New resistance gene against maize rough dwarf disease identified in cereal crops

We identified *ZmGLK36*, a resistance gene against rice black-streaked dwarf virus (RBSDV), in maize. *ZmGLK36* mediates resistance by regulating jasmonic acid (JA) biosynthesis and JA-mediated defence response; it also grants resistance to RBSDV to other cereal crops, such as rice and wheat.

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The mission

RBSDV[1] and maize rough dwarf virus (MRDV)[2] are closely related members of the *Fijivirus* genus in the *Reoviridae* family, and they can infect almost all major cereal crops (including maize, rice, wheat and barley) in Asia, Europe and South America. Whereas MRDV primarily infects maize in Europe and South America, RBSDV mainly infects maize in Asia, causing maize rough dwarf disease (MRDD). MRDD is characterized by dwarfing of plants; shortening of internodes, which are the lengths of the stem between two nodes (where buds, leaves or branches emerge); and thickened, short and stiff green leaves. MRDD ultimately results in heavy (30–100%) yield losses[2]. RBSDV is transmitted to plants via small brown grasshoppers, which act as a vector. Although adjustment of the sowing dates (so the crop planting time does not coincide with the grasshoppers' migration time) and insecticides (such as cypermethrin or γ-cyhalothrin) are commonly used to alleviate the disease and yield loss caused by RBSDV or MRDV, these practices are inefficient and harmful to the environment. Thus, identifying genes that confer resistance to RBSDV and breeding RBSDV-resistant cultivars (cultivated varieties) has remained the most effective and environment-friendly approach for MRDD management.

The solution

To fine map and clone *qMrdd2*, a previously reported major quantitative trait locus[3] (QTL, a DNA region associated with a specific phenotype) for RBSDV resistance in maize, we developed a pair of near-isogenic lines (NILs), one susceptible (NIL-S) and the other resistant (NIL-R) to RBSDV, and carefully examined MRDD symptoms in field conditions. Through de novo genome assembly, quantitative reverse transcription PCR and candidate gene association analyses, we pinpointed the candidate gene (*ZmGLK36*) and its causal (that is, resistance-associated) variation. Then, by using a suite of genetic (including overexpression and knockout mutant analyses), physiological and biochemical experiments (including yeast one-hybrid assay, transient expression assay in maize protoplasts and electrophoretic mobility shift assay), we dissected the molecular mechanisms of *ZmGLK36*-mediated resistance.

We show that *qMrdd2* includes a gene, *ZmGLK36* (which encodes a G2-like-transcription factor) that promotes resistance to RBSDV. RBSDV infection increases *ZmGLK36* expression and, in turn, ZmGLK36 promotes the transcription of *ZmJMT* (encoding jasmonate O-methyltransferase) and *ZmLOX8* (encoding linoleate 13S-lipoxygenase 8), which are involved in JA biosynthesis. This hormone mediates plant stress responses, including defence against pathogens[4]. We identified a 26-bp indel sequence located in the 5′UTR of *ZmGLK36* that is present in susceptible plants but absent in resistant plants and, therefore, contributes to the differential expression of *ZmGLK36* and the resulting resistance to RBSDV in maize NIL-S and NIL-R. In fact, ZmDBF2, an APETALA2/ethylene-responsive element binding protein (AP2/EREBP) transcription factor, directly binds to the 26-bp indel and represses *ZmGLK36* expression (Fig. 1). Finally, by using transgenic or marker-assisted breeding (in which DNA markers are associated with desirable traits to facilitate the selection of the wanted offspring), we demonstrate that *ZmGLK36* has a conserved role in conferring resistance to RBSDV in rice and wheat as well.

Future directions

This study identified a resistance gene against RBSDV and revealed a mechanism of RBSDV resistance, in which the resistant maize cultivars suppress virus replication by activating JA biosynthesis and defence response. In addition, we show that transgenic rice and wheat plants overexpressing *ZmGLK36* exhibit a much-enhanced resistance to RBSDV, which suggests that *ZmGLK36* is likely to play a conserved part in mediating the response against RBSDV and related viruses (like MRDV and Southern rice black-streaked dwarf virus). Thus, *ZmGLK36* might have a broad utility in breeding cereal crops that are resistant against RBSDV and related viruses.

