The pepper Bs4C proteins are localized to the ER membrane and confer disease resistance to bacterial blight in transgenic rice

Jun Wang[1], Xuan Zeng[1], Dongsheng Tian[1], Xiaobei Yang[1], Lanlan Wang[1] and Zhongchao Yin[1,2]

[1]Temasek Life Sciences Laboratory, 1 Research Link, National University of Singapore, Singapore 117604, Republic of Singapore.

[2]Department of Biological Sciences, 14 Science Drive, National University of Singapore, Singapore 117543, Republic of Singapore.

Author for correspondence:

Zhongchao Yin

Tel: +65 68727420

E-mail: yinzc@tll.org.sg

Contact information of other authors

Jun Wang: wj366192751@163.com

Xuan Zeng: zengxuan@scbg.ac.cn

Dongsheng Tian: tiands@tll.org.sg

Xiaobei Yang: belinda0213@163.com

Lanlan Wang: wanglanlan19850228@163.com

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Summary

Transcription activator-like effector (TALE)-dependent dominant disease resistance (*R*) genes in plants, also referred to as executor *R* genes, are induced upon infection by phytopathogenic bacteria of the genus *Xanthomonas* harbouring the corresponding TALE genes. Unlike the traditional R proteins, the executor R proteins do not determine the resistance specificity and may function broadly in different plant species. The executor *R* gene *Bs4C-R* in the resistant genotype PI 235047 of the pepper species *Capsicum pubescens* (*CpBs4C-R*) confers disease resistance to *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) harbouring the TALE genes *avrBsP/avrBs4*. In this study, the synthetic genes of *CpBs4C-R* and two other *Bs4C*-like genes, the susceptible allele in the genotype PI585270 of *C. pubescens* (*CpBs4C-S*) and the *CaBs4C-R* homolog gene in the cultivar "CM334" of *Capsicum annum* (*CaBs4C*), were characterized in tobacco (*Nicotiana benthamiana*) and rice (*Oryza sativa*). The *Bs4C* genes induced cell death in *N. benthamiana*. The functional Bs4C-eCFP fusion proteins were localized to the ER membrane in the leaf epidermal cells of *N. benthamiana*. The *Xa10* promoter-*Bs4C* fusion genes in transgenic rice conferred strain-specific disease resistance to *Xanthomonas oryzae* pv. *oryzae*, the causal agent of bacterial blight in rice, and were specifically induced by the *Xa10*-incompatible *Xoo* strain PXO99^{A}(pHM1avrXa10). The results indicated that the Bs4C proteins from the pepper species function broadly in rice and the Bs4C protein-mediated cell death from the ER is conserved between dicotyledonous and monocotyledonous plants, which can be utilized to engineer novel and enhanced disease resistance in heterologous plants.

Key words: *Bs4C*, TAL effector, *R* gene, rice, bacterial blight, *Xa10*

Introduction

Transcription activator-like effectors (TALEs) comprise a large family of bacterial type III effectors with sequence-specific DNA binding activity found in many species and pathovars of the genus *Xanthomonas*, with more distant orthologs in *Ralstonia solanacearum* and *Burkholderia rhizoxinica* where they play important roles in host-pathogen interactions (Boch & Bonas, 2010, Lange *et al.*, 2013, Juillerat *et al.*, 2014, Lange *et al.*, 2014). TALEs share a highly conserved tripartite protein structure (Schreiber *et al.*, 2015, Boch & Bonas, 2010). The N-terminal region of TALEs harbours signals for secretion through type III secretion system and translocation into the plant cell. The C-terminal region contains a host transcription factor binding domain (Yuan *et al.*, 2016), nuclear localization signals and an acidic activation domain. TALEs differ mostly in the central DNA-binding domain which is composed of nearly identical tandem repeats of typically 34 amino acids with repeat-variable di-residue (RVD) at positions 12 and 13 that determine DNA binding specificity (Boch *et al.*, 2009, Moscou & Bogdanove, 2009, Deng *et al.*, 2012, Mak *et al.*, 2012). TALEs bind specifically to short DNA elements, also termed as effector binding elements (EBE), in the promoters of the targeted host genes in a "one RVD to one nucleotide" manner and activate gene expression (Boch et al., 2009,

Moscou & Bogdanove, 2009). The bacteria usually use TALEs to target host susceptibility (*S*) genes for disease development (Yang *et al.*, 2006, Sugio *et al.*, 2007, Antony *et al.*, 2010, Yu *et al.*, 2011, Zhou *et al.*, 2015, Streubel *et al.*, 2013, Kay *et al.*, 2007, Cernadas *et al.*, 2014, Hu *et al.*, 2014). On the other hand, plants co-evolve disease resistance (*R*) genes to counteract bacterial infection by taking advantage of TALE activity. Three types of TALE-dependent *R* genes have been reported, which confer recessive, dominant non-transcriptional and dominant transcriptional based resistance. TALE-dependent recessive resistance occurs in plants with DNA polymorphisms of EBEs in *S* gene promoters, which enable the *S* genes to avoid being targeted by TALEs (Chu *et al.*, 2006, Zhou et al., 2015). The dominant non-transcriptional based resistance is represented solely by the classical NBS-LRR resistance gene from tomato, *Bs4*, which was identified as the cognate *R* gene to the TALE genes *avrBsP/avrBs4* (Bonas *et al.*, 1993, Schornack *et al.*, 2004). The transcriptional activity of the TALEs is not required for *Bs4* resistance elicitation as the truncated versions of AvrBs4 also trigger disease resistance (Schornack et al., 2004). The dominant transcriptional based *R* genes, also referred to as executor *R* genes for cell death (Bogdanove *et al.*, 2010), are directly targeted and activated by TALEs and the expressed R proteins trigger hypersensitive response (HR) for disease resistance (Tian *et al.*, 2014, Römer *et al.*, 2007, Gu *et al.*, 2005, Strauß *et al.*, 2012, Wang *et al.*, 2015). It should be noted that the specificities of the executor *R* gene-mediated disease resistance are determined by the EBEs in the promoters of *R* genes rather than by the R proteins, and new resistance specificities could be engineered by adding multiple EBEs to the promoters of executor *R* genes (Zeng *et al.*, 2015, Hummel *et al.*, 2012).

