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Rising from the dead: the power of genome editing

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To protect themselves against diverse pathogens, plants have developed sophisticated defense mechanisms. Hypersensitive response or the rapid localized death of plant cells is a major defense strategy deployed by plants to kill the invading pathogens (Wu et al., 2014). In the last several decades, scientists have identified many mutants that form lesions on leaves in the absence of infections due to misregulation of cell death. These mutants are classified as lesion mimic mutants (LMMs) (Kang et al., 2021). LMMs often confer broad-spectrum disease resistance. However, due to spontaneous cell death, these LMMs frequently come with severely reduced crop yield.

To identify novel rice LMM genes, Sha et al. (2023) visually screened more than 1,500 fast-neutron mutagenized lines in the rice variety Kitaake (Figure 1A). Among the six newly identified LMMs, the *rbl1* mutant showed robust resistance to both the rice blast fungal pathogen *Magnaporthe oryzae* and the rice bacterial blight pathogen *Xanthomonas oryzae* pv. oryzae (*Xoo*) through generation of reactive oxygen species, accumulation of the plant defense hormone salicylic acid, and upregulation of plant defense genes (Figure 1B) (Sha et al., 2023). Even though the *rbl1* mutants exhibited enhanced resistance to rice pathogens, the crop yield was unfortunately reduced by 20-fold compared with the wild-type control (Figure 1C).

The *RBL1* gene encodes a cytidine diphosphate diacylglycerol (CDP-DAG) synthase, which converts phosphatidic acid and cytidine triphosphate to CDP-DAG (Figure 1D) (Sha et al., 2023). CDP-DAG reacts with myo-inositol to produce phosphatidylinositol via phosphatidylinositol synthases. Different phosphatidylinositol phosphates, including PtdIns3P, PtdIns4P, and PtdInsP_2, can then be synthesized by adding a varied number of phosphate groups to phosphatidylinositol. In *rbl1* mutants, the levels of phosphatidylinositol and its derivative phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) are significantly reduced compared with those in the wild-type Kitaake. Notably, in rice, PtdIns(4,5)P_2 aggregates at the fungal infection site and is implicated as a disease-susceptibility factor (Sha et al., 2023).

Although the *rbl1* mutant line exhibits broad spectrum disease resistance, the crop yield is extremely low (Sha et al., 2023). To overcome this major deficiency, Sha et al. (2023) designed guide RNA and used a multiplexing genome-editing strategy to modify the *RBL1* gene (Figure 1E). Among the 57 T0-generation edited lines, 38 showed clear LMM phenotype while the other 19 displayed significantly reduced lesion or even no lesion.

Sha et al. (2023) then focused on $RBL1^{\Delta 12}$ with a 12 bp deletion. Strikingly, the $rbl1^{\Delta 12}$ plants conferred resistance to 10 *M. oryzae* strains, 5 *Xoo* strains, and 2 rice false smut *Ustilaginoidea virens* strains. Surprisingly, the $rbl1^{\Delta 12}$ plants showed no reduction in crop yield in small-scale field trials (Figure 1F). The $rbl1^{\Delta 12}$ plants are extremely useful in fields with high incidence of rice diseases. The yield of the $rbl1^{\Delta 12}$ plants was 5.3-fold more than the control Kitaake in a rice

