How to get high-quality RNA from chemically complex plants

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Ask any molecular plant biologist about RNA extractions and you might just open up the floodgates to the woes of troubleshooting. RNA extraction is a notoriously tricky and sensitive lab procedure. New protocols out of the University of Florida are quicker, more effective, and more reliable than previous methods.

"Obtaining pure and intact RNA samples is essential for sequencing the active genes, or the transcriptome, of a plant," explains botanist Ingrid Jordon-Thaden, who developed the protocols.

The protocols are given in bench-ready form with detailed notes and a troubleshooting guide in a recent issue of *Applications in Plant Sciences*. The most successful approach combines the TRIzol reagent and TURBO DNA-free kit manufactured by Ambion, Life Technologies, with additional steps tested by the researchers.

"We needed something that would consistently give us good quality RNA across a wide range of plant types," explains co-author Andre Chanderbali. "Compounds such as flavonoids, tannins, waxes, and other secondary metabolites found in plant tissues can make it difficult to extract RNA."

The researchers were working with a diverse array of woody, aromatic, and aquatic plants while contributing work with a major international transcriptome sequencing project, the 1000 Plants Initiative. They conducted 382 separate RNA extractions to test different techniques on

various plant species.

A key ingredient in the new protocols is the same foaming agent that is commonly used in shampoos, sodium lauroyl sarcosinate. Nicknamed sarkosyl, scientists have been using it since the 1970s because it breaks apart cellular fatty membranes to release the contents of a cell.

"Combined with TRIzol and the TURBO kit, sarkosyl helps extract high-quality and -quantity RNA from most plant species," explains Jordon-Thaden. "Adding a CTAB step improved extraction success for even the most stubborn species. Despite the success of the protocols, however, our methods were still not successful for some plants that contain high amounts of mucilage."

In the lab, RNA extractions have a reputation for causing agonizing failed days at the bench. RNA is extremely fragile and sensitive to degradation by enzymes called RNAses that exist everywhere in nature, including the air. Workspaces must be sterilized because RNAses can quickly turn intact RNA strands into a soup of nucleic acids not fit for sequencing.

"As soon as we collect a plant tissue, such as a leaf clipping, for RNA extraction, we flash-freeze it in liquid nitrogen to prevent degradation," says Jordon-Thaden. "We then grind it up and mix it into a cocktail of reagents followed by the washing away of plant fibers and other cellular components. The longer the process takes, the greater the chances of RNA degradation."

"Bad quality RNA equates to bad transcriptome sequencing," says Jordon-Thaden.

Extraction protocols have come a long way since the discovery of nucleic acids, but there is still a long way to go as sequencing projects

expand. Commercial kits provide ready-made ingredients and step-by-step instructions that ease the process, but as more labs work with complex non-model plant species, better extraction methods are needed.

The iteration process of troubleshooting 382 extractions and making modifications to manufacturer kits will serve as a valuable resource. As more and more botanists explore the genetic nature of the biodiversity of plants on Earth, these protocols will provide them with an extraction method that can handle almost any plant.

More information: Ingrid E. Jordon-Thaden, Andre S. Chanderbali, Matthew A. Gitzendanner, and Douglas E. Soltis. 2015. Modified CTAB and TRIzol protocols improve RNA extraction from chemically complex Embryophyta. *Applications in Plant Sciences* 3(5): 1400105. DOI: 10.3732/apps.1400105

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