

## DESIGN AND TRANSFER OF *OsSWEET14*-EDITING T-DNA CONSTRUCT TO BAC THOM 7 RICE CULTIVAR

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### ABSTRACT

Bac thom 7 rice (BT7 rice) is one of the major elite rice cultivars in Vietnam with superior productivity and quality but very susceptible to bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*. The gene *OsSWEET14*, belonging to the *OsSWEET* family which encodes sugar transport proteins, is considered to be a susceptible gene involved in the virulence of *Xoo*. At least three *cis*-elements (EBE - Effector-binding element), including *Tal5*, *PthXo3* and *AvrXa7* on BT7 *OsSWEET14* promoter, bind specifically to well-known transcription activator-like effectors (TALEs) of many Asian *Xoo* bacterium strains. In this study, a T-DNA construct which expressed the protein-RNA complex CRISPR/Cas for editing three EBEs position on the *OsSWEET14* promoter was designed. The recombinant binary vector was tested by PCR, restriction enzyme and finally sequencing and then successfully transferred to Bac thom 7 rice through *Agrobacterium tumefaciens*. 28 of 30 hygromycin-resistant regenerated rice lines that grew and developed normally under nethouse conditions were selected by PCR with specific primers. Among these, twelve transgenic rice lines were identified carrying one copy of the T-DNA construct. The presence of CRISPR/Cas9-induced mutations of the targeted promoter in the transgenic plants were confirmed by T7EI assay. These results provide the basis to determine the role of *OsSWEET14* in the susceptibility of Bac thom 7 rice to *Xanthomonas oryzae* pv. *oryzae* - caused disease, towards the further goal of generating an improved Bac thom 7 rice variety with broad-spectrum bacterial leaf blight resistance using *CRISPR/Cas9* technology.

**Keywords:** Bacterial leaf blight, Bac thom 7, *CRISPR/Cas9*, *OsSWEET14*, *Xanthomonas oryzae*.

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## INTRODUCTION

Bac thom 7 rice (BT7 rice) is an inbred rice variety originating from China, and recognized according to Decision No. 1224 QD/BNN-KHCN dated April 21, 1998. With some valuable traits such as high yields and good quality, BT7 had been ranked first among popular rice varieties for more than 20 years with over 40 crops in Vietnam. However, its productivity is seriously affected by the bacterial leaf blight disease (BLB), especially in the seasons with heavy rains and strong winds.

BLB disease is caused by the *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Borkar, Yumlemban, 2016). The *OsSWEET14* gene, a member of the *OsSWEET* family which encodes sugar transport proteins, is considered an important susceptible gene for *Xoo* bacteria in rice plants (Streubel et al., 2013). The role of *OsSWEET14* in the virulence of several representative *Xoo* strains collected in the Northern provinces of Vietnam had also been determined through artificial infection experiments (Sam et al., 2019). At the molecular level, *Xoo* bacteria secrete TAL proteins which activate the targeted gene through the specific binding to *cis* elements, named as effector-binding element (EBE), located on the promoter region. Some studies on producing rice varieties resistant to *Xoo* through editing the promoter *OsSWEET14* have been published (Li et al., 2012; Blanvillain-Baufumé et al., 2017). These results show that the application of gene editing tools, including the CRISPR/Cas9 system to creating a BLB-resistant BT7 rice variety via editing the susceptible *OsSWEET14* gene is a promising research direction.

In the previous study, we already isolated, sequenced and identified 4 EBEs located on the *OsSWEET14* promoter region of the rice variety BT7 (referred to as *SW14-BT7*), including *TalC*, *AvrXa7*, *PthXo3* and *Tal5* (Sam et al., 2019). However, several studies have demonstrated that EBE *TalC* is more related to the virulence of African *Xoo* strains than Asian ones (Blanvillain-Baufumé

et al., 2017). Therefore, in this study, to determine the role of the three EBEs *AvrXa7*, *PthXo3* and *Tal5* in the infection process of Vietnamese *Xoo* strains on BT7, a binary vector that carries the construct expressing the modified protein-RNA complexes (*SW14-BT7*) has been designed and transferred to BT7 rice through *A. tumefaciens*. This study will be the prerequisite for the generation of an improved broad-spectrum BLB-resistant BT7 rice variety by CRISPR/Cas9 technology.

