Shiraishi Foundation of Science Development

Newsletter

No. 2301

[Presentation of 2023 Grant-funded research]

Published: October 30, 2023

[2023 Grant-funded research]

Professor Dr. Masatsugu TOYOTA

"Long-range, Rapid Ca^{2+} Signal Transduction in Plants"

Recently, a spate of discoveries highlighting the amazing capabilities of plants has attracted a great deal of attention. A study that has attracted worldwide attention regarding the discovery of the "astonishing cleverness" of plants has also been selected from this fiscal year's grant-funded researches promoted by our foundation. We decided to present the study in this "newsletter". Learn the secrets on the role of calcium in these wonderful plants, and enjoy the observation of the plant kingdom.

1. Discover the secrets of plants!

(Cosmos, flower in autum)

In forests and fields, trees and flowers rustle in the wind, gently and unperturbed giving us a sense of refreshment and peace.

On the other hand, it is painful to see vegetables and trees in kitchen gardens and public parks attacked by insects. But, don't plants feel anything when they are attacked?

Recently, the group led by Prof. Toyota discovered that, when plants are bitten by an insect, "calcium signals, triggered from the wound site, propagate at high-speed throughout the plants' body to allow them to sense attacks from insects". In particular, Prof. Toyota was the first to capture real time images of calcium signals traveling at high-speed through the plant's body; a major accomplishment that took the world by storm[1]. His study demonstrated that, similarly to what happens in humans and other animals, transmission of biological information in plants also occurs via calcium signaling. Still more surprisingly, the study showed that calcium signals propagating in undamaged leaves promote the synthesis and storage of insect repellent substances, such as jasmonate, which allow plants to mount a preemptive defense against future attacks.

This wonderful discovery may lay the foundation for substantial contributions from across the academic world, such as the development of safe food production technology without the use of agrochemicals (e.g., pesticides), or the development of evolutionary theories focusing on the similarities between animals and plants[2]. This fiscal year's grant-funded research will provide more basic and detailed information about the "generation and high-speed propagation mechanisms of calcium signals in plants". In this "newsletter", we will introduce, in an accessible manner, and based on a Prof. Toyota's commentary[2,3], which prompted this research, "how calcium signals propagate through plants' body and the discovery of plants' cleverness".

2. Observation of calcium signals propagation in plants

Calcium signaling (hereinafter abbreviated as Ca signaling) plays an important role in transmitting biological information inside our bodies. A well-known example of this is its role in muscle contraction. When your brain sends the command "move your arm!", calcium in muscle cells of your arm transmits this information via a neural signal generated by motor neurons, causing the whole muscle to stretch and contract. Prof. Toyota, who has studied Ca signaling in humans and plants for many years and has a broad experience in Ca signaling imaging, successfully captured images of Ca signals propagating through plants with a novel real-time imaging

(Arabidopsis thaliana)

system. His study demonstrated that, similarly to what happens in humans and other animals, biological information in plants is transmitted via Ca signaling. For starters, we will briefly introduce a method for observing propagation of Ca signals in plants.

[Observation of calcium signaling] An engineered protein called GCaMP, which fluoresces green upon binding to calcium ions (Ca^{2+}), was used to detect Ca signals traveling through the plant's body. The plant used in the experiment was an Arabidopsis thaliana, a member of the mustard (Brassicaceae) family, which also includes cultivated species such as cabbage. Before starting the experiment, the arabidopsis was genetically modified to stimulate internal production of the fluorescent protein GCaMP using recombinant DNA technology. Because of this, calcium ions in the plant cells increased and, upon binding to GCaMP, produced fluorescence in vivo (i.e., emitted directly from the plant's body). The fluorescence images can be examined using a fluorescence microscope to determine the movement of calcium ions within the plant's body. Then we will demonstrate this with an example.

[An example of experimental observation] Figure 1 shows how Ca signals propagate throughout the whole body of a healthy Arabidopsis thaliana (Fig. 1a), when the right leaf (indicated by a dashed line in Fig. 1b) is pinched with tweezers. When a leaf is injured with tweezers, the calcium ion concentration at the wound site immediately increases, producing a bright fluorescence. These fluorescence signals pass through leaf veins (indicated by white arrows) and propagate gradually to distal undamaged leaves (indicated by red arrows) to inform the plant's body that a "leaf has been injured". This transmission of biological information determined by changes in calcium ion concentration is called calcium (Ca) signaling.