Bacterial spot of pepper and tomato, caused by *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) (Thieme *et al.*, 2005), and bacterial blight of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Salzberg *et al.*, 2008), are two devastating diseases that severely affect commercial production of these crops. In both pathosystems, TALEs from *Xcv* or *Xoo* strains contribute to virulence by targeting to host *S* genes or interfere with host resistance response (Yang et al., 2006, Sugio et al., 2007, Antony et al., 2010, Yu et al., 2011, Zhou et al., 2015, Streubel et al., 2013, Kay et al., 2007, Ji *et al.*, 2016), whereas plants have co-evolved different types of *R* genes to counteract bacterial infection (Gu et al., 2005, Tian et al., 2014, Wang et al., 2015, Chu et al., 2006, Iyer & McCouch, 2004, Römer et al., 2007, Strauß et al., 2012, Schornack et al., 2004). Three executor *R* genes, *Xa10*, *Xa23* and *Xa27*, have been isolated from rice and their expression confers race-specific disease resistance to *Xoo* strains harbouring the cognate TALE genes, *avrXa10*, *avrXa23* and *avrXa27* (Gu et al., 2005, Tian et al., 2014, Wang et al., 2015). The *Xa27* gene encodes a compact executor R protein with unknown biochemical function, which is localized to the apoplast (Gu et al., 2005, Wu *et al.*, 2008). The gene products of the *Xa10* and *Xa23* form a small executor R protein family (Wang et al., 2015, Tian et al., 2014). They locate to the ER membrane by forming oligomers, where they trigger cell death by disrupting the ER and cellular Ca^{2+} homeostasis (Wang *et al.*, 2017, Tian et al., 2014). Unlike Xa27

that only triggers HR in rice, the Xa10/Xa23 family proteins induce cell death in both rice and *Nicotiana benthamiana* (Wang et al., 2017, Tian et al., 2014). Two executor *R* genes, *Bs3* and *Bs4C-R*, have been isolated from pepper (Römer et al., 2007, Strauß et al., 2012). The *Bs3* gene in the species *Capsicum annuum* encodes a flavin-dependent monooxygenase (Römer et al., 2007). The *Bs4C-R* gene in the genotype PI 235047 of the species *Capsicum pubescens* (*CpBs4C-R*) encodes a putative 164-amino acid protein that shares no significant homology to any other protein of known function (Strauß et al., 2012). The *Bs4C-R* gene in pepper mediates recognition of TALE protein AvrBs4 from *Xcv* (Strauß et al., 2012). As mentioned above, AvrBs4, acting as an avirulence gene product, also triggers *Bs4*-dependent disease resistance in tomato to *Xcv* (Schornack et al., 2004). Genomes of *solanaceous* species contain *Bs4C*-like genes, such as the susceptible allele in the genotype PI 585270 (*CpBs4C-S*) and the *Bs4C-R* homolog gene in *C. annuum* cultivar "CM334" (*CaBs4C*), and they were found to be under tight transcriptional control (Strauß et al., 2012). As part of an effort to characterize the functions of the *Bs4C* genes, here we report the results on the subcellular localization of the three Bs4C proteins (CaBS4C, CpBs4C-R and CpBs4C-S) in *N. benthamiana* and their function in heterologous and monocotyledonous rice plants for disease resistance to bacterial blight.

Results

The synthetic *Bs4C* genes induce cell death in *N. benthamiana*

The coding regions of the three *Bs4C* genes (*CaBs4C*, *CpBs4C-R* and *CpBs4C-S*) were chemically synthesized based on their genomic sequence published previously (Strauß et al., 2012). The codons of the synthetic *Bs4C* genes were optimized to facilitate their expression in rice. The deduced gene products of the synthetic *Bs4C* genes are identical to those encoded by the native *Bs4C* genes in *C. annum* (*CaBs4C*) or *C. pubescens* (*CpBs4C-R* and *CpBs4C-S*). To avoid redundancy, the synthetic *Bs4C* genes were still designated as *CaBs4C*, *CpBs4C-R* and *CpBs4C-S*, respectively. The gene products of the three *Bs4C* genes (CaBS4C, CpBs4C-R and CpBs4C-S) share high identity at amino acid level (Figure 1a). The *Bs4C* genes were fused with CaMV 35S promoter (P_{35S}) and nopaline synthase gene terminator (T_{Nos}) to generate fusion genes, P_{35S}*:CaBs4C:*T_{Nos}, P_{35S}*:CpBs4C-R:*T_{Nos} and P_{35S}*:CpBs4C-S:*T_{Nos}. The *Bs4C* fusion genes were transiently expressed in *N. benthamiana* by agroinfiltration. At 24 hours after infiltration (HAI), cell death was observed in *N. benthamiana* infiltrated with agrobacteria harbouring binary constructs containing P_{35S}*:CaBs4C:*T_{Nos}, P_{35S}*:CpBs4C-R:*T_{Nos} or P_{35S}*:CpBs4C-S:*T_{Nos} (Figure 2). No cell death was observed in *N. benthamiana* infiltrated with agrobacteria harbouring empty vector (Figure 2). The *Bs4C* gene-induced cell death in *N. benthamiana* was further confirmed by trypan blue staining (Figure 2). Previous reports demonstrated that the constitutive or inducible expression of *Bs4C-R* or *Bs4C-S* genes (*CpBs4C-R* or *CpBs4C-S* in this study) in *N. benthamiana* triggered cell death (Strauß et al., 2012). The results in this study

indicated that, like *CpBs4C-R* or *CpBs4C-S*, *CaBs4C* encodes a functional executor R protein that triggers cell death in *N. benthamiana* as well.

The Bs4C proteins are localized to the ER membrane

Protein structure prediction indicated that the Bs4C proteins are putative transmembrane proteins with each containing 4 predicted transmembrane helices and showing similar topography on membrane to that of the Xa10 protein, even though members of the two executor R protein families share no identity at amino acid level (Figure 1b) (Tian et al., 2014, Wang et al., 2017). To detect the subcellular localization of the three Bs4C proteins, the *Bs4C* genes were fused in-frame with enhanced cyan fluorescent protein gene (*eCFP*). The 35S-driven *Bs4C* fusion genes (P_{35S}*:CaBs4C-eCFP:*T_{Nos}, P_{35S}*:CpBs4C-R-eCFP:*T_{Nos} and P_{35S}*:CpBs4C-S-eCFP:*T_{Nos}) induced cell death in *N. benthamiana* (data not shown), indicating that the Bs4C-eCFP fusion proteins (CaBs4C-eCFP, CpBs4C-R-eCFP and CpBs4C-S-eCFP) were functional. The *Bs4C* fusion genes were then co-expressed with the ER membrane marker gene P_{35S}*:eYFP-RcDGAT2:*T_{Nos} in *N. benthamiana* through agroinfiltration (Tian et al., 2014). Confocal microscopy study demonstrated that the Bs4C-eCFP fusion proteins and eYFP-RcDGAT2 were co-localized to the ER membrane in leaf epidermal cells of *N. benthamiana* (Figure 3). The results indicated that the three Bs4C proteins are localized to the ER membrane.

Generation of transgenic rice plants containing synthetic *Bs4C* genes

The coding regions of the *Bs4C* genes were used to replace the open reading frame of the *Xa10* gene in binary construct pCS4671 to generate fusion genes P_{Xa10}*:CaBs4C:*T_{Xa10}, P_{Xa10}*:CpBs4C-R:*T_{Xa10} and P_{Xa10}*:CpBs4C-S:*T_{Xa10}, respectively (Tian et al., 2014, Zeng et al., 2015) (Figure 4). Each fusion gene is comprised of a 2456-bp *Xa10* promoter, a 495-bp open reading frame of the synthetic *Bs4C* genes, a 378-bp *Xa10* terminator and a 1456-bp downstream region. As the *Xa10* promoter contain EBE_{AvrXa10}, which is the specific binding element of AvrXa10 (Tian et al., 2014), the *Bs4C* fusion genes were expected to be specifically induced upon inoculation with the *Xa10*-imcompatible *Xoo* strain PXO99^{A}(pHM1avrXa10).

