EFFICIENCY OF RNA EXTRACTION PROTOCOLS IN DIFFERENT TYPES OF COFFEE PLANT TISSUES

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ABSTRACT: In order to use sensitive techniques of molecular biology, such as the study of differentially expressed genes, a high-quality RNA in suitable quantities is necessary. Due to the presence of several varieties and often expressive quantities of secondary compounds in plants, there is no standard method for the isolation of nucleic acids that can be used for all species. Polyphenols and polysaccharides are the compounds that interfere the most in the extraction process, and when they are present, a low-quality RNA is produced. Four RNA extraction methods (CTAB method, Hot Borate, CONCERT and Tri Reagent), in four different coffee tissues (root, leaf, flower and fruit) were tested in this work, aiming at determining which method is more efficient. It was observed that the CTAB and Hot Borate methods, in which PVP and/or β-mercaptoethanol were added and precipitation with LiCl was performed, presented more pure RNA, with no degradation observed in any of the tissues, being suitable for further gene expression analysis. High-quality RNA was not obtained from any tissue in the extraction with Tri Reagent, which includes the use of phenol, and thus expression analysis was disturbed. The CTAB macroextraction method presented samples with the highest RNA quality and largest quantities in all tissues. Future works need to be carried out aiming the standardization of this macroextraction method.

Index terms: Gene expression, *Coffea arabica*, RT-qPCR.

EFICIÊNCIA DE PROTOCOLOS DE EXTRAÇÃO DE RNA EM DIFERENTES TECIDOS DO CAFEEIRO

RESUMO: *Para a utilização de técnicas sensíveis de biologia molecular, como o estudo de genes diferencialmente expressos, é necessário a obtenção de um RNA de boa qualidade e em quantidades adequadas. Devido à presença de grandes variedades, e frequentemente grande quantidade de compostos secundários em plantas, não existe um método padrão para o isolamento de ácidos nucléicos que possa ser utilizado para todas as espécies. Os polifenóis e os polissacarídeos são os compostos de maior interferência no processo de extração, e quando presentes geram um RNA de baixa qualidade. Nesse trabalho foram testados quatro métodos de extração de RNA (Método CTAB, Borato quente, CONCERT e Tri Reagente), em quatro diferentes tecidos de café (raiz, folha, flor e fruto), objetivando-se determinar qual método é mais eficiente. Foi observado que os métodos, CTAB e Borato quente, que possuíam a adição PVP e/ou β-mercaptoetanol, e precipitação com LiCl, foram os que apresentaram RNAs mais puros e sem degradação em todos os tecidos, e puderam ser utilizados para a análise de expressão gênica. Com a extração utilizando o TriReagente, que tem como base o fenol, não foi obtido RNA de boa qualidade em todos os tecidos e consequentemente não foi possível a análise de expressão. O método de macroextração CTAB foi o que apresentou amostras com RNA de melhor qualidade e em grandes quantidades em todos os tecidos. Trabalhos posteriores precisam ser realizados a fim de padronizar esse método para microextração.*

Termos para indexação*: Isolamento de RNA, Coffee arabica, RT-qPCR.*

1 INTRODUCTION

Coffee is one of the most important and valuable agricultural commodities in the world, with Brazil being the largest producer and exporter (FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS - FAO, 2011). Although the Coffee genus encompasses ~100 species, only two

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of these have major economical significance: C. arabica (arabica) and C. canephora (robusta), with the former producing a drink of better quality and being responsible for most of the world's production, despite its more demanding production system.

Although coffee physiology has been extensively studied, with the objective to produce a coffee with higher productivity and quality of drinking (MARTINS NETO; MATSUMOTO, 2010; PAIVA et al., 2010; PEDROSO et al., 2009; REZENDE et al., 2010), there are production constraints which are still unresolved. This includes its sequential flowering pattern (i.e., flowering time is not simultaneous in the same plant) which causes uneven fruit maturation. As a result, berry harvest becomes more difficult and causes losses in yield and drink quality (CHALFOUN, 2010; FARNEZI et al., 2010). There are also a panoply of biotic factors that affect both productivity and the quality of coffee (DIAS et al., 2009; MIRANDA et al., 2010; SERA et al., 2010).

