Diagnostic kit for rice blast resistance

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Blight-resistant rice lines are the most effective solution for bacterial blast, caused by Xanthomonas oryzae pv. oryzae (Xoo). Key resistance mechanisms involve SWEET genes as susceptibility factors. Bacterial transcription activator-like (TAL) effectors bind to effector-binding elements (EBEs) in SWEET gene promoters and induce SWEET genes. EBE variants that cannot be recognized by TAL effectors abrogate induction, causing resistance. Here we describe a diagnostic kit to enable analysis of bacterial blast in the field and identification of suitable resistant lines. Specifically, we include a SWEET promoter database, RT–PCR primers for detecting SWEET induction, engineered reporter rice lines to visualize SWEET protein accumulation and knock-out rice lines to identify virulence mechanisms in bacterial isolates. We also developed CRISPR-Cas9 genome-edited Kitaake rice to evaluate the efficacy of EBE mutations in resistance, software to predict the optimal resistance gene set for a specific geographic region, and two resistant ‘mega’ rice lines that will empower farmers to plant lines that are most likely to resist rice blast.

Rice is the most important food crop in the world, but biotic and abiotic stresses can reduce yields. Xoo causes the rice disease bacterial blast, which results in substantial yield losses across Asia. Bacterial blast has been classified as the most serious bacterial disease of rice, with the highest social impact. Epidemics severely affect smallholders: about 70% of farms in India are ~0.39 ha (ref. ), roughly similar to 80% of farms in sub-Saharan Africa (<2 ha). Bacterial blast is a major problem in India that has increased in severity year on year since 2000 (ref. ). Increased severity has been attributed in part to climate change (increased rainfall and higher cyclone frequency). Modeling indicates that climate change might result in an increased effect of Xoo-mediated disease in Africa, where rice production is rising, and researchers have suggested that losses due to rice blast would eventually exceed those caused by rice blast. Moreover, the United States is concerned about the potential for introduction of Asian or African bacterial blast strains; currently, only low-virulence strains that lack TAL effector genes are present in the United States. Xoo is on the US Select Agent list as a potential bioterrorism agent.

Genetic resistance to disease reduces the need for pesticides. The best known plant resistance (R) genes encode proteins that interact with specific pathogen effectors and endow plants with dominant resistance to pathogens. More than 40 R genes for bacterial blast have been identified; a few of these have been cloned, and several have been associated with modular TAL effectors. Complete genome sequences of Xoo enable identification of TAL effectors, information that can then be used together with the TAL effector recognition code to identify the respective TAL EBEs throughout the rice genome. In some cases, however, resistance is recessive and caused by effectors that target susceptibility factors, as in the case of host sucrose transporter (SWEET) genes. Xoo produces TAL effectors that ectopically induce the SWEET genes that make rice susceptible to infection and disease.

DNA polymorphisms in EBEs can prevent TAL effectors from binding to target promoters, and the respective rice lines with altered EBEs in a SWEET promoter are resistant to bacterial blast. The first identified SWEET resistance variant was xa13, a naturally occurring promoter variant in SWEET11 (refs. , ). xa13 resistance is recessive and is used in rice breeding programs, as the xa13 promoter variants do not negatively affect yield. The TAL effector PthXo1, which is present in several Xoo strains (PXO99 and PXO71), binds an EBE in the SWEET11 promoter. The resistant rice line IRBB13 (xa13) carries a 38-bp deletion and a 252-bp insertion in the SWEET11 promoter, which abrogate binding of the EBE by PthXo1. Other resistant varieties carrying xa13 include Chinsurah Boro2, Tepa1, Aus274, AC-19-1-1, Long Grain (35023), Kalimekri77-5, Long Grain (64950) and BJ1 (ref. ). xa13 resistance can be overcome by Xoo strains that produce alternative TAL effectors that bind to the promoter of a different SWEET paralog. For example, SWEET14 promoter elements can be bound by PthXo3 or AvrXa7 (ref. ; Fig. 1a). Naturally occurring recessive resistance has also been identified for SWEET13 (xa25) and SWEET14 (xa41). Altogether, six EBEs in three SWEET genes that are targeted by naturally occurring TAL effectors (PthXo1 targeting SWEET11; PthXo2 and variants targeting SWEET13; PthXo3, AvrXa7, Talc and Talf targeting SWEET14) have been characterized. The TAL effectors target three of the five clade III SWEET genes; the other two SWEET genes can function as susceptibility (S) genes when artificially induced, but no Xoo strains targeting these genes have been identified. SWEET genes in other clades do not function as S genes.

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Results

Content of the SWEET kit 1.0. The SWEET kit 1.0 (Supplementary Table 1) contains a SWEET promoter database (SWEETpdb), SWEET primers for detecting mRNA accumulation for each SWEET gene, three SWEET-rice tester lines that report spatial SWEET protein accumulation during infection using translational reporter fusions, single- and combined-knockout SWEET mutants to identify which SWEET genes are required for susceptibility to individual Xoo strains, SWEETpR tester lines in the R-gene-free Kitaake background to evaluate whether particular EBE variants (or combinations thereof) are sufficient for resistance against an Xoo isolate, SWEET PathoTracer, a decision tool based on disease.
diagnostic surveys and population information to develop the most effective and most durable resistance for a specific region, and a total of 32 transgene-free EBE-edited lines of two mega varieties: IR64 and Ciherang-Sub1 (ref. 3).

