HortScience 39(1):177–180. 2004.

An Effective Method for Co-isolating Nucleic Acids from Horticultural Aroids

Stacie L. Aragon, Keng-Chang Chuang[2], and Adelheid R. Kuehnle[1]
University of Hawaii at Manoa, Department of Tropical Plant and Soil Sciences, 3190 Maile Way, Honolulu, HI 96822-2279

Additional index words. Araceae, *Anthurium, Colocasia,* DNA, RNA, *Spathiphyllum,* taro

Abstract. **Isolation of high quality nucleic acids from aroids can be difficult due to the presence of carbohydrates, phenolics, and other compounds that bind to and/or co-precipitate with the DNA or RNA. Methods previously used for marine algae, mango, and papaya were modified and successfully used for the simultaneous isolation of high quality genomic DNA and RNA from *Anthurium, Colocasia,* and *Spathiphyllum* leaves. Genomic DNA yields averaged 477 $\mu g \cdot g^{-1}$ fresh weight for *Anthurium* and 322 and 177 $\mu g \cdot g^{-1}$ fresh weight, respectively, for *Colocasia* and *Spathiphyllum*. Total RNA yields averaged 129 $\mu g \cdot g^{-1}$ fresh weight for *Anthurium* and 61 and 50 $\mu g \cdot g^{-1}$ fresh weight, respectively, for *Colocasia* and *Spathiphyllum*. This method may be useful in co-isolating high quality nucleic acids from additional aroids and other plants.**

Extraction of DNA and RNA from plant tissues is often accompanied by complex carbohydrates and phenolics that bind to or co-precipitate with nucleic acids and proteins (Loomis, 1974) and can interfere with subsequent analytical procedures. The CTAB (cetyltrimethylammonium bromide) protocol (Doyle and Doyle, 1990) is a popular method used to eliminate complex carbohydrates during simultaneous isolation of DNA and RNA from many plant species. To date the CTAB protocol has been effective for *Anthurium* and other *Araceae* DNA (Cho and Palmer, 1999; Kuehnle et al., 2001) but may not completely remove polysaccharides to produce the quantity and quality needed for downstream applications (Buldewo and Jaufeerally-Fakim, 2002). Other methods used in *Anthurium* DNA isolation utilize buffers containing SDS (Buldewo and Jaufeerally-Fakim, 2002; Chen and Kuehnle, 1996) or sarkosyl (Ranamukhaarachchi et al., 2001) and proved suitable for RAPD-PCR and Southern analyses. Utility of these methods with other horticultural aroids and for co-isolation of genomic DNA and RNA are unreported.

Standard protocols for RNA purification, including use of guanidine isothiocyanate, Trizol®, 2-butoxyethanol, and a method using polyvinylpyrrolidone (Champagne and Kuehnle, 2000), all yielded degraded or impure RNA. We report here that a newly modified protocol, originally utilized for marine algae (Su and Gibor, 1988) and modified for mango and papaya (Lopez-Gomez and Gomez-Lim, 1992; Mason and Botella, 1997), is highly reproducible for obtaining high quality nucleic acids from *Anthurium, Colocasia,* and *Spathiphyllum*. RNA is isolated without co-precipitation of a largely insoluble purple-pigmented mass of presumed polysaccharides and polyphenols; genomic DNA is co-isolated from the same tissue. The protocol can be easily scaled down for small tissue samples and for use in microcentrifuge tubes only.

Received for publication 16 May 2003. Accepted for publication 12 Sept. 2003.

[1]To whom reprint requests should be addressed. E-mail: heidi@hawaii.edu

[2]Current address: Floriculture Research Center, TARI, 1-10 Mayuan Village, Ku-Keng, Yun Lin, Taiwan 646, R.O.C.