The *Bs4C* fusion genes in binary constructs were used to produce transgenic rice in cultivar Nipponbare background *via Agrobacterium*-mediated transformation. The regenerated T_0 plants were inoculated with PXO99^{A}(pHM1avrXa10). The results of disease evaluation for resistance to bacterial blight were summarized in Table 1. In brief, 18 of the total 25 putative T_0 plants of P_{Xa10}*:CaBs4C:*T_{Xa10}, 83 of the total 115 putative T_0 plants of P_{Xa10}*:CpBs4C-R:*T_{Xa10} and 82 of the total 103 putative T_0 plants of P_{Xa10}*:CpBs4C-S:*T_{Xa10} were resistant to PXO99^{A}(pHM1avrXa10). It should be mentioned that most of the resistant transgenic plants had stress-related phenotypes, including lesion mimics, stiff leaves and retardation of growth and development. Molecular analysis indicated

that these stress-related phenotypes were resulted from constitutive or leaky expression of P_{Xa10}*:CaBs4C:*T_{Xa10}, P_{Xa10}*:CpBs4C-R:*T_{Xa10} or P_{Xa10}*:CpBs4C-S:*T_{Xa10} in transgenic plants (data not shown). The expression of the executor *R* genes in rice, such as *Xa10* and *Xa27*, are tightly controlled in the absence of corresponding TALEs, but their transgenes in transgenic plants were frequently found to show constitutive or leaky expression, possibly due to the position effect of the transgenes in rice genome (Zeng et al., 2015, Gu et al., 2005). Transgenic line 50 of P_{Xa10}*:CaBs4C:*T_{Xa10} (L50), line 64 of P_{Xa10}*:CpBs4C-R:*T_{Xa10} (L64) and line 120 of P_{Xa10}*:CpBs4C-S:*T_{Xa10} (L120) displayed normal morphological phenotype in growth and development and conferred disease resistance to PXO99^{A}(pHM1avrXa10). They were selected for further molecular and genetic studies. The T_0 plant of L50 contained at least 8 copies of T-DNA (data not shown). A T_3 plant of L50 (T_0-50/T_1-31/T_2-25/T_3-23 or L50/T_3-23) was identified to contain 3-4 copies of T-DNA detected by the *Hpt* probe (Figure 5; Figure S1). At least one copy of the T-DNAs contained the intact P_{Xa10}*:CaBs4C:*T_{Xa10} gene by producing the expected 4.7-kb *Pst*I-*Xba*I band, detected by the P_{Xa10} probe in southern blot analysis (Figure 5). The other copies of the T-DNAs in L50/T_3-23 produced 3 bands with molecular size larger than 4.7 kb (Figure 5), which might be resulted from DNA mutation or rearrangement during T-DNA integration. Using similar approach, a T_1 plant of L64 (L64/T_1-27) and a T_1 plant of L120 (L120/T_1-95) were identified to contain intact P_{Xa10}*:CpBs4C-R:*T_{Xa10} and P_{Xa10}*:CpBs4C-S:*T_{Xa10} genes, respectively (Figure 5; Figure S1). T_0-64/T_1-27 carried 2 copies of T-DNAs and one copy of the T-DNAs contained the intact P_{Xa10}*:CpBs4C-R:*T_{Xa10} gene (Figure 5). L120/T_1-95 carried one copy of T-DNA that contained the intact P_{Xa10}*:CpBs4C-S:*T_{Xa10} gene (Figure 5).

Transgenic *Bs4C* rice plants conferred AvrXa10-dependent disease resistance to *Xoo* strains

The progeny of L50/T_3-23, L64/T_1-27 and L120/T_1-95 that contained the intact 4.7-kb *Bs4C* fusion genes were inoculated with PXO99^{A}(pHM1) and PXO99^{A}(pHM1avrXa10), respectively. Transgenic *Bs4C* plants (T_4 plants of L50 of P_{Xa10}*:CaBs4C:*T_{Xa10} or L50/T_4, T_2 plants of L64 of P_{Xa10}*:CpBs4C-R:*T_{Xa10} or L64/T_2, and T_2 plants of L120 of P_{Xa10}*:CpBs4C-S:*T_{Xa10} or L120/T_2) conferred specific resistance to PXO99^{A}(pHM1avrXa10), but were susceptible or moderately susceptible to PXO99^{A}(pHM1) (Figure 6 and Table 2). In control experiment, Nipponbare was susceptible to both PXO99^{A}(pHM1) and PXO99^{A}(pHM1avrXa10) (Figure 6 and Table 2). The results indicated that the *Bs4C* genes under the control of *Xa10* promoter in the transgenic *Bs4C* rice plants conferred AvrXa10-dependent resistance to PXO99^{A}(pHM1avrXa10).

The AvrXa10-dependent *Bs4C* induction in transgenic rice plants were detected by quantitative RT-PCR. The expression of P_{Xa10}*:CaBs4C:*T_{Xa10} was almost undetectable in the non-inoculated L50/T_4 plants, whereas very low levels of gene expression were detected in the non-inoculated L64/T_2 plants of P_{Xa10}*:CpBs4C-R:*T_{Xa10} and L120/T_2 plants of P_{Xa10}*:CpBs4C-S:*T_{Xa10}, respectively (Figure 7). The expression of transgenic *Bs4C* genes was increased in the L50/T_4, L64/T_2

and L120/T_2 plants at 48 hours after inoculation with PXO99^A(pHM1avrXa10) (Figure 7). No gene induction of the *Bs4C* transgenes was detected in the three transgenic lines at 48 hours after inoculation with strain PXO99^A(pHM1) and their expression levels were similar to those of non-inoculated transgenic plants (Figure 7). The results indicated that the *Bs4C* transgenes in rice were specifically induced in the presence of AvrXa10 from PXO99^A(pHM1avrXa10). The results also demonstrated the disease resistance genes *CaBs4C*, *CpBs4C-R* and *CpBs4C-S* from dicot plant pepper functioned broadly in monocot plant rice.

Discussion

Except for Xa10 and Xa23, other executor R proteins show great diversity at the amino acid level (Gu et al., 2005, Römer et al., 2007, Strauß et al., 2012, Tian et al., 2014, Wang et al., 2015, Wang et al., 2017). As for the subcellular localization, Bs3 is a flavin-dependent monooxygenase and the GFP-tagged Bs3 (Bs3-GFP) protein was observed in both cytoplasm and nuclei of leaf cells of *N. benthamina* (Römer et al., 2007). Xa27 was found to be translocated to the apoplast of the plant cells and a signal-anchor-like sequence at the N-terminal region of Xa27 was required for its translocation and disease resistance to *Xoo* (Wu et al., 2008). The Xa10 proteins are localized to the ER membrane by forming hexamers or higher oligomers, so may be the Xa23 proteins (Tian et al., 2014, Wang et al., 2017). In this study, we found that the eCFP-tagged Bs4C proteins, CaBs4C-eCFP, CpBs4C-R-eCFP and CpBs4C-S-eCFP, were localized to the ER membrane (Figure 3). The ER is an essential organelle of eukaryotic cells that is involved in multiple cellular processes, including calcium homeostasis, protein secretion and lipid biosynthesis. A few ER resident proteins were identified as cell death regulators involved in plant innate immunity (Tian et al., 2014, Zhu *et al.*, 2010, Xu *et al.*, 2012, Liebrand *et al.*, 2012, Caplan *et al.*, 2009, Carvalho *et al.*, 2014). Our previous studies demonstrated that the overexpression of ER membrane-localized Xa10 or Xa23 in *N. benthamiana* induces the depletion of Ca^{2+} from the ER lumen (Tian et al., 2014, Wang et al., 2017). However, no such activity was detected with the three Bs4C proteins in this study (data not shown).

