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News & Views

A novel recognition-transmission-execution module in maize immunity

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Maize (*Zea mays* L.) stands as one of the most extensively cultivated crops worldwide [1]. With its widespread cultivation comes a plethora of challenges, including diseases caused by a wide range of pathogens [2]. Gray leaf spot (GLS) is a foliar disease that drastically reduces worldwide maize production. Since it was first identified in the US in the 1920s, the disease has spread around the globe, severely affecting corn production. GLS is predominantly instigated from infection by *Cercospora zeae-maydis* and *Cercospora zeina* [3]. In many parts of China, GLS is an important disease [4]. In severe cases, GLS can lead to a reduction of yield by up to 40%. Hence, it is urgent to control the spread of this disease [3]. Developing and utilizing pathogen-resistant crop cultivars is the most economical and environment-friendly measure for controlling plant diseases. Scientists have dedicated significant efforts to characterize GLS resistance genes in maize. To date, *ZmCCoAOMT2* (caffeoyl-CoA *O*-methyltransferase 2) and *ZmMM1* (Mexicana lesion mimic 1) are the only two genes that have been shown to confer resistance to GLS [5,6]. However, to facilitate the development of resistant cultivars, there is an urgent need for additional genes that are effective against GLS and a clearer understanding of the molecular mechanisms underlying the regulation of host plant resistance to GLS.

It is now well-accepted that the plant immune system consists of two layers: pattern-induced immunity (PTI) and effector-triggered immunity (ETI). PTI relies on cell surface-located pattern recognition receptors (PRRs) to sense pathogen invasion and initiate immune responses. On the other hand, ETI is an enhanced form of defense that occurs after the detection of avirulent pathogen effectors by host intracellular nucleotide-binding domain leucine-rich repeat (NLR) proteins, which often exhibit high race specificity [7,8]. Mounting evidence indicates that ETI and PTI reinforce each other to bolster robust defense responses [9]. Over recent years, wall-associated receptor kinase (WAK) and WAK-like kinases (WAKLs) have emerged as two new categories of PRRs that confer resistance to pathogens in plants [10,11]. For instance, WAK22 and ZmWAK-RLK1 have been identified as key players in providing resistance to *Fusarium* races in *Arabidopsis* and northern corn leaf blight caused by *Exserohilum turcicum*, respectively [12,13]. However, the specific molecular mechanism by which plants recognize pathogens, transmit immune signals, and execute defense responses remains largely unknown. Recently, an exciting study by Zhong et al. has shed new light on these mysteries [14].

By performing Quantitative trait loci (QTL) analysis using a genetic population derived from the inbred lines Y32 and Q11 that were resistant and susceptible to GLS, respectively, Zhong and colleagues mapped *qRgls1*, a major quantitative resistance site that enhanced GLS resistance by 19.70% to 61.28% [14]. Through fine mapping and transgene complementation verification, the investigators found a functional gene locus *ZmWAKL* encoding a WAK-like kinase. The Y32 and Q11 alleles of *ZmWAKL* (*ZmWAKL*^{Y} and *ZmWAKL*^{Q}, respectively) differed primarily in the extracellular domains of their deduced proteins, exhibiting a sequence identity of only 62.8%. A chimera gene was engineered by substituting the extracellular domain of a disease-resistant gene with that of a susceptible gene, which demonstrated the pivotal role of extracellular domain in controlling maize resistance to GLS [14].

