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Evaluation of extraction methods for obtaining high-quality RNA from sweet potato

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ABSTRACT. Considering the great economic and social importance of sweet potato and the few transcriptome studies carried out on this species so far, which can potentially lead to significant improvements in its production system at both productivity and quality levels, this study aimed to determine the most suitable methodology for extracting high-quality RNA from sweet potato's tuberous roots, branches, and leaves. The experiment was composed of three biological replicates, each one comprising three plants. 100 mg of ground tissue was used for isolating RNA through the CTAB and TRIzol methods, while 160 mg was used for the Hot Phenol Acid method. From the three tested protocols, all of them enabled the isolation of RNA at quantities above 250 ng/µL for the three different tissues, which is the minimal quantity required for conducting molecular assays such as RT-qPCR. However, in terms of RNA quality, evaluated through the A_{260}/A_{280} and A_{260}/A_{230} ratios, only the Hot Phenol Acid and CTAB methods generated satisfactory results, displaying values from 1.8 to 2.2. To conclude, the hot acid phenol and CTAB methods, not commonly used for transcriptional studies in sweet potato, are excellent choices for the RNA extraction from different sweet potato tissues.

Key words: *Ipomoea batatas*; Gene expression; Transcriptome; RT-qPCR

INTRODUCTION

Sweet potato is an important crop that is cultivated in several countries, since its tuberous roots are an excellent source of carbohydrates, vitamins, and minerals (Pessu et al., 2020), in addition to being used in the production of nutraceuticals (Zhang et al., 2017) and bioethanol (Rizzolo et al., 2021). More than 91 million tons of sweet potato is produced worldwide, with China, the world's largest sweet potato producer, being responsible for 57 % of this total (FAO, 2021). The Brazilian sweet potato production, which comes from 29 registered cultivars, has reached 350,512 tons (MAPA, 2019). Considering its adaptation to diverse edaphoclimatic conditions, the production levels and cultivation area of sweet potato have significantly increased throughout the Brazilian territory. According to the Brazilian Institute of Geography and Statistics (IBGE, 2019), the Northeast, South and Southeast regions of Brazil are the main production areas of this crop.

Considering the economic importance of sweet potatoes, various breeding studies, mainly performed through conventional methods, such as the selection of superior genotypes, have been carried out (Azevedo et al., 2014). However, the selection process and the improvements in the crop take long periods of time to be achieved, and require a high number of individuals and adequate reproductive systems, considering that sweet potato is a hexaploid species (2n = 6x = 90 chromosomes) (Srisuwan et al., 2006). Thus, biotechnology acts as complementary tool for the classical plant breeding methods, allowing the development of tolerant genotypes for different pathogens and diseases in several species, in addition to enabling the generation of plants that can better deal with environmental variations, optimizing their physiological responses to their cultivation conditions and directly improving the production system, from quality to productivity (Anwar and Kim, 2020; Basso et al., 2020). Gene expression analysis is of fundamental importance in these studies and it can be performed through different techniques, among which Reverse Transcription - quantitative Real Time PCR (RT-qPCR) stands out, considering its sensitivity, reproducibility, and precision (Derveaux et al., 2010). However, to perform studies using this technique, several parameters should be followed in order to get reliable results, such as using high-quality RNA, displaying good integrity levels, choosing the right method for the cDNA synthesis, good amplification efficiencies of the primers, and appropriate reference genes (Bustin et al., 2009; Derveaux et al., 2010). Among the previous mentioned parameters, high-quality RNA is the first step for conducting transcriptional studies.

Several studies have been performed in different plant species, such as citrus (Huma et al., 2020), brassica (Siles et al., 2020), cassava (Behnam et al., 2019), *Phaseolus vulgaris* (Acosta-Maspons et al., 2019), *Elaeis guineensis* (Ong et al., 2019), and coffee (Paula et al., 2012; Huded et al., 2018) aiming to establish the best method for obtaining high-quality RNA. Plant tissues may have compounds, such as polysaccharides and polyphenols, that can negatively interfere in isolating high-quality nucleic acids (Badai et al., 2020), turning this process many times unique for each plant tissue.