However, future studies focusing on genetic analysis can lead to a better comprehension of the coffee flowering regulation process, with potential implications for molecular breeding programs (BARRETO et al., 2012; LIMA et al., 2011), as well as for the development of cultivars resistant to biotic factors. Quality RNA isolation is a mandatory requirement for studies of gene expression, including reverse transcriptase (RT), real-time quantitative PCR (RT-qPCR), construction of cDNA libraries, or microarray analyses (CARDILLO; GIULIETTI; MARCONI, 2006).

The main problem is that plant tissues are characterized by a large variation in their composition, with RNA isolation being particularly difficult in some (GEUNA; HARTINGS; SCIENZA, 1998). An efficient RNA extraction method is extremely important for works that use different tissues, regardless of their composition, in order to minimize the variations that may exist when different protocols are used.

Due to the presence of secondary metabolites, polysaccharides, and polyphenols, standardization of a quality RNA extraction for different coffee plant tissues is very difficult; also, the obtainment of high-quality RNA may be complicated because of RNA susceptibility to RNase degradation. Furthermore, one of the essential stages is the complete elimination of any DNA present after the extraction, since a DNA copy is theoretically capable of originating a false positive in studies based on gene expression (BIRTIC; KRANNER, 2006).

Studies describe extraction techniques for polyphenol- or polysaccharide-rich plant tissues that present problems for the isolation of high-quality RNA in large quantities (AZEVEDO; LINO-NETO; TAVARES, 2003; SALZMAN et al., 1999). However, these methods have been developed for specific plant tissues, since each method depends on tissue composition, and some have been inadequate in the reduction of polysaccharide contamination.

Among the most reported extraction techniques for species with a high content of secondary metabolites are the methods that use soluble polyvinylpyrrolidone (PVP) and ethanol precipitation (SALZMAN et al., 1999), hot borate (WAN; WILKINS, 1994), phenol extraction (KOMJANC et al., 1999), calcium precipitation (DAL CIN et al., 2005), 2-butoxyethanol (MALNOY et al., 2001; MANNING, 1991), or cetyltrimethyl-ammonium bromide (CTAB) in the extraction buffer (MEISEL et al., 2005).

In these techniques, a few substances are added to eliminate contaminants, including antioxidant agents such as PVP, polyvinylpolypyrrolidone (PVPP), and β-mercaptoethanol, organic solvents such as chloroform and phenol, which separate RNA from proteins, guanidine thycyanate, widely used to inhibit RNase activity (CHOMCZYNSKI; SACCHI, 1987; VALENZUELA-AVENDAÑO et al., 2005), among others.

Aiming at obtaining an ideal protocol for quality RNA extraction for diverse coffee plant tissues, this work had the objective of identifying the most adequate methodology to isolate RNA samples from leaves, flowers, fruits, and roots for use in sensitive molecular studies.

2 MATERIAL E METHODS

Plant material

The plant material used was collected in the experimental field of the Federal University of Lavras (UFLA) in Lavras, MG. Four plant tissues of *Coffea arabica*, Rubi cultivar, collected in September 2008, were used for RNA extraction:

young leaves, flowers in the G4-G5 stages as described by Morais et al. (2008), fruits in different stages of development, and secondary roots. The material was collected in liquid nitrogen and stored at -80°C until the moment of extraction. The tissues were submitted to four different RNA extraction methods: Concert™ (Invitrogen), TRI Reagent® (Sigma), hot borate (BIRTIC; KRANNER, 2006; WAN; WILKINS, 1994), and CTAB (CHANG; PURYEAR; CAIRNEY, 1993).

All the materials used in the extractions were treated with a 0.5% diethylpyrocarbonate solution (DEPC) to inactivate RNases, and the solutions used were prepared with distilled, autoclaved, RNase-free water.

