Improving DNA amplification from problematic plants

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The polymerase chain reaction (PCR) is a common technique used to amplify, or copy, pieces of DNA. Amplified DNA is then used in genetic analyses for everything from medicine to forensics. In plant research, PCR is a vital step in detecting and sequencing genes, and its applications are endless. However, compounds found in plants often inhibit PCR. Researchers at the University of Southern Mississippi discovered that the use of an additive allows PCR to successfully amplify DNA from once problematic plants.

PCR is widely used in plant sciences but is not 100 percent reliable. Many plant researchers encounter roadblocks when implementing PCR. For example, many plant species contain phenolic compounds that deter herbivores. These compounds are often extracted along with plant DNA and can stop PCR from working.

Graduate student Tharangamala Samarakoon and colleagues have found a technique to overcome many of these inhibitory plant compounds. They added a reagent to the PCR mixture that contains three ingredients: trehalose, bovine serum albumin, and polysorbate-20 (all three abbreviated TBT-PAR). "Unlike several other studies, TBT-PAR works at the PCR stage instead of at the DNA extraction stage, so it has promise for pigeon-holed and half-forgotten extractions that previously failed to be amplified using PCR," says Samarakoon. The authors published their research in the January issue of *Applications in Plant Sciences*.

Samarakoon tested the TBT-PAR reagent on DNA extracted from tropical and temperate species across four plant families, including Achariaceae, Asteraceae, Lacistemataceae, and Samydaceae. PCR with TBT-PAR successfully amplified DNA for all species, whereas standard DNA extraction and PCR techniques consistently failed.

TBT-PAR enhanced PCR for DNA extracted from fresh, silica-dried, and herbarium plant material. "Since we study tropical plants, many of which are geographically restricted or rare," explains Samarakoon, "herbarium material is sometimes all that we have available for DNA extraction, and curators are gracious to allow even a small destructive sampling for a single extraction attempt. We want that one attempt, of course, to be successful." Samarakoon predicts that inhibitory plant compounds could be the underlying cause of many PCR failures in herbarium specimens and hopes TBT-PAR will have widespread benefits in herbarium specimen DNA amplification.

TBT-PAR was first used in the PCR detection of a shrimp virus by co-author Shiao Wang and his colleagues. "The additive has also been helpful in a colleague's lab where they had trouble amplifying DNA from gopher tortoise ticks, so its utility extends beyond plants," comments Samarakoon. TBT-PAR has the potential for broad use in PCR techniques across DNA samples, species, and taxa.

The article will be published in the first issue of *Applications in Plant Sciences* (*APPS*), a new journal released by the Botanical Society of America. Theresa Culley, Editor-in-Chief of *APPS*, describes the new journal as a venue to "expedite the dissemination of innovative information encompassing all areas of the plant sciences, including but not limited to genetics, structure, development, evolution, systematics, and ecology." *APPS* publishes new methods in plant sciences—an important niche to fill in an age of rapid technological advances.

More information: Culley, T. M. 2013. Changing technologies offer new opportunities in the plant sciences. *Applications in Plant Sciences* 1(1): 1200008. doi:10.3732/apps.1200008

Samarakoon, T., S. Y. Wang, and M. H. Alford. 2013. Enhancing PCR amplification of DNA from recalcitrant plant specimens using a trehalose-based additive. *Applications in Plant Sciences* 1(1): 1200236. doi:10.3732/apps.1200236

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