

***In vitro* tissue culture as a tool for Croatian grapevine germplasm management**

Zvezdana Marković^{1*}, Željko Andabaka¹, Domagoj Stupić¹, Iva Šikuten¹, Petra Štambuk¹, Edi Maletić¹, Jasminka Karoglan Kontić Jasminka¹, Darko Vončina², Darko Preiner¹

¹ University of Zagreb, Faculty of Agriculture, Department of Viticulture and Enology, Zagreb,

² University of Zagreb, Faculty of Agriculture, Department of Plant Pathology, Zagreb,

Corresponding author: zmarkovic@agr.hr

Abstract

In vitro culture makes it possible to carry out specific studies that would not be possible with whole plants grown in the field or in a greenhouse. Cryopreservation allows long-term preservation without metabolic changes in the plant material and cryotherapy can be efficient in virus elimination, which is a major scientific challenge.

The preculture media of cryopreservation protocols were evaluated on three Croatian grape varieties with different antioxidants (salicylic acid, ascorbic acid and glutathione). The highest growth *in vitro* was achieved on the medium with the addition of glutathione and the lowest with the addition of salicylic acid. Growth *in vitro* and regeneration after cryopreservation depended on genotype and health status. The cryopreservation protocols tested (as part of cryotherapy) did not result in sufficient regeneration after cryopreservation (10-15%) in the varieties tested.

Virus elimination was tested for economically important viruses by meristem tip culture in 18 Croatian varieties. *In vitro* regeneration ranged 6.82-53.22%, but virus elimination was achieved in only three cultivars and was very low (23.8%). In addition, two new grapevine viruses (GVG and GBV-1) were tested. The results showed a low percentage of virus elimination (2%) by meristem tip culture in three-month-old tissue cultures.

Preliminary research activities were carried out by micrografting with Croatian grape varieties. The survival rate was good, but regeneration was difficult to achieve. A more detailed study is in progress. Overall, the presented methods of meristem tip culture, cryopreservation and micrografting should be further evaluated for the Croatian grapevine germplasm to enable wider application.

Keywords: *Vitis vinifera* L., cryopreservation, preculture with antioxidants, virus elimination, meristem culture, regeneration