# GOOD QUALITY *VITIS* RNA OBTAINED FROM AN ADAPTED DNA ISOLATION PROTOCOL

## OBTENTION D'ARN DE MEILLEURE QUALITÉ DE VITIS PAR MODIFICATION D'UN PROTOCOLE D'ISOLATION DE L'ADN

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**Abstract** : Grapevine is a woody plant, whose high carbohydrate and phenolic compound contents usually interferes with nucleic acid isolation. After we tried several protocols for isolating RNA from the *Vitis* rootstock Richter-110 (R-110) with little or no success, we adapted a method reported to be satisfactory for grapevine DNA isolation, to extract RNA. With slight protocol modifications, we succeeded to obtain polysaccharide- and phenolic-free RNA preparations from all vegetative tissues, without excessive sample handling. RNA isolated by the reported method permitted to obtain highly pure mRNA (messenger RNA) to construct a cDNA (complementary DNA) library and allowed gene transcription analysis by reverse Northern, which guarantees RNA integrity. This method may also be suitable for other plant species with high polysaccharide or phenolic contents.

**Résumé** : La vigne est une plante ligneuse dans laquelle les teneurs élevées en sucres et en composés phénoliques gènent généralement l'extraction des acides nucléiques. Après avoir essayé différents protocoles peu ou non satisfaisants d'extraction d'ARN des racines de *Vitis* Richter-110 (R-110), nous avons adapté une méthode d'extraction de l'ADN de la vigne à celle des ARNs. Par de légères modifications du protocole, nous avons obtenu de préparations d'ARN de tous les tissus végétatifs libres de contamination de polysaccharides et de composés phénoliques sans pour autant alourdir la méthodologie. Cet extrait d'ARN permet d'obtenir des préparations pures d'ARNm qui ont pu être utilisées pour constuire une banque d'ADNc (ADN complémentaire). Ainsi nous avons pu réaliser des analyses de transcription des gènes par la méthode du Northern inverse, ce qui montre que l'intégrité des ARNs est préservée. Par extension, cette méthode peut être utilisée pour d'autre matériel végétal qui contient des concentrations élevées en polysaccharides et composés phénoliques.

**Key words**: RNA extraction, RNA isolation, *Vitis*, grapevine, phenolics.

**Mots clés** : extraction d'ARN, purification d'ARN, *Vitis*, vigne, composés phénoliques.

## INTRODUCTION

Grapevine tissues are rich in polysaccharides and secondary metabolites like tannins and phenolics, which coextract with nucleic acids and therefore interfere with RNA purification.

Several protocols were tested in our laboratory for extracting RNA from *Vitis* rootstock Richter-110, but even those described for isolating RNA from *Vitis* tissues (FRANKE *et al.*, 1995; LOULAKAKIS *et al.*, 1996; TESNIÈRE and VAYDA, 1991) were unsatisfactory or tedious and time consuming. We eventually obtained degraded samples containing polysaccharides and polyphenols. Methods that are reported to obtain polysaccharide-free RNA preparations (FRANKE *et al.*, 1995; TESNIÈRE and VAYDA, 1991) or to remove phenolic compounds by binding them to polyvinylpyrrolidone or activated charcoal (VROH *et al.*, 1996) or by precipitating them (MANNING, 1991), also failed to provide good RNA quality. Finally, we adapted the *Vitis vinifera* DNA extraction method described by STEENKAMP *et al.* (1994) to isolate RNA from the R-110 rootstock. The yield was acceptable, there was rarely any degradation, and the quality of total RNA was good. The resulting RNA preparations served as substrate in reverse transcription to construct a cDNA library, synthesize radiolabelled cDNA probes for reverse Northern analysis, (BAIGES *et al.*, 2001) and be amplified by the polymerase chain reaction (PCR).

## MATERIALS AND METHODS

Our protocol includes some modifications to the one previously described (STEENKAMP *et al.*, 1994):

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*J. Int. Sci. Vigne Vin*, 2003, **37**, n°1, 59-61
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we used sand instead of glass beads, we replaced gentle mixing with vortexing and we removed DNA by LiCl precipitation or DNase treatment. Obviously, all the solutions and materials were RNase-free and gloves were worn at all times.

Plant tissues were quickly frozen in liquid nitrogen and stored at -80 °C. About 1 g of frozen plant material was fine-powdered in liquid nitrogen in a prechilled mortar containing about 0.5 to 1 g of washed sand. About 100 mg of homogenized tissue (containing sand) was transferred to each microcentrifuge tube preventing thawing. Tubes were kept frozen at -80 °C until the RNA was extracted. Then, 500 µL of extraction buffer [3 % CTAB (hexadecyltri-methylammonium bromide, SIGMA-ALDRICH, St. Louis, MO, USA); 1,4 M NaCl; 20 mM EDTA; 1 M Tris-HCl pH 8,6, filter-sterilized] and 1 µL of 2-mercaptoethanol were added to each homogenized sample and immediately transferred to a 60 °C water bath. Tubes were incubated at 60 °C for 30 min and alternately vortexed for 2 to 5 min throughout the time of incubation. One volume of chloroform/isoamyl alcohol [24:1 (v/v)] was added and the tubes were shaken vigorously for 10 to 15 min in an automatic shaker and vortexed from time to time. Samples were centrifuged at 10,000 g for 40 min at room temperature. Supernatants were transferred to a new tube and 0.7 volume of cold isopropanol were added. The tubes were centrifuged at 10,000 g for 5 min at 4 °C. The pellet was washed twice with cold 76 % ethanol and allowed to dry inverted on absorbing paper for 20 min. 100 µL of RNase-free water was added and incubated on ice until resuspended. A 5 µL aliquot was run in a 1 % Tris/Acetate/EDTA agarose gel containing ethidium bromide to check RNA quality and DNA contamination. We used two alternative protocols to remove DNA. When there were large amounts of DNA in the sample, 0.33 volume of 8 M LiCl were added and the mixture was incubated overnight at 4°C. RNA was selectively pelleted after centrifugation at 13,000 g for 30 min at 4 °C. The pellet was washed with ethanol, dried and resuspended in DEPC-water, as reported above, and 20 units of ribonuclease inhibitor (MBI FERMENTAS, GMBH, St.Leon-Rot, Germany) were added.