**SWEET promoter database.** To predict resistance against, or susceptibility to, any Xoo strain with TAL effector(s) that target SWEET promoters, the EBE sequences in SWEET promoters must be characterized. We analyzed EBE variations for SWEET11, SWEET13 and SWEET14 in 4,726 accessions (in the first 400 bp of the promoters) as well as 5 rice lines grown in India, Southeast Asia and Africa, and identified 15 sequence variants. One A/G variant in the TATA box of SWEET13 was characterized. We analyzed EBE variations for SWEET11, SWEET13 and SWEET14 double knockout grown in the greenhouse. No significant differences were identified. Length of lesions at 14 days after inoculation (DAI) caused by ME2 (negative control), PXO99 (positive control) and AXO1947 on single-, double- and triple-knockout (sweet11, sweet13 and sweet14) mutants relative to Kitaake wild type (mean ± s.e.m.; n = 10 inoculated leaves). The experiment was independently repeated twice with similar results. The difference observed for AXO1947 virulence between sweet14 and sweet11/t4 in a single experiment was not significant when compared over a larger number of experiments (Supplementary Fig. 14).

**SWEET^TM^–rice tester lines for SWEET protein accumulation.** First, we engineered transcriptional reporter lines to visualize SWEET RNA accumulation by histochemical β-glucuronidase (GUS) staining. However, reporter lines for all three promoters had nonspecific reporter activity (Supplementary Table 5 and Supplementary Note 1). SWEET mRNA levels were detected in uninfected leaves (Supplementary Fig. 4) and in infected leaves in a strain-specific manner (Fig. 1b). Quantitative RT–PCR (qRT–PCR) showed that SWEET13 mRNA levels were highest among the five clade III SWEET genes in uninfected leaves, with lower levels of SWEET14. SWEET13 may have a role in phloem loading, as shown for its maize homologs24. SWEET11 mRNA levels were very low in leaves, consistent with its roles in seed filling25. Validated primer pairs are available in the kit for testing new Xoo isolates.

**SWEET^TM^–reporter lines for SWEET transcriptional activity.** To monitor SWEET protein accumulation, translational reporter promoter reporter lines were engineered. The constructs included a 2-kb fragment from the SWEET promoter, the coding region of the SWEET gene including introns and a GUSPlus reporter (Supplementary Figs. 6–8). Consistent with the absolute mRNA levels measured by qRT–PCR, SWEET13 and SWEET14 translational promoter reporter activities were detected in uninfected leaves, whereas SWEET11 translational promoter fusion lines showed no detectable GUS activity (Fig. 2a–d). SWEET13 and SWEET14 translational promoter reporter lines showed vein-specific protein accumulation, consistent with the roles of SWEET13 and SWEET14 in phloem loading (Fig. 2b–d). Three reporter lines for SWEET11, SWEET13 and SWEET14, named SWEET^TM^–rice tester lines, were each infected with five Xoo strains (PXO61, PXO71, PXO86, PXO99 and PXO112), which are known to induce specific SWEET genes. The Xoo strain ME2, lacking TAL effectors for SWEET induction, was used as a control and did not trigger SWEET^TM^ reporter activity.
Table 1 | Resistance of sweet13;sweet14 double-knock-out mutants to Asian and African Xoo strains as determined by lesion length (in centimeters) from clipping assays

| Strain    | Origin     | Kitaake | sweet13:sweet14 |
|-----------|------------|---------|-----------------|
| ME2       | Lab        | 0.5 ± 0 | 0.6 ± 0.1       |
| ME2:PthXo2B | Lab        | 19.2 ± 0.4 | 0.7 ± 0.2       |
| ME2:TalC   | Lab        | 8.2 ± 0.7 | 0.6 ± 0.1       |
| PXO86     | PHL        | 12.4 ± 0.8 | 1.3 ± 0.2       |
| PXO61     | PHL        | 12.3 ± 0.9 | 1.3 ± 0.4       |
| PXO404    | PHL        | 13.6 ± 1.1 | 1.2 ± 0.2       |
| PXO364    | PHL        | 16.3 ± 0.7 | 0.9 ± 0.3       |
| PXO421    | PHL        | 16.9 ± 0.2 | 0.9 ± 0.2       |
| PXOS13    | PHL        | 12.5 ± 1.5 | 0.8 ± 1.1       |
| KXO85     | Korea      | 13.7 ± 1.7 | 1.7 ± 0.2       |
| JW89011   | Korea      | 16.2 ± 1.4 | 2.1 ± 0.3       |
| AXO1947   | Africa     | 13.1 ± 0.1 | 1.6 ± 0.3       |
| CFPB1998  | Africa     | 12.5 ± 2.3 | 1.8 ± 0.6       |
| CFPB1949  | Africa     | 15.2 ± 2.1 | 2.3 ± 0.8       |
| CFPB1951  | Africa     | 11.7 ± 0.9 | 2.1 ± 0.4       |
| CFPB1952  | Africa     | 12.0 ± 1.5 | 1.2 ± 0.3       |
| CFPB7319  | Africa     | 14.7 ± 2.6 | 2.0 ± 0.2       |
| CFPB7320  | Africa     | 14.3 ± 1.6 | 2.1 ± 0.2       |
| BA13      | Africa     | 15.5 ± 2.8 | 2.4 ± 0.3       |
| CFPB7322  | Africa     | 14.8 ± 1.7 | 1.2 ± 0.2       |
| CFPB7323  | Africa     | 17.8 ± 1.8 | 2.2 ± 0.2       |
| CFPB7324  | Africa     | 16.7 ± 0.9 | 1.9 ± 0.1       |
| MA11      | Africa     | 15.8 ± 1.1 | 1.1 ± 0.3       |
| CFPB7337  | Africa     | 16.7 ± 1.3 | 2.4 ± 0.6       |
| CFPB7340  | Africa     | 15.8 ± 2.7 | 2.4 ± 0.3       |
| CFPB8172  | Africa     | 11.7 ± 0.6 | 2.3 ± 0.3       |
| Dar16     | Africa     | 14.7 ± 1.8 | 2.1 ± 0.4       |
| T19       | Africa     | 16.8 ± 2.4 | 2.1 ± 0.5       |
| Ug11      | Africa     | 15.7 ± 1.3 | 1.4 ± 0.2       |