## MATERIALS AND METHODS

BT7 rice were supplied by Thai Binh Seed Corporation.

*Escherichia coli* strain DH5 $\alpha$  and *Agrobacterium tumefaciens* strain EHA105 were purchased from Thermo Fisher Scientific (USA) and Clontech Laboratories (USA), respectively. Vectors pENTR4/gRNA and pCas9 based on the pENTR4 vector (Marker Gene Technologies, USA) and pCAMBIA1300 vector (Invitrogen, USA) were provided by Dr. Sebastien Cunnac (Center for Development Studies, Montpellier, France).

The oligonucleotides (Table 1) used in the study were designed based on sequences published on GenBank and ordered from Invitrogen (USA) and Sigma (USA).

### Construction of binary vector for SW14-BT7 modification

The gRNA sequence simultaneously targeting 3 positions of *AvrXa7*, *PthXo3* and *Tal5* EBEs on *SW14-BT7* was designed using the CRISPR-P v2.0 software (<http://crispr.hzau.edu.cn/CRISPR2/>). The rice genomic DNA fragments homologous to the gRNAs were identified using CCTop software (<https://crispr.cos.uni-heidelberg.de/>). The designed gRNA sequence was synthesized by Invitrogen (USA).

Two 24-nucleotide fragments (carrying 20 nucleotides of gRNA and 4 added nucleotides for sticky 5'-end), namely *SW14-gRNA-Fw* and *SW14-gRNA-Rv* were annealed (95 °C for 10 min., 4 °C- $\infty$ ) to create a double stranded

crRNA (*SW14-gRNA*). The *SW14-gRNA* DNA fragment was inserted into the *BsaI* position between the *OsU6* promoter region and *tracRNA* on the pENTR4/gRNA vector (Fig. 1). The recombinant vector was confirmed by PCR with the pEN-Fw/pEN-Rv (1.4 kb) and U6-Fw/gRNA-SW14-Rv (276 bp) primer pairs. Next, the fragment containing the [*U6*:

*gRNA-SW14*] construct (451 bp) was ligated into the pCas9 binary vector using the Gateway Cloning kit (Invitrogen). The recombinant vector was tested by PCR with Ubi-Fw/NOS-Rv (4.3 kb), Cas9-Fw/Cas9-Rv (4.1 kb), U6-Fw/Ter-Rv (451 bp), U6-Fw/gRNA-SW14-Rv (276 bp) primers and finally by sequencing.

Table 1. Oligonucleotides used in the study

Name	Sequence (5'-3')	PCR product size (bp)	Gene/vector
Actin-Fw	TGATGGTGTTCAGCCACACT	350	<i>Actin1</i>
Actin-Rv	TGGTCTTGGCAGTCTCCATT		
HPT-Fw	AAGGAGGTGATCCAGCC	400	<i>HPT</i> (pCas9)
HPT-Rv	GAGTTTGATCCTGGCTCAG		
Ubi-Fw	CCCTGCCTTCATACGCTATT	4315	[ <i>Ubi:Nos</i> ] (pCas9)
NOS-Rv	AGACCGGCAACAGGATTCAA		
Cas9-Fw	CCATGGCCCCAAAGAAGAAG	4108	<i>Cas9</i> (pCas9)
Cas9-Rv	TCAATCGCCGCCGAGTTG		
Cas9-t-Rv	GCCTCGGCTGTCTCGCCA		<i>Cas9</i> (pCas9)
U6-Fw	GGATCCGGATCATGAACCAACG	451	pENTR4/gRNA
Ter-Rv	GAATTCGATATCAAGCTT		
pEN-Fw	CTACAAACTCTTCCTGTTAGTTAG	1384	pENTR4/gRNA
pEN-Rv	ATGGCTCATAACACCCCTTG		
gRNA-SW14-Fw	GTGTGGCTTGATGAGCTTAGCAC		gRNA-SW14
gRNA-SW14-Rv	AAACGGTGCTAAGCTCATCAAGC		
SW14-t-Fw	AACAATAAAGCTAGCAGA	520	<i>SW14-BT7</i>
SW14-t-Rv	CTTCGCCTTTGGTCTCCTAG		

Note: Underlined sequences denote recognition sites of restriction enzymes.