Sweet potato shows high levels of polysaccharides (Guo et al., 2017) and polyphenols (Wang et al., 2018), and different methods for isolating high-quality RNA have been tested in this species, including TRIzol (Meng et al., 2018; Ma et al., 2020) and

commercial kits (Guoliang et al., 2020). However, so far, there are no studies comparing the efficiency of these different methods.

Thus, considering the importance of transcriptional studies and the difficulties in isolating high-quality RNA, as well as, the absence of studies comparing RNA isolation methods in sweet potato, this study aimed to determine the best method for isolating high-quality RNA from three different sweet potato tissues: tuberous roots, branches, and leaves.

MATERIAL AND METHODS

Plant material

In order to determine the best RNA isolation method for sweet potato tissues, an experiment was carried out at the research station of the Palmas University Campus, of the Federal University of Tocantins (Latitude: 10°10'40" S; Longitude: 48°21'43"O; Altitude: 220 m), and all molecular analysis were performed in the Laboratory of Molecular Analysis (LAM/Human-Health-UFT). The experiment was conducted using five-months-old sweet potato (*Ipomoea batatas* cv. Duda) plants, using three biological replicates, each one comprising three plants. Tuberous roots, branches, and leaves (young and fully expanded) from the three plants of each biological replicate were collected and immediately frozen in liquid nitrogen and then stored at -80 °C until RNA extraction.

RNA extraction methods

In order to determine the RNA isolation methods to be tested for sweet potato tissues, a search was first carried out on the Web of Science (https://www.webofknowledge.com) and Scopus (https://www-scopus.ez6.periodicos.capes.gov.br/) databases, using the key-words gene expression, RNA, protocols, RT-qPCR, and sweet potato / *Ipomoea batatas*. Three different methods were chosen: hot acid phenol, CTAB (Cetyltrimethylammonium Bromide) (Chang et al., 1993), and TRIzol (Invitrogen).

Hot acid phenol method

The RNA extraction through the hot phenol acid methods was performed according to the protocol described by De Souza et al. (2021), with the following modifications: 160 mg of plant material, grinded in liquid nitrogen, was added to each microtube and 650 µL of acid Phenol were added, followed by homogenization. Then, 650 µL of TES (10 mM Tris-HCl pH 7.5, 10 mM EDTA and 0.5% SDS) were added and samples were homogenized. After incubating the samples on ice for five min and then on a dry bath incubator for one hour at 60 °C, with sample homogenization at every 10 min, samples were centrifuged for 10 min at 14,000 rpm and 4 °C. The supernatant (600 µL) was transferred to a new microtube and 600 µL of Acid Phenol was added, followed by homogenization. Samples were once again incubated on ice for five min and then centrifuged (10 min / 14.000 rpm / 4 °C). After centrifugation 300 µL of the supernatant were transferred to a new microtube (for tuberous roots, the supernatant of two tubes from each replicate were added to a single tube in order to increase the RNA amount to be extracted) for RNA precipitation, which was

carried out by the addition of 600 µL and 350 µL of isopropanol to the tuberous roots and other tissues, respectively. Samples were incubated for one hour at - 20 °C and then centrifuged (30 min / 14.000 rpm / 4 °C), with the supernatant being discarded and the RNA pellet washed with 700 µL of cold 70 % ethanol, followed by a last centrifugation step (8 min / 14.000 rpm / 4 ° C). Finally, the RNA pellet was resuspended in 20 µL of nuclease-free water and samples were stored at -80 °C.