Materials and Methods

Plant materials. Fully expanded leaf blades were harvested from greenhouse-grown wild-type *Anthurium* 'Paradise Pink' Hort., a Hawaiian taro, *Colocasia esculenta*, of the 'Lehua' group, and *Spathiphyllum* 'Petite'. Tissue was also used from *Anthurium* 'Paradise Pink' Hort. transgenic for Shiva-1 (T.M. Fujii, 2002). Samples were frozen immediately in liquid nitrogen and either stored at –80 °C until extraction was performed or used immediately. Samples were weighed out prior to grinding in liquid nitrogen in 1-g or 50-mg quantities for large-scale and minipreps, respectively.

Reagents and equipment. Chemicals were purchased from Fisher Scientific (Fairlawn, N.J.) unless otherwise noted. All reagents were made with distilled deionized water (ddH_2O). Reagents used for RNA procedures were made with ddH_2O that was treated with 0.05% v/v diethylpyrocarbonate (DEPC) and then autoclaved. All prepared solutions were autoclaved before use with the exception of those containing ethanol.

Nucleic acid extraction buffer was 150 mM Tris base, 50 mM EDTA, and 2% w/v SDS, adjusted to pH 7.5 with boric acid. Fresh saturated phenol (Fisher #BP1750I, pH 7.9 for combined RNA/genomic DNA preps; Fisher #BP1751; pH 4.3 for RNA only preps) was used for organic extractions. For large-scale preparations, centrifugation was performed in a Sorval RC-5B centrifuge (Beckman Coulter, Fullerton, Calif.) using a SS-34 rotor and Nalgene Oakridge polypropylene tubes (Fisher #05-529-1D) at 4 °C. Small-scale centrifugations were performed in an Eppendorf 5417R (BioRad, Hercules, Calif.) at 4 °C. All equipment, including Oakridge tubes, mortar and pestles, and spatulas, used in this protocol were washed with 1% v/v Absolve (NEN Life Science Products, Boston), rinsed with DEPC-ddH_2O, and autoclaved prior to use. A 10× MOPS stock was prepared with 0.2 M MOPS [3-(*N*-morpholino) propanesulfonic acid], 0.05 M sodium acetate, 0.01 M Na_2EDTA, adjusted to pH 7.0 with NaOH, filter sterilized, and stored at 4 °C. Glass beads were purchased from Biospec Products (Bartlesville, Okla.; #11079125).

The absorbance of DNA and RNA preps was determined using a Shimadzu UV160U UV-Vis scanning spectrophotometer (Allometrics, Baton Rouge, La.).

Nucleic acid extraction protocols

Large-scale nucleic acid isolation.

1) Per 1 g of aroid leaf tissue, 9.9 mL of nucleic acid extraction buffer and 0.1 mL 2-mercaptoethanol (1% v/v final concentration) was aliquoted into an Oakridge tube.

2) Tissue was ground to a fine powder, added to the extraction buffer, inverted gently but thoroughly for 1 min. Ethanol (0.25 vol) and 5 M potassium acetate (0.11 vol) were added directly to the sample and inverted for 1 min. This was followed by the addition of 1 vol 24 chloroform : 1 isoamyl alcohol to the tube, with gentle inversion for an additional minute. The tubes were then centrifuged at 39,000 *g* (maximum) for 30 min.

3) Supernatant was transferred to a clean tube, and extracted with an equal volume of phenol : chloroform : isoamyl alcohol, then inverted gently but thoroughly for 1 min. Phases were separated by centrifugation and followed by 3 to 4 additional phenol : chloroform : isoamyl alcohol extractions until no precipitate was visible at the interface. If a significant loss of aqueous volume occurred during the extractions, the volume was replenished with additional nucleic acid extraction buffer.

4) The supernatant was transferred to a clean tube, 2.25 vol ethanol added, mixed well, and incubated for 2 h at –20 °C.

5) The sample was then centrifuged, the supernatent discarded, and the pellet washed with 10 mL 70% v/v ethanol. The sample was centrifuged again and washed for a second time using another 10 mL 70% v/v ethanol.

