

Press Release

Discovery of compounds that keep plants fresh

~ Controlling plant pore openings for drought tolerance and delay in leaf withering ~

April 9, 2018

A team of scientists at Nagoya University has discovered new compounds that can control stomatal movements in plants. Some of the compounds have shown to prevent leaves from drying up and suppress withering when sprayed onto rose and oat leaves. Further investigation could lead to the development of new compounds that can be used to extend the freshness of cut flowers and flower bouquets, reduce transportation costs for plants and be applied as drought resistance agents for crops.

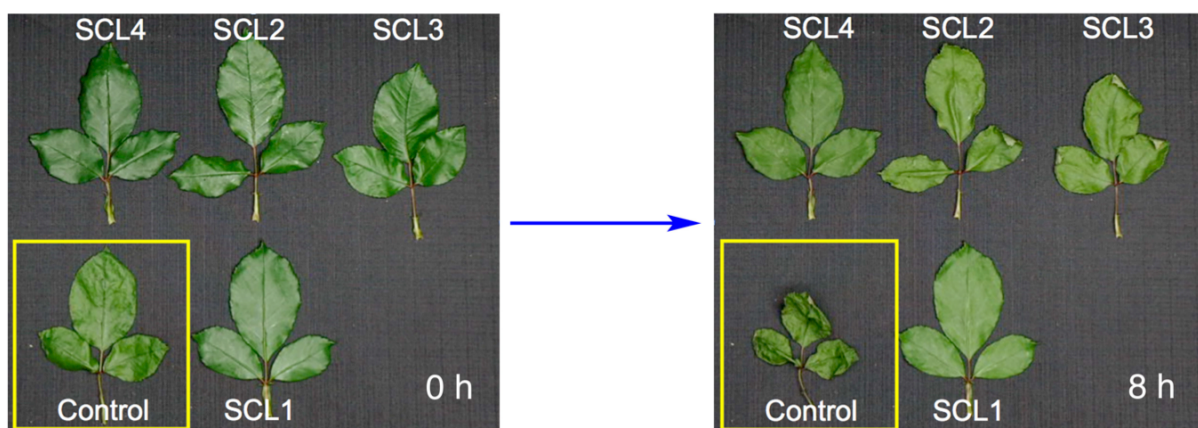
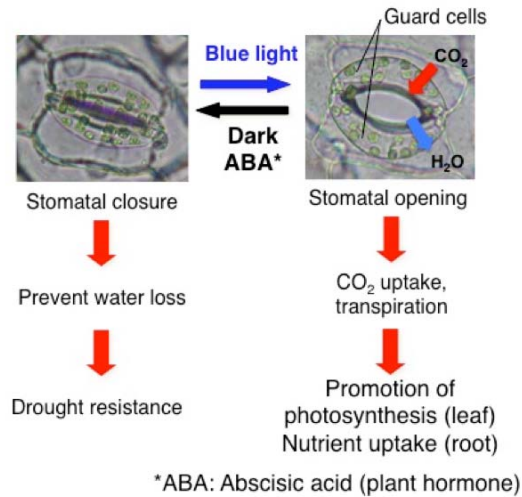


Photo of rose leaves (after 0 hours and 8 hours) sprayed with various stomata closing compounds. Leaves sprayed with stomatal closing compounds (SCLs) have stayed fresh for longer compared to the control leaves, which were untreated and shriveled with time.

Nagoya, Japan – A group of plant biologists and a chemist at the Institute of Transformative Bio-Molecules (ITbM), Nagoya University and Tohoku University, has used chemical screening to discover new compounds that can control stomatal movements in plants, according to a study reported in the journal *Plant & Cell Physiology*. They have succeeded in finding compounds exhibiting stomata closing activity, which are expected to be useful for drought tolerance to suppress withering of plants, and also stomata opening activity, which would lead to increased carbon dioxide uptake by plants, thus contributing to a low carbon society. This study explores a new chemical approach that does not rely on classical genetic methods to regulate the stomatal openings in plants.

