Molecular method promises to speed development of food crops

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Plant breeding efforts, like the Illinois field trial shown here, will benefit from fast and precise technologies to evaluate transgenics. Credit: Haley Ahlers, University of Illinois.

The first human farmers needed hundreds of years and a lot of good luck

to shape the first domesticated crops. Modern plant breeders wait weeks or months, not centuries, to discover what the literal fruits of their labors might be; now, a study led at Illinois and supported by the Bill & Melinda Gates Foundation has explored the strengths of a molecular method that reduces this wait time to a few days.

The new study, published in *Plant, Cell and Environment*, addresses a central challenge of transgenic plant development: how to reliably evaluate whether genetic material has been successfully introduced. Researchers at the University of Illinois, the Polish Academy of Sciences, the University of Nebraska-Lincoln and the University of California, Berkeley compared the traditional method to several new ones that have emerged from advances in genomic technology and identified one that is much faster than the standard approach, yet equally reliable. The study was led by Illinois postdoctoral fellows Kasia Glowacka and Johannes Kromdijk.

"For plants with long life cycles, such as our food crops, this will greatly speed the time between genetic transformation or DNA editing, and development of pure breeding lines," said Long, Gutgsell Endowed Professor of Crop Sciences and Plant Biology and the principal investigator for the study. Long is also a member of the Genomic Ecology of Global Change and Biosystems Design research themes and the Energy Biosciences Institute at the Carl R. Woese Institute for Genomic Biology.

To meet the food and fuel needs of an ever-growing global population, researchers benefit from transgenic technologies to develop crops with higher yields and greater resiliency to environmental challenges. None of the technologies used to introduce new genetic material into plants work with 100 percent efficiency. Plants and their offspring must be screened to identify those in which gene transfer was successful.

Traditionally, this was done in part by testing successive generations of plants to see if the desired traits are present and breed true over time. In addition, plant scientists can use one of several molecular methods to determine if a gene or genes have actually been successfully introduced into the plant genome. The "tried and true" method, the Southern blot, yields precise data but is slow and unwieldy. It requires isolating relatively large amounts of plant DNA, using fluorescent or radioactive dye to detect the gene of interest, and performing a week's worth of lab work for results from just a few samples at a time.

The team compared the Southern blot technique with several that use variations of a chemical process called polymerase chain reaction (PCR). This process allows researchers to quantify specific pieces of the introduced DNA sequences by making many additional copies of them, and then estimating the number of copies—somewhat like estimating the amount of bacteria present in a sample by spreading it on a petri dish and letting colonies grow until they are visible. These methods are much faster than Southern blotting, but if the DNA in each sample does not "grow" at exactly the same rate, the resulting data will be imprecise—size won't be a perfect indicator of the starting quantity.

One method examined by Long's group, digital drop PCR (ddPCR), is designed to overcome this weakness. Rather than using the PCR process to amplify all the DNA in a sample, this method first separates each individual fragment of DNA into its own tiny reaction, much like giving each bacterium its own tiny petri dish to grow in. PCR then amplifies each fragment until there are enough copies to be easily detected, and the total number of tiny reactions are counted. Because this method, unlike others, separates the growth-like step from the quantification step, it can be very precise even when the reaction isn't perfect. Results can be obtained in less than two days, and many samples can be processed simultaneously.

Long hopes that his group's demonstration that ddPCR is a "reliable, fast and high throughput" technique will help it to become the new standard for those developing transgenic crops. "I believe it will become widely adopted," he said. Although ddPCR is currently more expensive than the other methods, Long said the cost would likely drop quickly, as have the costs of other genomic technologies.

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