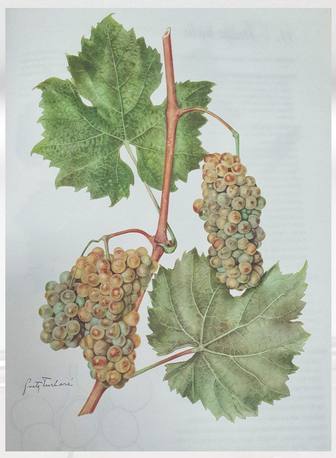
Preculture with SA+BAP



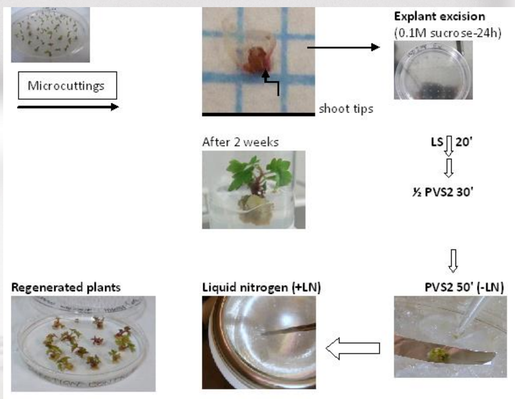
Growth of MC without SA+BAP

Cryopreservation after preculture with SA:

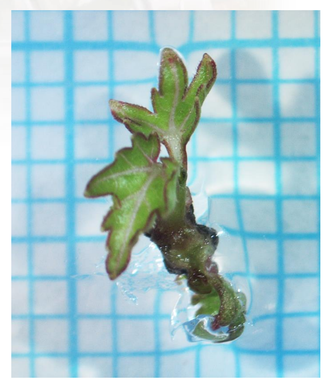
- higher regeneration of control than cryopreserved explants
- low regeneration after freezing (22%)
- no influence of antioxidant (SA) on regeneration after cryopreservation



Cultivar Graševina (Welschriesling, Riesling italico)

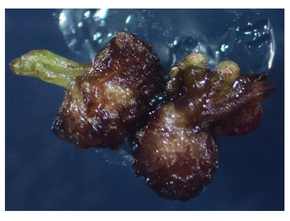


Droplet-vitrification protocol



Conclusion: *For cultivar Graševina better selection of antioxidant concentrations and adjustement of droplet-vitrification protocol should be made*

4 weeks-old explant_PVS2 control



4 weeks-old explant after freezing

Regenerated control plant

Acknowledgments

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