The Crop Journal 13 (2025) 115–124

Contents lists available at ScienceDirect

The Crop Journal

journal homepage: www.keaipublishing.com/en/journals/the-crop-journal/

Enhancing resistance to bacterial blight in rice using CRISPR-based base editing technology

Chenhao Li [a,b], Bo Liu [a], Hansong Dong [b], Bing Yang [a,c,*]

[a] Division of Plant Science and Technology, Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, USA
[b] Department of Nanjing Agricultural University, Nanjing 210095, Jiangsu, China
[c] Donald Danforth Plant Science Center, St. Louis, MO 63132, USA

ARTICLE INFO

Article history:
Received 20 May 2024
Revised 15 August 2024
Accepted 19 September 2024
Available online 21 September 2024

Keywords:
Base editing
Base editors CBE and ABE
Rice improvement
Bacterial blight resistance

ABSTRACT

Bacterial blight (BB), caused by *Xanthomonas oryzae* pathovar *oryzae* (*Xoo*), poses a significant threat to rice production, particularly in Asia and West Africa. Breeding resistance against BB in elite rice varieties is crucial to advancing rice breeding program and supporting smallholder farmers. Transcription Activator-Like effectors (TALes) are key virulence factors in *Xoo*, with some targeting the susceptibility (*S*) genes such as the sugar transporter *SWEET* genes in rice. Among these, *SWEET14* is an important *S* gene, with its promoter bound by the TALe TalC which exists across all sequenced African *Xoo* isolates. In the present study, we utilized CRISPR/Cas9-based cytidine and adenine base editors to alter the effector binding element (EBE) of TalC in the promoter of *SWEET14* in rice cultivars Kitaake, IR24, and Zhonghua 11. Mutations with C to T changes in EBE led to reduced *SWEET14* induction by TalC-containing *Xoo* strains, resulting in resistance to African *Xoo* isolates reliant on TalC for virulence. Conversely, A to G changes retained *SWEET14* inducibility and susceptibility to *Xoo* in edited lines. Importantly, no off-target mutations were detected at predicted sites, and the edited lines exhibited no obvious defects in major agronomic traits in Kitaake. These results underscore the effectiveness of base editing systems for both molecular biology research and crop improvement endeavors.

1. Introduction

Rice (*Oryza sativa* L.) stands as a vital staple crop globally, serving as the prime food source for over half of the world's population. The long-term food security in many developing countries hinges on rice production [1]. *Xanthomonas oryzae* pathovar *oryzae* (*Xoo*) is the causal agent of rice bacterial blight (BB), a devastating disease prevalent in Asia and increasingly significant in the rice-growing regions of sub-Saharan Africa, posing a challenge to rice cultivation in these areas [2]. Efforts to elucidate the underpinning genetic basis and molecular mechanisms of BB resistance against African *Xoo* are currently limited. Moreover, there is an urgent need for the development and strategic deployment of rice varieties with durable and broad resistance to BB in African countries. Using genetic tools, particularly advanced genome editing technologies, will play a pivotal role in clarifying the molecular genetics and mechanisms of rice bacterial blight susceptibility and resistance. This knowledge will enable us to design and engineer powerful and effective BB resistant rice germplasm.

Xoo utilizes a group of type III effector proteins known as Transcription Activator-Like effectors (TALes) as crucial determinants of pathogenicity. These TALes are secreted and delivered into host cells via a type III secretion system (T3SS), a mechanism prevalent in Gram-negative bacteria. TALes mimic eukaryotic transcriptional activators within host cells by employing a specific DNA binding structure and an activation domain [3]. Upon entry into a plant cell, TALes exploit the host nucleocytoplasmic transport process to gain access to the nucleus [4,5]. Within the nucleus, TALes bind to specific promoter elements known as effector binding elements (EBEs) of host genes, triggering host gene expression, reshaping the plant transcriptome, and altering the physiological conditions of the intracellular environment [6]. These physiological alterations typically favor pathogen proliferation and disease progression. However, the host has developed various mechanisms to counteract the activities of TALes [7,8].

The advancement of next-generation sequencing technology has enabled the determination of TALomes (the complete set of TALes in a genome) in numerous *Xoo* strains, revealing varying

* Corresponding author.
E-mail address: yangbi@missouri.edu (B. Yang).

https://doi.org/10.1016/j.cj.2024.09.003

TALe numbers across different geographic *Xoo* populations. Asian *Xoo* strains typically possess 10–20 TALes, African *Xoo* strains have 9 TALes, while North American *Xoo* strains lack any TALes, and *Xoc* (*Xanthomonas oryzae* pathovar *oryzicola*) strains contain 20–29 TALe copies [9]. The TALe gene counts vary among strains of the two *Xanthomonas oryzae* pathovars found in rice-growing regions [10]. African *Xoo* strains exhibit smaller and more conserved TALomes than their Asian relatives with only three TALes—TalC, TalF (formerly known as Tal5), and TalB—confirmed as virulence determinants of the pathogen [11–13]. TalB's virulence contributions appear to vary depending on the genetic context of rice varieties. The targets of these virulent TALes are known as disease-susceptibility (*S*) genes, with major TALes targeting the three members of the sugar transporter *SWEET* gene family [14]. The interaction between *SWEET S* genes and their corresponding TALes plays a crucial role in bacterial blight, as disrupting either component leads to incompatibility between the pathogen and the host [14–16]. Specifically, three rice *SWEET* genes from the clade III SWEET family – *SWEET11a* (also known as *Os8N3* or *Xa13*), *SWEET13* (*Xa25*), and *SWEET14* (*Os11N3*) – have been identified as key players in disease development [15–18]. Among African *Xoo* strains, it appears that only *SWEET14* of rice is targeted by TALes [19]. Previous research indicates that TalC and TalF directly bind to different sites in the promoter of *SWEET14*, with *SWEET14* induction contributing to blight development [11,13]. Blocking the binding of *SWEET* EBEs by individual TALes has proven to be an efficient strategy in preventing disease development [19–26].

The specificity and functionality of a TALe in pathogenesis (virulence and avirulence) are determined by the interaction between the cognate TALe of the pathogen and the host EBE DNA sequence. This DNA recognition is facilitated by the central tandem repeats, each consisting of 33 to 35 amino acid residues, where the repeat variable diresidues (RVDs) at positions 12 and 13 specify the nucleotide for each repeat to interact with [27]. Crystal structures of TALes bound to their targets have been reported, including the natural PthXo1 and artificially engineered dHax3 [27,28]. Understanding the interaction details between RVDs and individual DNA bases has helped TALEN (TALe nuclease) application, with researchers enhancing the specificity of TALe-based genome editing by broadening the RVD repertoire [29]. *SWEET14* has been a prominent target edited using both TALEN and CRISPR/Cas9 technologies in various rice cultivars [19,23,26]. Many edited plants exhibit significant deletions at the EBE site, effectively preventing binding by the corresponding TALe. While information about the impact of minor modifications of the EBE sites on resistance breeding is scarce, studies have shown that even a small deletion of one or two base pairs at the 5′ end of the TalF EBE binding site can confer resistance to the virulent *Xoo* strains [19]. However, the effectiveness of a single base pair change in preventing TAL effector binding and the precise contribution of RVDs binding to base pairs during the TAL effector-target DNA interaction remain unclear.

Engineered CRISPR/Cas9 and its variants have emerged as the predominant genetic tools for conducting fundamental and applied research in plant biology and agriculture. CRISPR/Cas-based genome editing technologies are known for their flexibility, high efficiency, and versatility. Typically, CRISPR/Cas9 is utilized to induce double-stranded breaks (DSBs) in genomic DNA at predetermined loci, leading to DNA insertions, deletions, and substitutions through *in vivo* DSB repair mechanisms. Additionally, enzymatically modified Cas9 variants such as nickase Cas9 and dead Cas9 have been repurposed as single-base editors capable of creating base pair conversions without the need for DSBs. These base editors encompass cytosine base editors (CBE) and adenine base editors (ABE). CBE comprises a cytidine deaminase fused with nCas9 (D10A) and an uracil glycosylase inhibitor, enabling the conversion of targeted cytosine to uracil at guide RNA-directed genomic sites, with subsequent replacement by thymine during DNA replication [30]. Similarly, ABE facilitates A to G conversions in genomic DNA [31]. Several research groups have successfully employed both systems for genome editing in rice [32–34]. The benefits of base editing systems have been evident in their applications for plant breeding and crop enhancement [35,36].