6) Pellets were air-dried for 20 min at room temperature, then resuspended in 10 mL DEPC-ddH_2O. LiCl was added to give a final concentration of 2 M LiCl and incubated at –20 °C overnight.

7) After centrifugation, supernatant was transferred to a fresh tube to continue with the genomic DNA preparation, step 8a. The pellet was used to isolate RNA, step 8b.

Genomic DNA isolation. Note: Gentle inversion was used for all mixing steps.

8a) A 2.5 vol 95% v/v ethanol was added to the step 7 supernatent and incubated at –20 °C for 1 h.

9a) The sample was than centrifuged at 27,000 *g* for 30 min and the pellet washed 2× with 70% v/v ethanol.

10a) The pellet was air-dried at room

temperature for 20 min, resuspended in 398 μL H_2O, and transferred to a 1.5-mL microcentrifuge tube.

11a) Two microliters of RNAseA (10 mg·mL^{-1}) was added followed by a 15-min incubation at 37°.

12a) Sodium acetate (0.1 vol, 3 M, pH 5.5) was added, mixed well, and 2.5 vol ethanol added. The samples were then incubated at –20 °C for 30 min.

13a) After incubation, the samples were centrifuged at 27,000 *g* for 30 min; then the pellet was washed 2× with 70% v/v ethanol.

14a) Pellets were air-dried a final time at room temperature for 20 min. The genomic DNA pellet was then resuspended in 200–300 μL TE buffer.

RNA isolation. Note: If only RNA was to be isolated, saturated phenol pH 4.3 was used in step 3. This decreased any genomic DNA contamination and increased the RNA quality. "RNA only" preps were shaken vigorously in steps 1–7, rather than gently inverted.

8b) Pellets from step 7 were washed 2× with 70% v/v ethanol.

9b) The RNA pellets were then air-dried at room temperature for 20 min, resuspended in 0.4 mL DEPC-ddH_2O, and transferred to a 1.5-mL microcentrifuge tube.

10b) A 0.1 vol 3 M sodium acetate pH 5.5 was added, mixed well, followed by the addition of a 2.5 vol ethanol. The sample was then incubated at –80 °C for 20 min.

11b) After centrifugation at 27,000 *g* for 30 min, the pellet was washed 2× with 70% v/v ethanol.

12b) The RNA pellet was air-dried at room temperature for 20 min and resuspended in 25 μL of DEPC-ddH_2O.

RNA and genomic DNA small-scale preparation. Starting material consisted of 50 mg of tissue ground in liquid nitrogen in 1.5-mL microcentrifuge tubes using micropestles and glass beads. The reagent ratios, centrifuge speeds, and timing were kept identical to those of the large-scale preparation; however, the reagent volumes were scaled down to accommodate the usage of 1.5-mL microcentrifuge tubes for the entire procedure. The final pellets for RNA and genomic DNA were resuspended in 10 and 30 μL DEPC-ddH_2O, respectively.

Determination of DNA and RNA quantity and quality. DNA or RNA was diluted 1:500 and the absorbance measured as a continuous scan from 200 through 300 nm. Absorbance at 260 nm was used to calculate DNA or RNA quantity. The absorbance at 260 was then compared to the absorbance at 230 and 280 nm as indicators of purity.

Genomic DNA was examined by electrophoresis in a 0.6% w/v agarose gel using standard TAE electrophoresis protocols (Sambrook and Russell, 2001). The DNA quantity and quality obtained by co-isolation were compared to results from the CTAB method (Doyle and Doyle, 1990) and commercial kits by Qiagen (DNeasy Plant Kit; Valencia, Calif.) and Promega (Wizard Genomic DNA Purification Kit; Madison, Wis.). A minimum of four samples were used for each method, except for two samples used per each kit, to determine average values. Total RNA was examined by electrophoresis in a 1.5% w/v denaturing agarose gel using the method outlined in Champagne and Kuehnle (2000). The RNA quantity and quality obtained by co-isolation were compared to results from a method using polyvinylpyrrolidone (Champagne and Kuehnle, 2000). A minimum of four samples was used for each method to determine average values. RT-PCR was performed using a standard RT-PCR protocol from Sambrook and Russell (2001). Primers to the Shiva-1 peptide coding sequence were used (T.M. Fujii and A.R. Kuehnle, unpublished).

