Latest News

People's Daily Online: Non-invasive micro-test technology improves research on rice disease resistance

Contributed by Yunqi Liu, Edited by Xuefei Li

Editor's note:

How to Predict a Rice Blast Ahead of Time? Gene mapping determines the identity of a "disease-resistant hero" in this article that was published on People's Daily Online. "A cyclic nucleotide-gated channel mediates cytoplasmic calcium elevation and disease resistance in rice" was first published by Academician Wan Jianmin of the Chinese Academy of Agricultural Sciences in Cell Research in 2019. There are 6 data graphs in this document, 3 of which are of Ca²⁺ flux data detected by non-invasive micro-test technology (NMT). This proves that non-invasive micro-test technology provided key evidence for the identification of CNGC9, an important calcium channel in the rice disease resistance process. The editor reviews this article here for the benefit of readers.



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Basic Information

Topic: The molecular mechanism of calcium ions to activate the immune system

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Title: A cyclic nucleotide-gated channel mediates cytoplasmic calcium elevation and disease resistance in rice

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Test sample: rice mesophyll cells

Ion/molecule detection indicators: Ca²⁺

Ca²⁺ flux experimental treatment method: rice seedlings, 10µM chitin or 10µM flg22 peptide transient flux

Ca²⁺ flux test liquid composition: 0.2mM CaCl₂, 0.1mM NaCl, 0.1mM MgCl₂ and 0.1mM KCl, pH 5.2

Abstract: The transient elevation of cytoplasmic calcium is essential for pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI). However, the calcium channels responsible for this process remain unknown. The results showed that rice *CDS1* (*CELL DEATH and SUSCEPTIBLE to BLAST 1*) encoding OsCNGC9, a cyclic nucleotide-gated channel protein, positively regulates the resistance to rice blast disease. The results indicated that OsCNGC9 mediates PAMP-induced Ca²⁺ influx and that this event is critical for PAMPs-triggered ROS burst and induction of PTI-related defense gene expression. The study further showed that a PTI-related receptor-like cytoplasmic kinase OsRLCK185 physically interacts with and phosphorylates OsCNGC9 to activate its channel activity. These results suggest a signaling cascade linking pattern recognition to calcium channel activation, which is required for initiation of PTI and disease resistance in rice. The Ca²⁺ influx of rice mesophyll cells under different treatments was detected by non-invasive micro-test technology (NMT). The discovery that OsCNGC9 can mediate Ca²⁺ influx in rice PTI lays the foundation for understanding the role of Ca²⁺ signaling in rice disease resistance.

Key words: non-invasive micro-test technology; calcium ion flux; rice mesophyll cells; transient flux

1. Experimental results of ion/ molecule flux

The study used NMT technology to investigate whether OsCNGC9 could mediate Ca^{2+} influx in PTI by measuring the dynamic changes of Ca^{2+} flux in mesophyll cells after treatment with two PAMPs (chitin or flg22). Previous studies have shown that these PAMP elicitors can trigger PTI signaling in plants. Upon chitin or flg22 stimulation, wild type (WT) mesophyll cells (but not rice mutant, *cds1 (cell death and susceptible to blast 1)* mesophyll cells) exhibited robust and fast Ca^{2+} influx (Figure 1a,b). These results suggest that OsCNGC9 can mediate Ca^{2+} influx in rice PTIs, whereas this ability is impaired in the *cds1* mutants. Ca^{2+} flux analysis showed that Nipponbare mesophyll cells (but not *Osrlck185/55* double mutant mesophyll cells) exhibited fast Ca^{2+} influx upon chitin stimulation (Figure 2e). In addition, after chitin treatment of *Oscerk1* knockout mutants, no obvious Ca^{2+} influx was observed (Figure 2f).

After PAMPs stimulation, mesophyll cells of OsCNGC9-OE transgenic plants showed stronger Ca^{2+} influx compared with Kitaake plants (Figure 3f,g).



Figure 1. OsCNGC9 is required for Ca^{2+} influx in response to PAMPs. Positive values represent Ca^{2+} efflux, negative values represent Ca^{2+} influx.

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Figure 2. *Osrlck185/55* double mutant plants show reduced chitin-induced Ca^{2+} influx. Positive values represent Ca^{2+} efflux and negative values represent Ca^{2+} influx.



Figure 3. Comparison of Ca^{2+} influx in the mesophyll cells of Kitaake and *OsCNGC9-OE* transgenic plants after chitin or flg22 treatment. Positive values represent Ca^{2+} efflux, negative values represent Ca^{2+} influx.