RNA isolation

Concert Reagent™

The RNA extraction protocol with Concert was performed according to the Concert™ Plant RNA Reagent (Invitrogen) manual. Microtubes containing approximately 100 mg of ground tissue and 500 µL of cold Concert reagent (4°C) were homogenized in a vortex mixer and incubated for 5 minutes at room temperature in a horizontal position in order to maximize RNA extraction. After this period, the material was submitted to centrifugation for 2 minutes at room temperature and speed of 12,000 x g, and the supernatant was transferred to a new tube. After the addition of 100 µL of 5 M NaCl and homogenization in a vortex mixer, 300 µL of chloroform were added and the tubes were mixed by inversion. For phase separation, the samples were submitted to centrifugation for 10 minutes at 4°C (12,000 x g) and the superior aqueous phase was transferred to a new tube. Next, isopropanol was added in a volume equivalent to the aqueous phase (approximately 400 µL) and the tubes were shook in a vortex mixer for 5 seconds. The samples were then kept at room temperature for 10 minutes and subsequently submitted to centrifugation for 10 minutes at 4°C (12,000 x g). After centrifugation, the supernatant was discarded, the pellet was washed with 1 mL of 75% ethanol, and the tubes were submitted to another centrifugation for one minute at room temperature (12,000 x g). The residual liquid was removed from the tube with a pipette and the RNA was resuspended in 20 µL of autoclaved ultrapure water. Finally, the samples were stored at -20°C.

Tri Reagent®

Total RNA was obtained using Tri reagent® (Sigma), according to manufacturer's recommendations. Microtubes containing approximately 100 mg of ground tissue and 1 mL of Tri Reagent were homogenized in a vortex mixer and incubated for 5 minutes at room temperature. After this period, the material was submitted to centrifugation for 10 minutes at 4°C and speed of 12,000 x g. The supernatant was then transferred to a new tube, which received 200 µL of chloroform and was homogenized for 15 seconds. For phase separation, the samples were submitted to centrifugation for 20 minutes at 4°C (12.000 x g), and the upper aqueous phase was transferred to a new tube (approximately 750 µL). Next, 500 µL of isopropanol were added and the tubes were homogenized by inversion and incubated for 60 minutes at -20°C. The samples were then centrifuged for 10 minutes at 4°C (12,000 x g) and the supernatant was discarded. The pellet was washed with 1 mL of 75% ethanol and the tubes were submitted to an additional centrifugation for 5 minutes at 4°C (12,000 x g). The residual liquid was removed from the tube with a pipette and the RNA was resuspended in 20 µL of autoclaved ultrapure water. Finally, the samples were stored at -20°C.

Hot borate method

The hot borate method was adapted from the protocol described by Birtic and Kranner (2006), where the extraction buffer is formed by 0.2 M sodium tetraborate decahydrate, 30 mM EGTA - ethylene glycol bis (2-aminoethyl ether)-N,N,N2 ,N2 - tetraacetic acid, 1% sodium dodecyl sulfate (SDS), and 1% sodium deoxycholate, adjusted to a pH of 9.0 with sodium hydroxide. The addition of 0.06 g of polyvinylpyrrolidone (PVP) and 2% of dithiothreitol (DTT) was made for every 1 mL of buffer, which was heated for the complete dissolution of the reagents. Approximately 100 mg of each tissue sample were ground in liquid nitrogen with the addition of 1 mL of extraction buffer, immediately followed by the addition of 1 mg of Proteinase K, agitation, and water bath at 45°C for 90 minutes. Afterwards, 80 µL of 2 M potassium chloride were added and the samples were kept on ice for 45 minutes, followed by centrifugation for 20 minutes at 4°C (12.000 x g) and

collection of the supernatant. Cold 8 M lithium chloride was added at one-third of the supernatant volume, and the samples were incubated at -20°C for at least one hour for RNA precipitation. After this period, the samples were centrifuged for 20 minutes at 4°C (12.000 x g) and the supernatant was discarded. The pellet was washed with 750 µL of 2 M lithium chloride twice, followed by a final centrifugation for 10 minutes, removal of the residual liquid with a pipette, and RNA resuspension in 20 µL of autoclaved ultrapure water. Finally, the samples were stored at -20°C.