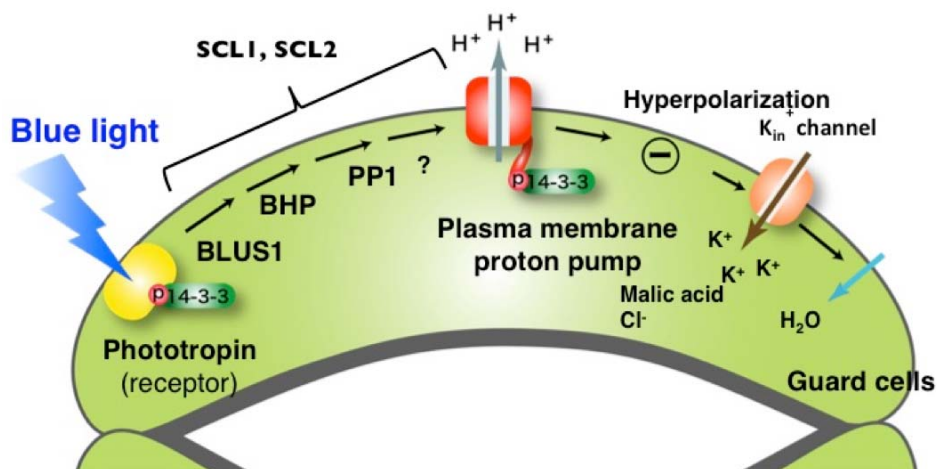
Stomata are small pores present in the surface of plants, including leaves, flower petals and other organs, and are responsible for gas exchange with the atmosphere. Through these pores, plants uptake the carbon dioxide necessary for photosynthesis and release water by transpiration, which enhances uptake of nutrients from the roots. Therefore, regulation of stomatal openings is essential for plant growth as well as survival in response to various environmental conditions.

Stomata consist of a pair of guard cells and open in response to the blue light present in sunlight. Opening of stomata leads to carbon dioxide uptake, explaining why photosynthesis occurs during the day. When plants are under dark conditions and/or drought stress, a plant hormone, abscisic acid (ABA) is biosynthesized and induces stomata closure. Stomata are closed during the night to prevent water loss from the plant.



Opening/closure of stomata and their purposes. Stomata open in response to blue light, and close in the presence of abscisic acid (ABA), which is biosynthesized when the plant is under dark conditions and/or drought stress. Stomata are essential for the uptake of carbon dioxide (CO₂) in order for the plant to carry out photosynthesis.

When the guard cells in the stomata detect blue light, the photoreceptor, phototropin is activated and induces signaling within the cell. As a result of the signaling, the enzyme, plasma membrane proton pump (PM H⁺-ATPase) is activated, leading to the uptake of potassium ions. This triggers water uptake and cell swelling that opens the stomata. Although activation of PM H⁺-ATPase is known to be significant for stomatal opening, the full mechanism of stomatal opening is yet to be clarified.



Proposed signaling pathway of stomata opening in response to blue light. 1) Phototropin receptors in the guard cells of stomata receives blue light; 2) Signal transmission by BLUS1, BHP and PP1 (proposed signaling components); 3) Activation of the plasma membrane proton pump (PM H⁺-ATPase); 4) Potassium (K⁺) ion uptake; 5) Accumulation of K⁺ ions increases the osmotic pressure in the guard cells; 6) Water uptake and swelling of cells; 7) Stomatal opening. This study has shown that compounds SCL1 and SCL2 act upon the signaling factors between phototropin and PM H⁺-ATPase.

“As we were interested in revealing the mechanism of stomatal opening, we decided to use a chemical library to explore molecules that affect stomatal opening,” says Professor Toshinori Kinoshita, a plant biologist at ITbM and the leader of this study. “We started this research in 2014, and were keen in using a chemistry-based approach instead of classical genetic techniques in order overcome drawbacks such as lethality and redundancy of the genes. We also thought that using compounds to regulate stomatal movements would be useful for developing agrochemicals.”

ITbM, which officially started in 2013, has a chemical library managed by Dr. Ayato Sato, consisting of both commercial compounds and compounds synthesized by ITbM's chemists. Kinoshita considered this to be a good opportunity to start a new approach.