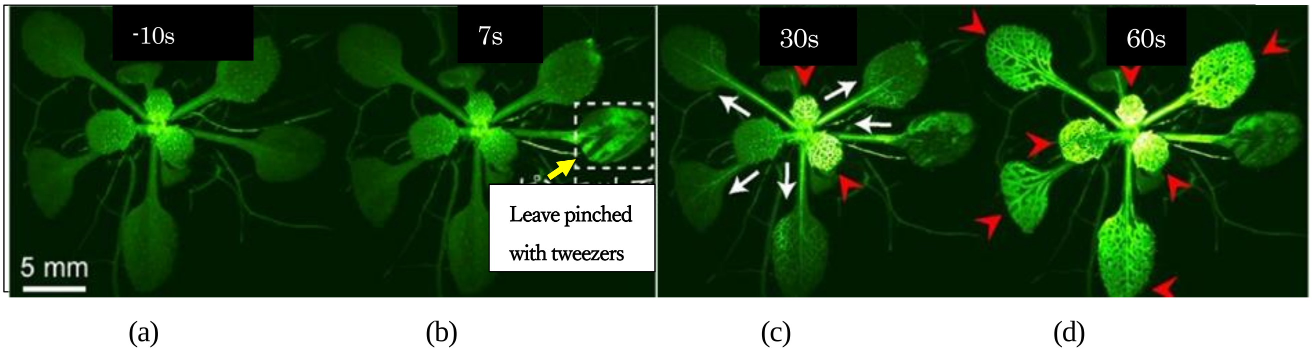


Fig.1　Calcium signals emitted from the wound site when an arabidopsis leaf is pinched with tweezers
(The times shown in the figure indicate the time elapsed from the moment the leaf was pinched with tweezers)

[Facts learned from the observation of calcium signaling] The following points were made clear from this and other similar experimental observations.

◎ Ca signaling was also observed in an experiment with arabidopsis leaves damaged by larvae of the cabbage white. In other words, arabidopsis can sense damage by insects throughout its whole body via Ca signaling.

◎ Propagation of Ca signals in plants can be observed regardless of whether leaf injury is caused by organisms, such as larvae, or is mechanically induced by pinching with tweezers. In short, leaf injury activates Ca signaling in plants.

◎ Even more surprising is the fact that Ca signals propagating to undamaged leaves induce instant biosynthesis and storage of insect repellent substances such as jasmonate.

◎ In other words, the plant's ability to sense damage by insects throughout its whole body via Ca signaling allows undamaged leaves to protect themselves from future attacks by storing substances which repel insects.

◎ Since, unlike humans, plants do not possess nerves, Ca signals propagate through the sieve tube (see glossary at the end), whose main function is to provide water and nutrients to the plant.

3. Glutamate in plant leaves activates calcium signaling

Although commonly known as a flavor enhancer in food products, glutamate is also the principal excitatory neurotransmitter in the human brain. Glutamate receptors (see glossary at the end) are proteins found in the nervous system, which, upon activation by glutamate, allow calcium ions to flow into neuronal cells to convey information to nerves.

In Arabidopsis, which contains 20 glutamate receptors, the glutamate released from the wound site of insect-damaged leaves is thought to trigger Ca signaling via the activation of glutamate receptors on cells membrane which allow calcium ions to flow into the plant's cells.

This means that glutamate, via activation of glutamate receptors, should trigger Ca signaling even when applied exogenously to undamaged leaves. Therefore, as shown in Fig.2, when a glutamate solution was applied to one leaf of the arabidopsis (indicated by the red arrow), our assumption was confirmed and propagation of Ca signals was observed

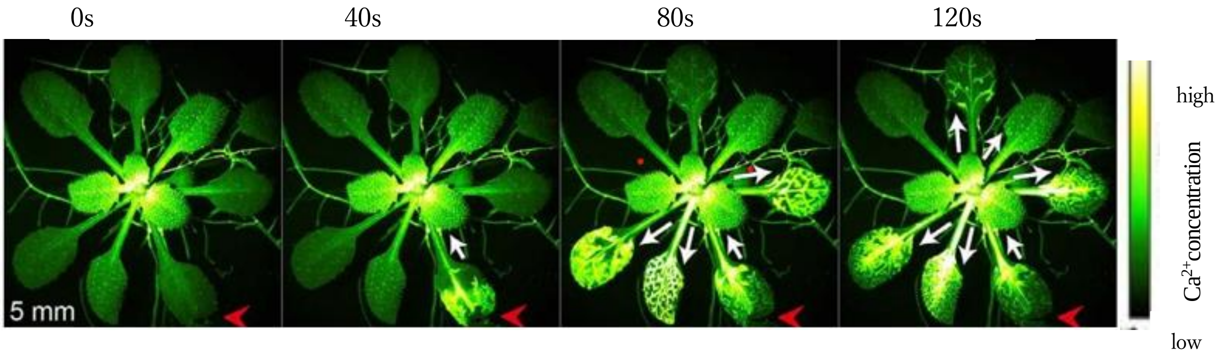


Fig.2 Triggering of calcium signaling when one leaf is fed with a glutamate solution (indicated by a red arrow)[3]

throughout the whole plant's body, as shown by the white arrows. Moreover, Ca signals propagating to leaves induce biosynthesis and storage of insect repellent substances such as jasmonate. This suggests that safe and high-quality food production without the use of pesticides may be possible if agrochemicals that trigger glutamate receptors activity are developed[2)].