Table 1. Comparison of genomic DNA yields and purity based on absorbance ratios for the isolation of genomic DNA from *Anthurium*, *Colocasia*, and *Spathiphyllum* by various method.

	Co-isolation[z]	CTAB[y]	Commercial kits[x]
DNA yield ug·g^{-1} fresh weight tissue			
Anthurium	477	75	13
Colocasia	322	50	nd
Spathiphyllum	177	77	nd
Absorbance 260/280 ratio			
Anthurium	1.9	1.9	1.5
Colocasia	1.9	1.9	nd
Spathiphyllum	2.0	1.9	nd
Absorbance 260/230 ratio			
Anthurium	2.0	1.2	0.9
Colocasia	1.9	1.3	nd
Spathiphyllum	1.9	1.6	nd

[z]DNA/RNA Co-isolation method.
[y]Cetyltrimethylammonium bromide method.
[x]Qiagen DNeasy Plant Kit and Promega Wizard Genomic DNA Purification Kit.
nd = not determined.

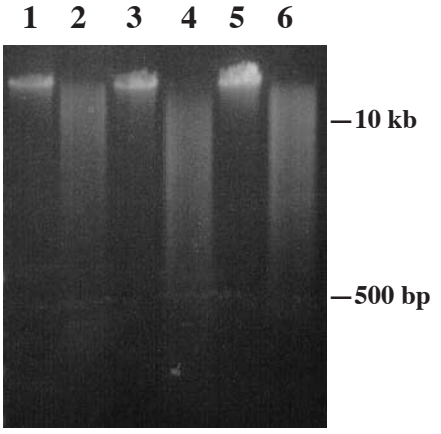

Fig. 1. Electrophoresis of genomic DNA extracted from aroid leaf blades. Aroid genomic DNA (10 μg) was digested by restriction enzymes to demonstrate purity. Lane 1, *Anthurium* 'Paradise Pink' Hort. undigested; Lane 2, *Anthurium* 'Paradise Pink' Hort. EcoRI digested; Lane 3, *Colocasia esculenta* 'Lehua' undigested; Lane 4, *Colocasia esculenta* 'Lehua' EcoRI digested; Lane 5, *Spathiphyllum* 'Petite' Hort. undigested; Lane 6, *Spathiphyllum* 'Petite' Hort. EcoRI digested.

Results

DNA. The quantity of DNA was determined using an equivalence of 50 μg·mL^{-1} double-stranded DNA per absorbance unit at 260 nm (Sambrook and Russell, 2001). Comparison of DNA quantity of the co-isolation method with other methods shows that for *Anthurium* this method has a much higher yield at 477 μg·g^{-1} fresh weight than CTAB or commercial kits at 75 and 13 μg·g^{-1} fresh weight, respectively (Table 1). *Colocasia* also had high yields for the co-isolation method at 322 μg·g^{-1} fresh weight, with only 50 μg·g^{-1} for the CTAB method. *Spathiphyllum* yields were 177 μg·g^{-1} for the co-isolation method and 77 μg·g^{-1} for CTAB. Yields were considered so low for commercial kits when applied to *Anthurium* that the other aroids, *Colocasia* and *Spathyiphillum*, were not tested using these methods.