CTAB method

This RNA macroextraction method was first described by Chang, Puryear e Cairney (1993). For each tube containing 2 g of the material ground in liquid nitrogen, 25 mL of extraction buffer were added 2% (w/v) CTAB (cethyltrimethylammonium bromide), 2% (w/v) PVP, 100 mM Tris-HCL, 25 mM EDTA, 20 mM NaCl, and 2% β–mercaptoethanol).The samples were incubated for 20 minutes at 65°C and then 20 mL of chloroform were added to each tube. After a centrifugation step at 7500 x g for 5 minutes at room temperature, approximately 20 mL of the sample supernatant were transferred to a new tube and the same volume of chloroform was added. Then, samples were homogenized and the centrifugation step was repeated. The top aqueous phase, approximately 15 mL, was transferred to a new tube and 12 M lithium chloride was added for a final concentration of 2.5 M. The samples were left overnight at 4°C for precipitation. Posteriorly, the material was centrifuged during 30 minutes at 4°C (7,500 x g) and the supernatant was discarded. The pellet was washed with 2.5 M lithium chloride, followed by centrifugation for 15 minutes at 4°C (10,000 x g). After discarding the supernatant, the pellet was washed with 1 mL of 70% ethanol, transferred to microtubes and centrifuged for 10 min at 4ºC (11,000 g). The supernatant was discarded and the residual liquid was removed from the tube with a pipette. Finally, the RNA was resuspended in 50 µL of autoclaved ultrapure water and the samples were stored at -20°C.

Treatment with DNase

All samples were treated with DNA-free DNase (Ambion) in a reaction containing 10 µg of total RNA, 1 U of the rDNAseI enzyme, 0.1 volume of the 10 x DNaseI buffer, and DNase/RNase-free water to complete the volume to 50 µL. This reaction was incubated at 37 °C for approximately 30 min and 5 µL of DNase inactivation solution was added to inactivate DNase. The samples were incubated for 2 minutes, centrifuged at 10,000 x g for 1.5 minutes, and their supernatants (approximately 50 µL) were transferred to new tubes.

In order to evaluate the integrity of the extracted samples, RNA was submitted a 1.2% agarose gel electrophoresis stained with 2 µL of ethidium bromide (0.5 µg/mL). Posteriorly, the gels were visualized under ultraviolet light and photographed with the Kodak® Photodocumentation EDAS 290 system. The samples were quantified in a Nanodrop® ND-1000 Spectrophotometer at A_{260} nm with the objective of determining quantity and quality.

cDNA Synthesis and RT-qPCR

cDNA synthesis and RT-qPCR analysis were only performed with the objective of determining if the extracted RNAs possess good enough quality to be submitted to more sensitive analysis.

The *High-Capacity cDNA Reverse Transcription Kit* (Applied Biosystems) was used for cDNA synthesis. Initially, RNA was prepared at a concentration of 1 µg in a final volume of 10 µL. After this step, a mix containing 2 µL of the 10x enzyme buffer, 2 µL of the 10X RT Random Primers primer, 0.8 µL of 100 mM dNTP mix, 1 µL *MultiScribe™* Reverse Transcriptase, and water for a final volume of 10 µL per sample was prepared and 10 µL were added to the prepared RNA solution. The tubes were submitted to an *Eppendorf Mastercycler gradient* thermal cycler programmed for three steps: 10 min at 25°C for primer annealing, 2 h at 37°C for enzyme action, and 5 min at 85°C for enzyme inactivation. The samples were then stored in a freezer at -20°C.

cDNA was used as template for analysis of quantitative gene expression through the ABI PRISM 7500 Real-Time PCR (Applied Biosystems) using the SYBR Green detection system.

First, an absolute quantification assay was performed, with the determination of the standard curve, primer efficiency, and best dilution presented by the samples. cDNAs were diluted at 1:10, 1:25, and 1:50.