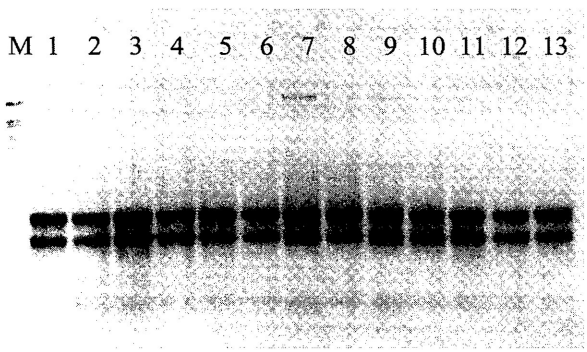



**Fig. 1 - Total RNA from different *Vitis* R-110 tissues.**
Lane M, λ Eco91I marker DNA; lane 1, root cortex; lane 2, root tip; lane 3, root stele; lane 4, bud; lane 5, petiole; lane 6, stem; lane 7, mature leaf; lane 8, young leaf; lane 9, expanding leaf; lane10, leaf mesophyll; lane 11, main foliar ribs; lane 12, main roots; lane 13, lateral roots. RNA was isolated as described and selectively separated from DNA by LiCl precipitation. Resolution on a 1 % Tris/Acetate/EDTA/ethidium bromide agarose gel.

**ARN total extraits de différents tissus de *Vitis* R-110.**
Ligne M, λ Eco91I marqueur ADN; ligne 1, cortex de racine; ligne 2, région d'élongation de la racine; ligne 3, racine médulaire; ligne 4, œil; ligne 5, pétiole; ligne 6, tige; ligne 7, feuille adulte, ligne 8, feuille jeune; ligne 9, feuille en croissance; ligne 10, mésophylle de feuille; ligne 11, nervure principale de la feuille; ligne 12, racine principale; ligne 13, racines latérales. ARN extrait selon la méthologie décrite dans matériels et méthodes, et séparé de l'ADN par précipitation au LiCl. Migration sur gel d'agarose (1 %) dans un tampon Tris/Acétate/EDTA/ bromure d'éthydium.




**Fig. 2 - Poly (A)+ mRNA isolated from total RNA of *Vitis* R-110 leaves.**
Lane 1, 1 µg leaf poly (A)+ mRNA; lane M, λ Eco91I marker DNA. Resolution on a 1 % Tris/Acetate/EDTA/ethidium bromide agarose gel.

**ARNm poly (A)+ extrait de l'ARN total des feuilles de *Vitis* R-110.**
Ligne 1, 1 µg poly (A)+ ARN m de feuille; ligne M, λ Eco91I marqueur ADN. Migration sur gel d'agarose (1 %) dans un tampon Tris/Acétate/EDTA/ bromure d'éthydium.

When there were small amounts of DNA in the sample, the DNA was digested according to AUSUBEL *et al.* (1995). RNA was then purified using the RNA Clean-up protocol of the RNeasy Mini Kit (QIAGEN® GMBH, Hilden, Germany).

RNA integrity and DNA digestion were both checked on an agarose gel. RNA purity was estimated by the A260/A280 and A260/A230 ratios as a measure of protein/polyphenols and polysaccharides contamination, respectively. We calculated the extraction yield spectrophotometrically after the A260.

## RESULTS AND DISCUSSION

We used this protocol successfully to extract RNA from all root and shoot tissues of hydroponically cultivated grapevines (fig. 1), from roots and leaves of *in vitro* cultured plants, and from leaves of grapevines cultivated in the vineyard. As the protocol does not require excessive handling, 6 to 8 samples can be pro-


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cessed simultaneously in 3 to 5 hr. When LiCl precipitation is performed, pure RNA preparations are ready in 2 days. If necessary, isopropanol precipitation can be omitted and the aqueous phase can be directly precipitated with 2 M LiCl to save time. This method usually obtained A260/A230 ratios exceeding 2 and A260/A 280 ratios of between 1.8 and 2, which indicates that there was no interference from polysaccharides, proteins or polyphenols. The average yield of RNA extractions depended on the tissue and ranged from 80 to 700 µg of RNA /g of plant material. This yield was considered acceptable when compared with other reported values.

A cDNA library was constructed from leaf RNA isolated as described here and used to sequence grapevine aquaporin genes (BAIGES *et al.*, 2001). Total RNA was enriched for poly(A)+ RNA (fig. 2) using the Oligotex' mRNA Kit (QIAGEN® GMBH, Hilden, Germany) and the yield was 1.4 %. The A260/A230 and A260/A 280 ratios were 2.57 and 2.07 respectively, which indicates high RNA quality. All the cDNAs we analyzed were full length, which guarantees RNA integrity.

## CONCLUSIONS

This paper shows that good DNA extraction methods appropriate for certain plants or tissues can be adapted to extract RNA. The method we have described is reliable for extracting RNA from all types of *Vitis* vegetative tissues, and may also be suitable for grape berries and other plant species with high polysaccharide or phenolic contents.

Acknowledgements : This work was supported by a grant from Ministerio de Ciencia y Tecnología AGL2001-0467, Spain.

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*Manuscrit reçu le 28 octobre 2002 ; accepté pour publication le 6 janvier 2003*


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