The quality of the genomic DNA recovered was first assessed by its 260/280 and 260/230 absorbance ratios. The absorbance ratios were 1.9 or higher for the co-isolation method. The CTAB method had values of 1.9 for the 260/280, indicating the samples were relatively free of protein, but the 260/230 ratios indicated polysaccharide or polyphenolic contamination, with values of 1.2 through 1.6. The commercial kits (only *Anthurium* tested) had lower values of 1.5 for 260/280 and 0.9 for 260/230. Although the values of the commercial kits are low and indicative of low quality, this was not the case when the samples were visualized on a gel or when used for downstream applications (data not shown). DNA was also assessed by the appearance of uncut and enzyme-digested nucleic acids on an agarose gel (Fig.1). A band of high molecular weight was visualized for the uncut genomic DNA, while the digested genomic DNA showed an absence of the same high molecular band and a corresponding smear from high to low molecular weights, as would be expected for well-digested DNA. The high-speed spins employed during isolation served to accumulate the contaminating material more tightly at the phenol-chloroform interface, significantly reducing their carryover in subsequent steps.

RNA. The quantity of RNA was determined using an equivalence of 38 μg·mL^{-1} RNA per absorbance unit at 260 nm (Sambrook and Russell, 2001). RNA yields were greatly improved with the co-isolation method, compared with the polyvinylpyrrolidone method (Table 2). The average amount of total RNA isolated using the co-isolation method was 129 μg·g^{-1} fresh weight for *Anthurium*, 61 μg·g^{-1} for *Colocasia*, and 50 μg·g^{-1} for *Spathiphyllum*. In compari-

Table 2. Comparison of RNA yields and absorbance ratios, as an indicator of purity, between two different methods of RNA isolation from *Anthurium*, *Colocasia*, and *Spathiphyllum*.

	Co-isolation[z]	Polyvinylpyrrolidone
RNA yield $\mu g \cdot g^{-1}$ fresh weight tissue		
Anthurium	129	20
Colocasia	61	10
Spathiphyllum	50	12
Absorbance 260/280 ratio		
Anthurium	1.9	0.8
Colocasia	2.0	1.6
Spathiphyllum	1.9	1.0
Absorbance 260/230 ratio		
Anthurium	2.0	0.7
Colocasia	1.9	1.0
Spathiphyllum	2.1	1.0

[z]DNA/RNA co-isolation method.

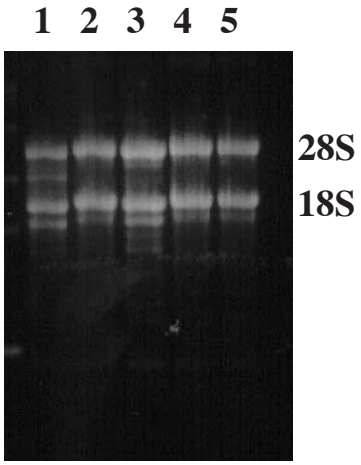

Fig. 2. Electrophoresis of total RNA extracted from aroid leaf blades. Total RNA (10 μg) was separated on a denaturing agarose gel, with the 28S and 18S ribosomal RNA bands indicated. Lanes 1–2, *Colocasia esculenta* 'Lehua' RNA; Lane 3, *Anthurium* 'Paradise Pink' Hort. RNA; Lanes 4–5, *Spathiphyllum* 'Petite' Hort. RNA.

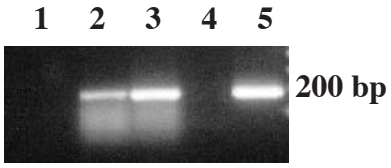

Fig. 3. RT-PCR of wild-type and transgenic anthurium cultivars using primers for the Shiva-1 transcript. Lane 1, Wild-type *Anthurium* 'Paradise Pink' Hort.; Lanes 2 and 3, *Anthurium* 'Paradise Pink' Hort. Shiva-1 transformants; Lanes 4, PCR negative control, no nucleic acid; Lane 5, PCR positive control, plasmid pBPRS1.

son, only 20 $\mu g \cdot g^{-1}$ for *Anthurium,* 10 $\mu g \cdot g^{-1}$ for *Colocasia,* and 12 $\mu g \cdot g^{-1}$ for *Spathiphyllum* were obtained using the polyvinylpyrrolidone method. Co-isolation preparations using the more acidic phenol (pH 4.3) resulted in an average decrease of 30% in total yield compared to the pH 7.9 phenol preparations. This decrease in yield still makes the co-isolation method much better than the polyvinylpyrrolidone method. Other methods using SDS, sarkosyl, and TRIZOL® (Invitrogen, Carlsbad, Calif.) produced very low yields, highly degraded RNA, and often impure RNA; therefore, they were not pursued.

