Optimized protocol for high-quality RNA extraction from grape tissues using sorbitol pre-wash.

Annalisa Prencipe[1], Antonella Salerno[1,2], Marco Vendemia[2], Carlo Bergamini[2], Margherita D'Amico[2], Lucia Rosaria Forleo[2], Teodora Basile[2], Maria Francesca Cardone[2], Antonio Domenico Marsico[2], Riccardo Velasco[2], Mario Ventura[1], Flavia Angela Maria Maggiolini[2*]

[1] Department of Biosciences, Biotechnology and Environment, University of Bari "Aldo Moro", 70125 Bari, Italy.
[2] Council for Agricultural Research and Economics - Research Center Viticulture and Enology (CREA-VE), Via Casamassima 148-70010 Turi (Ba), Italy.

[*]Corresponding author: flavia.maggiolini@crea.gov.it

Obtaining high-quality RNA from grape tissues, including berry pulp, berry skins, stems, rachis, or roots, is challenging due to their composition, which includes polysaccharides, phenolic compounds, sugars, and organic acids that can negatively affect RNA extraction. For instance, polyphenols and other secondary metabolites can bind to RNA, making it difficult to extract a pure sample. Additionally, RNA can co-precipitate with polysaccharides, leading to lower extraction yield. Also, sugars and organic acids can interfere with the pH and ionic properties of the extraction buffer. To address these challenges, we optimized a protocol for RNA isolation from grape tissues. Although commercial kits can provide a rapid extraction, they were inefficient for these plant materials. Similarly, protocols that work well for other vegetal tissues were also inefficient and time-consuming on grape tissues. To overcome these limitations, we added a sorbitol pre-wash step to both a three-day long protocol based on LiCl precipitation and a commercial kit. Our results showed that the addition of a sorbitol pre-wash improved multiple parameters: the A260/280 absorbance ratio, integrity and quality (IQ), and RNA integrity number (RIN). Sorbitol played a crucial role in ensuring high-quality RNA extraction from grape tissues. It inhibits RNase, thereby preserving RNA integrity and stability. It also helps in disrupting cellular membranes, facilitating the release of RNA, and maintains the osmotic pressure through hypertonicity, which is beneficial to RNA extraction. By using sorbitol, commercial kits can be used to extract RNA from challenging grape tissues, leading to an efficient and time-saving procedure.

Keywords: *Vitis vinifera*, RNA, sorbitol, extraction protocol.