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For Immediate Release

A universal RNA extraction protocol for land plants

New method will facilitate next-generation sequencing and comparative studies of gene expression

RNA, a nucleic acid involved in protein synthesis, is widely used in genetic research to study patterns of gene expression in different organisms. The types and quantities of RNA present in an organism indicate which genes are expressed, providing insight on the genes responsible for particular phenotypes.

Many tools, such as next-generation sequencing and quantitative PCR, are available for studying gene expression. However, these tools rely on the extraction of high-quality RNA from the organism of interest, and this can be a challenging task. Compared to genomic DNA, RNA is more delicate and prone to degradation. Additionally, many plant tissues are infused with starch, fibers, or secondary compounds that inhibit the isolation of RNA of sufficient quality and/or quantity.

Although numerous protocols for RNA extraction have been developed, most of these are plant-specific, with many tailored for particular crop plants or model organisms (e.g., *Arabidopsis*), making their utility for non-model plant species, which constitute the vast bulk of plant diversity, somewhat limited.

Researchers at the University of California, Berkeley, have developed a new protocol for RNA extraction that can be used across land plants, which comprise over 300,000 species. The protocol is available for free viewing in the December issue of *Applications in Plant Sciences* (http://www.bioone.org/doi/pdf/10.3732/apps.1300070).

According to Chelsea Specht, associate professor in the Department of Plant and Microbial Biology at UC Berkeley and senior author of the study, this protocol will greatly facilitate RNA-based studies of non-model plant species.

“Using this protocol, we can successfully extract high yields and high-quality RNA from tissues of any type from plants across the diversity of land plants, including tissues that are mechanically difficult to grind, rich in starch, or laden with secondary compounds.”

Lead author Roxana Yockteng, Specht, and their colleagues tested the protocol on a wide variety of land plant species (one moss species, three gymnosperm species, and numerous angiosperm species) as well as different tissue types (e.g., leaves, flowers, and cones). They were able to consistently recover large quantities of high-quality RNA from the samples tested, demonstrating the broad utility of the protocol.

Specht says the efficacy of the protocol lies in its flexibility; there are numerous steps in the protocol that can be readily modified to accommodate variations in plant chemistry and structure.

“You can micromanage your RNA extraction and make small changes that work for you, regardless of what lab you’re in or what plant you are working with.”

The protocol also includes an optional cleanup step at the end, which permits the acquisition of clean, high-quality RNA from problematic samples (e.g., those rich in lignin and/or secondary metabolites) without a significant loss in RNA quantity. This step is a modification of a similar method designed for cleaning DNA.

According to Specht, the new protocol will have numerous applications in plant genetic research. Studies of gene expression involving reverse transcriptase PCR, quantitative PCR, or RNA-sequencing (transcriptome profiling) can use this protocol to extract the requisite high-quality RNA. Additionally, researchers can use transcriptomes generated from next-generation sequencing to develop probes for targeted sequence capture for phylogenetic and phylogeographic studies.

The ability to use this protocol on an array of plants and tissue types will also facilitate comparative analyses of transcriptomes across diverse lineages, says Specht, permitting researchers to investigate a variety of interesting evolutionary questions. This was challenging before, as existing protocols are generally too taxon- or tissue-specific for use across numerous phylogenetically divergent species.

Roxana Yockteng, Ana M. R. Almeida, Stephen Yee, Thiago Andre, Colin Hill, and Chelsea D. Specht. A method for extracting high-quality RNA from diverse plants for next-generation sequencing and gene expression analyses. *Applications in Plant Sciences* 1(12): 1300070. doi:10.3732/apps.1300070.

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