Of note, the phenotypic effect of *qMrdd2* (which explains 8.64–11.02% of the total phenotypic variance) is lower than that of *qMrdd8* (which encodes *RabGDIα* and explains up to 24.2% of the phenotypic variance)[5,6]. Thus, it will be worthy including both *qMrdd2* and *qMrdd8* to achieve increased resistance to RBSDV in maize production.

In addition to ZmDBF2, we also identified ZmbHLH74 (a putative helix-loop-helix DNA-binding protein) as a potential upstream regulator of *ZmGLK36* expression. It will be interesting to investigate how ZmDBF2 and ZmbHLH74 act co-ordinately to regulate *ZmGLK36* expression and RBSDV resistance.

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EXPERT OPINION

"There is very little molecular knowledge on the quantitative resistance against viral pathogens, and this manuscript advances our understanding of the action of one specific QTL contributing additively to resistance. The study is technically very well done and methodologically cutting edge. The work represents a major advance in our molecular understanding of virus resistance in plants, specifically in cereal crops. Its translational aspects will be of broad relevance for cereal resistance breeding." **Beat Keller, University of Zurich, Zurich, Switzerland.**

FIGURE

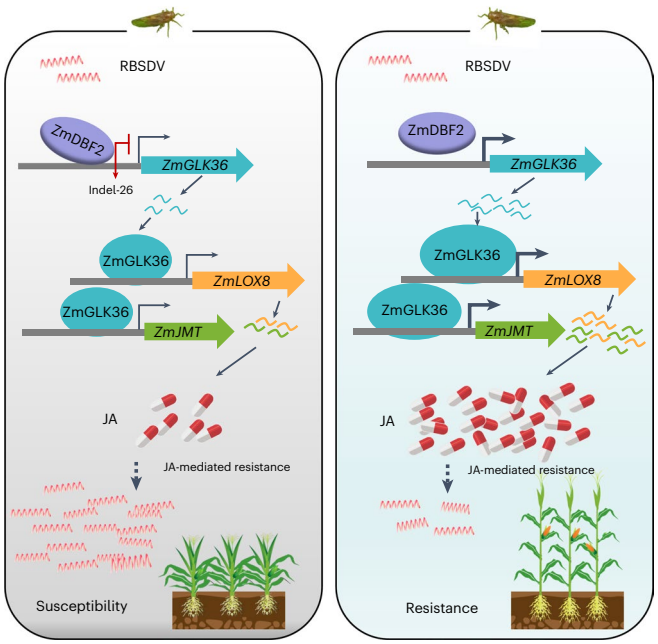

Fig. 1 | Model of ZmGLK36-mediated resistance to MRDD. In the resistant inbred lines, the expression of *ZmGLK36* is rapidly induced by RBSDV. Subsequently, ZmGLK36 promotes resistance to RBSDV by enhancing JA biosynthesis and JA-mediated defence response. However, in the susceptible inbred lines, *ZmGLK36* is repressed by binding of ZmDBF2 to the 26-bp indel in the 5′UTR, resulting in susceptibility to RBSDV. © 2023, Xu, Z. et al.

BEHIND THE PAPER

MRDD is one of the most destructive maize diseases, but resistant germplasm is relatively rare in China. We screened 260 inbred maize lines and mapped and cloned the resistance gene *ZmGDIa-hel* based on the resistant parent line X178. We found that another resistant line, Qi319, did not contain the *ZmGDIa-hel* resistance haplotype, which suggested that Qi319 might contain a new resistance gene. Thus, we reconstructed a new recombinant inbred line population using Qi319 and Ye478 (a susceptible line) over four years. In the absence of reference genomes for Qi319 and Ye478, we could not be sure of the genetic variation observed in the new population. Thus, we conducted a de novo assembly of Qi319 and Ye478 genomes. We spent months screening a yeast one-hybrid library to identify ZmDBF2, and with the improvement and update of the database, we eventually identified ZmDBF2 using a combination of bioinformatics. **X.L.**

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FROM THE EDITOR

"This work stands out because it reports the functional and mechanistic characterization of a new gene that contributes to maize resistance to an important viral disease. More importantly, given the fact that the disease-causing virus infects many cereal crops, the researchers showed that this gene can be harnessed to improve resistance in rice and wheat, and, therefore, has broad applications." **Jun Lyu, Senior Editor, *Nature Plants*.**