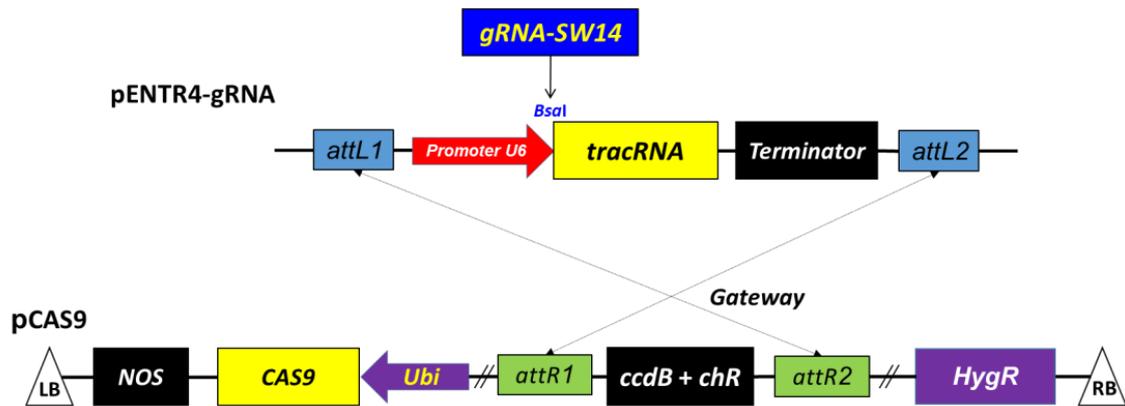


Figure 1. Diagram of constructing pCas9/gRNA-SW14 vector

### **Transformation of DNA into *A. Tumefaciens***

The binary vector was transformed into *A. tumefaciens* EHA105 by heat shock method (Wang, 2006). Plasmid DNA (1 µg) was added to 100 µL of competent cells and heat-shocked at 37 °C for 5 minutes. The reaction mixture was plated on LB medium containing 50 µg/mL spectinomycin, rifamicin 20 µg/mL; plates were incubated at 28 °C for 2–3 days. The transformants were then screened by colony PCR method (Sahoo et al., 2011) using the U6-Fw/Ter-Rv specific primers.

### **Rice transformation through *A. Tumefaciens***

The rice transformation experiment was performed according to Hiei et al. (1994), with some modifications. Calli formed from BT7 rice endosperm after 5–7 days of culture on MS medium were co-cultured with *A. tumefaciens* bacteria transformed vector pCas9/gRNA-SW14 for three days. Then, the calli were selected on culture media containing 20 mg/L and 50 mg/L hygromycin. The surviving calli were transferred in turn to the pre-regeneration medium (MS medium with 5.0 mg/L ABA, 100 mg/L Cefotaxime and 50 mg/L Hygromycin), shoot regeneration medium (MS medium with 0.5 mg/L Kinetin and 30 mg/L Hygromycin) and rooting medium (basic MS medium). The regenerated plants were grown in potting soil under nethouse conditions.

### **Genotyping of transgenic BT7 rice**

Total DNA of transgenic plants was extracted according to Doyle & Doyle (1990), using 2% CTAB solution.

The presence of transgenic expression construct in transgenic BT7 rice plants was confirmed by PCR (Sambrook & Russel, 2001), with primers Actin-Fw/Actin-Rv, HPT-Fw/HPT-Rv, Cas9-Fw/Cas9-t-Rv and U6-Fw/gRNA-SW-Rv. The PCR was performed with 35 cycles of 94 °C, 30 s; 56 °C, 30 s and 72 °C, 30 s.

The number of transgene copies was identified by qPCR according to Zhang et al.

(2003). The qPCR performed with 40 cycles of 94 °C, 15 s; 60 °C, 30 s and 72 °C, 30 s using HPT-Fw/HPT-Rv primers.

