P51 – Virome of Slovenian grapevine candidate clones and production of healthy plants by thermotherapy and meristem tip micrografting

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Abstract

The presented research was focused on virus screening using small RNA sequencing (sRNA-seq) technology, to get an overview of viruses and virus-like organisms that are present in preclonal candidates of six autochthonous and local grapevine varieties (*Vitis vinifera* L.) in Primorska wine-growing region in Slovenia. During the process of viral infection, the virus- and viroid-derived small RNAs (sRNAs) accumulate abundantly in plants and can be detected by deep sequencing of infected plants. sRNAs were isolated, twelve libraries were constructed, and sequenced on IonTorrent System. The sRNA-seq data were analyzed using the open-source bioinformatics pipeline VirusDetect. The used method revealed the presence of: grapevine fanleaf virus (GFLV), grapevine leafroll-associated virus 3 (GLRaV-3), grapevine rupestris stem pitting-associated virus (GRSPaV), grapevine fleck virus (GFkV), grapevine red globe virus (GRGV), grapevine rupestris vein feathering virus (GRVFV), grapevine Syrah virus-1 (GSyV-1), grapevine Pinot gris virus (GPGV), raspberry bushy dwarf virus (RBDV), hop stunt viroid (HSVd), and grapevine yellow speckle viroid 1 (GYSVd-1). In silico results were validated by RT-PCR and Sanger sequencing. Biotechnological approach in vivo thermotherapy combined with in vitro meristem tip (0.1-0.2 mm) micrografting onto in vitro growing seedling rootstocks of Vialla (*Vitis labrusca* x *Vitis riparia*) was used to study the elimination efficiency from selected samples infected with abovelisted viruses and viroids (except GRGV). The medium used for the growth and root development of micrografts and micropropagated plants (1/2 MS with vitamins, 30 g/L sucrose, and 8 g/L agar) proved to be highly efficient. The overall regeneration rate was very low (8.5%), but it is sufficient to obtain one virus-free regenerated plant per candidate that further can be micropropagated. The regenerated in vitro plants were tested with RT-PCR. Elimination success was 100% for all viruses, while for the viroids, HSVd and GYSVd-1, the elimination rate was significantly lower, 39.2% and 42.6%, respectively.

Keywords: *Vitis vinifera* L., viruses and viroids, *in vivo* thermotherapy, *in vitro* micrografting