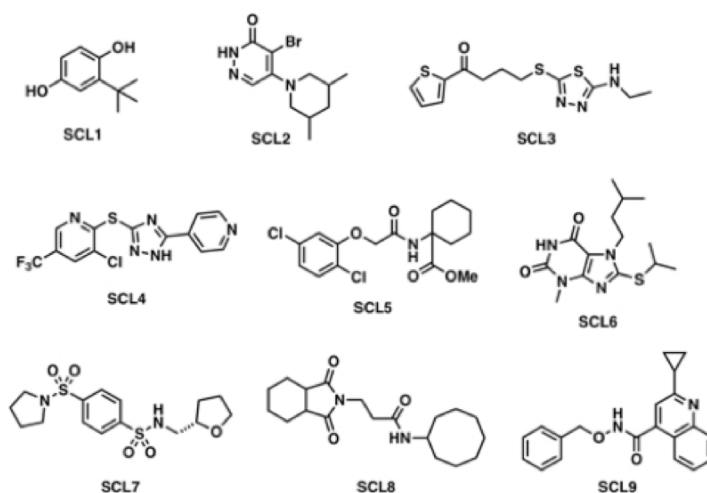
Using the herb, Benghal dayflower as a model plant, Dr. Shigeo Toh, Mr. Shinpei Inoue, and Dr. Yosuke Toda, established experimental conditions to screen over 20,000 compounds. They managed to find hit compounds after a year of random screening of the chemical library. This includes 9 compounds that suppress light-induced stomatal opening by more than 50%, and 2 compounds that induce stomatal opening even in the dark.

"The key to this research was to screen and find hit compounds as efficiently as possible from a vast pool of compounds," describes Kinoshita.



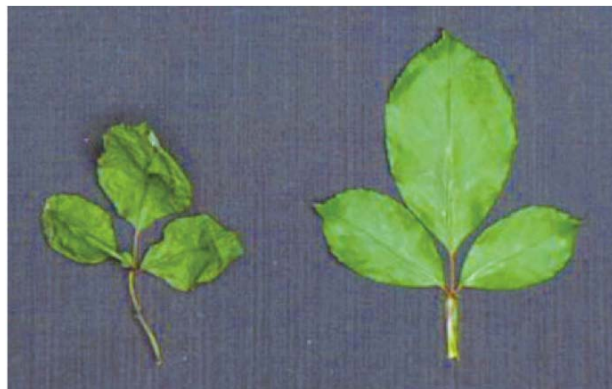
Photo of Benghal dayflower (*Commelina benghalensis*).

Further analysis of **stomatal closing** compounds (SCLs), SCL1 and SCL2, revealed that they inhibit the signaling components between the phototropin receptor and the PM H⁺-ATPase enzyme, thus inhibiting light-induced activation of PM H⁺-ATPase and leading to suppression of stomatal opening.



A series of **stomatal closing** compounds (SCLs), which suppress light-induced stomatal opening.

The researchers also sprayed SCL1 on rose and oat leaves and found that withering of the leaves was successfully suppressed over a certain time period.



Rose leaves sprayed with (right) and without (left) SCL1, left after 8 hours. Withering of the leaves sprayed with SCL1 (right) was suppressed compared to the control leaves (left).