CTAB method

RNA extraction through the CTAB method was carried out according to the protocol described by Chang et al. (1993), adapted to microtubes and with minor modifications. 450 µL of the extraction buffer [2% (w/v) CTAB (Cethyltrimethylammonium Bromide), 2% (w/v) PVP, 100 mM of Tris-HCL, 25 mM of EDTA, 20 mM of NaCl], 450 µL of TES (10 Mm de TRIS-HCL Ph 7,5, 10 mM de EDTA e 0,5% SDS), and 100 µL of β-mercaptoethanol were first added to 100 mg of ground material. After sample homogenization, samples were incubated for one hour at 65 °C on a dry bath incubator, with sample homogenization at every 10 min. Then, samples were centrifuged for 10 min at 11,000 rpm and 4 °C, with 800 µL of the supernatant being then transferred to microtubes containing 800 µL of chloroform. After homogenizing the samples, samples were again centrifuged (10 min / 11.000 rpm / 4 ° C), and 400 µL of the supernatant then were added to new microtubes containing 400 µL of isopropanol for RNA precipitation, which occurred through the incubation of the samples for 60 min at -20 °C. The RNA pellet was generated after centrifuging the samples for 30 min at 13,800 rpm and 4 °C, the supernatant was discarded, and the pellet washed with 800 µL of cold 75 % ethanol. After centrifuging the samples for 8 min at 13,800 and 4 °C, ethanol was discarded and the pellets dried for approximately 5 min on an oven at 37 °C, being finally resuspended in nuclease-free water and then stored at -80 °C.

TRIzol method

RNA extraction using the TRIzol (Invitrogen®) reagent was performed according to the manufacturer's instructions (https://assets.thermofisher.com/TFS-Assets/LSG/manuals/trizol_reagent.pdf).

DNase treatment and cDNA synthesis

RNA samples (5 µg) were treated with DNase I using the Turbo DNA-free kit (Ambion) for elimination of residual DNA contamination. RNA content, as well as quality (A_{260}/A_{280} and A_{260}/A_{230}) were accessed by spectroscopy (Nanodrop® ND-1000), and its integrity was visually analyzed in 1.0 % agarose gel. Considering the difficulties found in isolating RNA from sweet potato tuberous roots, due to their high starch content, RNA integrity for this tissue was also evaluated with the Agilent 2100 Bioanalyzer.

One µg of the total RNA was reverse transcribed into cDNA using the High- Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, USA), according to manufacturer's protocol, and subsequently stored at -20 ◦C.

Primer design and Real-Time quantitative PCR (RT-qPCR)

Gene expression analysis through RT-qPCR consisted in the evaluation of the expression profile of the gene *IbSRD1* (Storage Root Development-related 1), which was chosen due to its crucial role on sweet potato root development (Noh et al., 2010), on sweet potato tuberous roots, leaves, and branches, using the RNA obtained through the three extraction methods evaluated in this study. *IbSRD1* primers were designed using the OligoPerfect software (https://apps.thermofisher.com/apps/oligoperfect/), and their quality was evaluated through the OligoAnalyzer tool (https://www.idtdna.com/calc/analyzer).

RT-qPCR analyses were carried out on a ABI PRISM 7500 fast Real-Time PCR thermalcycler (Applied Biosystems), using SYBR-green detection system and the cDNA obtained from the RNA extracted from the three sweet potato tissues and extraction methods. Reactions were carried out in 10 µL reaction volume: 5 µL of SYBR-green (Master Mix PowerUp SYBR green UDG with ROX) (Invitrogen), 0.2 µL (0.2 M final concentration on the reaction) of forward and reverse gene-specific primers (see Table 1 for primer sequences and gene amplification efficiencies), 1 µL of cDNA (80 ng), and 3.6 µL of nuclease free water. Three biological replicates for each treatment were used, reactions were run in triplicates, and amplification was performed with the following reaction conditions: 2 min at 50 °C, followed by 10 min at 95 °C, then 40 cycles 95 °C for 15 s followed by 1 min at 60 °C, and completed with a melting curve analysis to access specificity of the reaction by raising the temperature from 60 to 95 °C, with 1 °C increase in temperature every 5 s. Relative fold differences were calculated based on the ΔΔCT method (Pfaffl, 2001), using *IbAlfaTub* (Alpha tubulin) e *IbELF* (translation initiation factor eIF-2B) as references genes (Yu et al., 2020), and were calculated relative to a calibrator sample (Biological replicate I from branch samples extracted through the hot acid phenol method).