4. Activation mechanisms of calcium signaling

In order to exploit the benefits of Ca signaling in plants, its mechanisms have been investigated in detail.

In plain words, when a plant is damaged by an insect: ① glutamate is released from the cells and tissues of the damaged leaves. ②This glutamate triggers activation of glutamate receptors in the sieve tube and parenchyma cells, and ③ Ca^{2+} ions flow into the plant's cells. ④ The Ca signals generated by the damaged leaves propagate rapidly, through plant's parts such as the sieve tube, to distal leaves which ⑤ synthesize and store jasmonate to protect

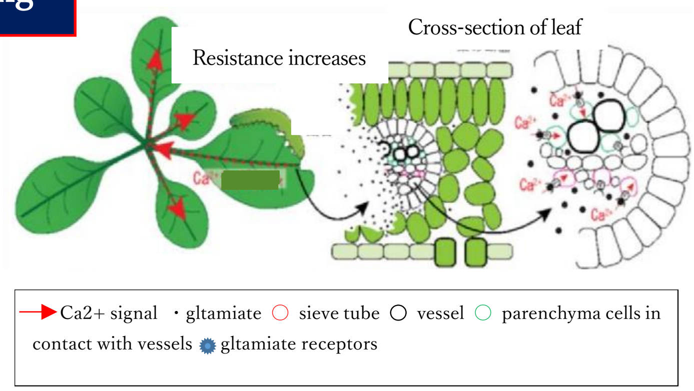


Fig.3 Generation mechanism of Ca^{2+} signal

themselves from future attacks by insects.

5. Potential applications of the research findings

Plants do not seem to sense anything while rustling quietly in the wind, but in fact, they can sense attacks from insects all over their body via Ca signaling. In some respect, the biosynthesis and storage of jasmonate triggered by Ca signals can be likened to a "plant-specific intelligent defense system" which repels insect attacks.

Moreover, it also became clear that, even when plants were not attacked or damaged by insects, external application of glutamate to leaves is enough to activate this plant-specific system. This suggests that, if amino acid-based agrochemicals capable of triggering glutamate receptors activity in plants can be developed, it would be possible to produce safe and high-quality food without harming both animals and humans and avoid the use of massive amounts of pesticides.

In recent years, a massive outbreak of locusts in the African desert led to grain losses and severe famine. With global warming, locusts migrated to the north, and the great famine has spread to the deserts of India. Maybe the day is not far off when these global challenges will be resolved without the massive use of pesticides.

[References]１）Toyota,M. *et al.*.; Science,361,1112(2018)、2)Toyota M. ; Seibutsu-butsuri , 62,56-57(2022)、3)Toyota M. : Kagaku to Seibutsu、58,(2)70-72(2020)

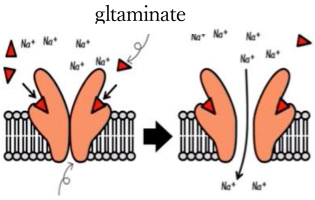
Introduction of Researcher

Profwssor Dr. Masatsugu TOYOTA
Graduate school of science, Saitama University, Saitama Japan
Honorary Fellow、University of Wisconsin-Madison

[Glossary]

[Sieve tube] A plant organ responsible for the transport of nutrients made during photosynthesis to leaves

[Glutamate receptors] There are various types of glutamate receptors, but here we will focus on ionotropic glutamate receptors (i.e., receptors that function by directly opening ion channels). The binding of glutamate to glutamate receptors located in the cell membrane (indicated by red triangles in illustration) triggers the opening of selective channels which allow only Ca^{2+} cations (in the illustration, Na^{2+}cations are shown) to flow into the cells, resulting in a high intracellular Ca^{2+} ion concentrate(This illustration of glutamate receptors is from TEKIBO youtube channel's high school study video "Biology Lesson 7" - signal transduction and receptors-)



Gltaminate receptor

Closing remarks / Notice

If we want to live in harmony with nature, we cannot turn our gaze away from the array of discoveries that shed light on the astonishing capabilities of plants, including those that clarified the mechanisms by which they communicate with other plants.

Meanwhile, amazing secrets are revealed in the animal kingdom which also includes humans. In the next "newsletter" we will present a study titled "Involvement of Ca^{2+} signaling in the mechanisms of lifespan regulation" from this fiscal year's selected researches. Don't miss it.