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# Distinctive outlook into the mechanism of resistance to rice blast

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## Abstract

Rice blast, caused by the filamentous fungus *Magnaporthe oryzae* (also known as *Pyricularia oryzae*), is the most severe disease threatening rice yield. Sakuranetin, an important plant phytoalexin, exhibits inhibitory effects on rice blast fungus growth. However, the underlying molecular mechanism by which sakuranetin enhances plant resistance against pathogens remains unclear. A recent study published in *Nature Communications* by Jiang et al. (*Nat. Commun.*, 2023, <https://doi.org/10.1038/s41467-024-47746-y>) elucidated from a cytological perspective how sakuranetin enhances resistance to rice blast disease. The research revealed that elevated accumulation of sakuranetin attenuates the endocytosis of *M. oryzae* effectors into the cytoplasm of rice host cells. The inhibitory effect of sakuranetin is dosage-dependent and targets clathrin-mediated endocytosis (CME). This study provides novel insights into understanding plant immune mechanisms underlying weakening pathogen virulence weapons.

In the ongoing battle against pathogen invasion, plants have evolved sophisticated and intricately orchestrated immune mechanisms known as the innate immune system (Medzhitov, 2007; Dodds and Rathjen, 2010). Serving as the primary line of defense against invading pathogens, this system is activated by pathogen-associated molecular patterns (PAMPs), effectively intercepting the majority of intruders in what is commonly referred to as PTI (PAMP-triggered immunity) (Chisholm et al. 2006; Jones and Dangl, 2006; Boller and Felix, 2009). However, the interaction between plants and pathogens is characterized by a dynamic interplay of mutual antagonism. Pathogens deliver

effector proteins into and around plant cells to counteract plant PTI and facilitate infection. Upon entering into host cells, these effector proteins trigger the secondary tier of the plant immune system, recognized as ETI (effector-triggered immunity), facilitated by the intracellular resistance receptors NLR (nucleotide-binding domain and leucine-rich repeat receptors) (Chisholm et al. 2006; Jones and Dangl, 2006). When rice blast spores adhere to rice leaves, they generate appressorial cells and subsequently penetrate rice cells with hyphae, triggering the reprogramming of a large set of defense-related genes. Recent research has uncovered that the rice blast fungus secretes two endoglucanases (MoCell12A and MoCell12B) to damage the rice cell wall, aiding its penetration (Yang et al. 2021). This results in the release of specific oligosaccharides. The rice OsCEBip and OSCERK1 recognize these oligosaccharides to activate downstream immune responses in rice cells (Yang et al. 2021). Meanwhile, the effector protein Bas83 of rice blast recruits fragments of plant cell membranes into a specialized biotrophic interfacial complex (BIC) during the infection process. Subsequently, cytoplasmic effector proteins enter the host cell cytoplasm through