Table 1. Gene names, GenBank accession numbers, RT-qPCR primer sequences, and gene amplification efficiencies of the analyzed genes from sweet potato.

Gene	GenBank Accession number	Primer sequence (5' – 3')	Amplicon (pb)	Amplification efficiency (%)
IbSRD1	FJ237529.1	Fw: GCACAAGTGACCCAAATGC Rv: GCTCTGGCTAGATCGCTG	124	90
IbELF	XM_019343175.1	Fw: ATCTCTTTGACGGCTGGTTG Rv: CTCTGCACGCTCAAGAAGG	111	98
IbAlfaTub	BM878762.1	Fw: CAACCGGCTTCAAATGTGG Rv: GTGGTCGATGCGTGAGAA	137	100

RESULTS

RNA extraction methods

The searches on the Web of Science and Scopus databases resulted in 12 studies that conducted RT-qPCR assays in sweet potato (Table S1). The analysis of the methodologies described in each paper allowed one to observe that two methods, TRIzol and commercial kits, comprised 54 % and 46 % of the studies, respectively (Figure 1). Thus, based on the results obtained from these databases, the TRIzol method was included in this study as a reference, since it is the method more commonly used for isolating RNA from sweet potato. The two other methods on the other hand, CTAB and Hot Acid Phenol, were chosen because they are often used in transcriptional studies in different organisms.

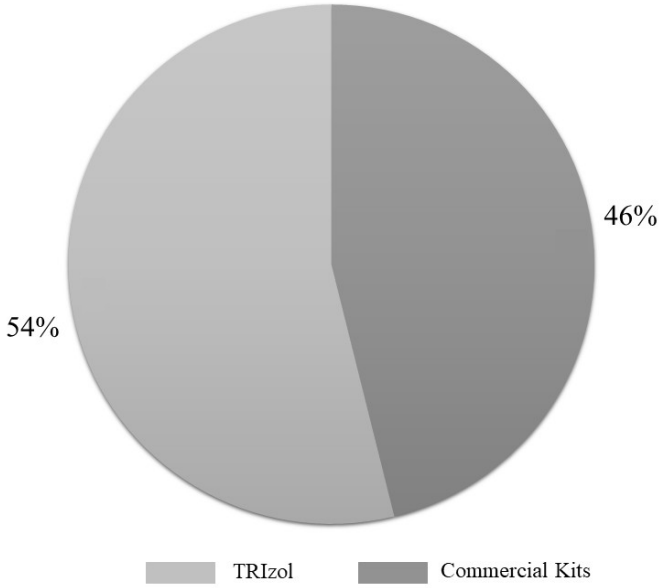

Figure 1. RNA isolation methods used in RT-qPCR studies conducted on sweet potato according to the Web of Science and Scopus databases.

RNA quantity and purity

The three RNA isolation methods analyzed here generated sufficient RNA for performing sensitive molecular studies (Table 2). Comparing the RNA quantities among each extracting method, one can observe that the hot acid phenol, the CTAB, and the TRIzol methods displayed mean values of 297.07 ng/µL to 730.73 ng/µL, 334.53 ng/µL to 608.77 ng/µL, and 315.30 ng/µL to 518.23 ng/µL, respectively (Table 2).

Table 2. Comparison of the RNA quantity and purity obtained from different sweet potato tissues through different RNA isolation methods.

RNA extraction method	Tissues	Quantity RNA (ng/µL)	Purity A260/A280	Purity A260/A230
Hot acid phenol	Tuberous roots	297.07 ± 14.90	1.93 ± 0.03	2.23 ± 0.01
	Branches	362.87 ± 25.68	1.92 ± 0.01	2.24 ± 0.05
	Leaves	730.73 ± 191.56	2.00 ± 0.03	1.99 ± 0.21
CTAB*	Tuberous roots	334.53 ± 34.04	2.16 ± 0.01	1.80 ± 0.05
	Branches	608.77 ± 41.12	2.10 ± 0.01	2.09 ± 0.05
	Leaves	592.20 ± 177.25	2.07 ± 0.06	1.91 ± 0.20
TRIzol	Tuberous roots	315.30 ± 96.03	1.96 ± 0.01	0.41 ± 0.03
	Branches	461.30 ± 80.23	2.04 ± 0.04	0.56 ± 0.05
	Leaves	518.23 ± 240.55	1.89 ± 0.07	0.37 ± 0.08