Given that PRRs have been demonstrated to engage with a coreceptor for substrate recognition and signal transduction [15], the researchers conducted immunoprecipitation assays utilizing extracts from transgenic plants overexpressing *ZmWAKL*^{Y}*-GFP*, followed by mass spectrometry analysis [14]. A plasma membrane-localized leucine-rich repeat receptor-like serine/threonine protein kinase, ZmWIK, was found to interact with ZmWAKL. Two distinct maize transgenic lines engineered to overexpress *ZmWIK* exhibited significantly reduced symptoms of GLS compared to the wild type (WT) inbred line B73. Conversely, *ZmWIK* null mutant plants, generated using ethyl methanesulfonate (EMS), showed increased susceptibility to GLS in field trials when compared to WT plants. Furthermore, the ZmWAKL^{Y} protein encoded by a disease-resistant allele had the ability to form dimers and interacted with its co-receptor ZmWIK located on the plasma membrane, thereby forming the ZmWAKLY/ZmWIK immune complex. This complex enhanced the phosphorylation levels of both proteins. Given the capacity of ZmWAKL^{Y} to recognize GLS pathogens, the phosphorylation levels of this complex likely play a role in regulating kinase-mediated resistance in maize (Fig. 1).

To date, two prominent receptor-like cytoplasmic kinases (RLCKs), *Botrytis*-induced kinase 1 (BIK1) and PBL1 (avrPphB susceptible 1-like 1), have been identified as key components downstream of PRRs, responsible for transmitting defense signaling in plants [16]. During the investigation into whether a similar RLCK could contribute to ZmWAKL-mediated immunity, a cytoplasmic

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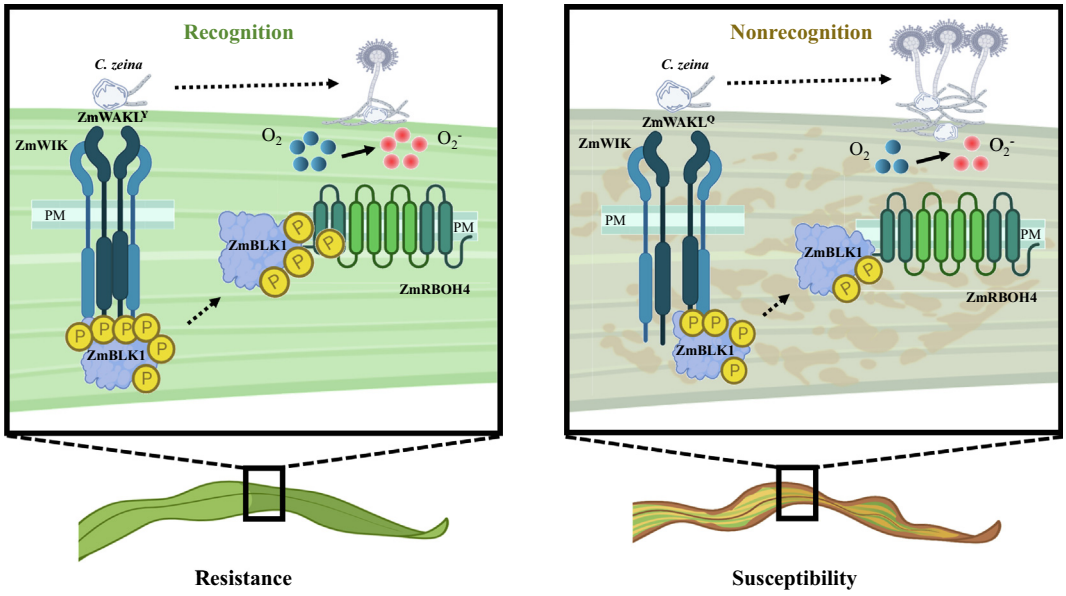

Fig. 1. A proposed model illustrating the functioning of the ZmWAKL-ZmWIK-ZmBLK1-ZmRBOH4 module in maize defense against gray leaf spot disease. (a) ZmWAKL^{Y} forms homodimers and interacts with the coreceptor ZmWIK at the plasma membrane. Upon recognition of the maize gray leaf spot pathogens, the ZmWAKL^{Y}/ZmWIK complex rapidly increases its phosphorylation activity, transmitting immune signals to ZmBLK1 and enhancing its phosphorylation. Eventually, ZmBLK1 interacts with and phosphorylates ZmRBOH4, triggering ROS burst to defend against the pathogens. (b) In contrast, ZmWAKL^{Q} is unable to form homodimers and exhibits weak interaction with ZmWIK. Consequently, upon pathogenic challenge, there is insufficient enhancement in phosphorylation activity, leading to an inadequate defense response against the pathogens. PM denotes the plasma membrane. The figure was created with the software BioRender (BioRender.com).