*SW14-BT7* mutation was detected by T7 Endonuclease I (T7EI) (Zischewski et al., 2017). A 711-bp fragment of the *SW14-BT7* promoter was amplified by PCR (Sambrook and Russel, 2001), using the SW14-t-Fw/SW14-t-Rv primer pair. PCR products from transgenic and non-transgenic rice plants were mixed at a ratio of 1:1 to create heterozygous double strands for detection of mutation (Zischewski et al., 2017). The T7EI enzyme has activity of cutting heterozygous double strands at the mismatch point, which forms two or more DNA fragments. The mixture was processed with T7EI at 37 °C for 30 minutes. The reaction product was checked by electrophoresis on 2% agarose gel. PCR products which were cut into 2 DNA bands (410 bp and 301 bp in lengths) were determined as carrying the mutation.

## **RESULTS**

### **Designing the expression construct of the *OsSWEET14* promoter-editing complex**

The CRISPR/Cas9 gene editing system works based on the site-specific double-stranded DNA cutting activity of the Cas9 protein (endonuclease) guided by the single guide RNA (sgRNA) molecule. The specificity of the CRISPR/Cas9 complex depends on the 20-nucleotide sequence (crRNA) on the sgRNA molecule (Bortesi & Fischer, 2015). The designing sgRNA sequence determines the efficiency of the CRISPR/Cas9 complex. In order to demonstrate the role of the three EBE *AvrXa7*, *PthXo3* and *Tal5* on the *SW14* promoter in the virulence of *Xoo* in BT7 rice, the crRNA sequence was designed to induce mutation overlapping the sites of these EBEs.

Using CRISPR-P v2.0 and CCTOP software, 3 crRNA sequences were identified for targeting the position of 3 EBEs, which

probably form sgRNA molecular with stable secondary constructs (Table 2); of which, crRNA-3 is predicted to be the most effective (total on-score and off-score is 106.3) (Fig. 2).

The crRNA-3 was designed with an additional sticky 3'-end (Table 1) and synthesized artificially (called *gRNA-SW14*) for designing *SW14-BT7* - editing T-DNA construct.

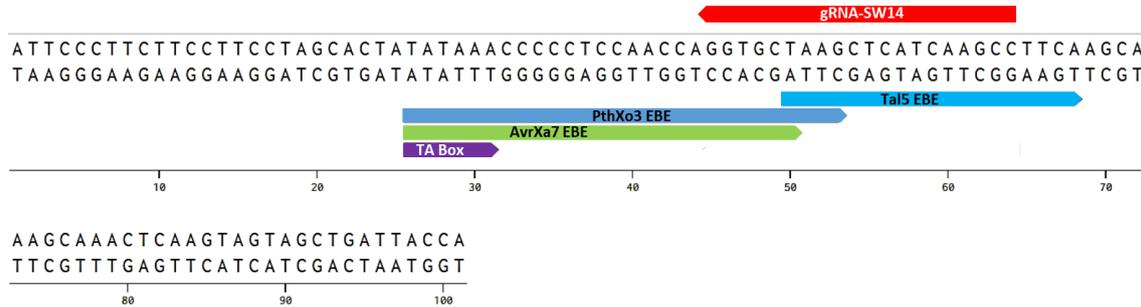


Figure 2. Position of crRNA sequence editing the three EBEs AvrXa7, PthXo3 and Tal5 on promoter SW14-BT7

Table 2. The crRNA for editing SW14-BT7 promoter

Name	Sequence (5'-3')	% GC	TSL	GSL	CBP	TBP	IBP	On-Score	Off-score
crRNA1	GCTTAGCACCT GGTTGGAGG	60	3	0	6	10	0	50,6	48,1
crRNA2	GAGCTTAGCAC CTGGTTGGA	55	3	0	6	10	0	50,3	49,0
crRNA3	GGCTTGATGAG CTTAGCACC	55	3	0	6	11	0	57,9	48,4

Note: TSL: total number of loops per sgRNA; GSL: number of loops per crRNA sequence; CBP: number of base pairs that are continuously paired between a crRNA sequence and another sequence on sgRNA; TBP: total number of base pairs paired on sgRNA; IBP: number of base pairs paired in the crRNA sequence; On-score: gRNA activity score (0-100); Off-score: specific score of sgRNA (0-100).