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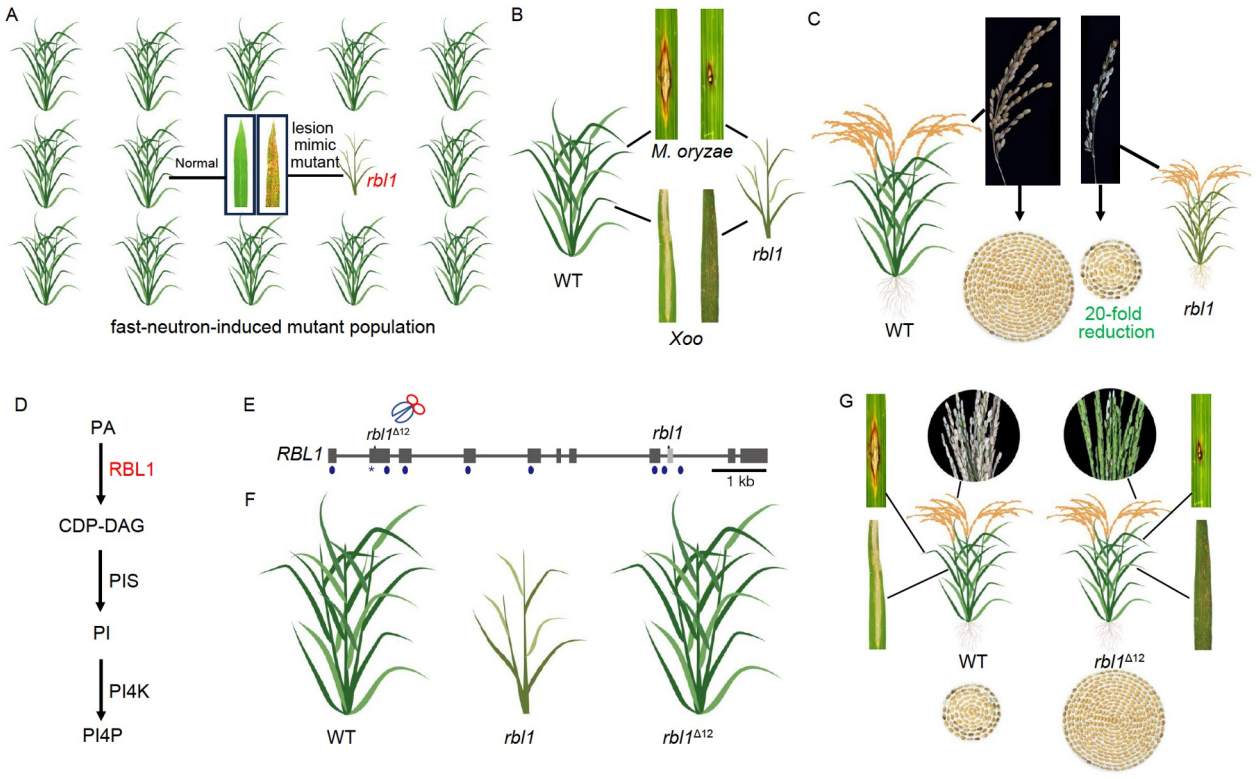

Figure 1 The *RBL1*$^{\Delta 12}$ allele created through targeted genome editing of a rice CDP-DAG synthase gene confers broad-spectrum disease resistance without a reduction in crop yield. A, Identification of *rbl1* as a lesion mimic mutant through screening more than 1,500 whole-genome-sequenced fast-neutron mutagenized lines in the rice variety Kitaake. B, The *rbl1* mutant exhibits resistance to both the rice blast fungal pathogen *M. oryzae* and the rice bacterial blight pathogen *Xoo*. C, The *rbl1* line displays a dramatic reduction in yield. *rbl1* mutant showed obvious lesion mimic phenotypes and reduced seed sets, resulting in seriously reduced yield. D, RBL1 acts as a cytidinediphosphate-diacylglycerol synthase. PA, phosphatidic acid; PIS, phosphatidylinositol synthase; PI, phosphatidylinositol; PI4K, phosphatidylinositol 4-kinases; PI4P, phosphatidylinositol 4-phosphate. E, Genome editing of the *RBL1* gene. Guide RNA sites are indicated by dots. The position of the scissors shows the edited site in *rbl1*$^{\Delta 12}$. F, The *rbl1*$^{\Delta 12}$ plants show only tiny hypersensitive response-like lesions and exhibit normal growth. G, The *rbl1*$^{\Delta 12}$ plants display broad-spectrum resistance to multiple rice diseases without yield penalty. The figure was created with the software BioRender (BioRender.com).

field with severe rice blast disease (Figure 1G).

