Exploring cell-to-cell signals in plants that trigger photosynthesis and defense mechanisms

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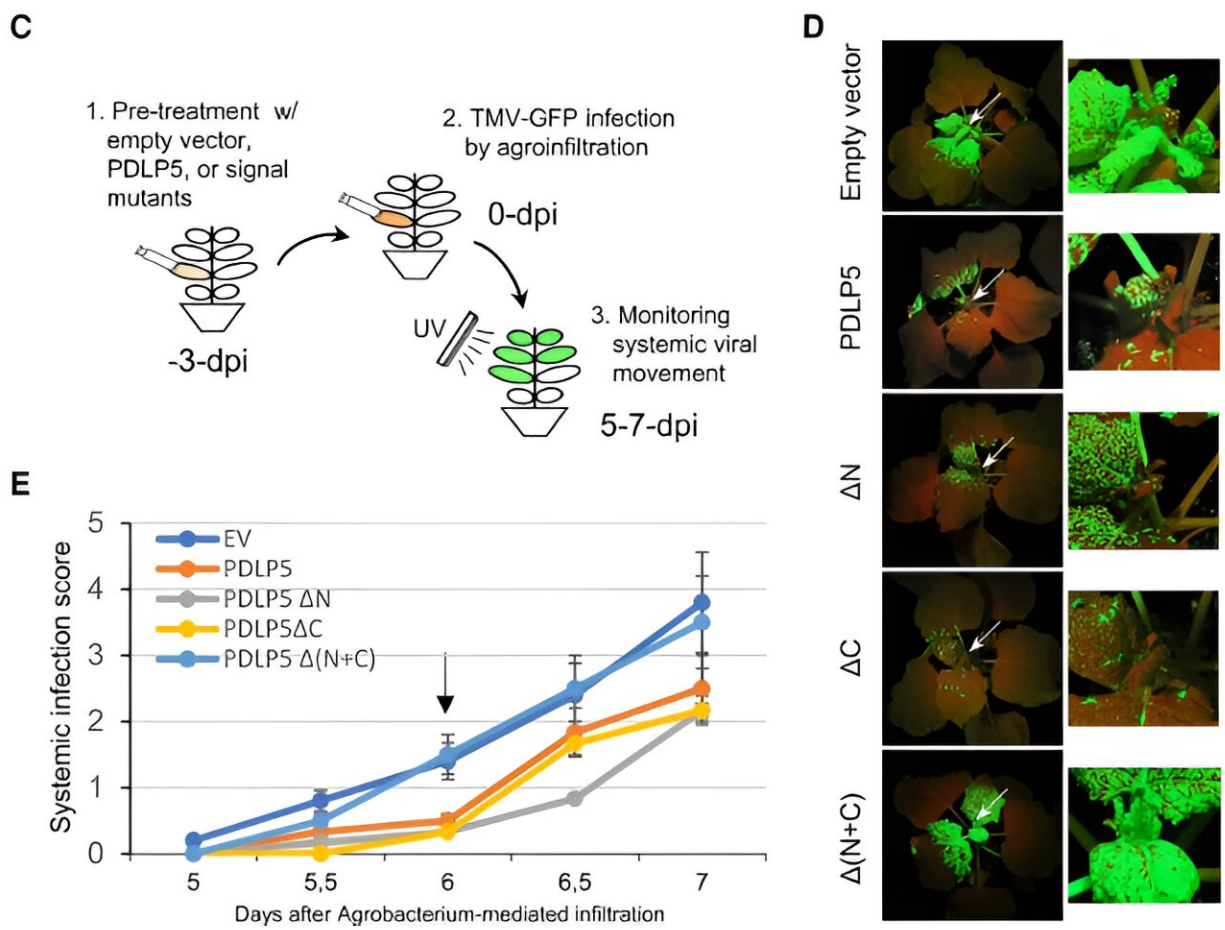

Functional analyses of ΔN, ΔC, and Δ(N + C) compared to intact PDLP5 by viral movement assays. C) Cartoon illustrating the experimental setup. D) Representative plant photographs showing the extent of systemic TMV-GFP movement (left, low magnification; right, close-up views of the shoot tips). E)

Quantitative analysis of the percentages of plants showing TMV-GFP systemic infectivity over 5 to 7 days after Agrobacterium-mediated infiltration of the virus. Experiments were performed 3 times using at least 5 plants per treatment. Credit: *The Plant Cell* (2023). DOI: 10.1093/plcell/koad152

Traffic lights signal to cars and buses when to stop, slow and go. Much like traffic lights, plant cells send signals to each other to perform photosynthesis to grow or fight off destructive viruses and pathogens.

Plant cells produce plasmodesmata, tiny tiny tubes that act as communication channels, allowing those signals to move from cell to cell. The plasmodesmata will open and close in response to various signals that activate protein regulators such as PDLP5.