To design the *sgRNA* expression construct for targeting *SW14-BT7*, the *gRNA-SW14* DNA fragment (Fig. 1) was ligated into the *pENTR4-gRNA* vector pre-treated with *BsaI* (Fig. 3A, lane 2) by T4 DNA ligase. The recombinant vector was examined by PCR with two different primer pairs. The results showed that DNA bands with expected sizes of about 1.4 kb (Fig. 3B, lane 1) and 0.28 kb (Fig. 3B, lane 3) were observed in PCR products using specific primers for the vector *pENTR4-gRNA* (*pEN-Fw/pEN-Rv*) and the *gRNA-SW14* - expression construct (*U6-Fw/gRNA-SW14-Rv*), respectively.

*gRNA-SW14* expression construct (*U6::gRNA-SW14*) was ligated into the binary vector *pCas9* carrying *Cas9* protein

expression construct (*[Ubiquitin:Cas9:Nos]*) by Gateway system (Fig. 1). The received vector *pCas9/gRNA-SW14* was then examined by PCR with primer pairs specific for protein expression construct (*[Ubiquitin: Nos]* (*Ubi-Fw/NOS-Rv*), *Cas9* (*Cas9-Fw/Cas9-Rv*), *sgRNA* expression construct (*U6-Fw/Ter-Rv*) and *gRNA-SW14* (*U6-Fw/gRNA-SW14-Rv*). The electrophoresis analysis showed that each amplicon corresponding primers has a single 4.3 kb, 4.1 kb, 0.45 kb and 0.28 kb DNA band (Fig. 3C, lanes 1, 3, 5 and 7) on 1% agarose gel, which were the expected products from the amplified DNA fragments. These results demonstrated that the obtained plasmid carried the *Cas9* gene

expression construct (controlled by the *Ubiquitin* promoter) and the *gRNA-SW14* expression construct (controlled by the *U6*

promoter). The recombinant vector was sequenced (Fig. 4) to ensure the correct insertion of the constructs.

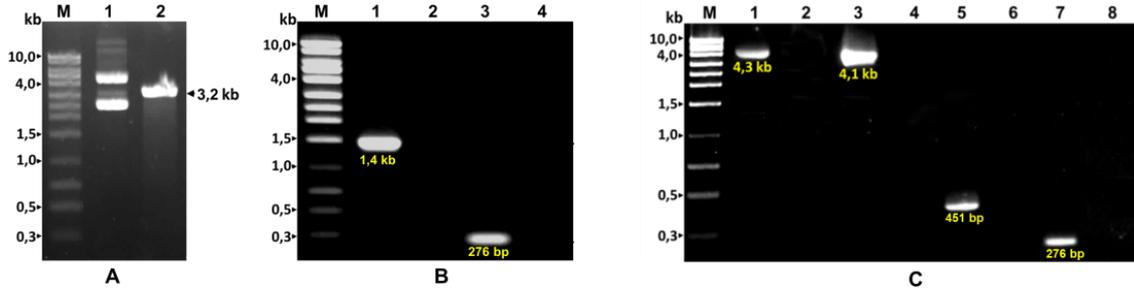


Figure 3. Insertion of gRNA-SW14 into the expression construct of T-DNA

Note: (A) Restriction of pENTR4/gRNA by *Bsa*I; lane 1: un-cut vector, lane 2: cut vector. (B) Testing pENTR4/gRNA-SW14 by PCR; lanes 1 and 3: recombinant vector; lanes 2 and 4: negative control (without DNA template); lanes 1 and 2: PCR with pEN-Fw/pEN-Rv primers; lanes 3 and 4: PCR with gRNA-SW14-Fw/gRNA-SW14-Rv primer. (C) Testing vector pCas9/gRNA-SW14 by PCR; lanes 1, 3, 5, 5 and 7: pCas9 / gRNA-SW14; lanes 2, 4, 6 and 8: negative control (without DNA template); lanes 1 and 2: PCR with Ubi-Fw/NOS-Rv primers; lanes 3 and 4: PCR with Cas9-Fw/Cas9-Rv primers; lanes 5 and 6: PCR with U6-Fw/Ter-Rv primers; lanes 7 and 8: PCR with U6-Fw/gRNA-SW14-Rv primers. Lane M: 1 kb DNA ladder.