*CTAB (cethyltrimethylammonium bromide)

In relation to RNA purity, the analysis of the A_{260}/A_{280} and A_{260}/A_{230} ratios showed that the RNA isolated through the CTAB and hot acid phenol methods displayed values above 1.8, indicating the high purity standards of these samples. On the other hand, for the

TRIzol method, RNA from all tissues analyzed showed values below 1.0 for the A_{260}/A_{230} ratio, indicating the contamination of the samples (Table 2).

RNA integrity

The analysis of RNA integrity through agarose gel allowed the clear visualization of the two ribosomal RNA subunits (28S and 18S) for the three RNA isolation procedures analyzed, except for the RNA (RNA partially degraded) from leaves (Biological replicate II - L2) extracted through the CTAB method and the TRIzol method (RNA completely degraded).

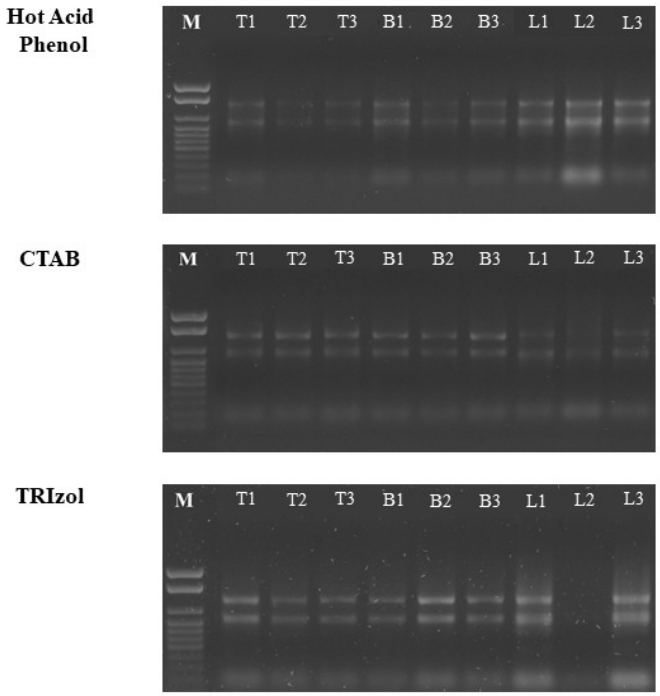

Figure 2. Integrity analysis of the RNA isolated from different sweet potato tissues (T: tuberous roots; S: stem; L: Leaves) through the hot acid phenol, CTAB, and TRIzol methods. Agarose gels (1.0%) were stained with Sybr safe. M - 1 Kb molecular weight marker.

About the RIN (RNA Integrity Number) analysis, carried out on tuberous roots samples, it could be observed, for the three RNA isolation methods, good integrity levels, with RIN values above 6.5 (Figure 3), which is in accordance with the results from the integrity analysis through agarose gels. However, the analysis of the electropherograms generated from each biological replicate allowed the observation that the RNA samples obtained through the TRIzol method showed an elevated basal line (Figure 3), possibly a result of the contaminants related to the A_{260}/A_{230} ratio, which showed values below 1.0 (Table 2).

Gene expression analysis

The analysis of the relative expression of the *IbSDR1* gene in the three sweet potato tissues, using the RNA obtained from the three different isolating methods tested in this study,

showed that *IbSDR1* was more expressed in tuberous roots, except when the RNA was extracted through the TRIzol method (Figure 4). The relatively higher expression on tuberous roots was an expected result, considering that this gene is related with the development of tuberous roots in sweet potato. In relation to the low expression levels of *IbSDR1* when RNA was isolated through the TRIzol method, this result was possibly related to the contamination of the samples, as observed in Table 2 and the noise present through the entire electropherogram of these samples (Figure 3).