The quality of RNA recovered was first assessed by 260/280 and 260/230 absorbance ratios. Absorbance ratios for the co-isolation method were 1.9 or higher, indicating high quality. In contrast, the polyvinylpyrrolidone method had very poor ratios, with 0.8 to1.6 for 260/280 and 0.7 to 1.0 for 260/230. The RNA was also assessed by its appearance on a denaturing agarose gel (Fig. 2). The denaturing agarose gel reveals that the quantity of 28S ribosomal RNA in each sample is greater than the 18S ribosomal RNA, as expected, and together these make up the majority of RNA. The quality of the polyvinylpyrrolidone isolated RNA was poor when visualized on a gel (data not shown). The quality of the co-isolated RNA was also assessed by RT-PCR (Fig. 3). RT-PCR yielded a single band of 200 bp, the expected size, for each transgenic sample tested but not for the non-transgenic wild-type controls.

Discussion

Previously we had employed several standard methods to prepare RNA from *Anthurium* leaves without success. Additional methods designed to overcome the plant-specific difficulties of high carbohydrate and polyphenol contamination were also attempted and included the Hall et al. (1978) protocol using sodium borate, lithium chloride, and ethanol precipitation, and another using polyvinylpyrrolidone (Champagne and Kuehnle, 2000). These procedures all produced degraded RNA and were unsuitable for downstream applications.

Recovery of nucleic acids from aroid leaf tissue is difficult, owing to the elevated quantities of polysaccharides and polyphenols. We found that by modifying a method originally developed for marine algae (Su and Gibor, 1988), we were successful in recovering RNA and DNA from *Anthurium* and other aroids. Using a ratio of 1 g of leaf tissue per 9.9 mL of extraction buffer yielded more DNA or RNA than 2 g of leaf tissue per 9.9 mL extraction buffer. Reducing the ratio to 0.5 g leaf tissue per 9.9 mL of buffer increased quality and yields per gram fresh weight; however, the total nucleic acid obtainable per preparation was decreased. The yield of total RNA in young green leaves of *Anthurium* was up to three times higher than that of mature green leaves (data not shown). This is not surprising as younger leaves are still expanding and may contain more cells per gram of tissue. It is also easier to finely powder younger leaves in liquid nitrogen than the tougher, more mature leaves, resulting in better extraction of cell contents. As many as eight 1-g samples can be simultaneously processed using this method. Regardless of the maturity of the leaves, each prep yielded a quantity of DNA and/or RNA sufficient for multiple (replicated) applications. In contrast, commercial kits, such as Qiagen and Promega, yielded a lower total quantity of nucleic acids, sufficient for only a one-time analysis.

The 260/230 absorbance ratios of 1.9 and higher, as obtained by co-isolation, indicate that leaf extracts for RNA and genomic DNA were free of polyphenolics and polysaccharides (Manning, 1991; Su and Gibor, 1988). The CTAB method had consistently low 260/230 absorbance ratios. The 260/280 absorbance ratios of 1.9 and higher, obtained with the co-isolation method, indicate that the extracts are relatively free of protein (Sambrook and Russell, 2001). The CTAB method could yield 260/280 ratios as high as the co-isolation ratios obtained here, but with much lower yields and low 260/230 ratios. Electrophoresis also demonstrated that the co-isolated genomic DNA was of high molecular weight, was not degraded, and that the DNA was easily digested using restriction enzymes. The CTAB genomic DNA had some degradation and was not always easily digested by restriction enzymes (data not shown). The RNA isolated was not degraded, as evidenced by the intact ribosomal bands and lack of smearing. The nucleic acids derived from the modified procedure reported here were used successfully for Northern blotting (Fujii, 2002, and A.R. Kuehnle et al., unpublished) and RT-PCR. The quality of the total RNA was such that only 25% (250 ng) was needed in RT-PCR for detection of positives, compared to the amount needed using previous total RNA isolation methods. This RNA could be stored for over one year at –80 °C and still be useful in RT-PCR analysis. The co-isolation protocol is effective in recovering genomic DNA and RNA of sufficient quantity and quality from *Anthurium* and other aroid leaves for further analysis.