Genome editing technologies have proven to be invaluable and powerful tools for crop improvement, as evidenced across various plant species [35]. Previous studies have often targeted *SWEET* genes for genome editing. Directly knocking out *SWEET* genes is not always ideal due to their essential physiological roles in rice, especially under specific conditions [17,37]. Initially, the *SWEET14* promoter was modified using TALEN technology to disrupt the activation of *SWEET14* by the TALes AvrXa7 and PthXo3, resulting in successful engineering BB resistance [23]. Similarly, essential genes *SWEET11a* and *SWEET13* were targeted in their promoter EBEs using CRISPR/Cas9 technology, leading to BB resistance while preserving other important agronomic traits [18,19,21,22]. Recently, a broad-spectrum bacterial blight resistance was achieved by simultaneously modifying EBEs in the promoters of three *SWEET* genes in diverse rice cultivars [24,25]. These findings hold promise for engineering rice cultivars with BB resistance through genome editing. However, the feasibility of utilizing the base editing system to confer BB resistance remains uncertain.

In this study, we utilized CBE and ABE to modify the TalC EBE in the promoter of *SWEET14*, a region targeted by at least four TALes in the *japonica* rice cultivars Kitaake and Zhonghua 11 as well as the *indica* rice IR24. The lines edited with CBE, resulting in homozygous C to T alterations, exhibited significantly increased resistance to *Xoo* strains reliant solely on TalC for virulence, including African *Xoo* field isolates, compared to the ABE-induced A to G conversions. Additionally, we analyzed the crystal protein structure to understand the basis of preventing TALe binding and subsequent changes in disease phenotypes. Our findings revealed varying impacts based on single base-pair editing, indicating that the A to G change may not be advantageous due to the binding flexibility of RVDs for A and G. This study introduces a robust and effective base editing system for conferring BB resistance and facilitating transgenic-free breeding during subsequent generations.

2. Materials and methods

2.1. Plant materials, bacterial strains, medium, and growth conditions

The rice varieties utilized in this study were *Oryza sativa* L. *japonica* Kitaake, Zhonghua 11 and *indica* IR24. The rice plants were grown in growth chambers at 30 °C for a 12-h light period and at 28 °C for a 12-h dark period, maintaining a relative humidity of 60%–75%. *Escherichia coli* was cultured in Luria-Bertani (LB) medium supplemented with appropriate antibiotics at 37 °C. *Agrobacterium tumefaciens* was grown at 30 °C in the dark. *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) was cultured at 28 °C in TSA medium (10 g L^{-1} tryptone, 10 g L^{-1} sucrose, 1 g L^{-1} glutamic acid). Antibiotics were used at the following concentrations for bacterial growth: 50 μg mL^{-1} kanamycin, 100 μg mL^{-1} spectinomycin, and 100 μg mL^{-1} ampicillin.

2.2. CRISPR-Cas9 based editing design and constructs

To generate the guide RNA, a double-stranded DNA oligonucleotide (dsOligo) targeting the *SWEET14* EBE for TalC was created by annealing two complementary oligonucleotides and ligated at the *Btg*ZI-restricted site in pENTR-gRNA1 [38]. The *Btg*ZI enzyme cutting site is positioned downstream of the U6 promoter and upstream of the guide RNA scaffold sequences. For multiple guide

RNAs targeting other *SWEET* EBEs, individual tRNA-gRNA modular units [39] were used to insert the dsOligos and assembled in another intermediary vector named pENTR4-U6.1P-ccdB [24,38]. The DNA sequences of the positive clones were validated through Sanger sequencing. Finally, the new gRNA cassettes were integrated into the CBE and ABE systems using Gateway LR clonase (Thermo Fisher Scientific, Waltham, MA, USA) as previously described [38].

To make two gRNAs, we utilized the previously employed intermediate vector [39]. In brief, the *BtgZ*I or *Bsa*I cutting site was positioned downstream of the U6 promoter and utilized to connect the guide RNA scaffold sequences for gRNA1 (for *SWEET11a*) or gRNA2 (for *SWEET13*). These cassettes were synthesized by GenScript and then cloned into pENTR4 (Thermo Fisher Scientific) through *BamH*I and *EcoR*I restriction and DNA ligation methods separately, resulting in a series of pENTR-gRNA vectors. These vectors allowed for the mobilization of the guide RNA cassettes to both CBE and ABE systems using the Gateway LR clonase (Thermo Fisher Scientific). The sequences of the oligonucleotides are provided in the supplementary data (Table S1).

2.3. Generation of transgenic rice lines

The *Agrobacterium tumefaciens* strain EHA105 harboring the specific CRISPR constructs was employed for genome editing in Kitaake, Zhonghua 11 and IR24. Genomic DNA was isolated, and PCR amplification was conducted on fragments of nCas9 or gRNA to detect transgenic events. Mature embryo-derived callus cells from Kitaake, Zhonghua 11 and IR24 were utilized for tissue culture and transformation following a modified protocol based on the method described by Yukoh Hiei in 1994 [40]. Subsequently, individual transformant callus lines were selected, cultured, and regenerated into complete plants (T0) for further analysis.

2.4. Genotyping of primary and progeny plants

Leaf tissues obtained from individual T_0 samples were homogenized in liquid nitrogen, and genomic DNA was extracted using the CTAB method. PCR products from the targeted regions in each line were initially examined using the *Sph*I restriction enzyme assay and then selectively subjected to Sanger sequencing.

To genotype the T_1 plants, multiple seeds from independent transgenic lines (T_0) were grown in the greenhouse and allowed to self-pollinate. Genomic DNA was isolated, and PCR amplification for nCas9 or gRNA was performed. PCR products from the targeted region in individual plants were assessed using *Sph*I, *Pvu*II, or *BsrG*I enzyme digestion to ascertain the zygosity (homozygous or heterozygous) of the progeny plants. Subsequently, selected PCR products from the targeted site underwent Sanger sequencing for further analysis.

2.5. Off-target analysis

Potential off-target effects of the base editing on the edited target genes were investigated. Off-target sites with up to three base mismatches to the sgRNA target region were identified utilizing the CRISPR-GE (https://skl.scau.edu.cn) software. Specific primers were designed for the target sequence in alignment with the Kitaake genome. Three off-target loci were amplified from the transgenic lines and subjected to Sanger sequencing for the detection of any unintended mutations.

2.6. Disease assays

Fully expanded leaves of rice plants (6–8 weeks old) were inoculated using a leaf-tip clipping method. *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) stocks, stored in a −80 °C freezer, were streaked onto TSA plates and incubated at 28 °C for 2–4 d. The *Xoo* cells were then harvested from the plates and suspended in sterilized distilled water to achieve an optical density at 600 nm (OD600) of 0.5, approximately 10^8 colony-forming units (CFU) per milliliter. Scissor blades were immersed in the *Xoo* suspension and used to clip the leaves approximately 2 cm from the leaf tips. The lengths of the lesions were measured 14 d post-inoculation, and lesion data were recorded for each test plant. Each strain was inoculated with more than three replications, with five plants per replicate.

2.7. Evaluation for major agronomic traits of rice disease-resistance lines

The selected mutant lines were evaluated in a controlled greenhouse environment with a minimum of three replications. Upon reaching maturity, parameters such as plant height, panicle length, percentage of reproductive tillers (number of tillers with panicle/total number of panicles per plant), and fertility percentage (number of filled grains/total number of grains) were recorded. The data are presented as mean ± standard error of the mean (s.e.m.), with the specific number of measurements indicated in the figure legends or graphs. One-way analysis of variance (ANOVA) was performed on all metrics, followed by Tukey's honestly significant difference test for pairwise comparisons to determine significance (set at $P < 0.05$).