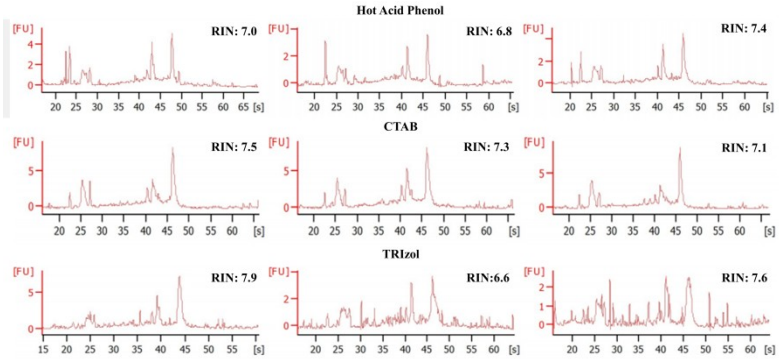

Figure 3. Electropherograms generated from the chip electrophoresis, performed with Bioanalyzer 2100, of the RNA samples isolated from sweet potato tuberous roots through the hot acid phenol, CTAB, and TRIzol methods.

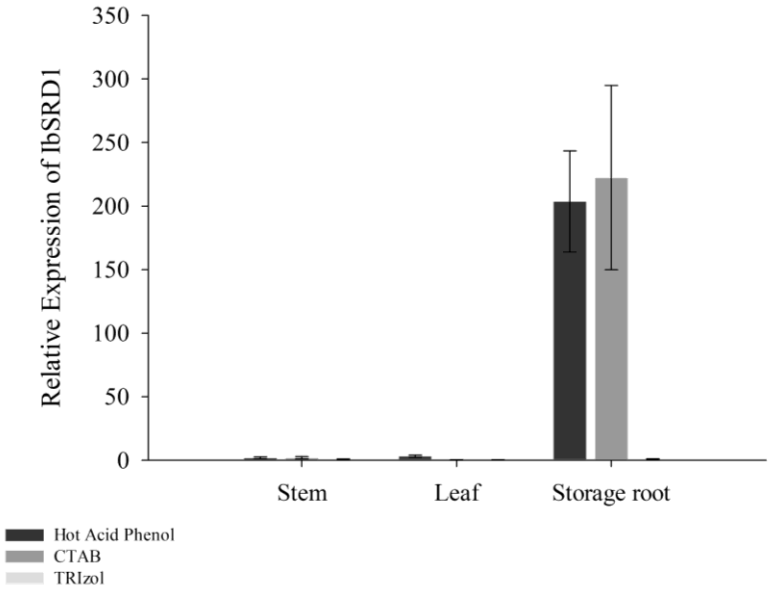

Figure 4. Relative quantitative expression profile of *IbSDR1* in sweet potato stem, leaves, and tuberous root, using the RNA obtained from three different methods (hot phenol acid, CTAB, TRIzol). Columns represent the fold difference in gene expression among the different RNA extraction methods for each tissue, relative to a calibrator sample (Biological replicate I from branch samples extracted through the hot acid phenol method). The relative quantification was obtained by the formula $E_{target}^{\Delta CTtarget} / E_{reference}^{\Delta CTreference}$ (Pfaffl, 2001), using *IbELF* and *IbAlfaTub* as reference genes. Expression values for each biological sample were obtained from three biological repetitions and error bars represent the standard error for them.

DISCUSSION

RNA extraction methods

In this study, the evaluation of three different RNA extraction methods, hot acid phenol, CTAB and TRIzol, for obtaining high-quality RNA from different tissues from sweet potato, showed that, although all of them generated RNA at sufficient quantities for molecular studies, the RNA obtained from TRIzol method displayed lower quality when compared to the other methods.