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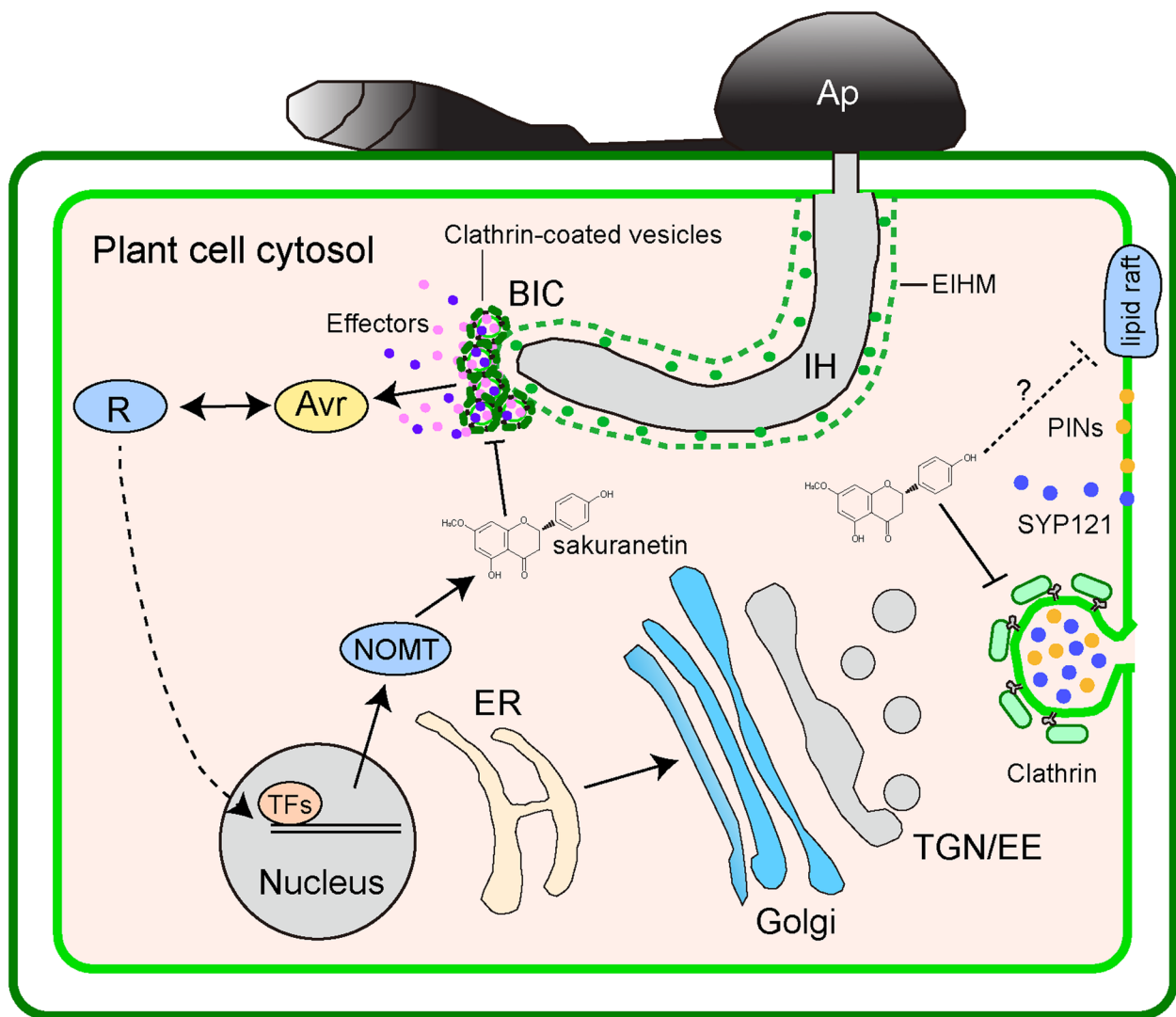


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clathrin-mediated endocytic pathways (Oliveira-Garcia et al. 2023). All of these processes involve an essential biological mechanism known as vesicle trafficking, responsible for transporting cargos to appropriate cellular compartments at the right time (Wang et al. 2016). Plants have the ability to produce phytoalexins to resist the invasion of pathogenic microorganisms, among which sakuranetin has been shown to have superior efficacy compared to momilactone A (a major rice diterpenoid phytoalexin), exhibiting potent defense activity against rice blast fungus (Hasegawa et al. 2014). How does sakuranetin defend against rice blast fungus at the cellular level? Does it achieve

this by inhibiting the endocytosis of effector proteins mediated by clathrin? The questions remain yet to be investigated.

The study by Jiang et al. (Nat. Commun, 2023, <https://doi.org/10.1038/s41467-024-47746-y>) sheds light on the mechanism of sakuranetin in rice defense from a cytological perspective (Jiang et al. 2024). Jiang et al. found that rice near-isogenic lines (NILs) containing various resistance genes exhibited higher resistance to *M. oryzae* than the susceptible rice line. Meanwhile, the NILs have higher levels of sakuranetin than the susceptible rice line. Furthermore, root cells of the NILs exhibited attenuated internalization of the