Bold font indicates resistance. Lesion lengths (mean ± s.e.m.; n = 10) were derived from ten leaves at 14 DAI. The disease assay was repeated twice independently with similar results. PHL, the Philippines.

Induction of SWEET11 was detected upon infection with PXO71 and PXO99 (both of which harbor PthXo1) but not with the other strains. SWEET13 was induced only upon infection with PXO61 (harboring PthXo2B), whereas SWEET14 was induced upon infection with PXO61 (PthXo3), PXO86 (AvrXa7) and PXO112 (PthXo3; Fig. 2e). Infection with ME2 strains harboring PthXo1 (targeting SWEET11), PthXo2B (targeting SWEET14) or PthXo3, TalC, AvrXa7 and TalF (targeting SWEET14) further confirmed specific SWEET isoform induction by this set of reporter lines (Supplementary Fig. 9).

SWEET™ knock-out lines to detect Xoo SWEET targets. Tools that could identify the SWEET genes targeted by a specific Xoo strain, without previous knowledge of the targeted EBE, would inform efforts to engineer resistance. This is especially important because variant Xoo strains can target either different promoter elements or other clade III SWEET paralogs. Moreover, it is conceivable that promoter-edited lines or variants could impair yield, if the promoter variations affect normal gene function in uninfected plants.

Knowledge of the specific defects is thus important to judge possible negative effects of such mutations on plant performance. SWEET-mutant lines could also provide insight into the role(s) of SWEET genes in resistance and yield. To diagnose which SWEET was targeted by any Xoo strain and to predict the yield effects of alterations in SWEET genes, knockout mutants were created for four of the five described clade III SWEET genes (SWEET11, SWEET13, SWEET14 and SWEET15) using CRISPR–Cas9 (Supplementary Fig. 10 and ref. 21). In all cases, lines containing frameshift mutations in the sequence corresponding to transmembrane domain I (TM I) were identified (Supplementary Table 6). Frameshifts that result in early termination should create nonfunctional transporters. For example, premature termination in the last transmembrane domain, TM VII, of OsSWEET11 results in defective transporters21. Sweet13 mutant lines had reduced SWEET13 mRNA levels, which often occur concurrently with early termination (Fig. 3b). Knockout mutants for SWEET11 and SWEET15 were reported to have defects in seed filling22. Although SWEET11 and SWEET15 have important roles in seed filling in rice, widely used promoter variants, such as the resistance-conferring xa13 variant (SWEET11), do not seem to affect yield23,24.

Because maize ZmSWEET13 paralogs have roles in phloem loading11, the role of rice SWEET13 was investigated. SWEET13 is the most highly expressed SWEET gene in rice leaves, and the encoded protein localizes to the plasma membrane and, in common with SWEET14, transports sucrose (Supplementary Figs. 4 and 11)11,24. Analysis of GUS reporter fusions showed that SWEET13 accumulates in the phloem (Fig. 2b,d). SWEET14 also accumulated in the phloem but had substantially lower mRNA levels in leaves than SWEET13 (Fig. 2c; transcript levels not shown). Nevertheless, CRISPR–Cas9 sweet13 and sweet14 knockout mutant lines did not show detectable growth or yield defects under greenhouse conditions (Fig. 3a,c and Supplementary Fig. 12), nor were obvious differences observed in a single-season field experiment (based on visual inspection during the growth period and after harvest). To identify potential compensatory activity from other SWEET genes in the knockout mutants, the expression levels of other sucrose-transporting SWEET genes were analyzed. Only the weakly expressed SWEET14 and SWEET15, and none of the other clade III SWEET genes, showed substantial increases in mRNA accumulation in the leaf blade of sweet13 knockout lines (Supplementary Fig. 13). To test whether upregulation of SWEET14 could compensate for the loss of SWEET13 and thereby restore apoplastic phloem loading, sweet14 single-knockout and sweet13;sweet14 double-knockout lines were generated. Double mutants did not show obvious growth differences relative to controls in the greenhouse (Fig. 3d). Because mutant lines had no clear defects in plant growth or yield, EBE-edited lines in which the normal promoter function of SWEET13 and SWEET14 is affected are not hypothesized to have yield penalties. Further, our data indicate that apoplastic phloem loading in rice, in contrast to maize and Arabidopsis, either is not crucial to plant performance or does not entirely depend on SWEET function25,26.