We have found this procedure to be very useful for the isolation of both RNA and DNA in large- and small-scale preps from several horticultural aroids. This procedure, or modifications of it, may be useful with other tissues that do not respond well to the newer phenol-free procedures for nucleic acid extraction.

Literature Cited

Buldewo, S. and Y.F. Jaufeerally-Fakim. 2002. Isolation of clean and PCR-amplifiable DNA from *Anthurium andraeanum*. Plant Mol. Biol. Rptr. 20:71a–71g.

Champagne, M.M. and A.R. Kuehnle. 2000. An effective method for isolating RNA from tissues of *Dendrobium*. Lindleyana 15:165–168.

Chen, F.C. and A.R. Kuehnle. 1996. Obtaining

transgenic *Anthurium* through *Agrobacterium*-mediated transformation of etiolated internodes. J. Amer. Soc. Hort. Sci. 121:47–51.
Cho, Y. and J.D. Palmer. 1999. Multiple acquisitions via horizontal transfer of a group I intron in the mitochondrial coxl gene during evolution of the *Araceae* family. Mol. Biol. Evol. 16:1155–1165.
Doyle, J.J. and J.L. Doyle. 1990. Isolation of plant DNA from fresh tissue. Focus 12:13–15.
Fujii, T.M. 2002. Evaluation of transgenic *Anthurium* expressing the shiva-1 gene encoding a synthetic antimicrobial peptide. MS Thesis, Univ. of Hawaii, Honolului.
Hall, T.C., B.U. Buchbinder, J.W. Pyne, S.M. Sun, and F.A. Bliss. 1978. Messenger RNA for G1 protein of french bean seeds: Cell-free translation and product characterization. Proc. Natl. Acad. Sci. (USA) 75:3196–3200.
Kuehnle, A.R., F.C. Chen, and N.C. Sugii. 2001. Transgenic *Anthurium*, p. 3–15. In: Y.P.S. Bajaj (ed.). Biotechnology in agriculture and forestry, Vol. 48, Transgenic crops. Springer Verlag, Berlin.
Loomis, W.D. 1974. Overcoming problems of phenolics and quinones in the isolation of plant enzymes and organelles. Meth. Enzymol. 31: 528–545.
Lopez-Gomez, B. and M.A. Gomez-Lim. 1992. A method for extracting intact RNA from fruits rich in polysaccharides using ripe mango mesocarp. HortScience 27:440–442.
Manning, K. 1991. Isolation of nucleic acids from plants by differential solvent precipitation. Anal. Biochem. 195:45–50.
Mason, M.G. and J.R. Botella. 1997. Identification and characterisation of two 1-aminocyclopropane-1-carboxylate (ACC) synthase cDNAs expressed during papaya (*Carica papaya*) fruit ripening. Aust. J. Plant Physiol. 24:239–244.
Ranamukhaarachchi, D.G., R.J. Henny, C.L. Guy, and Q.B. Li. 2001. DNA fingerprinting to identify nine *Anthurium* pot plant cultivars and examine their genetic relationship. HortScience 36:758–760.
Sambrook, J. and D.W. Russell. 2001. Molecular cloning a laboratory manual. 3rd ed. Cold Spring Harbor Lab. Press, Woodbury, N.Y.
Su, X. and A. Gibor. 1988. A method for RNA isolation from marine macro-algae. Anal. Biochem. 174:650–657.