After determining the 1:10 dilution and primer efficiency between 94 and 97%, the relative expression assay was performed through the comparative C_T method. The samples were processed in triplicates with three biological repetitions. Expression of the genes GAPDH and 14.3.3 was analyzed, both genes considered efficient endogenous controls for coffee according to Barsalobres-Cavallari et al. (2009). Expression of the gene GAPDH was normalized with the control gene 14.3.3, and expression of the gene 14.3.3 was normalized with the control gene GAPDH.

The thermal conditions of the reaction were as follows: 2 min at 50°C, 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C, finalizing with 1 s at 95°C. The data were analyzed in the program 7500 *Fast Software* (Version 2.1). For each reaction, 1 µL of cDNA, 1.5 µM of each *primer*, and 5 µL of *Master Mix SYBR Green* (Applied) were used for a final volume of 10 µL per sample.

To calculate the expression, each sample was first normalized with the endogenous control using the equation $\Delta C_T = C_T$ (target gene) - C_T (endogenous control), while the relative quantification was obtained through the formula $2^{-\Delta\Delta CT}$. Threshold was defined manually.

3 RESULTS AND DISCUSSION

The same collected material was used in the extraction procedures and the same quantity of tissue was standardized in the microextractions. All the methods yielded enough quantity of RNA for posterior works. As a macroextraction, the CTAB method was the only one that used a larger quantity of plant tissue and thus yielded a high amount of RNA. However, only sample quality is considered when comparing methods.

For a successful RNA isolation, it is necessary to prevent that polysaccharides and polyphenols, which are found in different quantities in different tissues of distinct species, bind to the nucleic acids, contributing to the determination of RNA quantity, quality, and integrity (SUZUKI; KETTERLING; MCCARTY, 2003; ZAMBONI; PIERANTONI; FRANCESCHI, 2008). Therefore, these parameters were evaluated to determine the most efficient RNA extraction method for coffee.

Purity relations and RNA concentrations from all the evaluated extraction methods are on Table 1. To evaluate purity, the absorbance levels at 280 nm, 260 nm, and 230 nm are observed. The A260/A230 absorbance ratio indicates potential contamination with polysaccharides and polyphenols, while the A260/A280 ratio indicates potential contamination with proteins (LOGEMANN; SCHELL; WILLMITZER, 1997; MANNING, 1991). According to Asif et al. (2006), when these values are between 1.8 and 2.1, they indicate decontamination of the samples.

Comparison of the methods described in this work shows that the hot borate and CTAB methods generally present a better sample quality, especially the CTAB method. As can be observed in Table 1, this method presented the best results with ideal absorbance relations, indicating samples with little or no contamination with proteins and polysaccharides.

This may be related to the use of substances like PVP and β-mercaptoethanol, which have the function of reducing oxidant substances and phenolic compounds (JAAKOLA et al., 2001), and to the precipitation with lithium chloride, which has the function of retaining protein substances, thus aiding in sample decontamination. The use of lithium chloride was described by Rubio-Piña and Vázquez-Flota (2008) as the best substance for RNA precipitation protocols. The use of the CTAB detergent in the extraction buffer in the CTAB method is the basis for separating polysaccharides from nucleic acids (CHANG; PURYEAR; CAIRNEY, 1993; ROGERS; BENEDICH, 1994), as well as being a differential for the obtainment of high-quality RNA.

According to the absorbance value ratios, the Concert and Tri Reagent methods presented proteins and polyphenols contaminated samples, with lower values than those recommended, especially the Tri method (Table 1). Low yield among tissues and, consequently, high contamination are observed in the extraction using the Tri Reagent according to the manufacturer's recommendations, possibly due to the presence of proteins and polyphenolic compounds. A similar result was observed in *Vaccinium myrtillus* fruits by Jaakola et al. (2001). This reagent is composed of phenol, which may cause damage to the poly-A tail of the extracted mRNA (AZEVEDO; LINO-NETO; TAVARES, 2003) and thus hinder the synthesis of DNA and future analyses. Nevertheless, it may be observed in the individual tissue analyses (Table 1) that leaf tissue presents the best quality in almost all the methods, with the presence of few polyphenols.