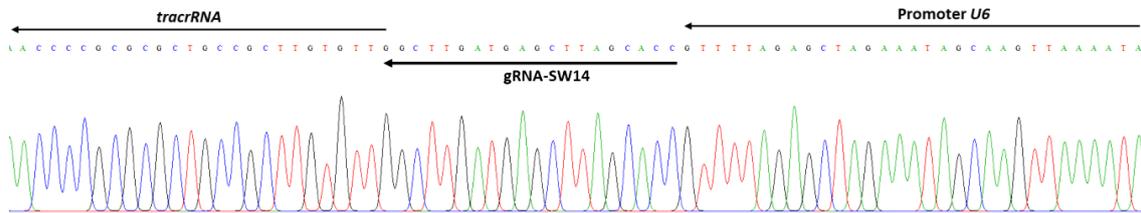


Figure 4. pCas9/gRNA-SW14 sequence.

Note: A part of pCas9/gRNA-SW14 vector was sequenced with the Ter-Rv primer. The 20-bp sequence gRNA-SW14 was ligated at the position between U6 promoter and tracrRNA.

In similar studies, the sgRNA expression construct can be engineered in a variety of ways, the most common of which was using overlapping-PCR method. To express the Cas9-sgRNA complex in rice cells, the authors typically design the sgRNA expression construct under the control of the either *U3* or *U6* promoter and the *Cas9* expression construct under the control of the *Ubiquitin* promoter, which were located on the same binary vector frame to facilitate the transformation into host cells. This vector system has been shown to be stable, efficient

and capable of inducing the inheritable mutations (Ma et al., 2015). In this study, the sgRNA expression construct controlled by the *U6* promoter simultaneously targeting 3 EBEs *Tal5*, *AvrXa7* and *PthXo3* on the *SW14 – BT7* was generated by using *Bsa*I enzyme and T4 DNA ligase. The construct [*U6: gRNA-SW14*] and the construct [*Ubiquitin: Cas9: NOS*] (expressing the *Cas9* protein) were cloned into pCas9 together in order to create a plant transformation vector carrying the construct which expressed the protein-RNA complex modifying promoter *SW14 – BT7*.

### Transformation of the *OsSWEET14* promoter-editing construct into BT7 rice

To create material for rice transformation experiment, vector pCas9/gRNA-SW14 was transformed into *A. tumefaciens* EHA105 cells by heat shock method. The transformants were screened by colony PCR with specific primers U6-Fw/Ter-Rv. The electrophoresis results of PCR products from the colonies all gave a single DNA band (Fig. 5, lanes 1 - 5) with a size of about 0.45 kb similar to the positive control reaction (Fig. 5, lane 7). This proved that all colonies carried pCas9/gRNA-SW14 construct. A transformant was randomly used for the BT7 rice transformation.

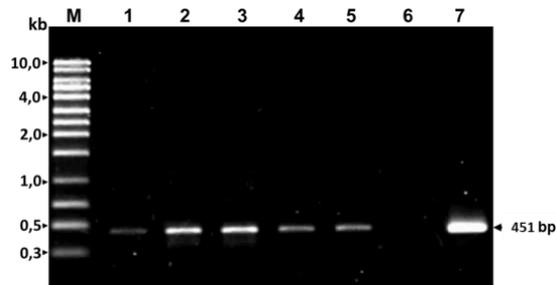


Figure 5. Colony PCR of *A. tumefaciens* transformants

Note: PCR products with U6-Fw/Ter-Rv primers were electrophoresed on 1% agarose gel. Lane M: 1 kb DNA ladder; lanes 1 - 5: colonies transformed with pCas9/gRNA-SW14; lane 6: negative control (untransformed colonies); lane 7: positive control (pCas9/gRNA-SW14).

*A. tumefaciens* cells transformed the construct expressing the *SW14-BT7*-editing CRISPR/Cas9 complex were co-cultured with BT7 rice calli on *in vitro* medium supplemented with Acetosyrigone to increase the efficiency of gene transformation. The hygromycin-resistant cell lines growing on selection and shoot-regeneration media were transferred to root regeneration medium to create complete seedlings (Fig. 6). The results of BT7 rice

transformation presented in table 3 showed that the survival rate of calli was quite high, reaching over 75% in most stages of the gene transfer process. In particular, the regeneration rate of plants after 3 selections reached 54.29% (38/70 lines regenerated completely). However, only 30 of 38 regenerating plantlets survived after transferring to potting soil. Finally, from 300 original BT7 rice callus samples, 30 regenerated plants were obtained which grew normally under the nethouse conditions; the achieved regeneration efficiency was 10%. This shoot regeneration rate of BT7 rice callus was similar to that of *Pusa Basmati 1* indica rice variety using the same transgenic protocol (Phuong et al., 2015).