Knockout lines can serve as diagnostic tools for testing whether Xoo strains require specific SWEET genes. The knockout mutants proved to be valuable tools for characterizing the virulence of a collection of 95 different Xoo strains with diverse geographic origins24. In this analysis, we observed that an African strain, AXO1947, which contains the effector TalC and can induce SWEET14, was still able to infect a Kitaake mutant line edited in the TalC EBE present in the SWEET14 promoter21. Although technically weakly virulent on lines carrying TalC EBE variants, AXO1947 showed substantially reduced virulence in the quintuple-mutant promoter lines, which are likely sufficiently resistant in field conditions24. The quintuple-mutant line was moderately resistant, with lesion lengths of 5–7 cm upon infection by AXO1947, as compared to 18–25 cm in controls. A systematic screen for resistance
using sweet13 and sweet14 single-knockout and sweet13 sweet14 double-knockout mutants showed that AXO1947 lost some virulence in sweet14 knockout lines but was unable to infect sweet13 sweet14 double mutants (Fig. 3e and Supplementary Fig. 14). These data demonstrate the utility of knockout lines for testing resistance (Fig. 3e, Table 1 and Supplementary Table 7). Co-dependence of strain AXO1947 on both sweet13 and sweet14 function is under investigation. Further characterization is needed, as dependence on sweet13 is not understood, given that sweet13 induction by AXO1947 was not detected. Notably, whereas in leaves basal sweet11 mRNA levels are low and induction is easily detectable, sweet13 and sweet14 are expressed in leaves; thus, a further increase in expression in a few cells in the xylem is difficult to detect against this background. SWEET targeting and sweet tester lines are thus better suited to detect SWEET dependence.

SWEETp, genome-edited EBE tester lines for Xoo genotyping. Rice varieties have different numbers and types of R genes. The only known R gene for bacterial blight in the japonica rice variety Kitaake is a cryptic resistance gene similar to the recessive xa25, which interacts with the major TAL effector PthXo2 (ref. 18). Thus, Kitaake is a useful reference line for testing Xoo compatibility. In a parallel study24, a series of EBE variants of SWEET11, SWEET13 and SWEET14 in Kitaake were engineered by genome editing, and resistance/susceptibility to Xoo strains was validated24. These 20 genome-edited Kitaake tester lines (named SWEETp) are available for genotyping Xoo isolates and function similarly to R-gene line panels for race characterization3 (e.g., Kitaake line 11.1-45 was resistant to strains containing the TAL effectors PthXo1 and AvrXa7, and line 12.2-12 was resistant to strains containing PthXo2B, PthXo3 and AvrXa7 (ref. 24)) (Supplementary Tables 8 and 9).

SWEET PathoTracer visualization. Geographic Information System (GIS)-based platforms that incorporate pathogen monitoring and resistance profiles of rice varieties are useful for management of local disease outbreaks26. We integrated the near-isogenic IR64 and Ciherang-Sub1 lines into the PathoTracer platform (http://webapps.irri.org/pathotracer/index.html). PathoTracer displays the predicted involvement of SWEET11, SWEET13 and SWEET14 on the basis of the Xoo population that is present in geographic regions and suggests effective edited variants for planting in the next season. For example, a dataset containing analyses about the ability of Xoo strains to infect rice accesses to support strategic deployment of varieties in the region. Tester-strain-based prediction of SWEET targets is provided here for SWEET14, using Xoo populations collected from 1972 to 2012 in Laguna, a disease-endemic area in the Philippines (n = 1,294 isolates). A screenshot of the full interface with the same map is shown in Supplementary Fig. 15.
the R-gene near-isogenic IRBB lines\(^1\) was compared to the proportion of endemic strains from an area of the Philippines that might activate SWEET\(14\) (Fig. 4 and Supplementary Fig. 15). On the basis of this information, 47% of the strains in the Xoo population are predicted to be controlled by one or more of the SWEET\(14\) EBE variants. By planting the recommended varieties, farmers can minimize the risk of infection and the resulting yield losses in the next season.

**Genome-edited Xoo-resistant mega variety lines.** Mega rice varieties are defined as varieties planted on more than 1 million hectares. Although genome-edited bacterial-blight-resistant SWEET\(pR\) Kitaake lines can be used by breeders, SWEET\(pR\) mega variety lines would reduce the need for further breeding efforts. This is of particular relevance because breeding efforts are more extensive in this context, owing to the recessive nature of SWEET-based resistance. CRISPR–Cas9 was used to edit five of the six EBE sites in the three SWEET promoters in widely used indica rice mega variety IR64 and in Ciherang-Sub1, a new flooding-tolerant elite line\(^24,33,34\). We generated edited lines with alterations in single or multiple EBEs. Together, 32 Cas9-free lines were produced, encompassing 35 single variations in the three SWEET promoters. Agronomic assessment and pathogenicity trials validated resistance against single or multiple Xoo strains (Fig. 5 and Supplementary Table 10)\(^11\).

**Discussion**

Genetically narrow germplasm and extensive mono-cropping are two hallmarks of modern agriculture that favor disease and its spread\(^1\). Genome editing could provide efficient tools for rapid engineering of pathogen resistance in cropping lines. However, detection of pathogen strains, their virulence factors and cropping line susceptibilities will be crucial if we are to effectively reduce the impact of plant diseases. Here we present a diagnostic kit to enable breeders, crop management teams and farmers to reduce the effect of bacterial blight on rice yields worldwide.

Genome editing can create large pools of R-gene variants, providing new ways to implement and manage long-term genetic resistance. Diseases that use TAL effectors are excellent candidates for reducing yield loss by engineering resistant plant lines, because TAL effecter binding depends on highly conserved and short cognate EBE sequences present in host S-gene promoters. One crucial finding in the fight against bacterial blight is that Xoo strains use different TAL effectors (eight effectors have been characterized to date) to directly target five EBEs in three SWEET gene promoters\(^1\). Pathogen-mediated induction of one of three SWEET genes is sufficient to cause disease. Extant Xoo strains harbor just eight SWEET-targeting TAL effectors, indicating that effectors with novel EBE recognition motifs may not evolve quickly. Therefore, genome editing could provide durable as well as broad bacterial blight resistance.