2.8. Gene expression and gRNA transcriptional detection

Two-week-old rice seedlings' leaves were infiltrated with *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) strain at OD_{600} = 0.5. At 24 h post-inoculation (hpi), total RNA was extracted from rice leaves using TRIzol reagent following the manufacturer's protocol (Thermo Fisher Scientific). The isolated total RNA (1 μg) was treated with DNase I and utilized for cDNA synthesis with the iScriptTM cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). The synthesized cDNA was employed for either reverse transcription polymerase chain reaction (RT-PCR) or quantitative RT-PCR (qRT-PCR), with RT-PCR following the standard PCR procedure with meticulous concentration control. qRT-PCR was conducted using an ABI Real-time PCR System (Applied Biosystems, Foster City, CA, USA) and PowerTrack SYBR Green Master Mix (Thermo Fisher Scientific). To assess *SWEET14* expression in rice, a 3′ fragment of the *SWEET14* transcript was amplified using specific primers (Table S1).

For quantitative RT-PCR of individual guide RNAs (gRNAs), the DNase I treated total RNA were reverse-transcribed into cDNAs using a specific primer, gRNA-R, and iScript reverse transcriptase (Bio-Rad) following the manufacturer's guidelines. Real-time PCR was conducted using an ABI Real-time PCR System (Applied Biosystems) and PowerTrack SYBR Green Master Mix (Thermo Fisher Scientific). The rice actin gene served as the internal reference for quantification. Please refer to Table S1 for the primer sequences.

3. Results

3.1. SWEET14 is the target of TalC in AXO1947

Previous research has established TalC as the major virulence TALe in all African *Xoo* isolates, including AXO1947, known to be virulent to the *japonica* cultivar Kitaake [9]. TalC directly interacts with an effector binding element (EBE) located in the promoter region of *SWEET14* (Fig. 1A), a prevalent susceptibility gene in rice [17]. TalC comprises twenty-two central repeats that correspond to

22 EBE nucleotides within the promoter of *SWEET14*. The EBE sequence targeted by TalC is consistent across various rice cultivars examined, indicating a universal vulnerability of *SWEET14* to TalC and demonstrating the feasibility of conserved targeting [24]. To investigate the impact of TalC on virulence, we introduced TalC isolated from the African *Xoo* strain AXO1947 into the ME2 strain, a master mutant strain used for detecting virulent TALe. Notably, lesions exceeding 15 cm in length developed 14 d post-inoculation, in contrast to the ME2 strain that exhibited no clear lesion formation (Fig. 1B). RT-PCR results indicated a robust induction of *SWEET14* by TalC 24 h post-inoculation (Fig. 1C). Modifying a single base pair within this EBE domain could disrupt the binding between TalC and the target DNA sequence, potentially impacting the rice chromosome minimally. This alteration could lead to stable *SWEET14* expression during disease progression, imparting disease resistance to rice (Fig. S1). Based on this hypothesis, we meticulously selected a target region within the EBE site, which includes six cytosines and five adenines. For practicality, we designated TGG (297 bp to 295 bp) upstream of the transcription start site of *SWEET14* as the protospacer adjacent motif (PAM) for nicking Cas9 and guide RNA targeting. A carefully designed 20 bp guide RNA (gRNA) sequence was employed for the editing system. The target site features an *Sph*I restriction enzyme site, facilitating subsequent genotyping of the plants (Fig. 2B).

3.2. Generation of CBE/ABE-expression transgenic rice and selection of ideal candidate lines

To implement the precise base editing system in rice, we selected rBE9 (CBE) and rBE14 (ABE) as the construct backbone, as validated for efficient performance previously [33,34]. The methodology section briefly outlines the vector construction process, resulting in a final vector structure containing a specific guide RNA (gSWEET14p) and other functional components (nCas9-deaminase genes and selection marker gene). Agrobacterium-mediated plant transformation was employed for rice base editing, encompassing the entire process from Agrobacterium inoculation with base editors to the generation of transgenic rice, taking approximately 90 d (Fig. S2). Fifteen transgenic lines were regenerated from CBE editing transformation, along with 15 lines from the transformation of Kitaake with the ABE system (Fig. S3). Both edited and wild-type lines were cultivated in the containment greenhouse. Genomic DNA was isolated using the CTAB method to assess the functionality of the base editing system. Target regions were PCR-amplified with gene-specific primers and the amplicons were subjected to restriction enzyme (SphI) mutation screening. The regions were subsequently analyzed via Sanger sequencing. The PCR amplicons of the 361 bp target region from T_0 underwent *Sph*I digestion, revealing the expected bands (approximately 235 bp and 126 bp) in the wild-type lines. Conversely, mutant plants (homozygous or heterozygous) should exhibit no or partial appearance of the two smaller bands, confirming editing events in several lines (Fig. S3). Remarkably, Sanger sequencing results indicated that all plants carrying CBE-gSWEET14p were edited, with most plants displaying bi-allelic mutations (14/15) and large deletions observed in several plants (9/15) (Fig. S4A). Notably, one distinct mutant with a base-pair alteration within TalC EBE (CBE-gSWEET14p-4#) was obtained, where the C_0 site of TalC EBE converted to T_0 and C_4 of TalC EBE changed to T_4 (Fig. S4A). Additionally, all 15 plants derived from ABE-sgRNA were successfully obtained, albeit with an editing efficiency of only 20% (3/15). Three heterozygotes and two homozygotes with varying genetic profiles were identified through Sanger sequencing analysis (Fig. S4B). From the 15 CBE-sgRNA-transformed rice plants, nine lines were selected, while 14 lines were chosen from the 15 ABE-sgRNA-modified rice plants for further characterization. Analysis of mutation transmission and segregation in the T_1 generation involved enzyme digestion of the PCR products from the T_1 plant genomic DNA samples, demonstrating inheritance consistent with classical Mendelian patterns (Table S2). Following self-pollination and progeny segregation, two additional mutation types were identified in T_1 generation plants. One variant, CBE-18#-B8, featured a one-base deletion upstream of the PAM and was already transgenic-free (Fig. 2B). The other variant, CBE-5#-2B3, involved a cytosine to thymine change at the C_0 site of TalC EBE and a cytosine to guanine substitution at the C_4 site of TalC EBE (Figs. 2B, S4A). The C to G substitution was likely due to host plant base excision repair during the editing process. At this stage, homozygous individuals and transgenic-free were selected for subsequent disease assessment (Fig. 2).

3.3. SWEET14 promoter is the exclusive target of TalC on the Kitaake background

We inoculated the edited plants with ME2 (*TalC*) and ME2 strain as a negative control to assess the performance of the obtained

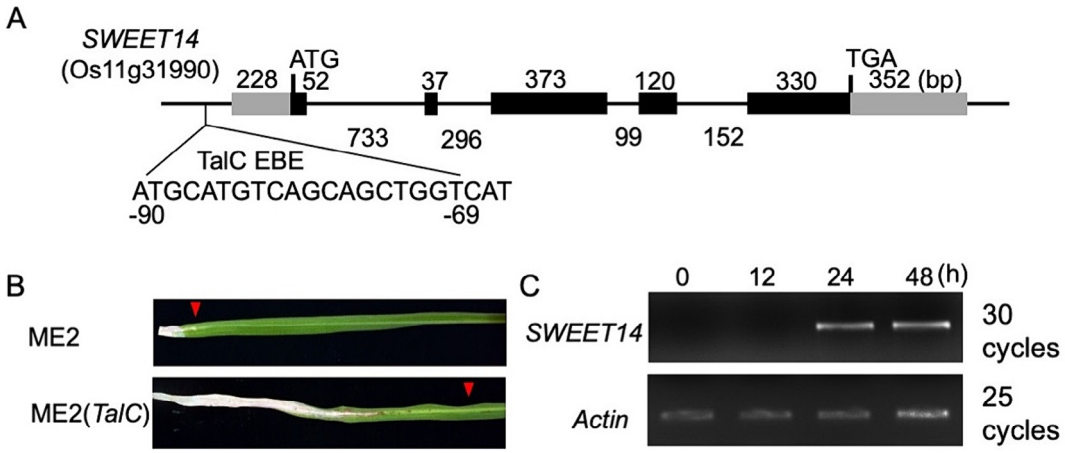

Fig. 1. The virulence of TalC depends on the induction of *SWEET14* in rice. (A) The diagram of the *SWEET14* gene structure. Black blocks indicate the exons, black lines between exons indicate the introns, and grey blocks indicate the untranslated regions. The numbers indicate the lengths of individual regions. The EBE sequence for TalC is shown. (B) The disease phenotypes of Kitaake are caused by ME2 and the *TalC*-containing strain ME2(*TalC*). Representative diseased leaves were photographed 14 d after inoculation, and red arrowheads indicate the edges of the lesions. (C) The gel images of semi-quantitative RT-PCR with *SWEET14* after inoculation with ME2(*TalC*), with the rice actin gene used as an internal control.