The three RNA isolation methods analyzed here showed that the hot acid phenol method has not been used so far in transcriptional studies of this species (Table S1), even though it is considered an efficient RNA extraction method that has been used in other organisms (Scholes and Lewis, 2020; De Souza et al., 2021). For the CTAB method, according to the search performed on the Web of Science and Scopus databases, this method also has not been used in transcriptional studies of sweet potato. However, studies that are not present on these databases, aiming the optimization of the RNA isolation method for obtaining high-quality RNA, have been carried out using this method in sweet potato (Suzuki et al., 2008; Zhou et al., 2009). However, these optimizations are related to macro-extractions, which requires more plant material and reagents. In relation to the TRIzol method, it was shown to be the most commonly used method for RNA isolation from sweet potato so far (Table S1). Park et al. (2017) and Meng et al. (2018), for instance, used this method to isolate RNA and evaluate the gene expression profile of *High-affinity K+ transporter 1* (*IbHKT1*) (involved in salinity tolerance) and genes from the NAC family (involved in the response of several abiotic stresses), respectively, through RT-qPCR. Hou et al. (2021) used the TRIzol method to isolate RNA and analyze genes from the B-galactosidase family, which are involved in the response of biotic and abiotic stresses. In addition, the TRIzol method has also been used on the transcriptome sequencing of sweet potato (Tao et al., 2012), and on the characterization of *ERD15*, a gene that seems to be involved in the defense response to water stress in sweet potato (Shao et al., 2014), to identify water deficit responsive genes in RNAseq and RT-qPCR studies (Arisha et al., 2020), in the validation of reference genes for the expression normalization of miRNAs (Liu et al., 2020), and for the evaluation of genes associated to starch and sucrose metabolism (Ma et al., 2020; Qin et al., 2021).

RNA quantity and purity

The challenge of extracting high-quality RNA is in part related to the need of preventing RNA contamination with polysaccharides and polyphenols, which can be found in different amounts according to the tissue and species and can bind to nucleic acids, affecting their quality and integrity (Behnam et al., 2019; Badai et al., 2020). Thus, these parameters were evaluated here to determine the most efficient method for isolating RNA from sweet potato.

An RNA quantity commonly used for DNase treatment in transcriptional studies using the RT-qPCR technique is 5 µg, with 1 µg being then used for the cDNA synthesis (Freitas et al., 2019; Daude et al., 2020; De Souza et al., 2021), with these quantities varying according to the kits that are used. Thus, a concentration of at least 250 ng/µg, in a

20 µL volume (as used in this study), is required to perform the further analysis, and as one can observe in Table 2, all RNA isolation methods tested in this study generated sufficient RNA quantities to perform the gene expression analysis.

In relation to the purity of the samples, the absorbance at 230 nm, 260 nm, and 280 nm are evaluated. The A_{260}/A_{230} ratio indicates the contamination of the RNA sample with polysaccharides or polyphenols, and the A_{260}/A_{280} ratio indicates the contamination with proteins (Liu et al., 2018). The analysis of the results generated here allowed the observation that the RNA from the three sweet potato tissues analyzed here generated satisfactory results towards the A_{260}/A_{230} and A_{260}/A_{280} ratios, except when the TRIzol method was used, where the A_{260}/A_{230} ratio was significantly reduced in all tissues (Table 2).

One of the procedures that have helped in obtaining high-quality RNA for the hot acid phenol and CTAB methods was the use of the TES buffer (10 Mm de TRIS-HCL Ph 7,5, 10 mM de EDTA e 0,5% SDS). This buffer contains the reagent SDS (Sodium Dodecyl Sulfate), which according to Behnam et al., (2019), it is an anionic detergent that, in addition to solubilizing proteins and lipids, facilitates sample lysis. Different RNA extraction protocols have been optimized for the use of SDS, providing higher RNA quantities and elevated purity ratios (Behnam et al. 2019; Vennapusa et al., 2020).

For the CTAB method, in addition to using TES in the RNA extraction procedure, the RNA isolation uses chloroform for removing proteins, resulting in a colorless RNA and, through the use of NaCl (2M) in the buffer, it is possible to remove polysaccharides (Chang et al., 1993). Thus, the CTAB method is commonly used for the RNA isolation from tissues with high polysaccharide contents (Ong et al., 2019; Qadri et al., 2019). However, it is a method not commonly used for RNA extraction from sweet potato tissues.