Although the Bs4C and Xa10 proteins might work through different cell death signalling from the ER, the ER-localized executor R proteins function broadly across monocotyledonous and dicotyledonous plants, which made it possible for us to engineer AvrXa10/$EBE_{AvrXa10}$-dependent and Bs4C-mediated transgenic resistance in rice. Transgenic resistance has long been used as a method for controlling plant diseases (Cillo & Palukaitis, 2014, Saharan *et al.*, 2016). Successfully genetic engineering by introducing *R* genes from unrelated plant species has been reported to generate broad-spectrum resistant plants (Zhao *et al.*, 2005, Thilmony *et al.*, 1995, Whitham *et al.*, 1996, Tai *et al.*, 1999). Among which, the *R* gene *Rxo1* from maize was demonstrated to confer resistance against *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*), the causal agent of bacterial leaf streak of rice, when introduced into rice (Zhao et al., 2005). The generation of controlled cell death through genetic

engineering to mimic the HR of plant cells for disease resistance is another approach to generate transgenic resistance. A successful example is the inhibition of fungal sporulation of the fungus *Phytophthora infestans*, the causal agent of potato late blight, by the inducible cell death controlled by barnase and bastar in transgenic potato plants (Strittmatter *et al.*, 1995). The native function of TALE-dependent *R* gene products as the executors of HR or programmed cell death in plants make them as ideal candidates for engineering controlled cell death in distantly related plant species. In this study, the transgenic rice plants carrying the *Bs4C* genes conferred specific disease resistance to PXO99^{A}(pHM1avrXa10) and the *Bs4C* transgenes in transgenic plants were specifically induced by AvrXa10 from the incompatible pathogen. Among which, the interaction between AvrXa10 and $EBE_{AvrXa10}$ in the *Xa10* promoter determined recognition specificity, while the induced Bs4C proteins triggered HR and disease resistance. This proof-of-concept study, together with the previous studies (Tian et al., 2014, Wang et al., 2015, Wang et al., 2017), indicates that the executor R proteins, such as Bs4C and Xa10, can be used to engineer transgenic resistance in distantly related plant species against phytopathogenic bacteria that rely on TALE or TALE-like effectors for virulence. By changing and adding multiple EBEs in the promoters of the executor *R* genes, novel and broad-spectrum resistance specificity could be generated in desirable plant species (Zeng et al., 2015, Hummel et al., 2012).

Experimental procedures

Rice line and growth condition

Nipponbare is a cultivar of japonica rice, which is susceptible to many *Xoo* strains. Rice plants were grown in greenhouse at a temperature of 30°C for 12.5 h (light) and 26°C for 11.5 h (dark) with average humidity at 84%.

Genes and constructs

The DNA sequences of the coding regions of the *CaBs4C*, *CpBs4C-R* and *CpBs4C-S* genes were synthesized by GenScript® (Piscataway, NJ 08854, USA) after codon optimization for gene expression in rice. The coding regions of the synthetic *CaBs4C*, *CpBs4C-R* and *CpBs4C-S* genes were fused with the *Xa10* promoter through PCR amplification. The 1196-bp *Bam*HI-*Apa*I fragments of the fusion genes were used to replace the corresponding *Bam*HI-*Apa*I fragment of the *Xa10* gene in pCSA4671 (Tian et al., 2014) to generate binary constructs pC4671-CaBs4C, pC4671-CpBs4C-R and pC4671-CpBs4C-S, which contain P_{Xa10}*:CaBs4C:*T_{Xa10}, P_{Xa10}*:CpBs4C-R:*T_{Xa10} and P_{Xa10}*:CpBs4C-S:*T_{Xa10} genes, respectively. The coding regions of the *CaBs4C*, *CpBs4C-R* and *CpBs4C-S* genes were also used to replace the *GUSPlus* gene in pCAMBIA1305.1 to generate binary constructs pC35S-CaBs4C, pC35S-CpBs4C-R and pC35S-CpBs4C-S, which contain P_{35S}*:CaBs4C:*T_{Nos}, P_{35S}*:CpBs4C-R:*T_{Nos} and P_{35S}*:CpBs4C-S:*T_{Nos} genes, respectively. Similarly, the coding regions of the *CaBs4C*,

CpBs4C-R and *CpBs4C-S* genes were fused with the open reading frame of enhanced cyan fluorescent protein gene (*eCFP*) and the fusion genes were used to generate binary constructs pC35S-CaBs4C-eCFP, pC35S-CpBs4C-R-eCFP and pC35S-CpBs4C-S-eCFP, which contain P_{35S}*:CaBs4C-eCFP:*T_{Nos}, P_{35S}*:CpBs4C-R-eCFP:*T_{Nos} and P_{35S}*:CpBs4C-S-eCFP:*T_{Nos} genes, respectively. The binary constructs were introduced into *A. tumefaciens* strains AGL1 for rice transformation or GV3101 for infiltration of *N. bentahmiana*. The cosmid construct pHM1avrXa10, which harbours the *avrXa10* gene under the *LacZ* promoter, has been reported in previous study (Tian et al., 2014). The constructs used in this study are listed in Table S1.

Rice transformation

Agrobacterium-mediated transformation of Nipponbare was conducted according to the method described previously (Zeng et al., 2015).

