Bursts of fluorescence caught on video reveal how and why the sensitive plant Mimosa pudica moves its leaves rapidly

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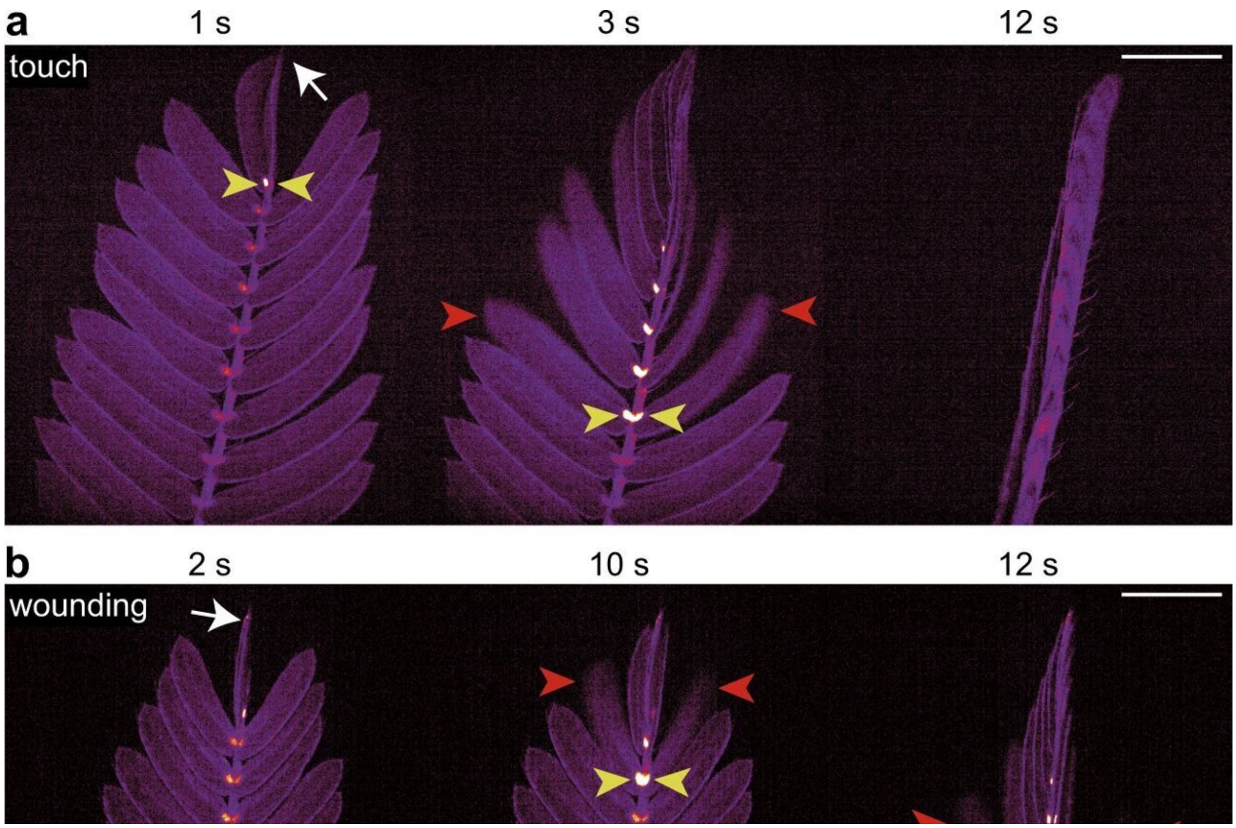