Traditionally, nucleotide-binding and leucine-rich repeat (NLR) resistance genes have been widely used in improving crop disease resistance (van Wersch et al., 2020), but NLR resistance genes often provide narrower resistance and can lose resistance due to changes in field pathogen population because they activate plant defence by recognizing specific pathogen effectors. In contrast, *RBL1*$^{\Delta 12}$ provides broad-spectrum resistance and likely confers durable resistance, which will provide tremendous value to rice improvement through molecular breeding. Secondly, the *rbl1*$^{\Delta 12}$ line created through genome editing is transgene-free, which could make it more likely to be commercialized than genetically modified rice with a transgene. Last but not least, preliminary data have shown that the *RBL1* gene is highly conserved in different crops, including rice, wheat, tomato, lettuce, and maize. Therefore, genome editing of the *RBL1* gene in these crops could reduce or eliminate lesions and create a mutant similar to the *rbl1*$^{\Delta 12}$ rice plant, which will then be used to control diseases in these crops while maintaining a normal yield.

Rice is an extremely important crop and the major food source for more than half of the world's population. However, rice plants can be infected by a wide variety of pathogens, causing severe economic losses. In this paper, Sha et al. (2023) found that the *RBL1*$^{\Delta 12}$ allele exhibited robust resistance to rice blast and false smut fungal diseases and bacterial blight disease. It is worthwhile to investigate whether the *RBL1*$^{\Delta 12}$ allele also confers resistance to the rice sheath blight fungal pathogen *Rhizoctonia solani*, sheath-rot fungal pathogen *Sarocladium oryzae*, bacterial leaf streak pathogen *Xanthomonas oryzae* pv. oryzicola, southern rice black-streaked dwarf virus, and rice stripe virus, etc. (Gnanamanickam, 2009). The outcomes of these investigations will guide more effective uses of *RBL1*$^{\Delta 12}$ allele in future rice improvement.

It will be very interesting to figure out the underlying mechanism of the lesion mimic phenotype in the fast-neutron-generated *rbl1* mutants. It is also not clear why the *RBL1*$^{\Delta 12}$ allele generated through deletion of 12 bp in the *RBL1* gene phenotypically shows broad-spectrum resistance without a visible yield reduction. RBL1 acts as CDP-DAG

synthase and plays a key role in the biosynthesis of certain phospholipid molecules in the biological membrane (Sha et al., 2023). Future studies on the roles of phospholipid compounds in mediating growth and immunity may help us fill these important knowledge gaps (2023).

More than fifty different LMMs have been reported (Bruggeman et al., 2015). Among them, one group belongs to *accelerated cell death* (*acd*) mutants, e.g., *acd1*, *acd2*, *acd5*, *acd6*, and *acd11*. LMMs also include the *constitutive expressor of PR genes* (*cpr*) mutants *cpr5* and *cpr22*, the *suppressor of SA insensitivity* (*ssi*) mutants *ssi1*, *ssi2*, and *ssi4*, the *enhanced disease resistance* (*edr*) mutants *edr1*, *edr2*, and *edr3*, the *death no death* (*dnd*) mutants *dnd1* and *dnd2*, and many others, e.g., *exocyst 70b1* (*exo70b1*), *catalase2* (*cat2*) and *lesion simulating disease resistance response 1* (*lsd1*) mutants (Bruggeman et al., 2015; Lorrain et al., 2003). These LMMs provide excellent tools for dissecting defense and cell death pathways in plants (Lorrain et al., 2003; Moeder and Yoshioka, 2008). Based on extensive research from many different labs, LMMs affect several common pathways that involve sphingolipids and fatty acids, reactive oxygen species (ROS), ion flux, and membrane trafficking (Bruggeman et al., 2015; Moeder and Yoshioka, 2008). Similar targeted genome editing approaches could be used to modify these genes that are involved in lesion mimic phenotypes as well to significantly reduce or eliminate lesions, achieving normal yield while maintaining broad-spectrum durable disease resistance.

Besides achieving plant disease resistance, genome editing has already been broadly used to create better crops (Cardi et al., 2023; Razzaq et al., 2019). These traits include but are not limited to improved oil quality, tolerance to biotic and abiotic stresses, and improved grain yield and crop quality. The CRISPR/Cas9 system has emerged as a powerful tool for precise and efficient plant genome editing. This breakthrough discovery, which magically created a broad-spectrum disease resistant *RBL1*$^{\Delta 12}$ allele without reducing crop yield by deleting 12 bp in the CDP-DAG gene *RBL1*, suggests that we might underestimate the power of genome editing (Sha et al., 2023). Without a doubt, genome editing will play more and more important roles in improving crops in the era of climate changes.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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