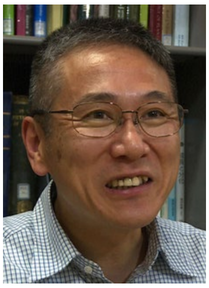

# Title of Project : Studies on the molecular mechanisms and evolution of the generation and transmission of action potential in plants

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## 【Purpose and Background of the Research】

Plants lack blood flow and nerves, but have evolved unique long-distance intercellular signaling mechanisms. Signals using plant hormones, peptides, proteins, and slow calcium waves have been well elucidated; however, the molecular mechanism of long-range, rapid, intercellular signaling by action potentials with fast calcium waves, which evolved in parallel to similar signaling mechanisms in animals, remains largely unknown. Rapid transmission of action potentials has been reported in specific tissues of the sensitive plant *Mimosa pudica*, the Venus flytrap *Dionaea muscipula*, and the sundew *Drosera rotundifolia*. Here, we propose to use these three species, as well as *Arabidopsis thaliana*, to study the molecular mechanisms of action potential generation and transmission. We have obtained genome sequences for these species and established techniques for transformation. We will screen for genetic factors responsible for fast intracellular and intercellular electrical signaling by action potentials, analyze their molecular characteristics such as effects on ion permeability and intracellular localization, and perform genetic gain- and loss-of-function experiments. This will allow us to understand the general mechanisms of action potential transmission in plants and the evolutionary process that resulted in diversity of transmission velocity, which is adaptive in the three plants.

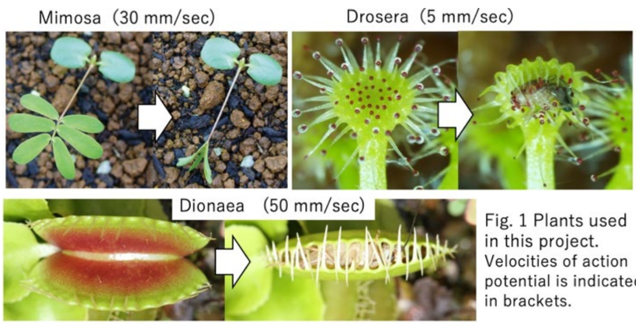

Fig. 1 Plants used in this project. Velocities of action potential is indicated in brackets.

## 【Research Methods】

Our objective is to understand the molecular mechanisms of the generation and transmission of high-speed electrical signals within and between cells and the diversity in transmission speeds. We will analyze the molecular aspects of the generation and transmission of action potentials using Mimosa, Dionaea, and Drosera as well as Arabidopsis as follows: (1) We will screen candidate ion channels that generate and transmit action potentials with comparing transcriptomes between movable and non-movable cells in the three movable plants. (2) We will characterize their functions with analyzing ion permeability, intracellular localization, and loss- and gain-of-function mutants. (3) We will characterize Arabidopsis genes orthologous to the candidate genes found in the movable plants. (4) We will swap the genes between the movable and non-movable plants to examine their functions and infer the evolutionary process. (5) A model of molecular mechanisms for the generation and transmission of action potentials in plants will be constructed and the process of molecular evolution that produced the diversity in velocity will be deduced.

## 【Expected Research Achievements and Scientific Significance】

How action potentials are propagated between cells remains particularly elusive. In animal neurons, action potentials are propagated synaptically between cells by chemicals. Plants, however, possess no synaptic structures between cells. Instead, cells are connected by plasmodesmata, channels 50 nm in diameter and 100 nm long. Isolation of plasmodesmata from plant tissues propagating action potentials has been unsuccessful, and plasmodesma-specific proteomic analysis is difficult to perform, so it is unclear whether ion channels exist to propagate action potentials. In addition, the $Ca^{2+}$ diffusion rate is simulated to be too slow with respect to the rate of electrical signaling (Evans *et al.* 2016, *Plant Physiol.* 171: 1771). Therefore, how $Ca^{2+}$ ions propagate through thousands of Dionaea cells with action potentials as long as 20 μm in length at a speed of 50 mm/sec remains a great mystery. We believe that the key to solving this mystery is to identify the channel proteins generating the electrical signals and observe their localization and dynamics.

## 【Publications Relevant to the Project】

・ Suda, H. et al. 2020. Calcium dynamics during trap closure visualized in transgenic Venus flytrap. Nature Plants 6: 1219-1224.
・ Palfalvi, G. et al. 2020. Genomes of the Venus flytrap and close relatives unveil the roots of plant carnivory. Current Biology 30: 2312-2320.

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