In relation to the TRIzol method, though it is widely used in transcriptional studies of sweet potato (Figure 1), the results found in this study were not satisfactory. Although this RNA extraction method has been used in other species (Acosta-Maspons et al., 2019; Behnam et al., 2019; Guan et al., 2019), the different optimizations present in these studies were not sufficient to improve the purity of RNA obtained.

RNA integrity

The RNA integrity can be evaluated through the use of agarose gels (Azizi et al., 2017; Murgan et al., 2020), as well as through the determination of the RIN (Siles et al., 2020; Vennapusa et al., 2020). However, the integrity analyzed by agarose gels depends on the interpretation of images by the user, which is prone to errors, differently the RIN approach, where an algorithm is used to standardize the RNA quality control, generating an integrity number (Schroeder et al., 2006).

The algorithm evaluates aspects such as: total RNA proportion (compares the fraction area of the 18S and 28S region with the total area), the 28S peak height, and the ratio between the fast region and marker height. By analyzing these parameters, the RNA integrity is classified into ten different categories (1 to 10), where an intact RNA receives a RIN of 10 and a totally degraded RNA displays a RIN of 1 (Schroeder et al., 2006). Fleige and Pfaffl (2006) consider RNA samples with good integrity levels for RT-qPCR studies those presenting RINs above 5.

In the present study, we obtained RIN≥ 6.6 for the RNA samples obtained through the three RNA isolation methods tested (Figure 3), what is similar to other studies present in the literature, such as transcriptional studies through qPCR from *Coffea arabica* (Freitas et al., 2017), *Pelargonium zonale*, and *Ramonda serbica* (Vidović and Ćuković, 2020), and *Manihot esculenta* (Behnam et al., 2019; Guan et al., 2019). For instance, in the study developed by Freitas et al. (2017), RINs ≥ 7.0 were obtained for different tissues in an assay for the validation of reference genes for RT-qPCR analysis of *Coffea arabica* somatic embryogenesis-related tissues.

RT-qPCR

IbSDR1 is a MADS-box gene that has an important role in storage roots (Noh et al., 2010). Considering this, the promoter region of this gene from sweet potato has been analyzed in Arabidopsis, carrot, and transgenic potato, aiming to direct the expression to storage organs (Noh et al., 2012). *IbSDR1* overexpression is able to double the number of sweet potato storage roots (Bae et al., 2013). In addition, *IbSDR1* seems to be involved in the regulation of the Indole-3-acetic acid concentration (Si et al., 2018), which is an auxin essential root growth (Han et al., 2019).

The results described in the literature corroborates with the one obtained here when the RNA from sweet potato tissues was extracted through the hot acid phenol and CTAB methods, since *IbSDR1* showed relatively higher expression levels in tuberous roots and displayed a basal expression level in the other tissues (stem and leaves) (Figure 4). On the other hand, when RNA was isolated through the TRIzol method, the results showed that the RNA quality directly affected the reaction, inhibiting the PCR, similar to what has been reported in other studies, as observed by Huma et al. (2020). In this study, where different RNA isolation methods were tested in citrus, low values for the A_{260}/A_{230} ratio directly affect the expression analysis, leading to the absence of amplification in the RT-PCR analysis.

CONCLUSIONS

The three RNA extraction methods tested here (hot acid phenol, CTAB and TRIzol) allowed the isolation of RNA with sufficient quantities for carrying out molecular studies in sweet potato. In relation to RNA quality, the hot acid phenol and CTAB methods generated satisfactory results for conducting transcriptional studies through RT-qPCR in sweet potato, while the TRIzol method must be optimized, since it can lead to imprecise results. To conclude, the hot acid phenol and CTAB methods, not commonly used for transcriptional studies in sweet potato so far, are excellent choices for the RNA extraction from different sweet potato tissues.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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