Southern blot analysis

About 2μg of rice genomic DNA was digested with appropriate restriction enzymes. The digested DNA samples were separated completely on 0.8% agarose gel and blotted to Hybond^{TM}-N+ nylon membrane (Amersham Biosciences, Piscataway, NJ 08855-1327, USA). DNA hybridization and detection of the interested genes or DNA fragments were conducted using the DIG DNA labelling and detection kit (Roche Applied Science, Penzberg, Upper Bavaria, Germany) in accordance with the manufacturer's instructions. The DNA probe for detecting the copy number of T-DNA was *hpt* probe derived from the coding region of the *Hpt* gene and the common DNA probe for detecting the fusion *Bs4C* genes was P_{Xa10} probe derived from the *Xa10* promoter (Figure 4). The oligo DNA primer pairs were Hpt-F(5'AAAAAGCCTGAACTCACCGCG3')/Hpt910-1(5' TACTTCTACACAGCCATCGGT3') for the *hpt* probe and PXa10-F(5'AGCTTACGAAGGTTGAGAGC3')/PXa10-R(5'GAGGAGTGAACGTGATTGCG3') for the P_{Xa10} probe.

qRT-PCR

Total RNA was extracted from rice leaf tissues using RNeasy Plant Mini Kit (Qiagen, 40724 Hilden, Germany). About 1 μg of total RNA was treated by DNase I. The first-strand cDNAs were synthesized using the iScript cDNA synthesis kit (Bio-Rad, Hercules, California 94547, USA) according to the manufacturer's instructions. The quantitative PCR was conducted using a CFX96 real-time PCR system (Bio-Rad). A standard reaction mixture (20 μl) contained 1 μl cDNA template, 10 μl KAPA SYBR® FAST qPCR Master Mix (2X) Universal (KAPABIOSYSTEMS, Boston, Massachusetts, USA) and 200 nM forward and reverse primers. The PCR reaction was conducted at an initial denaturing step of 95°C for 3 min, followed by 40 cycles of 95°C for 3 s, 60°C for 30 s.

PCR product specificity was confirmed by melting curve analysis and agarose gel electrophoresis to ensure that the PCRs were free of primer dimers. The expression of rice ubiquitin gene 5 (Os01g0328400, *OsUbi5*) was used as the internal control. The qRT-PCR experiments were conducted in triplicate and the data were presented as means ± SD. The oligo DNA primers were CaBs4C-Q-F(5'ACCACCCAATCATCAGAATACG3')/CaBs4C-Q-R(5'TTACCGATCCAACTTGAGCAGAGA3') for the P_{Xa10}*:CaBs4C:*T_{Xa10} gene, CpBs4C-R-Q-F(5'TACGACCTCAGCCGATCTTT3')/CpBs4C-R-Q-R(5'CAGAATCCTCTGCCGAAAAC3') for the P_{Xa10}*:CpBs4C-R:*T_{Xa10} gene, CpBs4C-S-Q-F(5'CATCACGATCACGCTCAGTT3')/CpBs4C-S-Q-R(5'GCGATTTGTTTGGGTCTCAT3') for the P_{Xa10}*:CpBs4C-S:*T_{Xa10} gene and UBQ5-F(5'AACCACTTCGACCGCCACT3')/UBQ5-R(5'GTTCGATTTCCTCCTCCTTCC3') for the *OsUbi5* gene.

Agroinfiltration of *N. benthamiana* and confocal microscopy

A. tumefaciens strain GV3101 harbouring binary constructs were cultured in 5 ml of LB liquid medium (10g/L tryptone, 10 g/L NaCl, 5 g/L yeast extract, pH7.0) with appropriate antibiotics at 28°C until the density of bacteria reached an optical density of 0.8 at 600 nm (OD_{600}), and then sub-cultured in 100 ml of AB liquid medium (3g/L K_2HPO_4, 1 g/L NaH_2PO_4, 1 g/L NH_4Cl, 0.3g/L $MgSO_4{\cdot}7H_2O$, 0.15 g/L KCl, 0.01 g/L $CaCl_2$, 0.0025 g/L $FeSO_4.7H_2O$, 5g/L glucose, pH7.0) at 28°C until the density of bacteria reached 0.8 at OD_{600}. The bacteria were collected and re-suspended in 11mM $MgCl_2$ to the density of 0.6~0.8 at OD_{600}. 2-(N-morpholino) ethanesulfonic acid (MES) and Acetosyringone (AS) were then added to the bacterial solution at the final concentration of 10mM and 200μM, respectively. *N. benthamiana* plants were grown in a growth room with 16 hours of light and 8 hours of dark at 25°C. Leaves of 4-week old *N. benthamiana* plants were used for infiltration as described previously (Kay et al., 2007). For confocal microscopy, leaf sections were examined on an LSM 510 Exciter Upright confocal microscope (Carl Zeiss, Oberkochen, Germany). The excitation/emission combinations were 405/475- to 525-nm band pass for eCFP, 514/560- to 615-nm band pass for eYFP and 514/610-nm band pass for autofluorescence of chlorophyll.

Trypan blue staining

The trypan blue staining solution was prepared by diluting trypan blue stock solution (10 g phenol, 10 ml glycerol, 10 ml lactic acid, 10 ml water and 0.02 g trypan) (Sigma-Aldrich, St. Louis, Missouri, USA) with 96% ethanol (1:2 v/v). Leaves of *N. benthamiana* were boiled in trypan blue staining solution for 1 min and left in the solution at room temperature for 24 hours. Stained leaves were subsequently de-stained in chloral hydrate solution (2.5 g of chloral hydrate dissolved in 1 ml of distilled water) for 72 hours.

Bacterial blight inoculation

Xoo strains were cultured in the PSA plate (10 g/L peptone, 10 g/L sucrose, 1 g/L glutamic acid, 16 g/L bacto-agar, and pH 7.0) at 28°C for 48 hours. Bacterial cells were collected and re-suspended in sterile water to the density of 0.5 at OD_{600}. Bacterial blight inoculation on fully expanded leaves of 6-week old rice plants was carried out using the leaf-clipping method (Kauffman, 1973). The lesion length (L.L.) was measured 14 days after inoculation. The disease symptom was scored as resistant (R, L.L. ≤ 3.0 cm), moderately resistant (MR, 3.0 cm < L.L. ≤ 6.0 cm), moderately susceptible (MS, 6.0 cm < L.L. ≤ 9.0 cm) and susceptible (S, L.L. > 9.0 cm). To detect the specific induction of transgenes upon inoculation with compatible or incompatible *Xoo* strains, leaves of four-week old transgenic plants were infiltrated with bacterial inoculum using a needleless syringe. Leaf tissues were collected at 48 hours after infiltration.

Protein analysis

The amino acid sequences of Bs4C proteins were aligned using the program ClustalW (https://embnet.vital-it.ch/software/ClustalW.html) and the output was shaded using GENEDOC software (Nicholas & Nicholas). The prediction of transmembrane helix of the Bs4C proteins was done with SOSUI program (http://harrier.nagahama-i-bio.ac.jp/sosui/).

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References

Antony, G., Zhou, J., Huang, S., Li, T., Liu, B., White, F., *et al.* (2010) Rice *xa13* recessive resistance to bacterial blight is defeated by induction of the disease susceptibility gene Os-*11N3*. *Plant Cell*, **22,** 3864-3876.

Boch, J. and Bonas, U. (2010) *Xanthomonas* AvrBs3 family-type III effectors: discovery and function. *Annu. Rev. Phytopathol.*, **48,** 419-436.

Boch, J., Scholze, H., Schornack, S., Landgraf, A., Hahn, S., Kay, S., *et al.* (2009) Breaking the code of DNA binding specificity of TAL-type III effectors. *Science*, **326,** 1509-1512.

Bogdanove, A. J., Schornack, S. and Lahaye, T. (2010) TAL effectors: finding plant genes for disease and defense. *Curr. Opin. Plant Biol.*, **13,** 394-401.

