Researchers report faster screening of photoprotection in crops

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RIPE postdoctoral research associate, Dhananjay Gotarkar, who led this study, collecting leaf disk samples from field-grown soybeans before transportation to a laboratory where NPQ relaxation is measured in a close PAM-flurometer. Credit: JoVE

Plants spend all day creating food from sunlight using photosynthesis—a 100+ step process that researchers have been working to improve its efficiency in crops to ultimately deliver more yield. When crop leaves

are in full sunlight, such as in the wide-open fields, their green chlorophyll molecules receive more light energy than they can use. If this energy isn't dissipated from the molecules, they will react with oxygen to produce bleaching agents that can destroy the leaf, costing plants up to 10–30% of their ability to photosynthesize.

Plants have, however, evolved to develop several photo-protective mechanisms to prevent damage, called non-photochemical quenching (NPQ) of the excited chlorophyll states. By speeding up the process of NPQ relaxation, researchers could potentially increase the efficiency of photosynthesis in crops.

In a recent study, published in the *Journal of Visualized Experiments*, RIPE researchers from the University of Illinois and the University of Cambridge reported a high-throughput method for screening rates of NPQ relaxation in field grown plants, with the potential to aid identification of genotypes with beneficial characteristics by developing a pulse amplitude modulated (PAM) chlorophyll fluorescence analysis.

"NPQ can be measured by using a variety of commercially available PAM fluorometers, from simple hand-held devices to more advanced closed systems," said Dhananjay Gotarkar, a postdoctoral researcher at Illinois, who led this work for a research project called Realizing Increased Photosynthetic Efficiency (RIPE). "There is a limitation to using several of these approaches due to its relatively low throughput, which makes screening large collections of plants challenging without multiple devices and a team of researchers."

RIPE is an international research project that aims to increase global food production by developing food crops that turn the sun's energy into food more efficiently with support from the Bill & Melinda Gates Foundation, Foundation for Food & Agriculture Research, and U.K. Foreign, Commonwealth & Development Office.

Building on previous research, the team used PAM chlorophyll fluorescence analysis of leaf disks for high-throughput screening of NPQ relaxation rates in field-grown soybean that has the key advantage compared to sequential analysis of individual plants.

"Since conventional measurements are done on a per-plant basis, using this procedure opens up the possibility to test hundreds of genotypes within a day and the potential to perform genome-wide association studies," said Steven Burgess, an author of this study. "This protocol is designed for analyzing field-grown soybean, but it can be modified for sampling and measurement of greenhouse-grown material or other higher plants."

In their paper, the team of researchers laid out their protocol from seed planting in the summer of 2021 to collecting leaf samples from the field and processing and measuring NPQ chlorophyll fluorescence data. The team found that to obtain reliable measurements of NPQ, several components need to be taken into consideration, such as the choice and handling of leaf disks. For example, rough handling with tweezers could introduce stress to the leaf tissue, and rates of NPQ activation and relaxation can vary with leaf age and plant developmental stage, all of which are elements to watch out for to reduce experimental noise.

"By identifying major bottlenecks that hinder photosynthetic efficiency, we can figure out how to help plants grow more productively," said Johannes Kromdijk, lecturer at the University of Cambridge, who contributed to this study. "This approach involves faster ways to analyze data and PAM chlorophyll fluorescence analysis is a powerful technique for measuring photosynthetic efficiency, providing us a means to measure many genotypes simultaneously."

More information: Dhananjay Gotarkar et al, High-throughput Analysis of Non-Photochemical Quenching in Crops using Pulse

Amplitude Modulated Chlorophyll Fluorometry, *Journal of Visualized Experiments* (2022). DOI: 10.3791/63485