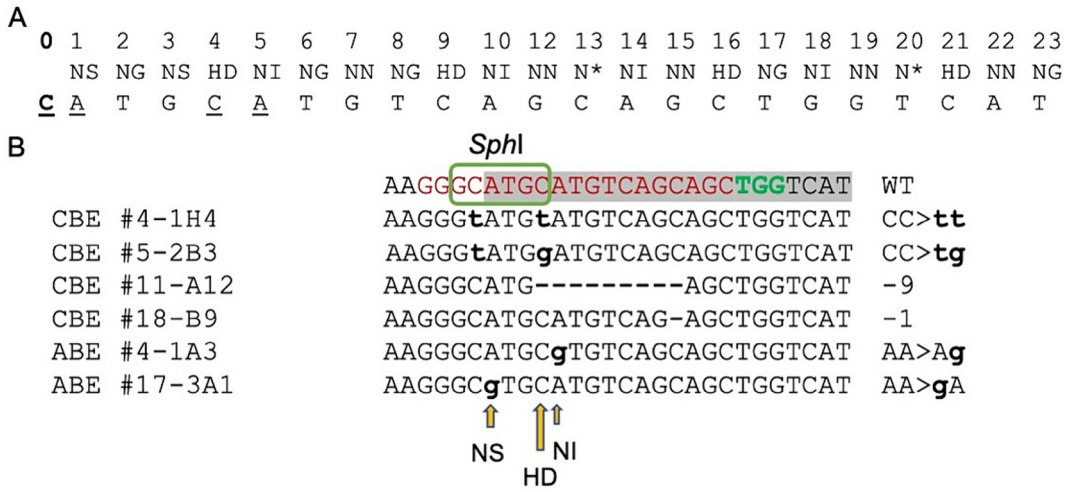

Fig. 2. The representative base-edited mutants of the T_1 generation. (A) The match between TalC RVDs and EBE sequence in the promoter of *SWEET14*. Nucleotides of intended changes are underlined. (B) The genotypes of selected mutants containing nucleotide changes within EBE. The protospacer adjacent motif (PAM) and protospacer sequences for guide RNA are highlighted in green and red, enzymatically recognized sequence of *Sph*I is highlighted in a green frame, respectively. The EBE sequence is shaded in grey. Base pair changes are indicated by lowercase letters and deletions by black dashed lines.

mutants in disease analysis. Concurrently, we selected one mutant (CBE-#11-A12) with a significant deletion around the TalC EBE domain as an additional negative control. The results demonstrate that the simultaneous alteration of two cytosines can notably impact TalC's virulence (Fig. 3A, B). There is no evidence comparing the differential effects of a C_0 to T_0 substitution, and editing a single C could elucidate this further. Some research suggests that T_0 may be more effective than C_0, especially in cases of mismatches during binding between RVDs and the EBE sequence [41]. We hypothesize that converting either C_4 to T_4 or G_4 may lead to most mismatches, while a 9-bp deletion and a 1-bp deletion primarily confer resistance due to alterations in the binding complementarity (Fig. 3). Interestingly, changing A_1 to G_1 or A_5 to G_5 did not result in unexpected resistance to TalC. This suggests varied performances or flexibility in the binding between TalC and EBE sites. In some instances, *in vitro* binding assays may not accurately reflect the biological activity in host cells, primarily due to the complex physiological processes in different plant cells.

As the virulence of TalC primarily hinges on the induction of *SWEET14* expression, the qRT-PCR results revealed that ABE-4#-1 and ABE-17#-3 plants exhibit a similar level of *SWEET14* expression to the WT Kitaake, with *SWEET14* expression induced nearly two hundred times compared to the negative control. Conversely, the expression of *SWEET14* in CBE-4#-1H4, CBE-5#-2B3, and CBE-18#-B9 lines did not reach higher levels as observed in ABE lines (Fig. 3C). It was observed that CBE-4# and CBE-5# lines still displayed an induction of approximately 15 times compared to the control. However, TalC might still be capable of partially binding to the EBE site of *SWEET14* in these cases, with the induction probably not providing sufficient benefits for sugar transportation or bacterial proliferation and eventually lesion progression. The level of induction is speculated to significantly impede the evolution of virulence in *Xoo* strains. Our findings affirm that the *SWEET14* promoter is the exclusive target of TalC in the Kitaake background during the disease progression. Furthermore, when testing the WT African *Xoo* on TalC-resistant lines, we observed that the plants exhibited moderate resistance to all African natural *Xoo* strains (Fig. 4). These results suggest an additional pathogenic pathway (involving other host factor or pathogen factor or both) by those African *Xoo* isolates aside from TalC vs *SWEET14*.

To find out the effect of host genetic background on the partial susceptibility or modest resistance of TalC EBE edits to TalC containing AXO strains as illustrated in the C to T edited plants of Kitaake, we generated base edited lines of Zhonghua 11 (referred to as ZH 11, a *japonica* rice) and IR24 (an *indica* rice) using the CBE construct carrying a single gRNA targeting the TalC EBE of *SWEET14*. Progeny plants of three lines (T_1#1, #6 and #13) of ZH 11 and two lines (T_1#1 and #4) of IR24 all homozygous for changes of C_0 to T_0 and C_4 to T_4 were generated and used to assess disease phenotypes in response to AXO1947. Like C to T edited Kitaake lines, the five T_1 lines of ZH 11 and IR24 were also modestly resistant, while the parental lines were highly susceptible to AXO1947 (Fig. 5). The results indicate the genetic background of the three rice genotypes may not be a factor to contribute the remaining host susceptibility to the natural *Xoo* isolates compared to that caused by ME2(*TalC*). It is worth to point out the knock out of ZH 11 led to highly resistant phenotype against AXO1947 [26].

3.4. Off-target base editing influence of CBE and ABE

Some studies have already demonstrated that cytosine base editors can induce significant genome-wide off-target mutations [42]. To evaluate the presence of insertions and off-target effects of the base editing system employed in this study, we meticulously selected potential genomic sites that could be targeted by the editing agents due to partial homology to the guide RNA. Three off-target loci were selected for analysis in the edited plants. Site-specific primers were designed, and the PCR products were sequenced. Among the identified potential off-target sites, two showed no editing, while amplification of the PCR product was not observed for the third site, possibly due to the presence of a transposon element (Table S4). These findings indicate that the CBE editing systems utilized in this study exhibit guide RNA-based high specificity and precision. It is important to note that off-target effects do significantly impact the application of animal disease therapy in agriculture. The ultimate breeding goal is to develop desirable and environmentally friendly traits; in cases where off-target effects occur, backcrossing based on marker selection can aid in purifying the genetic background. Considering the overall