"We knew that this protein is critical for plant defense," said Jung-Youn Lee, a University of Delaware professor of plant molecular and cellular biology and the interim director of the Delaware Biotechnology Institute. "But how does this protein get to the plasmodesmata?"

The question—how these protein regulators find their destination to fulfill their purpose and help a cell function—had been plaguing scientists. Until the University of Delaware got involved.

In new research that made the cover of the journal *The Plant Cell*, UD researchers found that the protein—PDLP5—that helps guard plants from the invasion of viruses and bacteria has not one, but two special targeting signals, or "zip codes" as Lee calls them, unexpectedly stationed outside of cells.

"It is almost like you have a zip code hidden on an unusual side of the envelope," Lee said. "We did more than just locate the zip code; we cracked the code. Now we understand where the zip code is and what it looks like."

An interdisciplinary research team of biologists and computational scientists developed machine-learning algorithms and introduced mutations into the protein sequence of PDLP5 and reintroduced into plant species Arabidopsis thaliana and Nicotiana benthamiana to examine whether PDLP5 would go to plasmodesmata in these plants or not and to find where the second zip code is. The team discovered even if they got rid of one zip code, PDLP5 would still go to the plasmodesmata just fine.

"It gave us a lot of headaches," Lee said. "We never really thought initially there are two zip codes right next to each other."

Two 'zip codes'

Historically, plants under a viral attack were considered "helpless losers," Lee said. But in 2011, Lee and her team of researchers discovered plants send signals through plasmodesmata for cells to "close their borders" to defend themselves from pathogens. This new insight was made possible through their study of the then newly identified protein, PDLP5.

They have wanted to know how plants lead those PDLP5 proteins to help plasmodesmata close off their channels.

Several years ago, Lee's former student and 2017 UD graduate Xu Wang had been working on his doctoral thesis studying plant cell-to-cell communication and the function of proteins that would go to plasmodesmata.

"I was trying to figure out which part of the protein that localized to plasmodesmata is important," Wang said, "and whether this part contains a more universal or common feature that can help us to understand the localization mechanisms for other proteins, not only for the proteins we're studying."

When Wang introduced various mutations into PDLP5 to try to chop it up, he was stunned by what happened next.

"Nothing changed," Wang said. "The mutated, or a shorthand form, always went to the plasmodesmata."

Wang felt his study and the last piece of his thesis was stuck.

Following Wang's graduation, Lee brought in computer scientists to develop machine-learning algorithms to help solve the mystery.

Li Liao, an associate professor of computer and information sciences, who worked with Lee and her then newly recruited postdoc Gabriel Robles Luna (currently a university faculty member in Argentina) on the research, said a computer model trained the machine-learning algorithms to make two types of predictions.

The model would predict whether a protein sequence was a PDLP5-targeting protein that would go to plasmodesmata or not and would predict where the targeting signals are in the protein sequence.

"One challenge was this problem that we had very limited training data, only eight such protein sequences," Liao said. "Now, machine learning is powerful because it can train on large amounts of data. It won't be easy if you have a small amount of data to train a model."

To overcome that, Liao and his then new doctoral student Jiefu Li, a 2021 UD graduate, had to train the model in a new way.

"We have developed some novel mechanisms, including revision of the standard training algorithm to handle the partial signals," Liao said. "If we know some tentative patterns, we can incorporate that into the training algorithm. This will allow us more importantly to do active learning."

Understanding the protein regulation within plant cells can ultimately help scientists genetically engineer new crops capable of quickly fighting off viruses and other microbial pathogens, Lee said. It's one more way to improve how plants and crops function.

"This becomes a cool new toolbox for scientists," Lee said. "We have mechanisms and molecules we can manipulate."

Wang agreed, adding that genetically engineering plants with plasmodesmata-located proteins that can open and close channels will help "manipulate the overall plant fitness or the plant's defense to potentially have some agricultural benefits."

The research doesn't stop here. Researchers have submitted another grant proposal to the National Science Foundation to continue their research. Now, the team wants to use machine-learning algorithms to know how the protein signals are being used. Like how a UPS driver might use a zip code to deliver a package. Lee said the team wants to know the ins and outs of the whole "delivery system," including which proteins are involved and any unknown players.

"If we just manipulate the zip code, it may work 50% of the time," Lee said. "But if we know who the delivery man is and improve or change the delivery man so a virus can't be transferred to plasmodesmata anymore, if we can change the system, plants will recognize the invaders better and know not to deliver them to plasmodesmata."

More information: Gabriel Robles Luna et al, Targeting of plasmodesmal proteins requires unconventional signals, *The Plant Cell* (2023). DOI: 10.1093/plcell/koad152

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