Fruits, with high levels of phenolic compounds, polysaccharides, and RNases (JAAKOLA et al., 2001), hinder the extraction process. These polysaccharides form a gelatinous structure that precipitates during extraction, affecting both the quantity and the quality of RNA (SHARMA; GILL; SINGH, 2003). However, only the hot borate and CTAB methods efficiently removed these contaminants, presenting A260/A230 ratios larger than 1 (Table 1) as found in the same tissues by Oliveira et al. (2010) due to the action of previously discussed substances added to these methods.

In terms of sample integrity, a relation between integrity and quality may be observed in Figure 1, showing that the methods with satisfactory quality also exhibited integrity of the 28S and 18S bands, characteristic of rRNA.

An expression profile was visible in the methods extracted with the Concert, hot borate, and CTAB methods, determining the quality of these samples (Figures 2 and 3). After treatment with DNase and cDNA synthesis, the samples extracted with the Tri Reagent, despite low quantity and quality, were good enough to perform RT-qPCR; nevertheless, expression patterns characteristic of degraded samples were observed in a few samples, possibly in consequence of the Tri Reagent inaptitude to remove all the contaminants from the sample, hindering the amplification of the desired fragment. Alternatively, the protocol was not efficient enough to wash away all the interfering substances for the reaction, considering that real-time PCR has high detection sensitivity and that these contaminants may interfere in observing expression.

Figures 2 and 3 show that the hot borate, Concert, and CTAB extraction methods efficiently eliminated any contaminant that could potentially obstruct the reaction, because the expression of genes GAPDH and 14.3.3 was observed (as expected for constitutive genes of coffee) (BARSALOBRES-CAVALLARI et al., 2009).

TABLE 1 – Comparison of the quality of RNAs isolated from different tissues through four methodologies. The data represent the SE of three biological replicates.

	Hot Borate			*CTAB*		
Tissues	**Quantity (ng/µL)**	**Purity A_{260}/A_{280}**	**Purity A_{260}/A_{230}**	**Quantity (ng/µL)**	**Purity A_{260}/A_{280}**	**Purity A_{260}/A_{230}**
Root	301 ± 52	1,5 ± 0,12	1,2 ± 0,01	176 ± 37	2,1 ± 0,02	2,1±0,18
Leaf	635 ±60	2,1 ± 0,02	2,0 ± 0,03	2796 ±761	2,0 ± 0,06	2,1 ± 0,08
Flower	782 ±179	2,0 ± 0,04	1,1 ± 0,07	4176 ± 52	1,4 ± 0,14	1,5 ± 0,16
Fruit	225 ± 46	1,9 ± 0,08	1,5 ± 0,19	3109± 74	2,1 ± 0,03	1,7 ± 0,08
	Concert™			*Tri Reagent®*		
Tissues	**Quantity (ng/µL)**	**Purity A_{260}/A_{280}**	**Purity A_{260}/A_{230}**	**Quantity (ng/µL)**	**Purity A_{260}/A_{280}**	**Purity A_{260}/A_{230}**
Root	776 ± 106	1,6 ± 0,04	0,6 ± 0,08	327 ± 53	1,3 ± 0,03	0,7 ± 0,13
Leaf	2045 ± 176	1,9 ± 0,06	1,1 ± 0,16	647 ± 80	1,6 ± 0,06	0,5 ± 0,09
Flower	1141 ± 20	1,9 ± 0,10	0,7 ± 0,02	1197± 149	1,3 ± 0,07	0,5 ± 0,03
Fruit	430 ± 33	1,4 ± 0,06	0,5 ± 0,11	481 ± 175	1,0 ± 0,03	0,7 ± 0,24