The success of genome-edited rice lines resistant to bacterial blight will be threatened by the emergence of pathogenic strains that have adapted to recessive resistance. The resistance endowed by SWEET promoter variants that prevent TAL effector binding can be overcome by the emergence of pathogenic Xoo strains. For example, resistance based on the natural promoter variant \(xal\) can be overcome by Xoo strains that produce alternative TAL effectors, such as PthXo3 or AvrXa7, to induce either the same or different SWEET genes\(^\text{20,25}\). SWEET promoter variants will likely need to be deployed in combination with other R genes, and Xoo strain emergence may, therefore, depend on multiple genetic alterations.

The underlying key to durable resistance may be preventing bacteria from multiplying in monocultures\(^\text{34}\). Genome editing enabled us to establish a portfolio of recessive resistance variants with different promoter sequences that are available as R-gene variants to use when a novel pathogen emerges. The portfolio allows us to swap between single- and combined-knockout SWEET\(pR\) tester lines to identify which SWEET genes are required for susceptibility and SWEET\(pR\) tester lines in Kitaake. We added a component to a web-based decision tool\(^3\) named PathoTracer, which uses population information, to aid development of the most effective and most durable R-gene combination for a specific region through breeding. We also include four SWEET\(acc\)-rice expert testers to detect which SWEET genes are deployed in adjacent R-gene variants and engineered promoter variants if TAL effectors evolve. Although only three of five clade III SWEET genes have been targeted\(^\text{34}\), we have also

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**Fig. 5 | Resistance of genome-edited rice lines to different Xoo strains.** Reactions of IR64 SWEET-promoter-edited lines to three representative Xoo strains (data from ref. \(^2\)). Lesion lengths were measured at 14 DA1 with strains PXO99A, PXO339 and PXO86. Infections were carried out at the maximum tillering stage by inoculating 3–6 leaf samples using a leaf clipping. Four replicate experiments with two plants each were performed per strain (four replicates per strain, two plants per replicate \((n = 8)\) and scored for 3–6 inoculated leaf samples per plant. The experiment was repeated three times independently.
developed sweet15 knockout mutants that have no obvious growth defects in greenhouse and field conditions. We plan to improve our kit by adding sweet knockout lines to cover all clade III SWEET genes. There is one report of an Xoo strain that does not induce SWEET genes, but it is challenging to prove a lack of induction, as exemplified here for AXO1947.

SWEET-based resistance also occurs for bacterial blights of cotton and cassava, so our approach of providing a disease diagnosis and management kit together with a suite of genome-edited lines may prove useful for other pathogens that decimate crops such as cassava and cotton.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at https://doi.org/10.1038/s41587-019-0268-y.

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References
1. Mew, T. W. Current status and future prospects of research on bacterial blight of rice. Annu. Rev. Phytopath. 25, 539–582 (1987).
2. Ke, Y., Deng, H. & Wang, S. Advances in understanding broad-spectrum resistance to pathogens in rice. Plant J. 90, 738–748 (2017).
3. Government of India. State of Indian Agriculture 2015–16 (Ministry of Agriculture & Farmers Welfare, Cooperation & Farmers Welfare Directorate of Economics & Statistics Department of Agriculture, 2016).
4. Laba, G. S. et al. Changes in Rice Disease Scenario in India: An Analysis from Production Oriented Survey (ICAR–Indian Institute of Rice Research, 2016).
5. Duku, C., Sparks, A. H. & Zwart, S. J. Spatial modelling of rice yield losses in Tanzania due to bacterial leaf blight and leaf blast in a changing climate. Clim. Change 135, 569–583 (2016).
6. Triplett, L. R. et al. Genomic analysis of Xanthomonas oryzae isolates from rice grown in the United States reveals substantial divergence from known X. oryzae pathovars. Appl. Environ. Microbiol. 77, 3930–3937 (2011).
7. Nature Editors. Rice pathogen is added to list of bioterror agents. Nature 455, 1163 (2008).
8. Vikal, V. & Bhatia, D. in Advances in International Rice Research (ed. Li, J. Q.) 175–213 (Intech Open, 2017).
9. Boch, J. et al. Breaking the code of DNA binding specificity of TAL type III effectors. Science 326, 1509–1512 (2009).
10. Moscou, M. J. & Bogdanove, A. J. A simple cipher governs DNA recognition by TAL effectors. Science 326, 1501 (2009).
11. Chen, L. Q. et al. Sugar transporters for intercellular exchange and nutrition of pathogens. Nature 468, 527–532 (2010).
12. Chen, L. Q. et al. Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. Science 335, 207–211 (2012).
13. Chu, Z. et al. Promoter mutations of an essential gene for pollen development result in disease resistance in rice. Genes Dev. 20, 1250–1255 (2006).
14. Yang, B., Sugio, A. & White, F. F. OsN3 is a host disease-susceptibility gene for bacterial blight of rice. Proc. Natl Acad. Sci. USA 103, 10503–10508 (2006).
15. Sathivel, K. et al. Host background of rice influences the resistance expression of a three genes pyramid (xa5 + xa13 + xa21) to bacterial blight (Xanthomonas oryzae pv. oryzae) pathotypes of Indian mainland and Bay islands. Plant Breed. 136, 357–364 (2017).
16. Antony, G. et al. Rice xa13 recessive resistance to bacterial blight is defeated by induction of the disease susceptibility gene Os-11N3. Plant Cell 22, 3864–3876 (2010).
17. Cheng, Q. et al. Characterization of a disease susceptibility locus for exploring an efficient way to improve rice resistance against bacterial blight. Sci. China Life Sci. 60, 298–306 (2017).
18. Zhou, J. et al. Gene targeting by the TAL effector PhXo2 reveals cryptic resistance gene for bacterial blight of rice. Plant J. 82, 632–643 (2015).
19. Hutin, M., Sabot, E., Ghesquière, A., Koebnik, R. & Szurek, B. A knowledge-based molecular screen uncovers a broad-spectrum OsSWEET14 resistance allele to bacterial blight from wild rice. Plant J. 84, 694–703 (2015).
20. Streubel, J. et al. Five phylogenetically close rice SWEET genes confer TAL effector-mediated susceptibility to Xanthomonas oryzae pv. oryzae. New Phytol. 200, 808–819 (2013).
21. Li, T., Liu, B., Spalding, M. H., Weeks, D. P. & Yang, B. High-efficiency TALEN-based gene editing produces disease-resistant rice. Nat. Biotechnol. 30, 390–392 (2012).
22. Bi, H. & Yang, B. Gene editing with TALEN and CRISPR/Cas in rice. Prog. Mol. Biol. Transl. Sci. 149, 81–98 (2017).
23. Septiningsih, E. M. et al. Accelerating the development of new submergence tolerant rice varieties: the case of Cihergang-Sub1b and PSB Rc18-Sub1b. Euphytica 202, 259–268 (2015).
24. Oliva, R. et al. Broad-spectrum resistance to bacterial blight in rice using genome editing. Nat. Biotechnol. https://doi.org/10.1038/s41587-019-0267-z (2019).
25. Yang, J., Luo, D., Yang, B., Frommer, W. B. & Eom, J.-S. SWEET11 and 15 as key players in seed filling in rice. New Phytol. 218, 604–615 (2018).
26. Dossa, G. S., Sparks, A., Cruz, C. V. & Oliva, R. Decision tools for bacterial blight resistance gene deployment in rice-based agricultural ecosystems. Front. Plant Sci. 6, 305 (2015).
27. 3,000 Rice Genomes Project. The 3,000 Rice Genomes Project. Gigasience 3, 7 (2014).
28. Zhao, H. et al. RiceVarMap: a comprehensive database of rice genomic variations. *Nucleic Acids Res* **43**, D1018–D1022 (2015).

