New screening method could lead to microbe-based replacements for chemical pesticides

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Plants have evolved unique immunity mechanisms that they can activate upon detecting the presence of a pathogen. Interestingly, the presence of

some nonpathogenic microorganisms can also prompt a plant to activate its systemic immunity mechanisms, and some studies have shown that pretreating agricultural crops with such "immunity-activating" nonpathogenic microorganisms can leave the crops better prepared to fight off infections from pathogenic microorganisms. In effect, this means that immunity-activating nonpathogenic microorganisms can function like vaccines for plants, providing a low-risk stimulus for the plant's immune system that prepares it for dealing with genuine threats. These are exciting findings for crop scientists because they suggest the possibility of using such pretreatment as a form of biological pest control that would reduce the need for agricultural pesticides.

However, before pretreatment with nonpathogenic microorganisms can become a standard agricultural technology, scientists need a way to screen microorganisms for the ability to stimulate plant immune systems without harming the plants. There is currently no simple method for evaluating the ability of microorganisms to activate plant immune systems. Conventional methods involve the use of whole plants and microorganisms, and this inevitably makes conventional screening a time-consuming and expensive affair. To address this problem, Associate Professor Toshiki Furuya and Professor Kazuyuki Kuchitsu of Tokyo University of Science and their colleagues decided to develop a screening strategy involving cultured plant cells. A description of their method appears in a paper recently published in *Scientific Reports*.

The first step in this screening strategy involves incubating the candidate microorganism together with BY-2 cells, which are tobacco plant cells known for their rapid and stable growth rates. The next step is to treat the BY-2 cells with cryptogein, which is a protein secreted by fungus-like pathogenic microorganisms that can elicit immune responses from tobacco plants. A key part of the cryptogein-induced immune responses is the production of a class of chemicals called reactive oxygen species (ROS), and scientists can easily measure cryptogein-induced ROS

production and use it as a metric for evaluating the effects of the nonpathogenic microorganisms. To put it simply, an effective pretreatment agent will increase the BY-2 cells' ROS production levels (i.e., cause the cells to exhibit stronger immune system activation) in response to cryptogein exposure.

To test the practicability of their screening strategy, Dr. Furuya and his colleagues used the strategy on 29 bacterial strains isolated from the interior of the Japanese mustard spinach plant (Brassica rapa var. perviridis), and they found that 8 strains boosted cryptogein-induced ROS production. They then further tested those 8 strains by applying them to the root tips of seedlings from the Arabidopsis genus, which contains species commonly used as model organisms for studies of plant biology. Interestingly, 2 of the 8 tested strains induced whole-plant resistance to bacterial pathogens.

Based on the proof-of-concept findings concerning those 2 bacterial strains, Dr. Furuya proudly notes that his team's screening method "can streamline the acquisition of microorganisms that activate the immune system of plants." When asked how he envisions the screening method affecting agricultural practices, he explains that he expects his team's screening system "to be a technology that contributes to the practical application and spread of microbial alternatives to chemical pesticides."

In time, the novel screening method developed by Dr. Furuya and team may make it significantly easier for crop scientists create greener agricultural methods that rely on the defense mechanisms that plants themselves have evolved over millions of years.

More information: Mari Kurokawa et al, An efficient direct screening system for microorganisms that activate plant immune responses based on plant–microbe interactions using cultured plant cells, *Scientific Reports* (2021). DOI: 10.1038/s41598-021-86560-0