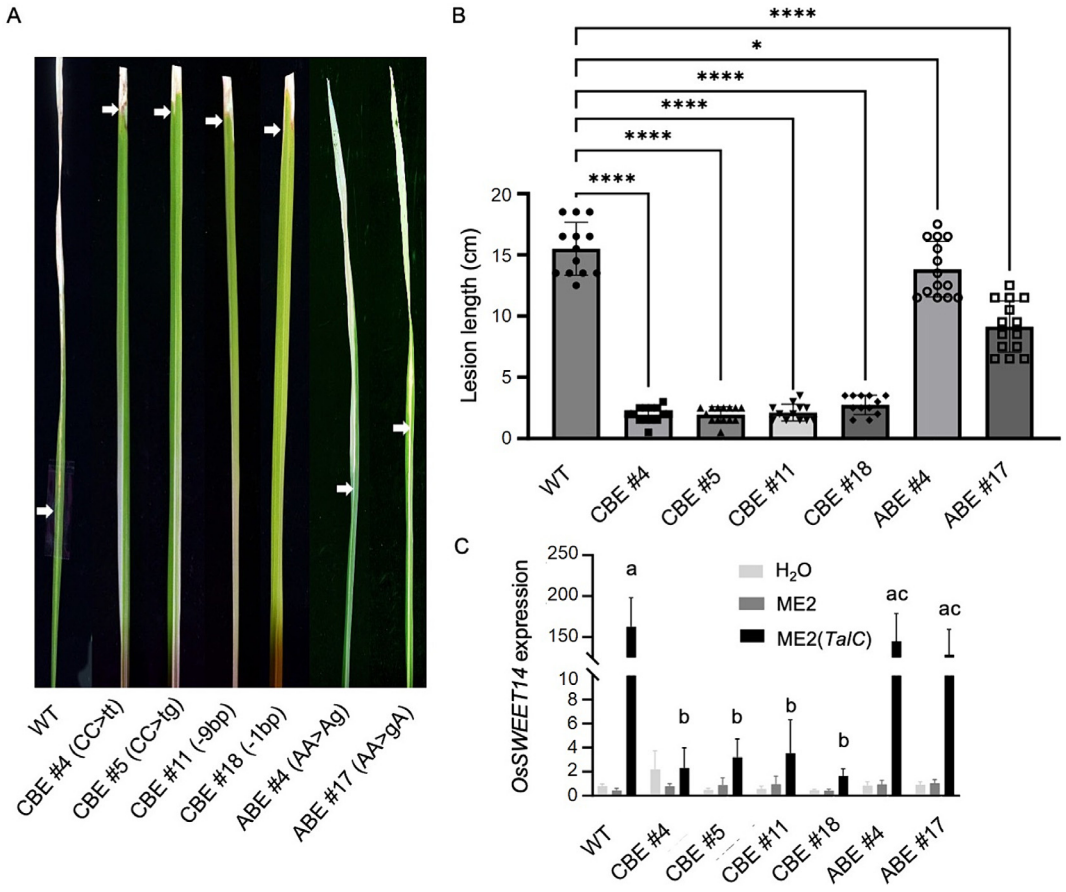

Fig. 3. Base-edited *SWEET14* promoters enable blight resistance to TalC-containing *Xoo* by blocking the induction of *SWEET14*. (A) Disease phenotypes of base-edited plants (specificed below each leaf) in response to inoculation of ME2(*TalC*). The inoculated leaves were photographed 14 d after inoculation. The white arrows indicate the edges of the lesions. (B) The lesion measurements based on edited lines were conducted 14 d post-inoculation. Error bars of graphs represent the standard deviation of at least ten leaves from individual lines. The statistical data were visualized with means ± SE using One-way ANOVA (*, $P < 0.05$; ****, $P < 0.0001$). (C) The expression of *SWEET14* after inoculation with H_2O, ME2, ME2(*TalC*) on the WT and base-edited lines as indicated below the graphs. Values are means ± SE. Different letters above the ME2(*TalC*) data points indicate significant differences (two-sided Welch's *t*-test, $P < 0.01$). WT is the parent Kitaake.

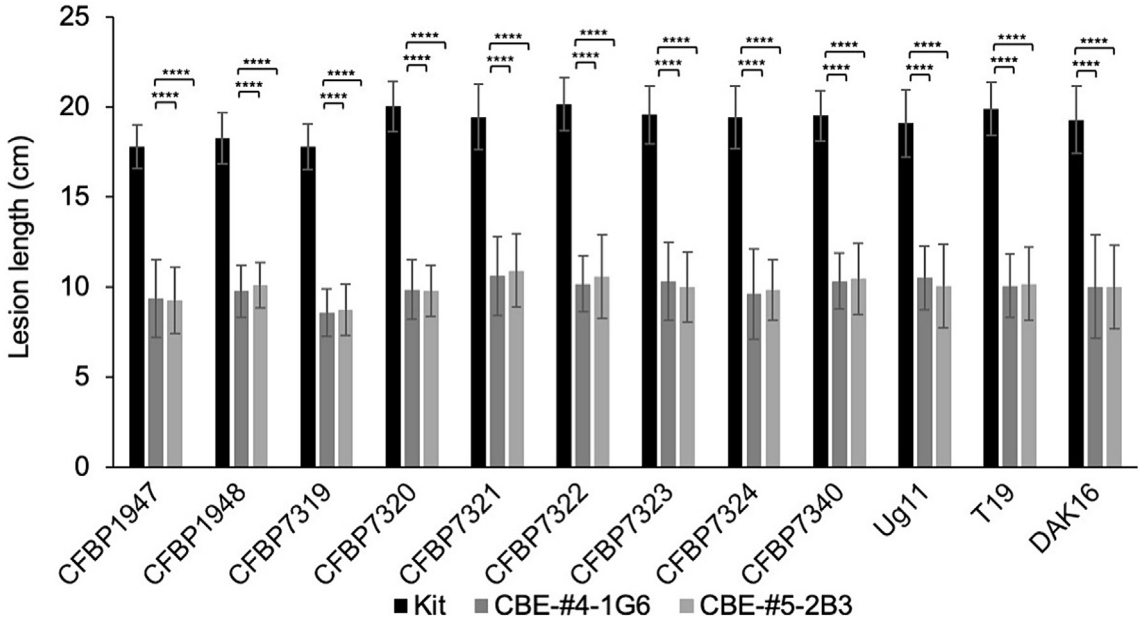

Fig. 4. Lesion lengths in two base-edited lines were measured 14 d after inoculation. Lesion lengths were caused by twelve African Xoo field isolates. All isolates used contain TalC but lack TalF. The statistical data were visualized with means ± SE using Student's *t*-test (****, $P < 0.0001$).

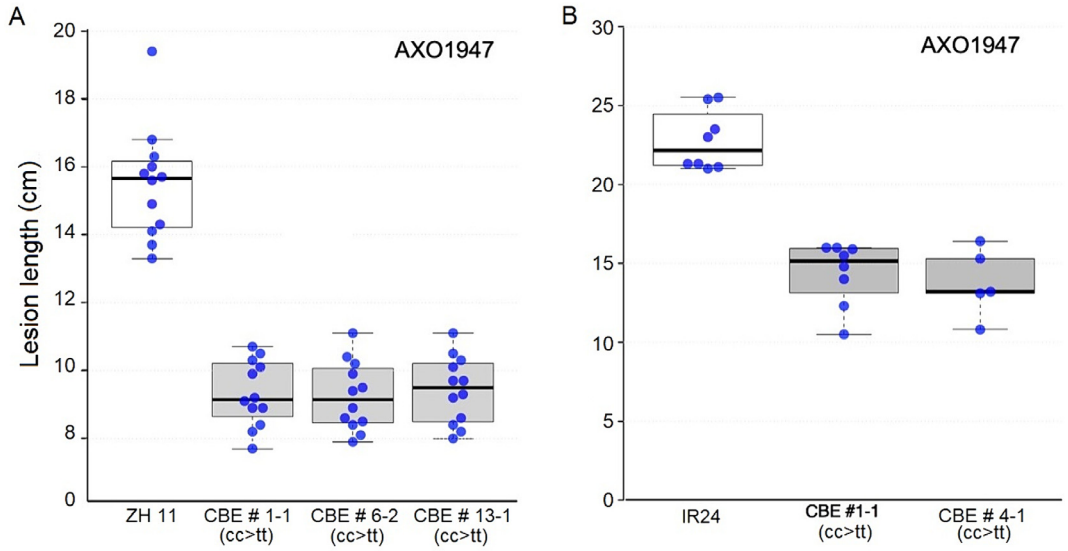

Fig. 5. Lesion lengths of base-edited Zhonghua 11 and IR24 plant leaves of T_1 generation caused by AXO1947. The lesion lengths were measured 14 d after inoculation. Each measurement was derived from fully-expanded leaves of five plants. Center lines show the medians, and box limits indicate 25th and 75th percentiles as determined by R software. Data points were plotted using BoxPlotR (http://shiny.chemgrid.org/boxplotr). Means for the wild types (ZH 11 and IR24) are significantly different from means for their derived base-edited lines ($P < 0.01$).

characteristics of the edited plants remains the key standard for assessment.