29. Zaka, A. et al. Natural variations in the promoter of OsSWEET13 and OsSWEET14 expand the range of resistance against Xanthomonas oryzae pv. oryzae. *PLoS ONE* **13**, e0203711 (2018).

30. Bezrutczyk, M. et al. Impaired phloem loading in zmSwee13a, b, c sucrose transporter triple knock-out mutants in *Zea mays*. *New Phytol.* **218**, 594–603 (2018).

31. Ogawa, T., Yamamoto, T., KFush, G. S. & Mew, T.-W. Breeding of near-isogenic lines of rice with single genes for resistance to bacterial leaf pathogen *Xanthomonas oryzae* (variety). *Jpn. J. Breed.* **41**, 523–529 (1991).

32. Quibod, I. L. et al. Effector diversification contributes to *Xanthomonas oryzae* pv. oryzae phenotypic adaptation in a semi-isolated environment. *Sci. Rep.* **6**, 34137 (2016).

33. Toledo, A. M. U. et al. Development of improved Ciherrang-Sub1 having tolerance to anaerobic germination conditions. *Plant Breed. Biotech.* **3**, 77–87 (2015).

34. Mackill, D. J. & Khus, G. S. IR64: a high-quality and high-yielding mega variety. *Rice* **11**, 18 (2018).

35. Webb, K. M. et al. A benefit of high temperature: increased effectiveness of a rice bacterial blight disease resistance gene. *New Phytol.* **185**, 568–576 (2010).

36. Vera Cruz, C. M. et al. Predicting durability of a disease resistance gene based on an assessment of the fitness loss and epidemiological consequences of avirulence gene mutation. *Proc. Natl Acad. Sci. USA* **97**, 13500–13505 (2000).

37. Mikaberidze, A., McDonald, B. A. & Bonhoeffer, S. Developing smarter host mixtures to control plant disease. *Plant Pathol.* **64**, 996–1004 (2015).

38. Rimbaud, L., Papaix, J., Rey, J.-F., Barrett, L. G. & Thrall, P. H. Assessing the durability and efficiency of landscape-based strategies to deploy plant resistance to pathogens. *PLoS Comp. Biol.* **14**, e1006067 (2018).

39. Camacho-Sanchez, M., Burraco, P., Gomez-Mestre, I. & Leonard, J. A. Preservation of RNA and DNA from mammal samples under field conditions. *Mol. Ecol. Resour.* **13**, 663–673 (2013).

40. Li, C. et al. An efficient method to clone TAL effector genes from *Xanthomonas oryzae* using Gibson assembly. *Mol. Plant Pathol.* [https://doi.org/10.1111/mpp.12820](https://doi.org/10.1111/mpp.12820)  (2019).

41. Carpenter, S. C. D. et al. A strain of an emerging Indian *Xanthomonas oryzae* pv. oryzae pathotype defeats the rice bacterial blight resistance gene xax13 without inducing a clade III SWEET gene and is nearly identical to a recent Thai isolate. *Front. Microbiol.* **9**, 2703 (2018).