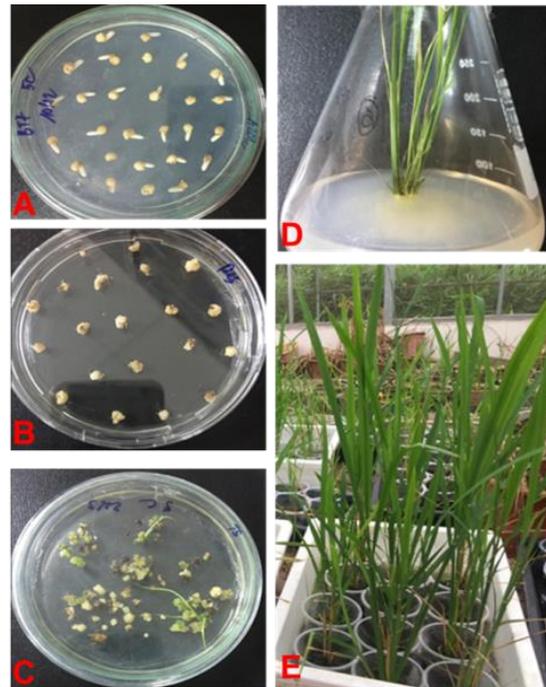


Figure 6. Transformation of SW14-BT7 - editing T-DNA construct into BT7 rice variety  
Notes: (A) Co-culture of calli with *A. tumefaciens* cells; (B) Selection of calli by 50 mg/L hygromycin; (C) Shoot regeneration from calli; (D) Root regeneration; (E) Regenerated plants grown in nethouse.

Table 3. Transformation of pCas9/gRNA-SW14 construct into BT7 rice

Stage	Total sample	Number of surviving/growing samples	Survival/growth sample rate (%)	Efficiency (%)
Callus induction	300	269	89,67	89,67
Co-culture	269	250	92,94	83,33
Selection I	250	200	80,00	66,67
Selection II	200	150	75,00	50,00
Selection III	150	80	53,33	26,67
Pre-regeneration	80	70	87,50	23,33
Regeneration, complete plant production	70	38	54,29	12,67
Nethouse growth	38	30	78,95	10,00

### Genotyping of transgenic BT7 rice plants

To determine the presence of pCas9/gRNA-SW14 constructs in the genome, total DNA from leaf tissue of 30 lines of regenerated BT7 rice were extracted and tested by PCR with specific primers (Table 4). The PCR analysis with specific primers for the internal standard gene *Actin1* showed positive rate of 100%; indicating the good quality of all extracted DNA samples for the PCR experiment. There were 28 of 30 regenerated rice lines showed positive PCR results with specific primers for *Hygromycin phosphotransferase (HPT)*, *Cas9* and [*U6: gRNA-SW14*] construct, reaching the rate of 93.33% (Table 4). This result suggested that the expression construct of *Cas9* protein and

*sgRNA* (carrying *gRNA-SW14*) were successfully integrated into BT7 rice genome; the gene transformation efficiency of the whole process reached 9,33%.

In order to ensure the stable function of T-DNA constructs, transgenic plants with more than one copy of the transgene are often removed in many plant transformation experiments. Therefore, in this study, regenerated rice plants showing positive PCR results with all four specific primer pairs were further analyzed by qPCR to determine the copy number of the T-DNA construct (through the copy number of *HPT* gene) in the genome. The analysis indicated that 14/28 regenerated plants had values of  $2^{\Delta Ct}$  from 0.4 to 0.67, corresponding to single copy of transgene in the genome (Table 4).