receptor-like kinase, ZmBLK1, was discovered to interact with the ZmWAKL^{Y}/ZmWIK complex. This interaction may result in the formation of an immune signaling complex at the plasma membrane. Moreover, it was observed that ZmBLK1 was phosphorylated directly by ZmWIK within the ZmWAKL/ZmWIK/ZmBLK1 complex, with ZmWAKL^{Y} enhancing the phosphorylation process. Conversely, ZmWAKL^{Q} did not exhibit this enhancing effect on phosphorylation (Fig. 1). These findings provide evidence for a molecular mechanism by which the ZmWAKL^{Y}/ZmWIK complex activates downstream defense signaling pathways through the phosphorylation of ZmBLK1.

In *Arabidopsis*, BIK1 plays a crucial role in enhancing immunity through the generation of a reactive oxygen species (ROS) burst, achieved by its direct binding to and phosphorylation of the NADPH oxidase RBOHD [17]. Does the execution of the defense response downstream of ZmBLK1 involve ROS burst? Zhong et al. [14] investigated this question and discovered that ZmBLK1 interacted with and phosphorylated the maize NADPH oxidase ZmRBOH4 on the plasma membrane. Knockout mutant plants of *ZmRBOH4* generated using CRISPR/Cas9 exhibited significantly increased susceptibility to GLS compared with WT plants. By integrating the different datasets generated, a recognition-transmission-execution module acting in maize resistance against GLS is proposed (Fig. 1). In essence, when ZmWAKL^{Y} detects a pathogenic invasion, its phosphorylation activity rapidly increases, initiating an immune signaling cascade from ZmWAKL^{Y} to ZmWIK, then to ZmBLK1, and ultimately to ZmRBOH4. This cascade leads to enhanced generation of ROS, culminating in a strong resistance against GLS (Fig. 1).

For the first time, Zhong et al. [14] showcased the characterization of the ZmWAKL-ZmWIK-ZmBLK1-ZmRBOH4 module in maize resistance against GLS. This breakthrough represents a significant stride in deciphering the intricate, yet finely tuned immunity mechanisms that dictate a plant's susceptibility or resistance to fungal pathogens. While this study sheds light on the role of ROS burst in this resistance, it remains to be explored whether other key defense processes, such as calcium influx, may be activated during the signaling mediated by ZmWAKL-ZmWIK-ZmBLK1. Another important issue is the specificity of ZmWAKL-ZmWIK-ZmBLK1. May it be broadly effective against multiple pathogens? Moreover, do other plant species have immune signaling modules similar to ZmWAKL-ZmWIK-ZmBLK1? Furthermore, previous studies have identified two genes that are effective against GLS. *ZmCCoAOMT2* regulates metabolite levels in the phenylpropanoid and lipoxygenase pathways, as well as the regulation of programmed cell death [5]. Additionally, the teosinte allele of *ZmMM1* enhances maize resistance to GLS without incurring plant growth or yield penalties [6]. Notably, both of these genes, along with this newly identified module, play crucial roles in regulating cell death during GLS defense. Therefore, it is imperative to explore the effects of different gene combinations in maize GLS resistance breeding, and to develop effective and targeted strategies for maintaining the delicate balance between plant disease resistance and growth. Further studies of these questions will not only deepen the understanding of plant immunity to pathogens but also pave the way for engineering innovative and effective resistance against the diverse diseases that frequently erupt and ruin precious agricultural crops

CRediT authorship contribution statement

Xiuyu Wang: Writing – original draft. **Ashline Matthew:** Writing – review & editing. **Daowen Wang:** Writing – review & editing. **Hongyuan Zheng:** Conceptualization, Writing – original draft. **Zhengqing Fu:** Conceptualization, Funding Acquisition, Writing – original draft.

Conflict of interest

The authors declare that they have no conflict of interest.

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