42. Cohn, M. et al. *Xanthomonas axonopodis* virulence is promoted by a transcription activator-like effector-mediated induction of a SWEET sugar transporter in cassava. *Mol. Plant Microbe Interact.* **27**, 1186–1198 (2014).

43. Cox, K. L. et al. TAL effector driven induction of a SWEET gene confers susceptibility to bacterial blight of cotton. *Nat. Comm.* **8**, 15588 (2017).

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Author contributions

J.-S.E., J.Y., R.O., C.V.C., B.S., F.F.W., B.Y. and W.B.F. conceived and designed experiments. C.I., J.-S.E., J.Y., V.T.L., S.N.C. and D.L. performed experiments. B.L. transformed Kitaake, J.-S.E., C.I., J.C.H.-T., G.A.-G., V.T.L., J.Y., B.Y. and W.B.F. analyzed the data. H.N. did informatics for PathoTracer. J.-S.E., S.M.S., R.O., S.M.S. and F.F.W. helped with revisions.

Competing interests

W.B.F., J.S.E., F.W., B.Y. and R.O. are inventors on US provisional patent application 62832300 that covers Kitaake, IR64 and Ciherrang-Sub1 EBE-edited lines and kit components described here.

Additional information

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Methods
Promoter variation analysis. Rice varieties having nucleotide variations in six EBEs (for the TAL effectors PthXo1, PthXo2, PthXo3, TaLC, AvrXa7 and TaLF) were found using the ‘Search for Variations in a Region’ and ‘Search for Genotype With Variation ID’ functionalities in RiceVarMap v2 (http://ricevarmap.ncpgr.cn/v2/). Two varieties were selected for each variation type as representative. Sequences of the first 400 bp of SWEET11, SWEET13 and SWEET14 promoters of the selected varieties were extracted from the 3K database (http://nsp-seek.irri.org/). Alignment was performed using ClustalW2.1 in Geneious 11.1.5 (https://www.geneious.com/).

Genotyping of rice plants. Rice genomic DNA was extracted using CTAB (http://gel.irri.org/services/dna-extraction-king-fisher-met). PCR was performed using ExTaq DNA polymerase (Clontech) with a melting temperature of 56 °C for SWEET11, SWEET13 and SWEET14 (primers listed in Supplementary Table 5). The PCR amplicons from the mutant alleles were validated by Sanger sequencing. Chromatograms were analyzed and aligned using Sequencer (https://www.genecodes.com/).

RNA isolation and transcript analyses. Total RNA was isolated using Spectrum Plant Total RNA kits (Sigma) or TRIzol (Invitrogen), and first-strand cDNA was synthesized using a QuantiTect Reverse Transcription kit (Qiagen). qRT–PCR was performed using LightCyrcle 480 (Roche), with the 2 −ΔΔC T method for relative quantification. Primers for SWEET11, SWEET13, SWEET14 and UBI1 are listed in Supplementary Table 5.

DNA constructs and plant transformation. Generation of GUS reporter constructs. The method for constructing pSWEET11-gSWEET11-GUSPlus was described44. In short, the promoter and coding region were fused in frame to the GUSPlus coding sequence with the NOS terminator, and the resulting constructs were PCR amplified and inserted into pc13.9000C (GenBank, AF290787.1). For tissue-specificity analysis, a 4,354-bp genomic clone of SWEET13 containing 1,919 bp of the 5′ region upstream of the translational start codon (ATG) and 2,435 bp of the entire coding region without a stop codon and a 4,365-bp genomic clone of SWEET14 containing 2,176 bp of the 5′ upstream region and 2,189 bp of the entire coding region without a stop codon were amplified by PCR using Kitaake genomic DNA as a template (primers are listed in Supplementary Table 5). The PCR amplicons were subcloned into pET211/blunt (Thermo Fisher), and resulting inserts were confirmed by DNA sequencing. Sequences are shown in Supplementary Table 11. The cloned fragments digested with Xbal and KpnI for SWEET13 or AvrII and Xmal for SWEET14 were subsequently inserted in front of the GUSPlus coding sequence of a promoterless GUSPlus coding vector42 restricted with Xbal and KpnI for SWEET13 and Xbal and Xmal for SWEET14. The resulting pSWEET11gSWEET13-GUSPlus and pSWEET14gSWEET14-GUSPlus constructs were used to transform Oryza sativa L. ssp. japonica Kitaake. Nine independent events were obtained for pSWEET13gSWEET13-GUSPlus and pSWEET14gSWEET14-GUSPlus. Whereas GUS activity levels were different in the independent lines, the GUS patterns were similar.

Kitaake was also used for CRISPR-Cas9- and TALEN-mediated genome editing of SWEET11, SWEET13 and SWEET14. The methods for the CRISPR-Cas9-induced mutant (sweet11-1) and the TALEN-induced mutant (sweet11-2) were described previously45. The knockout mutants sweet11-3, sweet13-1, sweet13-2, sweet14-1 and sweet14-2 were obtained with a CRISPR-Cas9 construct containing the sequences 5′-GCGTGTCCCTGCAGCATCCCTGG-3′ of SWEET13 and 5′-GCTAGTCTCTTACGATCTCTCG-3′ of SWEET14 (where underlining indicates the position of the protospacer-adjacent motif (PAM)) common to the first exon as previously described46. Double mutants (mutants sweet13-2sweet14-1) were created by crossing. SWEET13 RNA levels were analyzed in the sweet13 mutant and shown to be reduced (Fig. 3b).