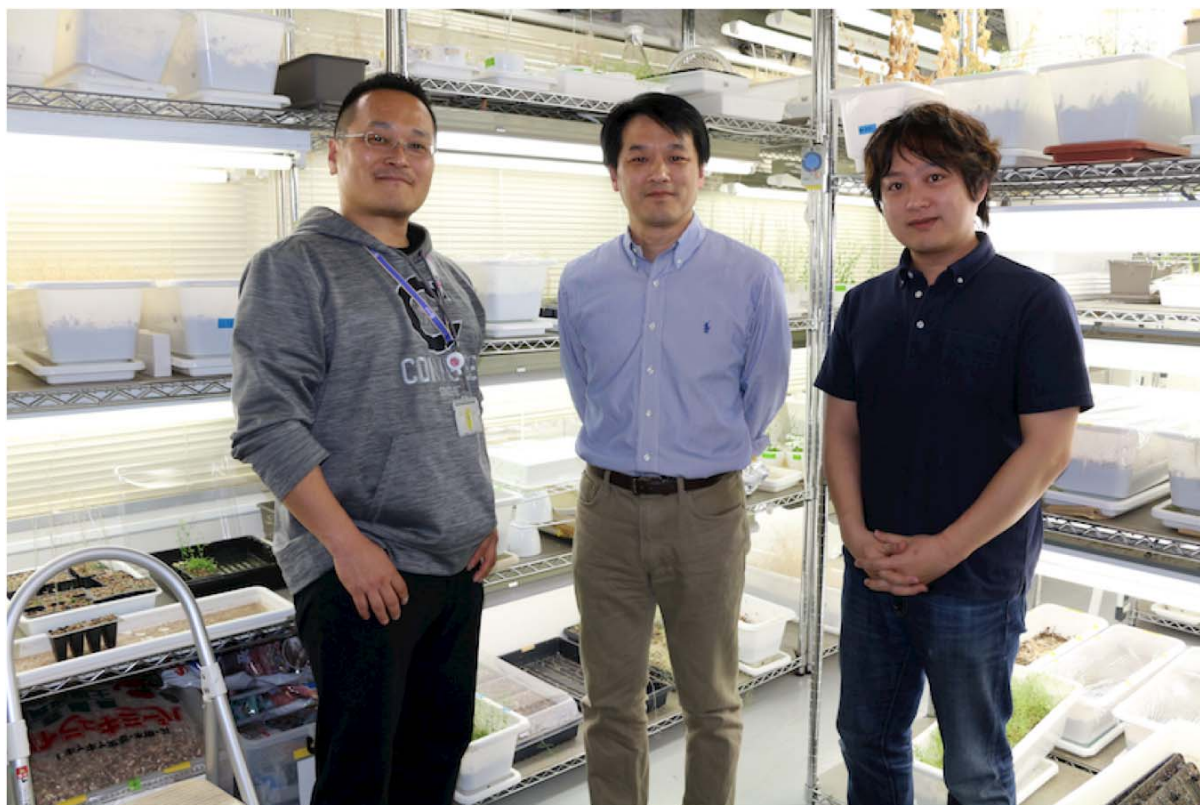
“This was the best moment in our research, to find that the molecules that we had discovered had an effect on suppressing the withering of leaves,” says Kinoshita. “The fact these compounds induce stomatal closure by a different mechanism to the plant hormone, ABA is important.”

Along with its stomatal closure effect in response to drought stress, ABA is also known to be an inhibitor for seed germination and plant growth. Although ABA and its derivatives are currently being developed as reagents to induce drought tolerance to plants, their side effects have been an issue. SCL1 currently shows to have an effect only on stomatal closure, so the team envisages that this is a good starting point for the development of new reagents for drought tolerance. They consider that these types of compounds would be useful for keeping cut flowers fresh and also reduce transportation costs, simply by spraying the compounds on the plants.

“We hope to be able to find partners to work on further elucidation of the stomatal opening mechanism and developing compounds together that can be applied for drought tolerance to various plants, including major crops and flowers,” says Kinoshita. “We also believe that by making leaves last for longer, this may lead to increasing the variation of leaves that can be used in the Japanese traditional flower arrangement, *Ikebana*, which currently has a tendency to avoid using simple leaves that can easily wither.”

This article “Identification and Characterization of Compounds that Affect Stomatal Movements” by Shigeo Toh, Shinpei Inoue, Yosuke Toda, Takahiro Yuki, Kyota Suzuki, Shin Hamamoto, Kohei Fukatsu, Saya Aoki, Mami Uchida, Eri Asai, Nobuyuki Uozumi, Ayato Sato & Toshinori Kinoshita is published online in *Plant & Cell Physiology*.

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From the left: Dr. Ayato Sato, Prof. Toshinori Kinoshita, and Dr. Yosuke Toda

About WPI-ITbM (<http://www.itbm.nagoya-u.ac.jp/>)

The Institute of Transformative Bio-Molecules (ITbM) at Nagoya University in Japan is committed to advance the integration of synthetic chemistry, plant/animal biology and theoretical science, all of which are traditionally strong fields in the university. ITbM is one of the research centers of the Japanese MEXT (Ministry of Education, Culture, Sports, Science and Technology) program, the World Premier International Research Center Initiative (WPI). The aim of ITbM is to develop transformative bio-molecules, innovative functional molecules capable of bringing about fundamental change to biological science and technology. Research at ITbM is carried out in a "Mix Lab" style, where international young researchers from various fields work together side-by-side in the same lab, enabling interdisciplinary interaction. Through these endeavors, ITbM will create "transformative bio-molecules" that will dramatically change the way of research in chemistry, biology and other related fields to solve urgent problems, such as environmental issues, food production and medical technology that have a significant impact on the society.

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