3.5. *SWEET14* promoter-edited plants do not exhibit abnormal phenotypes

We meticulously assessed the key characteristics of the bacterial blight resistant plants grown in a greenhouse, including plant height, flag leaf length, flag leaf width, number of productive panicles, panicle length, seedling setting rate, and 100-seed weight (Table S3). The results of all measurements indicate that the development of plants edited at the *SWEET14* promoter is normal and showed no discernible impact on yield and traits compared to wild-type Kitaake plants (Fig. 6).

3.6. The molecular basis of TalC resistance mutation

To understand the molecular mechanisms underlying TalC resistance base changes, we revisited crystal protein structure research and examined the chemical bonds between the Repeat Variable Diresidues (RVDs) and the target nucleotides. Our analysis focused on the interaction involving the RVD "HD" with cytosine. The aspartate residue in "HD" engages in van der Waals interaction with the edge of the corresponding cytosine base and forms hydrogen bonds with the cytosine N4 atom (Fig. S5A). When T_4 replaces C_4 in the *SWEET14* EBE of TalC, the N4 atom position in the benzene ring transitions to the O4 atom of thymine. The oxygen atom typically acts as an electron acceptor during hydrogen bond formation. The chemical bond between "HD" and thymine is disrupted, similar to the C_4 to G_4 substitution. In this case, the O6 atom of guanine takes the place of the N4 atom in the benzene ring of cytosine, preventing interaction with aspartate. This single base pair alteration can significantly affect the binding affinity of the entire TalC protein, elucidating how the base pair change confers resistance in edited rice. Moreover, it is essential to note that another change occurs at C_0 in the EBE site of TalC. Previous studies indicate that EBE sequences for most natural TALes from *Xanthomonas* typically precede the thymine (T_0) except for TalC with the C_0, with T_0 being more effective, especially in cases of mismatches during recognition between RVDs and the EBE sequence [41]. The EBE sequence recognized by TalC was nearly perfect, with G_{17} being a weaker target for the RVD "NI" based on the canonical model (Fig. S5B) [28]. Therefore, the mutation induced by CBE partially hinders the binding of TalC, directly altering the disease susceptibility element.

The change from A_1 to G_1 did not significantly impact the induction of *SWEET14* by TalC primarily because the RVD 'NG' can target four different bases (A, T, G, C) without a specific preference. Interestingly, although A_5 was originally targeted by RVD 'NI', which is known to preferentially target adenine, the substitution of A_5 to G_5 also did not affect the binding interaction. Through a review of protein crystal research, we discovered that during the binding

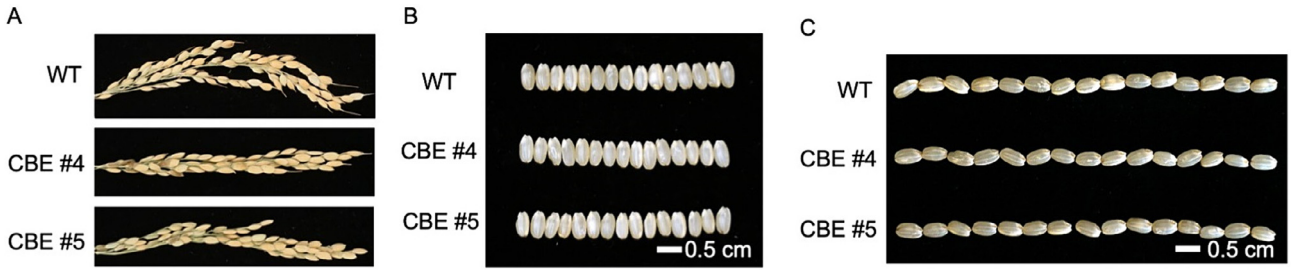

Fig. 6. The seed setting of two base-edited Kitaake lines. (A) The spikes of WT (wild type), CBE #4, and CBE #5. (B, C) The morphology of grains ((B) width and (C) length) from WT, CBE #4, and CBE #5.

between RVD 'NI' and the target adenine, the aliphatic side chain of the isoleucine residue forms non-polar van der Waals contacts with C_8 of the adenine purine ring [28]. This van der Waals interaction may play a secondary role in the binding of RVDs to their targets. The conversion from A_5 to G_5 likely seems not alter the positioning of C_8 in guanine, which may explain the observed phenotype (Fig. S5).

3.7. Inefficiency of editing by multiple gRNA depends less on gRNA and Cas9 expression

It is well-established that simultaneous mutation of the EBEs of three *SWEET* genes can confer broad-spectrum resistance to *Xoo* strains [24]. In this study, we aimed to explore the feasibility of simultaneous editing using the base editing system. Employing the same six-gRNA cassettes that were previously used [24] for the base editing system targeting six sites in *SWEET11a/13/14* promoters, and two gRNAs for *SWEET11a* and *SWEET13*, the rice transformation process proceeded normally with lots of plants regenerated. However, upon genotyping analysis, no discernible mutations were detected (Table S5). To investigate the potential reasons for the lack of mutations, we first confirmed the expression and functionality of nCas9 and gRNA. Several lines were selected based on positive PCR results for the nCas9 fragment and gRNA regions from genomic DNA (Fig. S6). The successful detection of nCas9 gene expression and individual gRNAs indicated that the expression of nCas9 and gRNA was not the cause of the mutation issue (Fig. S7). In the ABE system, the expression of individual gRNAs was even slightly higher compared to the CBE system (Fig. S8). Nevertheless, the ABE editing system exhibited lower efficiency than CBE based on previous editing outcomes and single gRNA editing in this study. It is possible that we did not screen a sufficient number of lines; however, achieving higher efficiency for simultaneous editing with multiple gRNAs remains a challenge.

4. Discussion

We have successfully implemented the base editing system (CBE and ABE) in the rice cultivar Kitaake, ZH 11 and IR24. The genetic transformation was carried out using the popular *Agrobacterium*-mediated method, followed by genotyping of T_0 and T_1 plants. Various base-pair edited plants were generated, involving substitutions from cytosine to thymine and adenine to guanine. These two systems demonstrated effective performance, even with larger editing windows than initially anticipated, with the CBE system resulting in some significant deletions. The occurrence of large deletions was primarily attributed to the incomplete enzyme activity of nCas9 (D10A). The base-edited mutations and phenotypes were inheritable through selfing and to the progeny due to the primary targeting of *SWEET14* by TalC, with the target sequence already well-defined from previous work [13]. In comparing the relationship between genotype and phenotype, we observed varying influences from single or dual base pair changes. For instance, a single adenine change may not sufficiently prevent TalC binding, as the A_1 to G_1 substitution had minimal impact, likely because the RVD 'NS' exhibits a similar affinity for both adenine and guanine. The conversion of A_5 to G_5 did not seem to affect the overall EBE binding capacity of the *SWEET14* promoter by TalC. The interaction between RVD 'NI' and adenine involves the aliphatic side chain of the isoleucine residue making non-polar van der Waals contacts with guanine, influencing slight positional changes in the purine ring [28]. Understanding these interactions can shed light on why the A_5 to G_5 conversion had no significant impact on binding and subsequently the phenotype associated with TalC-containing *Xoo* strains, suggesting that the CBE system may be more beneficial than ABE when applying base editing technology to DNA targets bound by proteins. Recent reports have confirmed that editing the *SWEET14* coding sequence, resulting in knockouts, in ZH 11 significantly enhances resistance to AXO1947 [26]. The choice of cultivar background can influence the application of genome editing technology, especially when considering mutations in the EBE site of TalC in other cultivars to confer resistance to African *Xoo* strains.