**Fig. 1** Working model for sakuranetin-enhanced resistance against *M. oryzae* invasion in rice. Rice expressing *R* genes have high levels of the phytoalexin sakuranetin. Sakuranetin acts on the clathrin-mediated endocytic pathway to attenuate the endocytosis of the effector proteins and reduce the secretion of avirulence proteins *Avr* from *M. oryzae* into host cells, thereby enhancing the plant's ability to resist rice blast. Ap, appressorial cell, BIC, biotrophic interfacial complex, EIHM, extrinsic hyphal matrix, ER, endoplasmic reticulum, IH, invasion hyphae, TFs, transcription factors

endocytic tracer FM4-64 compared with the susceptible line. There appears to be a correlation between fungal resistance, sakuranetin level, and endocytosis. Indeed, overexpression of *OsNOMT* (*NARINGENIN 7-O-METHYLTRANSFERASE*) increases sakuranetin level and enhances rice resistance, while the synthesis mutant *nomt-cc* is more susceptible to rice blast. To determine whether sakuranetin contributes to the reduced endocytosis in the NILs root cells, the authors examined its effect on the internalization of FM4-64 and plasma membrane-localized proteins, including OsPIN1, OsPIN2, OsPIN3, and syntaxin of plants 121 (OsSYP121). The results demonstrate that sakuranetin inhibits endocytosis of FM4-64 and these plasma membrane-localized proteins in a concentration-dependent manner. The authors further showed that sakuranetin hindered the endocytosis of *M. oryzae* effectors. These results suggest that sakuranetin may resist pathogen invasion by hindering the internalization of *M. oryzae* effectors into the cytoplasm of rice host cells (Jiang et al. 2024).

Plant cells host a complex endomembrane system, including the endoplasmic reticulum (ER), Golgi apparatus, trans-Golgi network/early endosome (TGN/EE), multivesicular body/prevacuolar compartment/late endosome (MVB/PVC/LE), and vacuoles (Cui et al. 2020). Secretory proteins are conventionally transported to the plasma membrane or other organelles through vesicular transport-mediated protein sorting pathways (Wang et al. 2016). Conversely, proteins residing on the plasma membrane undergo recycling through the TGN/EE or are directed to vacuoles by MVB/PVC for degradation (Wang et al. 2016). So, does sakuranetin affect the dynamics of intracellular vesicle trafficking? Transmission electron microscopy, endosome marker co-localization, and fluorescence recovery after photobleaching (FRAP) analyses showed that sakuranetin did not generally interfere with endosomal dynamics and plasma membrane fluidity. Subsequently, researchers analyzed how sakuranetin confers resistance to rice blast by weakening endocytosis. The researchers found that after inhibiting the CME pathway, sakuranetin no longer exerts an effect on endocytosis in rice cells. This suggests that sakuranetin's inhibition of endocytosis is dependent on the CME pathway. Consistently, knocking out of the rice *CLATHRIN HEAVY CHAIN* gene or treatment with CME inhibitors enhances rice blast resistance.

In summary, these findings suggest that sakuranetin conveys resistance to rice blast by weakening CME (Jiang et al. 2024). Based on this, the following model can be drawn: Rice plants expressing the resistance *R*

genes have high levels of sakuranetin. Sakuranetin acts on the CME pathway, thereby attenuating the endocytosis of the effector proteins, consequently enhancing the plant's ability to defend against rice blast (Fig. 1). These insights offer a unique perspective and novel ideas for understanding the mechanism of rice resistance to rice blast.

A crucial question that needs to be addressed is how sakuranetin affects CME. Sakuranetin, being a lipophilic compound, has the ability to integrate into model cell membranes, where it interacts with the phospholipids and potentially reduces the molecular order of the acyl chains (da Cruz Ramos Pires et al. 2022; de Souza et al. 2024). These properties share similarities with sterols, which also affect endocytosis (Men et al. 2008; Kim et al. 2017; Cui et al. 2018). Sterols play a role in promoting the formation of ordered membrane domains, known as lipid rafts, which are crucial for supporting endocytosis and signaling transduction (Kim et al. 2017). Upon treatment with flg22 (a PAMP), the plasma membrane-localized pattern recognition receptor (PRR) FLS2 is redistributed to lipid rafts, which facilitates its endocytosis (Cui et al. 2018). Further confirmation is required to determine whether sakuranetin can affect the abundance of pattern recognition receptors within lipid rafts.

Finally, an open-ended question is how sakuranetin inhibits endocytosis and how it is activated? It is worth the author's in-depth research.

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#### Authors' contributions

SM and JW had the idea for the article, SM and CT performed the literature search and wrote the draft of the manuscript, CT drew the figure and SM critically revised the work.

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#### Competing interests

The authors declare no competing financial interests.

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