Plant materials and growth conditions. Kitaake wild-type and mutant plants were grown either in field conditions (20 individual plants per genotype, paddy field known as a "growth center" in Kitaake), grown either in field conditions (20 individual plants per genotype, paddy field known as a "growth center" in Kitaake). Rice lines were grown in a small-scale field environment (28 days at 25°C during the day/23°C at night; 80–85% relative humidity) at IRRI.

Histochemical GUS analyses. Samples were collected in cold 90% acetone for fixation, vacuum infiltrated for 10 min and incubated for 30 min at room temperature. Leaf samples were vacuum infiltrated in GUS washing buffer (staining solution without 5-bromo-4-chloro-3-indole-b-glucuronide (X-Glucl) on ice for 10 min. The solution was changed to GUS staining solution (50 mM sodium phosphate pH 7.0, 10 mM EDTA, 20% (vol/vol) methanol, 0.1% (vol/vol) Triton X-100, 1 mM potassium ferrocyanide, 1 mM potassium ferricyanide and 2 mM X-Glucl dissolved in DMSO). Samples were incubated at 37°C. After 2 h of incubation, samples were cleared in an ethanol solution (20%, 35% and 50%) at room temperature for 30 min. Samples from Xoo-inoculated leaves were incubated in 0.1 M ethanol to remove the GUS substrate. Specimens were observed with a SteREO Discovery.V12 stereo microscope (Zeiss). For paraffin sections, samples were fixed using FAA for 30 min (50% (vol/vol) ethanol, 3.7% (vol/vol) formaldehyde and 5% (vol/vol) acetic acid). Dehydration was performed with an ethanol series (70%, 80%, 90% and 100%, 30 min each) and 100% tert-butanol. Samples were transferred and embedded in Histosec pastilles (Millipore). Sections (10 μm) were obtained with a rotary microtome (Jung RM 2025). Specimens were observed with an Eclipse e600 microscope (Nikon). GUS histochemistry experiments were performed at least two times with 12 individual plants, with similar results.

Xoo strains and infection protocols. The Xoo strains collected from different geographic regions were reported47. Plasmid-containing Xoo strains were obtained through electroporation of competent cells48 with respective pHM1-derived plasmids (e.g., pHM1/ZWpthXo1 for the pthXo1 gene). Infection experiments, bacterial inocula were prepared by growing bacterial cells on tryptone sucrose plates with appropriate antibiotics. Cells were scraped from the plates in 0.85% (wt/vol) NaCl suspended in sterile distilled water (OD 600 ~ 0.5). (i) Leaf clipping: the two youngest fully expanded leaves of 4- to 5-week-old rice plants were clipped about ~1–2 cm from the tip with scissors blades that were immersed in bacteria immediately before clipping. Five plants were used for inoculation of each strain (ten leaves in total). Lesion length (distance from the cut to the leading edge of (gray) symptoms) was measured for each inoculated leaf at 12–14 DAI. The mean lesion length of the ten leaves was used for each treatment. The Tukey test for analysis after ANOVA was used for statistical analyses. Leaf tissues were mounted in laminating film and photographed under white light. (ii) Syringe infiltration: bacterial suspensions were infiltrated into leaves from the bottom by pressing the opening of a needless syringe to the leaf. Leaf fragments with inoculated spots were cut off 4 h after inoculation for RNA extraction and GUS staining analysis.

Statistical analysis. Data were plotted using BoxPlotR (http://shiny.chemgrid.org/boxplotr/) or are presented as mean ± s.e.m as specified in respective tables or figures. One-sided ANOVA was conducted on measurements. The Tukey honestly significant difference test was used after ANOVA. Pairwise tests for significance, which was set at P-test = 0.05. Exact P values, the statistical test used and sample number n can be found in figure legends or graphs.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Materials will be made available for nonprofit research under a material transfer agreement (Supplementary Notes 2 and 3). We aim at obtaining freedom to operate for use by low-income farmers and will work with breeders to make the materials available to subsistence farmers. For commercial applications, accessibility will be negotiated from appropriate patent holders, and profits will be used to support dissemination to subsistence farmers. Edited IR64- and Cibangher SUH1-based materials can be obtained from R.O.; edited Kitaake lines can be obtained from B.Y.; and translational reporter lines can be obtained from W.B.F. Distribution of Xoo strains may be restricted because of regulations of Xanthomonas oryzae as a Select Agent by the US government, because of the Nagoya protocol, or because some strains were donated from other groups and thus these groups should be contacted directly (for details, see ref 3).

References
44. Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2 −ΔΔC T method. Methods 25, 402–408 (2001).
45. Ouwerkerk, P. B., de Kam, R. J., Hoge, H. J. & Meijer, A. H. Glucocorticoid-inducible gene expression in rice. Planta 213, 370–378 (2001).
46. Yang, B. & Bogdanove, A. in The Plant Cell, Vol. 19, no. 15, 2357–2369 (2007).
47. Yang, B. & White, F. F. Diverse members of the AvrB33/PthA family of type III effectors are major virulence determinants in bacterial blight disease of rice. Mol. Plant Microbe Interact. 17, 1192–1200 (2004).
48. Kaufman, H. E., Reddy, A. P. K., Hsieh, S. P. Y. & Merca, S. D. An improved technique for evaluating resistance of rice varieties to Xanthomonas oryzae. Plant Dis. Rep. 57, 537–541 (1973).
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Xoo genome sequences were deposited in GenBank under Bioprojects PRJNA497307 and PRJNA497605.

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