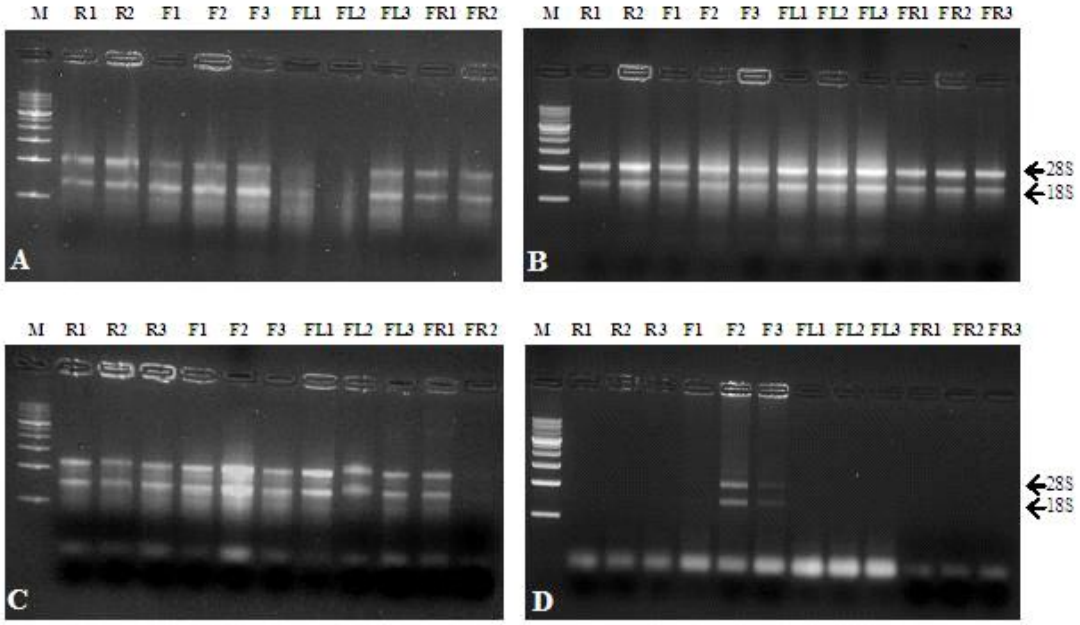

FIGURE 1 – 1.2% agarose gel stained with ethidium bromide. The used samples were treated with DNase and 2 µL were applied to the gel. A – extraction through the hot borate method; B – CTAB; C – Concert; D- Tri Reagent. M –1 Kb molecular marker; *Coffea arabica* tissues: 1-3 (biological repetitions). R (1-3) root; F (1-3) leaf; FL (1-3) flower; FR (1-3) fruit.

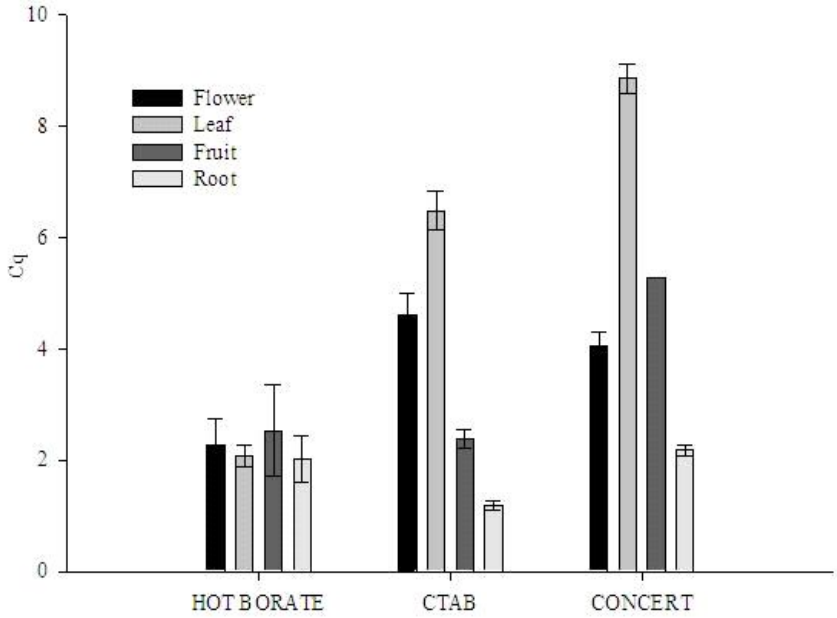

FIGURE 2 – Example expression pattern of the endogenous gene GAPDH, normalized with 14.3.3, in coffee tissues (flower, leaf, fruit, and root), using different extraction methods. The values represent the average of the expression values of the biological triplicates.

