Doing more with less in the study of plant chemical defense

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Plants can't run away to avoid being eaten, so instead they employ a variety of chemical defenses to keep herbivores at bay. Understanding plant chemical defenses is critical for keeping crops healthy, and for

answering a variety of more academic questions about ecology and evolution. However, current techniques for assessing plant chemical defenses are time consuming and require impractically large amounts of plant tissue. In research presented in a recent issue of *Applications in Plant Sciences*, Dr. Chandra Jack and colleagues devised a new technique for assessing plant chemical defenses that is less laborious and more practical for a variety of experimental applications.

Traditional methods of measuring defense compounds use light to measure different chemicals in leaves using an apparatus called a spectrophotometer. These methods require large tissue samples, meaning that multiple leaves must be pooled together, collapsing meaningful variation. The technique reported here, using a microplate reader, detected activity in samples smaller than 10 mg, or 2% of the traditional tissue sample weight.

"One of the biggest constraints is the amount of tissue that is needed for traditional spectrophotometer-based assays," said Dr. Jack, the lead author of the study. "Now, because researchers don't have to combine leaves from a single plant, they can better explore localized versus systemic responses, or monitor individual plant response over time... It allows us to measure population-level variation and to parse out the influence of environment on genes."

"The other constraint is the time needed to carry out these experiments," said Dr. Jack. The huge amounts of labor and time required to carry out assays using spectrophotometric methods put serious constraints on the number of samples that can be assessed. This limits feasible experimental designs, and consequently the types of questions one can hope to answer. Additionally, both the time and tissue demands of traditional methods make it more difficult to re-run a sample or reproduce a result. In this study, researchers were able to conduct a set of assays that would normally take 41 hours in only six hours.

Plant chemical defense is complex, involving the production of multiple compounds at once. While traditional techniques require separate extractions to assay each class of defense compounds, the technique presented here uses consolidated preparation buffers and protocols to extract multiple compounds at once. This makes it practical to measure a wider range of chemical responses, providing a more nuanced and higher-resolution picture of plant defense. "Plants produce so many secondary metabolites and enzymes in response to herbivore attack, that the story of plant defenses is not complete unless you assay multiple compounds," said Dr. Jack.

The method presented here, in which sample preparation buffers are consolidated and samples are run simultaneously on a microplate, vastly reduces the time and expense of assaying defense compounds. "For us it was a case of necessity being the mother of invention," said Dr. Jack. "I was planning to set up an experiment...and couldn't find high-throughput assays to accomplish what was needed." But in developing this technique, the authors opened up avenues of inquiry into plant defenses for others. Researchers can now investigate multiple defense compounds at once, look at localized and systemic responses, compare a larger sample of individuals, and replicate their findings much more feasibly than before.

"Originally, this wasn't going to be a stand-alone project," said Dr. Jack. "However, as we combed through the literature and invested so much time into the protocol, we realized that this would be useful for the scientific community and could impact so many different fields."

More information: Chandra N. Jack et al, A high-throughput method of analyzing multiple plant defensive compounds in minimized sample mass, *Applications in Plant Sciences* (2019). DOI: 10.1002/aps3.1210

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