The base editing (BE) system represents an advanced genome editing strategy with significant potential for application in crop improvement, with limitations related to the availability of PAM sequences and editing positions. In our study, successful modifications of the *SWEET14* EBE were achieved albeit with some difficulty due to PAM constraints being a critical factor. Several research groups have expanded the editing scope through various strategies, such as utilizing Cas9 variants, altering the position of the deaminase, adjusting the number and location of nuclear localization sequences, and employing diverse deaminase origins [32,44,45]. Recent advancements in base-editing-mediated gene evolution methods highlight the efficacy of the base editing system in plant applications [46]. The enhanced base editing system holds promise for enabling multiplex editing in the breeding of bacterial blight-resistant cultivars and evaluating the impact of base pair changes on crop traits.

The findings of this research underscore the significant role of single nucleotide polymorphism (SNPs) across the rice genome in mediating the interaction between rice and pathogens. These SNPs influence the host defense mechanism and susceptibility, offering valuable insights into basic understanding of disease biology and innovative strategies for future crop improvement. With the advancement of high-throughput sequencing technologies and state-of-the-art genome editing tools, we are poised to uncover even more critical information. On the other hand, the identification of Pan-phytopathogen SNPs broadens the scope of potential antipathogen targets (e.g., major TALe genes of virulence). This progression in biology and technology promises enhanced crop resilience and productivity, ultimately contributing to sustainable food security.

The primary concern with genome editing systems has been the issue of off-target effects, leading to the development of improved strategies to mitigate these effects. However, for crop enhancement, the manageability of plants poses less of a hindrance. Recent studies have indicated that the CBE system exhibits a higher off-target rate compared to ABE [42]. There is evidence suggesting that base editing results in fewer off-target genomic changes than the conventional CRISPR/Cas9 system [43]. Utilizing whole-genome sequencing technology and eliminating undesirable traits through backcrossing with wild-type plants and subsequent segregation process to obtain desired genotypes can enhance outcomes. We advocate for the public understanding of genome-edited plants, even though several countries have already exempted transgene-free plants produced through genome editing from GMO regulations. The urgency of global challenges, such as climate change and population growth, necessitates swift acceptance. However, striking a balance between public acceptance and compliance with relevant laws and regulations remains an ongoing issue. Nevertheless, genome-edited crops represent the optimal choice for addressing the challenges posed by climate change and population growth pressures.

CRediT authorship contribution statement

Chenhao Li: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft. **Bo Liu:** Methodology, Resources, Validation. **Hansong Dong:** Funding acquisition, Supervision, Writing – review & editing. **Bing Yang:** Conceptualization,

Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The work was partially supported by a sub-award to the University of Missouri from the Heinrich Heine University of Dusseldorf funded by the Bill & Melinda Gates Foundation (OPP1155704) (Bing Yang) and the China Scholar Council (Chenhao Li, as a joint Ph.D. student). The authors are grateful to the U.S. National Small Grain Collection for providing the rice accessions for this study. We thank Dr. Zhiyuan Ji for his critical reading of the manuscript.

Appendix A. Supplementary data

Supplementary data for this article can be found online at https://doi.org/10.1016/j.cj.2024.09.003.