Bonas, U., Conrads-Strauch, J. and Balbo, I. (1993) Resistance in tomato to *Xanthomonas campestris* pv *vesicatoria* is determined by alleles of the pepper-specific avirulence gene *avrBs3*. *Mol. Gen. Genet.,* **238,** 261-269.

Caplan, J. L., Zhu, X., Mamillapalli, P., Marathe, R., Anandalakshmi, R. and Dinesh-Kumar, S. (2009) Induced ER chaperones regulate a receptor-like kinase to mediate antiviral innate immune response in plants. *Cell Host Microbe,* **6,** 457-469.

Carvalho, H. H., Silva, P. A., Mendes, G. C., Brustolini, O. J., Pimenta, M. R., Gouveia, B. C., *et al.* (2014) The endoplasmic reticulum binding protein BiP displays dual function in modulating cell death events. *Plant Physiol.,* **164,** 654-670.

Cernadas, R. A., Doyle, E. L., Niño-Liu, D. O., Wilkins, K. E., Bancroft, T., Wang, L., *et al.* (2014) Code-assisted discovery of TAL effector targets in bacterial leaf streak of rice reveals contrast with bacterial blight and a novel susceptibility gene. *PLoS Pathog.,* **10,** e1003972.

Chu, Z., Yuan, M., Yao, J., Ge, X., Yuan, B., Xu, C., *et al.* (2006) Promoter mutations of an essential gene for pollen development result in disease resistance in rice. *Genes Dev.,* **20,** 1250-1255.

Cillo, F. and Palukaitis, P. (2014) Transgenic resistance. *Adv. Virus Res.,* **90,** 35-146.

Deng, D., Yan, C., Pan, X., Mahfouz, M., Wang, J., Zhu, J.-K., *et al.* (2012) Structural basis for sequence-specific recognition of DNA by TAL effectors. *Science,* **335,** 720-723.

Gu, K., Yang, B., Tian, D., Wu, L., Wang, D., Sreekala, C., *et al.* (2005) *R* gene expression induced by a type-III effector triggers disease resistance in rice. *Nature,* **435,** 1122-1125.

Hu, Y., Zhang, J., Jia, H., Sosso, D., Li, T., Frommer, W. B., *et al.* (2014) *Lateral organ boundaries 1* is a disease susceptibility gene for citrus bacterial canker disease. *Proc. Natl Acad. Sci. USA,* **111,** E521-E529.

Hummel, A. W., Doyle, E. L. and Bogdanove, A. J. (2012) Addition of transcription activator-like effector binding sites to a pathogen strain- specific rice bacterial blight resistance gene makes it effective against additional strains and against bacterial leaf streak. *New Phytol.,* **195,** 883-893.

Iyer, A. S. and McCouch, S. R. (2004) The rice bacterial blight resistance gene xa5 encodes a novel form of disease resistance. *Mol. Plant Microbe Interact.,* **17,** 1348-1354.

Ji, Z., Ji, C., Liu, B., Zou, L., Chen, G. and Yang, B. (2016) Interfering TAL effectors of *Xanthomonas oryzae* neutralize R-gene-mediated plant disease resistance. *Nat. Commun.,* **7,** 13435.

Juillerat, A., Bertonati, C., Dubois, G., Guyot, V., Thomas, S., Valton, J., *et al.* (2014) BurrH: a new modular DNA binding protein for genome engineering. *Sci. Rep.,* **4,** 3831.

Kauffman, H. (1973) An improved technique for evaluation of resistance of rice varieties to *Xanthomonas oryzae*. *Plant Dis. Rep.,* **57,** 537-541.

Kay, S., Hahn, S., Marois, E., Hause, G. and Bonas, U. (2007) A bacterial effector acts as a plant transcription factor and induces a cell size regulator. *Science,* **318,** 648-651.

Lange, O., Schreiber, T., Schandry, N., Radeck, J., Braun, K. H., Koszinowski, J., *et al.* (2013) Breaking the DNA- binding code of Ralstonia solanacearum TAL effectors provides new possibilities to generate plant resistance genes against bacterial wilt disease. *New Phytol.,* **199,** 773-786.

Lange, O. d., Wolf, C., Dietze, J., Elsaesser, J., Morbitzer, R. and Lahaye, T. (2014) Programmable DNA-binding proteins from Burkholderia provide a fresh perspective on the TALE-like repeat domain. *Nucleic Acids Res.,* **42,** 7436-7449.

Liebrand, T. W., Smit, P., Abd-El-Haliem, A., de Jonge, R., Cordewener, J. H., America, A. H., *et al.* (2012) Endoplasmic reticulum-quality control chaperones facilitate the biogenesis of Cf receptor-like proteins involved in pathogen resistance of tomato. *Plant Physiol.,* **159,** 1819-1833.

Mak, A. N.-S., Bradley, P., Cernadas, R. A., Bogdanove, A. J. and Stoddard, B. L. (2012) The crystal structure of TAL effector PthXo1 bound to its DNA target. *Science,* **335,** 716-719.

Moscou, M. J. and Bogdanove, A. J. (2009) A simple cipher governs DNA recognition by TAL effectors. *Science,* **326,** 1501-1501.

Nicholas, K. and Nicholas, H. J. GeneDoc: a tool for editing and annotating multiple sequence alignments. 1997. *Distributed by the author*.

Römer, P., Hahn, S., Jordan, T., Strauß, T., Bonas, U. and Lahaye, T. (2007) Plant pathogen recognition mediated by promoter activation of the pepper *Bs3* resistance gene. *Science,* **318,** 645-648.

Saharan, V., Jain, D., Pareek, S., Pal, A., Kumaraswamy, R. V., Jakhar, S. K., *et al.* (2016) Viral, Fungal and Bacterial Disease Resistance in Transgenic Plants. In: *Advances in Plant Breeding Strategies: Agronomic, Abiotic and Biotic Stress Traits.* (Al-Khayri, J. M., Jain, S. M. and Johnson, D. V., eds.). Cham: Springer International Publishing, pp. 627-656.

Salzberg, S. L., Sommer, D. D., Schatz, M. C., Phillippy, A. M., Rabinowicz, P. D., Tsuge, S., *et al.* (2008) Genome sequence and rapid evolution of the rice pathogen *Xanthomonas oryzae* pv. *oryzae* PXO99^{A}. *BMC genomics,* **9,** 204.

Schornack, S., Ballvora, A., Gürlebeck, D., Peart, J., Ganal, M., Baker, B., *et al.* (2004) The tomato resistance protein Bs4 is a predicted non- nuclear TIR-NB-LRR protein that mediates defense responses to severely truncated derivatives of AvrBs4 and overexpressed AvrBs3. *Plant J.,* **37,** 46-60.

Schreiber, T., Sorgatz, A., List, F., Blüher, D., Thieme, S., Wilmanns, M., *et al.* (2015) Refined requirements for protein regions important for activity of the TALE AvrBs3. *PLOS ONE,* **10,** e0120214.

Strauß, T., van Poecke, R. M. P., Strauß, A., Römer, P., Minsavage, G. V., Singh, S., *et al.* (2012) RNA-seq pinpoints a *Xanthomonas* TAL-effector activated resistance gene in a large-crop genome. *Proc. Natl Acad. Sci. USA,* **109,** 19480-19485.