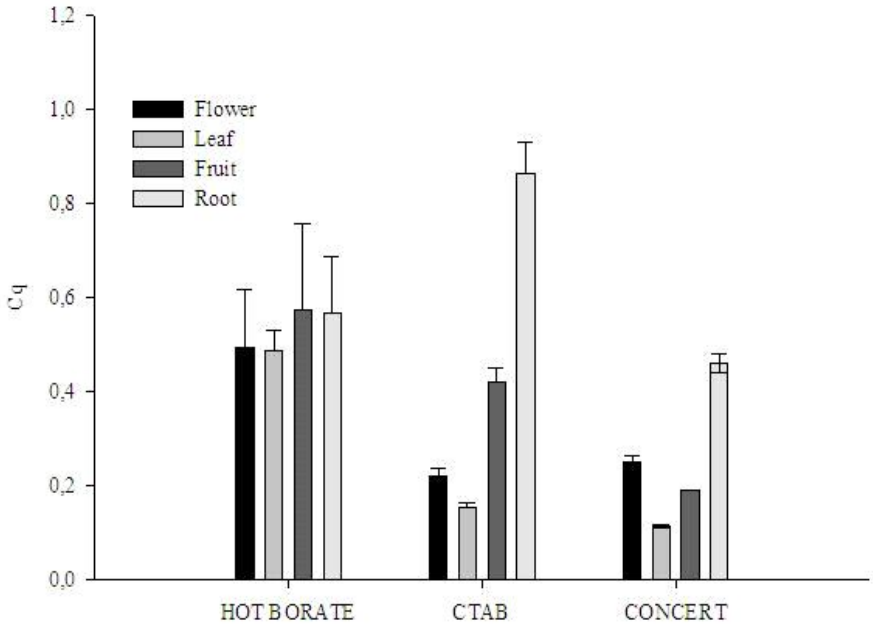

FIGURE 3 – Example expression pattern of the endogenous gene 14.3.3, normalized with GAPDH, in coffee tissues (flower, leaf, fruit, and root), using different extraction methods. The values represent the average of the expression values of the biological triplicates.

Although the extraction from a few coffee tissues using the Tri Reagnet method did not produce RNAs with good enough quality to be submitted to real-time PCR analysis, it was observed that the leaf RNA samples were sufficient for this analysis (data not shown). This may be explained by the fact that coffee leaf tissue does not present as many metabolic compounds and contaminants as the other tissues used in this work.

It may be inferred through the analysis of Table 1 and Figure 1 that the coffee leaf tissue generated quality RNA samples when all four protocols were used. However, when comparing the extraction methods for coffee leaf, flower, root, and fruit tissues, the best results were observed for the CTAB method. Considering it is a macroextraction method, the availability of a larger quantity of plant material is necessary. The Concert and hot borate methods were also efficient in quality RNA extraction for real-time PCR analysis, but besides being cheaper, the hot borate also presented samples with superior quality than those from the Concert extraction method.

4 CONCLUSIONS

The use of certain components in the hot borate, CTAB, and Concert extraction methods yielded RNAs of root, leaf, flower, and fruit with few contaminants, not interfering in the RT-qPCR quantitative analysis.

The CTAB method was considered as the one that generated RNAs with the best quality in all tissues and during the entire manipulation process.

Extraction with Tri Reagent presented contamination on most of the samples, being considered an inefficient method for obtaining high-quality coffee RNA.

All methods efficiently extract RNA from coffee leaf, including the Tri Reagent method, and may be used to obtain high-quality RNA.

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