References

[1] S. Mohanty, R. Wassmann, A. Nelson, P. Moya, S.V.K. Jagadish, Rice and climate change: significance for food security and vulnerability, IRRI Discussion Paper Series No. 49 (2013) 1–14.
[2] V. Verdier, C. Vera Cruz, J.E. Leach, Controlling rice bacterial blight in Africa: needs and prospects, J. Biotechnol. 159 (2012) 320–328.
[3] W. Zhu, B. Yang, N. Wills, L.B. Johnson, F.F. White, The C terminus of AvrXa10 can be replaced by the transcriptional activation domain of VP16 from the herpes simplex virus, Plant Cell 11 (1999) 1665–1674.
[4] D. Gürlebeck, B. Szurek, U. Bonas, Dimerization of the bacterial effector protein AvrBs3 in the plant cell cytoplasm prior to nuclear import, Plant J. 42 (2005) 175–187.
[5] S. Hui, Y. Shi, J. Tian, L. Wang, Y. Li, S. Wang, M. Yuan, TALE-carrying bacterial pathogens trap host nuclear import receptors for facilitation of infection of rice, Mol. Plant Pathol. 20 (2019) 519–532.
[6] A.L. Perez-Quintero, B. Szurek, A decade decoded: spies and hackers in the history of TAL effectors research, Annu. Rev. Phytopathol. 57 (2019) 459–481.
[7] M. Hutin, P.-Q. Alvaro, C. Lopez, B. Szurek, MorTAL Kombat: the story of defense against TAL effectors through loss-of-susceptibility, Front. Plant Sci. 6 (2015) 535.
[8] J. Zhang, Z. Yin, F. White, TAL effectors and the executor R genes, Front. Plant Sci. 6 (2015) 641.
[9] C. Li, C. Ji, J. Huguet-Tapia, F.F. White, H. Dong, B. Yang, An efficient method to clone TAL effector genes from genomes of *Xanthomonas oryzae* using Gibson assembly, Mol. Plant Pathol. 20 (2019) 1453–1462.
[10] N. Schandry, J.M. Jacobs, B. Szurek, A.L. Perez-Quintero, A cautionary TALE: how plant breeding may have favoured expanded TALE repertoires in *Xanthomonas*, Mol. Plant Pathol. 19 (2018) 1297–1301.
[11] J. Streubel, C. Pesce, M. Hutin, R. Koebnik, J. Boch, B. Szurek, Five phylogenetically close rice SWEET genes confer TAL effector-mediated susceptibility to *Xanthomonas oryzae* pv. *oryzae*, New Phytol. 200 (2013) 808–819.
[12] T.T. Tran, A.L. Perez-Quintero, I. Wonni, S.C.D. Carpenter, Y. Yu, L. Wang, J.E. Leach, V. Verdier, S. Cunnac, A.J. Bogdanove, R. Koebnik, M. Hutin, B. Szurek, Functional analysis of African *Xanthomonas oryzae* pv. *oryzae* TALomes reveals a new susceptibility gene in bacterial leaf blight of rice, PLos Pathog. 14 (2018) e1007092.
[13] Y. Yu, J. Streubel, S. Balzergue, A. Champion, J. Boch, R. Koebnik, J. Feng, V. Verdier, B. Szurek, Colonization of rice leaf blades by an African strain of *Xanthomonas oryzae* pv. *oryzae* depends on a new TAL effector that induces the rice nodulin-3 *Os11N3* gene, Mol. Plant-Microbe Interact. 24 (2011) 1102–1113.
[14] L.Q. Chen, SWEET sugar transporters for phloem transport and pathogen nutrition, New Phytol. 201 (2014) 1150–1155.
[15] Z. Chu, M. Yuan, J. Yao, X. Ge, B. Yuan, C. Xu, X. Li, B. Fu, Z. Li, J.L. Bennetzen, Q. Zhang, S. Wang, Promoter mutations of an essential gene for pollen development result in disease resistance in rice, Gene. Dev. 20 (2006) 1250–1255.
[16] B. Yang, A. Sugio, F.F. White, *Os8N3* is a host disease-susceptibility gene for bacterial blight of rice, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 10503–10508.
[17] G. Antony, J. Zhou, S. Huang, T. Li, B. Liu, F. White, B. Yang, Rice *xa13* recessive resistance to bacterial blight is defeated by induction of the disease susceptibility gene *Os-11N3*, Plant Cell 22 (2010) 3864–3876.
[18] J. Zhou, Z. Peng, J. Long, D. Sosso, B. Liu, J.S. Eom, S. Huang, S. Liu, C. Vera Cruz, W.B. Frommer, F.F. White, Gene targeting by the TAL effector PthXo2 reveals cryptic resistance gene for bacterial blight of rice, Plant J. 82 (2015) 632–643.
[19] S. Blanvillain-Baufume, M. Reschke, M. Sole, F. Auguy, H. Doucoure, B. Szurek, D. Meynard, M. Portefaix, S. Cunnac, E. Guiderdoni, J. Boch, R. Koebnik, Targeted promoter editing for rice resistance to *Xanthomonas oryzae* pv. *oryzae* reveals differential activities for SWEET14-inducing TAL effectors, Plant Biotechnol. J. 15 (2017) 306–317.
[20] Y. Gao, C. Zhang, X. Han, Z.Y. Wang, L. Ma, D.P. Yuan, J.N. Wu, X.F. Zhu, J.M. Liu, D.P. Li, Y.B. Hu, Y.H. Xuan, Inhibition of *OsSWEET11* function in mesophyll cells improves resistance of rice to sheath blight disease, Mol. Plant Pathol. 19 (2018) 2149–2161.
[21] Y.A. Kim, H. Moon, C.J. Park, CRISPR/Cas9-targeted mutagenesis of *Os8N3* in rice to confer resistance to *Xanthomonas oryzae* pv. *oryzae*, Rice 12 (2019) 67.
[22] C. Li, W. Li, Z. Zhou, H. Chen, C. Xie, Y. Lin, A new rice breeding method: CRISPR/Cas9 system editing of the *Xa13* promoter to cultivate transgene-free bacterial blight-resistant rice, Plant Biotechnol. J. 18 (2020) 313–315.
[23] T. Li, B. Liu, M.H. Spalding, D.P. Weeks, B. Yang, High-efficiency TALEN-based gene editing produces disease-resistant rice, Nat. Biotechnol. 30 (2012) 390–392.
[24] R. Oliva, C. Ji, G. Atienza-Grande, J.C. Huguet-Tapia, A. Perez-Quintero, T. Li, J.-S. Eom, C. Li, H. Nguyen, B. Liu, F. Auguy, C. Sciallano, V.T. Luu, G.S. Dossa, S. Cunnac, S.M. Schmidt, I.H. Slamet-Loedin, C. Vera Cruz, B. Szurek, W.B. Frommer, F.F. White, B. Yang, Broad-spectrum resistance to bacterial blight in rice using genome editing, Nat. Biotechnol. 37 (2019) 1344–1350.
[25] Z. Xu, X. Xu, Q. Gong, Z. Li, Y. Li, S. Wang, Y. Yang, W. Ma, L. Liu, B. Zhu, G. Chen, Engineering broad-spectrum bacterial blight resistance by simultaneously disrupting variable tale-binding elements of multiple susceptibility genes in rice, Mol. Plant 12 (2019) 1434–1446.
[26] X. Zeng, Y. Luo, N.T.Q. Vu, S. Shen, K. Xia, M. Zhang, CRISPR/Cas9-mediated mutation of *OsSWEET14* in rice cv. Zhonghua11 confers resistance to *Xanthomonas oryzae* pv. *oryzae* without yield penalty, BMC Plant Biol. 20 (2020) 313.
[27] D. Deng, C. Yan, X. Pan, M. Mahfouz, J. Wang, J. Zhu, Y. Shi, N. Yan, Structural basis for sequence-specific recognition of DNA by TAL effectors, Science 335 (2016) 720–723.
[28] A. Nga-Sze Mak, P. Bradley, R.A. Cernadas, A.J. Bogdanove, B.L. Stoddard, The crystal structure of TAL effector PthXo1 bound to its DNA target, Science 335 (2012) 716–719.
[29] J.P. Guilinger, V. Pattanayak, D. Reyon, S.Q. Tsai, J.D. Sander, J.K. Joung, D.R. Liu, Broad specificity profiling of TALENs results in engineered nucleases with improved DNA-cleavage specificity, Nat. Methods 11 (2014) 429–435.
[30] A.C. Komor, Y.B. Kim, M.S. Packer, J.A. Zuris, D.R. Liu, Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage, Nature 533 (2016) 420–424.
[31] N.M. Gaudelli, A.C. Komor, H.A. Rees, M.S. Packer, A.H. Badran, D.I. Bryson, D.R. Liu, Programmable base editing of A·T to G·C in genomic DNA without DNA cleavage, Nature 551 (2017) 464–471.
[32] K. Hua, X. Tao, J.K. Zhu, Expanding the base editing scope in rice by using Cas9 variants, Plant Biotechnol. J. 17 (2019) 499–504.
[33] B. Ren, F. Yan, Y. Kuang, N. Li, D. Zhang, X. Zhou, H. Lin, H. Zhou, Improved base editor for efficiently inducing genetic variations in rice with CRISPR/Cas9 guided hyperactive hAID mutant, Mol. Plant 11 (2018) 623–626.
[34] F. Yan, Y. Kuang, B. Ren, J. Wang, D. Zhang, H. Lin, B. Yang, X. Zhou, H. Zhou, Highly efficient A.T to G.C base editing by Cas9n-guided tRNA adenosine deaminase in rice, Mol. Plant 11 (2018) 631–634.
[35] K. Chen, Y. Wang, R. Zhang, H. Zhang, C. Gao, CRISPR/Cas genome editing and precision plant breeding in agriculture, Annu. Rev. Plant Biol. 70 (2019) 667–697.
[36] M. Marzec, G. Hensel, Targeted base editing systems are available for plants, Trends Plant Sci. 23 (2018) 955–957.
[37] D. Sosso, D. Luo, Q.B. Li, J. Sasse, J. Yang, G. Gendrot, M. Suzuki, K.E. Koch, D.R. McCarty, P.S. Chourey, P.M. Rogowsky, J. Ross-Ibarra, B. Yang, W.B. Frommer, Seed filling in domesticated maize and rice depends on SWEET-mediated hexose transport, Nat. Genet. 47 (2015) 1489–1493.
[38] H. Zhou, B. Liu, D.P. Weeks, M.H. Spalding, B. Yang, Large chromosomal deletions and heritable small genetic changes induced by CRISPR/Cas9 in rice, Nucleic Acids Res. 40 (2014) 10903–10914.
[39] K. Xie, B. Minkenberg, Y. Yang, Boosting CRISPR/Cas9 multiplex editing capability with the endogenous tRNA-processing system, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 3570–3575.
[40] Y. Hlei, S. Ohta, T. Komari, T. Kumashiro, Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA, Plant J. 6 (1994) 271–282.
[41] T. Schreiber, U. Bonas, Repeat 1 of TAL effectors affects target specificity for the base at position zero, Nucleic Acids Res. 42 (2014) 7160–7169.
[42] J. Shuai, Y. Zong, Q. Gao, Z. Zhu, Y. Wang, P. Qin, C. Liang, D. Wang, J.L. Qiu, F. Zhang, C. Gao, Cytosine, but not adenine, base editors induce genome-wide off-target mutations in rice, Science 364 (2019) 292–295.
[43] A.C. Komor, K.T. Zhao, M.S. Packer, N.M. Gaudelli, A.L. Waterbury, L.W. Koblan, Y.B. Kim, A.H. Badran, D.R. Liu, Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T: a base editors with higher efficiency and product purity, Sci. Adv. 3 (2017) eaao4774.

[44] C. Li, Y. Zong, Y. Wang, S. Jin, D. Zhang, Q. Song, R. Zhang, C. Gao, Expanded base editing in rice and wheat using a Cas9-adenosine deaminase fusion, Genome Biol. 19 (2018) 59.
[45] Y. Zong, Q. Song, C. Li, S. Jin, D. Zhang, Y. Wang, J. Qiu, C. Gao, Efficient C-to-T base editing in plants using a fusion of nCas9 and human APOBEC$_3$A, Nat. Biotechnol. 36 (2018) 950–953.
[46] Y. Kuang, S. Li, B. Ren, F. Yan, C. Spetz, X. Li, X. Zhou, H. Zhou, Base-editing-mediated artificial evolution of *OsALS1* in planta to develop novel herbicide-tolerant rice germplasms, Mol. Plant 13 (2020) 565–572.