Streubel, J., Pesce, C., Hutin, M., Koebnik, R., Boch, J. and Szurek, B. (2013) Five phylogenetically close rice *SWEET* genes confer TAL effector- mediated susceptibility to *Xanthomonas oryzae* pv. *oryzae*. *New Phytol.,* **200,** 808-819.

Strittmatter, G., Janssens, J., Opsomer, C. and Botterman, J. (1995) Inhibition of fungal disease development in plants by engineering controlled cell death. *Nat. Biotechnol.,* **13,** 1085-1089.

Sugio, A., Yang, B., Zhu, T. and White, F. F. (2007) Two type III effector genes of *Xanthomonas oryzae* pv. *oryzae* control the induction of the host genes *OsTFIIAγ1* and *OsTFX1* during bacterial blight of rice. *Proc. Natl Acad. Sci. USA,*, **104,** 10720-10725.

Tai, T. H., Dahlbeck, D., Clark, E. T., Gajiwala, P., Pasion, R., Whalen, M. C., *et al.* (1999) Expression of the *Bs2* pepper gene confers resistance to bacterial spot disease in tomato. *Proc. Natl Acad. Sci. USA,*, **96,** 14153-14158.

Thieme, F., Koebnik, R., Bekel, T., Berger, C., Boch, J., Büttner, D., *et al.* (2005) Insights into genome plasticity and pathogenicity of the plant pathogenic bacterium *Xanthomonas campestris* pv. *vesicatoria* revealed by the complete genome sequence. *J. Bacteriol.,* **187,** 7254-7266.

Thilmony, R. L., Chen, Z., Bressan, R. A. and Martin, G. B. (1995) Expression of the tomato *Pto* Gene in tobacco enhances resistance to *Pseudomonas syringae* pv *tabaci* expressing AvrPto. *Plant Cell,* **7,** 1529-1536.

Tian, D., Wang, J., Zeng, X., Gu, K., Qiu, C., Yang, X., *et al.* (2014) The rice TAL effector-dependent resistance protein XA10 triggers cell death and calcium depletion in the endoplasmic reticulum. *Plant Cell,* **26,** 497-515.

Wang, C., Zhang, X., Fan, Y., Gao, Y., Zhu, Q., Zheng, C., *et al.* (2015) XA23 is an executor R protein and confers broad-spectrum disease resistance in rice. *Mol. Plant,* **8,** 290-302.

Wang, J., Tian, D., Gu, K., Yang, X., Wang, L., Zeng, X., *et al.* (2017) Induction of *Xa10*-like genes in rice cultivar Nipponbare confers disease resistance to rice bacterial blight. *Mol. Plant Microbe Interact. ,* **30,** 466-477.

Whitham, S., McCormick, S. and Baker, B. (1996) The *N* gene of tobacco confers resistance to tobacco mosaic virus in transgenic tomato. *Proc. Natl Acad. Sci. USA,* **93,** 8776-8781.

Wu, L., Goh, M. L., Sreekala, C. and Yin, Z. (2008) XA27 depends on an amino-terminal signal-anchor-like sequence to localize to the apoplast for resistance to *Xanthomonas oryzae* pv. *oryzae*. *Plant Physiol.,* **148,** 1497-1509.

Xu, G., Li, S., Xie, K., Zhang, Q., Wang, Y., Tang, Y., *et al.* (2012) Plant ERD2-like proteins function as endoplasmic reticulum luminal protein receptors and participate in programmed cell death during innate immunity. *Plant J.,* **72,** 57-69.

Yang, B., Sugio, A. and White, F. F. (2006) *Os8N3* is a host disease-susceptibility gene for bacterial blight of rice. *Proc. Natl Acad. Sci. USA,,* **103,** 10503-10508.

Yu, Y., Streubel, J., Balzergue, S., Champion, A., Boch, J., Koebnik, R., *et al.* (2011) 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,** 1102-1113.

Yuan, M., Ke, Y., Huang, R., Ma, L., Yang, Z., Chu, Z., *et al.* (2016) A host basal transcription factor is a key component for infection of rice by TALE-carrying bacteria. *Elife,* **5,** e19605.

Zeng, X., Tian, D., Gu, K., Zhou, Z., Yang, X., Luo, Y., *et al.* (2015) Genetic engineering of the *Xa10* promoter for broad- spectrum and durable resistance to *Xanthomonas oryzae* pv. *oryzae*. *Plant Biotechnol. J.,* **13,** 993-1001.

Zhao, B., Lin, X., Poland, J., Trick, H., Leach, J. and Hulbert, S. (2005) A maize resistance gene functions against bacterial streak disease in rice. *Proc. Natl Acad. Sci. USA,,* **102,** 15383-15388.

Zhou, J., Peng, Z., Long, J., Sosso, D., Liu, B., Eom, J.-S., *et al.* (2015) Gene targeting by the TAL effector PthXo2 reveals cryptic resistance gene for bacterial blight of rice. *Plant J. ,* **82,** 632-643.

Zhu, X., Caplan, J., Mamillapalli, P., Czymmek, K. and Dinesh- Kumar, S. P. (2010) Function of endoplasmic reticulum calcium ATPase in innate immunity-mediated programmed cell death. *EMBO J.,* **29,** 1007-1018.

Supporting Information Legends

Table S1 Constructs used in this study.

Figure S1 Morphology of transgenic rice plants carrying different *Bs4C* genes. Images were taken at two months after sowing. Ni, Nipponbare; L50/T_3-23, T_3 plant of transgenic line 50 of P_{Xa10}*:CaBs4C:*T_{Xa10}; L64/T_1-27, T_1 plant of transgenic line 64 of P_{Xa10}*:CpBs4C-R:*T_{Xa10}; L120/T_1-95, T_1 plant of transgenic line 120 of P_{Xa10}*:CpBs4C-S:*T_{Xa10}.

Supplementary Sequence S1. Nucleotide sequence of the synthetic *Capsicum annuum Bs4C* gene (*CaBs4C*) and the deduced amino acid sequence of CaBs4C.

Supplementary Sequence S2. Nucleotide sequence of the synthetic *Capsicum pubescens* Bs4C-R gene (*CpBs4C-R*) and the deduced amino acid sequence of CpBs4C-R.

Supplementary Sequence S3. Nucleotide sequence of the synthetic *C. pubescens* Bs4C-S gene (*CpBs4C-S*) and the deduced amino acid sequence of CpBs4C-S.

Figure legends

Figure 1 Amino acid sequence alignment and outputs of transmembrane helix prediction of Bs4C proteins. (a) Amino acid sequence alignment of CaBs4C, CpBs4C-R and CpBs4C-S. The conserved amino acids among three or between two proteins are highlighted in black or grey, respectively. (b) The outputs of transmembrane helix prediction of CaBS4C, CpBs4C-R and CpBs4C-S.