Table 4. Genotyping analysis of regenerated BT7 rice lines

Experiment	Number of tested plants	Number of positive plants
PCR with <i>Actin</i> primers	30	30
PCR with <i>HPT</i> primers	30	28
PCR with <i>Cas9</i> primers	28	28
PCR with <i>gRNA-SW14</i> primers	28	28
qPCR identifying plants with one copy of T-DNA	28	14
T7EI assay	14	12

BT7 rice plants with single copy of transgene were used for evaluating the targeted mutation efficiency of the CRISPR/Cas9 complex using T7EI (T7 endonuclease I assay). The 711-bp fragment containing the recognition site of *gRNA-SW14*

amplified from *SW14-BT7* promoter of each transgenic and non-transgenic rice plant was amplified with SW14-t-Fw/SW14-t-Rv primers (Fig. 7A) and mixed together for T7EI assay (Fig. 7B). Agarose gel electrophoresis of the restriction products

showed 0.4kb and 0.3kb DNA bands in 12 of 14 tested transgenic plants (table 4). This result indicated that transformation of pCas9/gRNA-SW14 construct into BT7 rice induced the mutations in the targeted *SW14-BT7* promoter in transgenic plants, therefore suggested that the *SW14-BT7* promoter-

editing CRISPR/Cas9-gRNA complex was successfully expressed in BT7 rice cells. Gene editing efficiencies were 85.7% or 4% based on the number of transgenic plant harboring one copy of T-DNA (14 lines) or the total number of BT7 seed for callus induction (300 seeds), respectively.

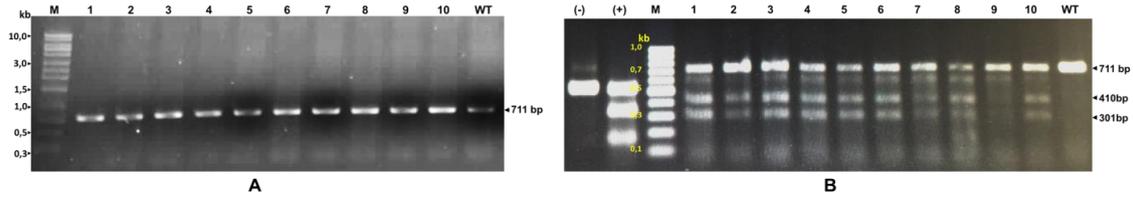


Figure 7. Identification of *SW14-BT7* mutation by T7EI.

Note: (A) Amplification of *SW14-BT7* fragment with *SW14-t-Fw/SW14-t-Rv* primers. (B) Mixture of amplicons from transgenic plant and wild-type plant was treated with T7EI. Lanes 1-10: transgenic BT7; lane WT: wild-type BT7; lane (-): negative control; lane (+): positive control; lane M: 1 kb (A) and 100 bp (B) DNA ladder.

In general, the success rate of gene transformation in indica rice varieties are much lower than that of japonica rice (Slamet-Loedin et al., 2014). Although several improved protocols have been published, the efficiency of genetic modification has not been significantly improved. In this study, for the first time, a CRISPR/Cas9-expressing T-DNA construct was successfully transformed into a Vietnamese popular (indica) rice variety (BT7 rice) through *A. tumefaciens*, using calli from mature embryos. In addition, the study has also demonstrated that CRISPR/Cas9 construct induced mutation at targeted site on *SW14-BT7* promoter of the transgenic BT7 lines carrying single copy of transgene. Our results showed that it is possible to apply gene editing systems for modifying susceptible genes involved in the virulence of *Xoo* to host rice plants, and, on that basis, generating a broad-spectrum BLB-resistant BT7 rice in particular and improving the important agronomic traits of Vietnamese major rice varieties in general.

## CONCLUSION

In this study, we have successfully designed protein-RNA complex expression construct for modifying the *OsSWEET14*

promoter of the Vietnamese elite BT7 rice variety. The gene-editing construct was successfully transformed into BT7 rice through *A. tumefaciens* with an efficiency of 9.3%. The regenerated BT7 rice lines have been identified to carry T-DNA construct in the genome and targeted mutation on the *OsSWEET14* promoter through PCR and T7EI analysis. These results are the premise for further studies on the function of *OsSWEET14* in the interaction between *Xoo* and BT7 rice as host plant, thereby leading to the generation of improved rice varieties with high yields and quality and resistance to BLB disease.

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