$[Ca^{2+}]_{cyt}$ increase at the pulvinus triggers rapid leaflet movement. a, b Touch (a) and wounding (b) (white arrows) caused $[Ca^{2+}]_{cyt}$ increases at the tertiary pulvini (yellow arrowheads) and leaflet movements (red arrowheads) that propagated toward the base of the rachilla. c, d Wounding triggered $[Ca^{2+}]_{cyt}$ increases at the tertiary pulvini that preceded the leaflet displacements in control (c) but not in La^{3+}-treated leaves (d). Dashed white and solid red lines indicate leaflet positions before and after leaflet movements, respectively. e, f $[Ca^{2+}]_{cyt}$ signatures at the tertiary pulvinus and leaflet angle in leaves pretreated with H_2O (e, n = 5) and 50 mM La^{3+} (f, n = 7). Mean ± SEM values are shown. Scale bars, 5 mm (a and b) or 1 mm (c and d). Credit: *Nature Communications* (2022). DOI: 10.1038/s41467-022-34106-x

Plants do not possess nerves and muscles that enable rapid movement in animals. However, Mimosa pudica, commonly called touch-me-not, shame or sensitive plant, moves its leaves by bending the motor organ "pulvinus" immediately in response to touch and wounds. Since the era of Charles Darwin, this spectacular leaf movement has been studied. However, the long-distance signaling molecules that trigger the rapid leaf movements and the physiological roles of this movement remain unexplored.

The team, led by Professor Masatsugu Toyota (Saitama University, Japan), revealed what signals travel long distances and trigger rapid movements in Mimosa pudica and why Mimosa pudica moves its leaves immediately.

The research is published on November 14 in *Nature Communications*. Takuma Hagihara led the work as a Ph.D. student in Toyota's lab and collaborated with researchers from Hasebe's lab at the National Institute for Basic Biology, Japan.

"To clarify the long-distance signals and physiological functions of the rapid leaf movements, we created transgenic 'fluorescent' and 'immotile' Mimosa pudica," says Toyota. The videos demonstrate that bursts of fluorescence travel rapidly throughout the leaves and trigger leaf movements. The fluorescent light tracks the cytosolic calcium in real time.

"Mimosa pudica closes its leaves just 0.1 seconds after the arrival of the Ca^{2+} signals in the motor organ pulvini," Toyota adds.

Previous studies have suggested that electrical signals, such as an action potential, are critical for the rapid leaf movements in Mimosa pudica.

"We developed a simultaneous recording system for the cytosolic Ca^{2+} and electrical signals to reveal the spatiotemporal relationship between these signals," says Toyota. Upon wounding the leaf, the Ca^{2+} and electrical signals propagated systemically at similar speeds and passed through the recording site at a similar time. Therefore, the long-distance Ca^{2+} and electrical signals were spatiotemporally coupled in Mimosa pudica.

Pretreating Mimosa pudica leaves with the Ca^{2+} channel inhibitors, La^{3+} and verapamil, and the Ca^{2+} chelating reagent, EGTA, blocked both Ca^{2+}/electrical signals and the leaf movements in response to the wound. These data support the idea that Ca^{2+} acts as a long-distance signaling molecule that triggers rapid leaf movements in Mimosa pudica.

"Mimosa pudica is one of the most famous plants due to its spectacular movements," says Toyota. "However, although there are many hypotheses for the physiological functions of the rapid leaf movements, why Mimosa pudica moves its leaves has not been scientifically elucidated."

Using the CRISPR/Cas9 genome editing technique, Toyota's team of scientists created an "immotile" elp1b mutant lacking motor organ pulvini. They compared the wild-type motile Mimosa pudica and the genetically and pharmacologically immotile Mimosa pudica and discovered that herbivorous insects, such as grasshoppers, consumed these immotile leaves more than wild-type leaves.

They also visualized the Ca^{2+} signals, the leaf movements, and the behavior of a grasshopper on the leaf under a microscope. Upon feeding by the grasshopper, the leaflets moved sequentially in parallel with the propagation of the Ca^{2+} signals, and thereafter, the grasshopper stopped the feeding and moved away.

"We finally obtained evidence that rapid movements based on propagating Ca^{2+} and electrical signals protect Mimosa pudica from insect attacks," says Toyota. "Plants possess various communication systems that are normally hidden from view; seeing is believing," he adds.

More information: Takuma Hagihara et al, Calcium-mediated rapid movements defend against herbivorous insects in Mimosa pudica, *Nature Communications* (2022). DOI: 10.1038/s41467-022-34106-x

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