Figure 2 Transient expression of *Bs4C* genes induced cell death in *N. benthamiana*. Images show cell death phenotypes (Left panel) and trypan blue staining (Right panel) of *N. benthamiana* leaves transiently expressing *CaBs4C*, *CpBs4C-R*, *CpBs4C-S* and empty vector at 24 hours after infiltration.

Figure 3 Subcellular localization of the Bs4C proteins in leaf cells of *N. benthamiana*.
ER membrane marker eYFP-RcDGAT2 was transiently co-expressed with eCFP, CaBs4C-eCFP, CpBs4C-R-eCFP or CpBs4C-S-eCFP in leaf epidermal cells of *N. benthamiana.* Images were taken at 24 hours after infiltration. Bars = 10 μm.

Figure 4. Schematic map of T-DNA region of binary construct for rice transformation (map not drawn to scale). Binary constructs used for rice transformation were constructed based on pCSA4671, which carrying 4671-bp genomic clone of the *Xa10* gene (Tian et al., 2014). The coding region of the *Xa10* gene has been replaced with the open reading frames of the synthetic *Bs4C* genes (*CaBs4C*, *CpBs4C-R* or *CpBs4C-S*). Restriction enzyme digestion sites and DNA probes for specific detection of the *Hpt* gene or *Xa10* gene promoter are indicated. *Hpt*, hygromycin phosphotransferase gene; LB, left border; P_{Xa10}, *Xa10* gene promoter; P_{35S}, CaMV 35S promoter; RB, right border; T_{Xa10}, *Xa10* gene terminator.

Figure 5 Detection of *Bs4C* genes in transgenic rice plants. Genomic DNA of transgenic rice plants were double digested with restriction enzymes *Pst*I and *Xba*I and subjected to southern blot analysis using *Hpt* probe (left panel) or P_{Xa10} probe (right panel). The expected 4748-bp fragments of the chimeric *Bs4C* genes is indicated with an arrow. M, lambda DNA/*Hind*III markers; Ni, Nipponbare; L50/T_3-23, T_3 plant of transgenic line 50 of P_{Xa10}*:CaBs4C:*T_{Xa10}; L64/T_1-27, T_1 plant of transgenic line 64 of P_{Xa10}*:CpBs4C-R:*T_{Xa10}; L120/T_1-95, T_1 plant of transgenic line 120 of P_{Xa10}*:CpBs4C-S:*T_{Xa10}.

Figure 6 Bacterial blight phenotype of transgenic rice plants carrying *Bs4C* genes.
Non-transgenic and transgenic rice plants were inoculated with *Xoo* strains PXO99^{A}(pHM1) and PXO99^{A}(pHM1avrXa10), respectively. The inoculated leaves were photographed at 14 days after inoculation. Ni, Nipponbare; L50/T_4, T_4 plant of transgenic line 50 of P_{Xa10}*:CaBs4C:*T_{Xa10}; L64/T_2, T_2 plant of transgenic line 64 of P_{Xa10}*:CpBs4C-R:*T_{Xa10}; L120/T_2, T_2 plant of transgenic line 120 of P_{Xa10}*:CpBs4C-S:*T_{Xa10}.

Figure 7 Expression of *Bs4C* genes in transgenic rice after inoculation with *Xoo* strains.
Transgene expression in uninoculated transgenic plants or transgenic plants at 48 hours after inoculation with PXO99^{A}(pHM1) or PXO99^{A}(pHM1avrXa10) was detected by qRT-PCR. The expression levels of transgenes were normalized against rice ubiquitin gene 5 gene (Os01g0328400). The qRT-PCR experiments were performed in triplicate, and the data are presented as means ± SD. L50/T_4, T_4 plant of transgenic line 50 of P_{Xa10}*:CaBs4C:*T_{Xa10} gene; L64/T_2, T_2 plant of transgenic line 64 of P_{Xa10}*:CpBs4C-R:*T_{Xa10}; L120/T_2, T_2 plant of transgenic line 120 of P_{Xa10}*:CpBs4C-S:*T_{Xa10}.

Table 1. Number of transgenic T_0 plants obtained from rice transformation with *Bs4C* genes

Gene	Number of T_0 plants*		
	Total	Resistant	Susceptible
P_{Xa10}*:CaBs4C:*T_{Xa10}	25	18	7
P_{Xa10}*:CpBs4C-R:*T_{Xa10}	115	83	32
P_{Xa10}*:CpBs4C-S:*T_{Xa10}	103	82	21

*Six-week-old transgenic T_0 plants were inoculated with PXO99^A(pHM1avrXa10) using leaf-clipping method and bacterial blight lesions were measured at 14 days after inoculation.

Table 2. Lesion length and disease phenotype of transgenic rice plants at 14 days after inoculation with PXO99^{A}(pHM1) and PXO99^{A}(pHM1avrXa10)

Plant	Gene	Lesion length (cm) and disease score*	
		PXO99^{A}(pHM1)	PXO99^{A}(pHM1avrXa10)
Nipponbare	N.A.	11.6±2.1 (S)	12.1±2.0 (S)
L50	P_{Xa10}:*CaBs4C*:T_{Xa10}	13.1±4.2 (S)	1.4±1.1(R)
L64	P_{Xa10}:*CpBs4C-R*:T_{Xa10}	8.7±2.5 (MS)	0.7±0.4 (R)
L120	P_{Xa10}:*CpBs4C-S*:T_{Xa10}	6.4±2.5 (MS)	0.6±0.3 (R)

*Six-week-old plants were inoculated with *Xoo* strains using leaf-clipping method and bacterial blight lesions were measured at 14 days after inoculation. The lesion length of bacterial blight is the average of 16 infected leaves from 4 inoculated plants. The standard deviation of the mean is indicated. MS, moderately susceptible; R, resistant; S, susceptible.

(a)

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CaBs4C :  MEFDLRYFILILANMLKSILS-ISNNWDPFHIIGNPIPSFMTFMNRLFFLFIFSFIFS :  57
CpBs4C-R: MEFDLRYLIILILANMLKSILS-ISDNWDPFHIFHD-HPSFIVFINKLFFLFIFSFIFS :  56
CpBs4C-S: MEFDLRYFILILLANMIKSILSSISDNWDPFHIFHD-HPSFIVFINKLFFLFIFSFIFS :  57

CaBs4C :  ITRMTLHHPIIRIRVSIT----FSKSFNILCLASLLLPQMLFWYFFFIIITLSLCSSW : 111
CpBs4C-R: ITRITLHHPNIRIRVRTTTSADLSKSFNILCLASLLLPQMLFWYFFVIIIALSSCSSW : 114
CpBs4C-S: ITRITLHHPNIRIRIRTTTSADLSKSFNILCLASLLLPQMLFWYFFFITITLSSCSSW : 115

CaBs4C :  IGNTWASFRQRILHIFSTIIFPAVSIFINVEFDRNNVISQQHETQTNHLLSLV : 164
CpBs4C-R: IGNTWASFRQRILHIFSTIIFPAVSIFINVEFDRNN-VSQQHETQTNRLLV-- : 164
CpBs4C-S: IGNAWASFRGRTLHIFST-IFPAVSIFINVEFDRNN-VSQQHETQTNRLLV-- : 164
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(b)