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Research Article

In Silico Designing of Xa13 Locus Specific TALENs for Introducing Bacterial Blight Resistance in Rice

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ABSTRACT

Bacterial blight disease in rice is caused by Xanthomonas oryzae pv. oryzae (XOO) through binding of type III secretion system effectors to susceptibility genes in host. PthXo1 effectors secreted by XOO binds to promoter region of sugar transporter gene Os8N3 thereby activating dominant allele of Xa13 susceptibility gene in rice. Introgression of recessive xa13 allele by molecular breeding programs have successfully imparted resistance against PthXo1 effector mediated disease in few rice cultivars. However, molecular breeding mediated introgression of disease resistance in hundreds of susceptible cultivars is a daunting task. Recent advancements in the field of genome editing technology by use of engineered nucleases ZFN, TALEN and CRISPR-Cas9 system allow fast and précised modifications in the genome. Therefore, the present study focus on designing of Xa13 locus specific TALENs for introducing bacterial blight resistance in Indica rice through TALEN mediated genome editing. Total three hundred thirty-three TALENs were designed against promoter region of Xa13. Out of these, One-hundred thirty-nine pairs of TALENs that follow Streubel's guidelines and having targets with >40% GC content were retained. Further, Screening of these selected TALENs on basis of distance of their putative cleavage site from PthXo1 effectors binding site in rice genome resulted into eleven TALEN pairs. These eleven TALEN pairs were further screened for their number of putative targets in host genome and on the TAL score. Finally only three pairs possess a unique target site and score below a cut-off of four. Best scoring TALEN pair was selected for designing of TALEN coding gene sequences codon-optimized for high level expression in rice. These TALEN coding genes can be used for introducing deletions in Xa13 promoter and impart resistance against bacterial blight disease in rice.

Keywords: *Genome editing, biotic stress, TALENs, computational biology, DNA-protein interaction, transcription regulation,Bacterial blight resistance,Xa13 locus.*

Abbreviations:

XOO: Xanthomonas oryzae pv. oryzae

BB: Bacterial blight

UPT: Upregulated by TALE

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INTRODUCTION

The food requirement for the growing global population is being further aggravated by different biotic and abiotic stresses. It is estimated that by the year 2050, the global population will reach to 870 million and to meet the food demand, the global food production has to increase by 60-110% (McGuire 2013; Tilman et al., 2011). Rice (*Oryza sativa*) is an important cereal crop (wheat and maize being other two important cereals consumed globally) with a global annual production of 745.7 million tons. Bacterial blight (BB)is one among the most destructive diseases of rice caused by *Xanthomonas oryzae pv. oryzae* (XOO) in Asia and Africa continents, the major zones of rice production. At global scale, XOO strains infect millions of hectares of rice annually leading to massive crop losses – upto 75% under severe infection conditions. (Savary et al., 2000; Verdier, Vera Cruz & Leach 2012). Plant susceptibility to disease depends upon developmental stage of the plant, genetic make-up of rice cultivar, pathogenic strains and environmental conditions. Rice plants are more prone to infection at seedling stage owing to wounds which may occur at the time of transplantation. Occurrence of infection at seedling stage hampers crop yield whereas at booting stage, seed quality is majorly affected trait.

BB is a vascular disease caused by pathogen invasion in plant either through hydathodes or wounds followed by xylem vessels colonization. XOO strains elicit infection in plants through type III effectors mediated activation of susceptibility genes by binding to their upstream/promoter region (Niño-Liu, Ronald, & Bogdanove 2006; White & Yang 2009). Different XOO strains cause infection in race-specific manner by secretion of specific effectors. These effectors bind to promoter region of sugar transporter genes in the host. Major susceptibility alleles, sugar transporter genes and their activators have been studied in detail(Jiang et al., 2020; Verdier et al., 2012; Vikal and Bhatia 2017; Wang et al., 2017). Dominant allele Xa13 is activated by binding of PthXo1 effector to UPT (Upregulated by TALE) box in promoter region of Os8N3 gene located at chromosome 8 in rice (T. Yuan et al., 2011). Activation of Xa13 locus makes the plant susceptible to disease.

Development of rice cultivars carrying resistance gene is the best approach to control BB disease(Verdier, Vera Cruz, and Leach 2012). Molecular breeding programmes have incorporated different resistance genes in multiple rice cultivars (Shanti et al., 2010). Incorporation of single resistance allele has found to provide race-specific resistance against disease(Ellur et al., 2016; Kameswara R. Kottapalli et al., 2007; Li et al., 2012; Sundaram et al., 2009). However, pyramiding of multiple resistance genes in host has resulted into broad spectrum resistance in different rice cultivars (Kameswara Rao Kottapalli, Narasu, and Jena 2010; Pradhan et al., 2015, 2016, 2019; Raina et al., 2019; Singh et al., 2001). Transgenic plants of an elite indica rice cultivar 'IR72' carrying Xa21 allele developed using particle bombardment method were found to be resistant against races 4 and 6 of XOO (Tu et al., 1998).

Although in last decade, molecular breeding programmes have succeeded in development of disease resistant rice cultivars and their commercialization in main rice growing countries(Arunakumari et al., 2016; Sundaram et al., 2008, 2009). However, fast evolution in bacterial strains, requirement of introgression of race-specific alleles and long-time taken in molecular breeding mediated introgression of alleles still pose major bottleneck in development of BB resistant cultivars of rice (Antony et al., 2010; M. Yuan et al., 2010).

Emergence of genome editing technologies (ZFN, TALEN and CRISPR-Cas9) have made it possible to introduce desired modification at the selected locus in diverse species(Shah et al., 2018; Yin, Gao, and Qiu 2017). A recent study reported CRISPR/Cas9 mediated knockdown of Os8N3 in rice to achieve resistance against BB (Kim, Moon, and Park 2019). Further, successful

development of broad spectrum BB resistance in rice cultivars Kitaake, IR64 and Ciherang-Sub1 by CRISPR/Cas9 genome editing technology through introduction of mutations in PthXo1 effector binding region in promoter of sugar transporter genes has been reported (Oliva et al., 2019).

Amongst ZFNs, TALENs and CRISPR-Cas9 systems of genome editing, TALENs are comparatively easy to design, have higher success rate than ZFNs and provide more target specificity (Less off target activity) than CRISPRs. Hence TALENs are a tool of choice when high target specificity is required. TALENs are generated by fusing a specific DNA recognition domain (TALE protein repeats) with a non-specific nuclease domain (FokI). DNA binding domain of TALE proteins is composed of 33–34 amino acids long repeats of nearly identical peptide sequence with two variable amino acids and each repeat binds specifically to one nucleotide in the DNA. Minor difference at 12^{th} & 13^{th} position (repeat variable di-residues or RVD) of amino acid sequence within each repeat unit determines target nucleotide. To provide high specificity in sequence recognition, a combination of 15–20 TALE repeat units is enough. Pairing of two such fusion proteins will cause dimerization and activation of FokI, which introduces a double strand break at the target locus.

xa13, also known as Os8N3 – a member of MtN3 gene family, is a recessive gene for BB resistance in rice (Chu, Fu, et al., 2006; Yang, Sugio, and White 2006). Promoter region of dominant allele of xa13 (Xa13) confers binding site (UPTPthXo1) box for type III effector PthXo1 secreted by XOO (Römer et al., 2010). Recessive allele xa13 cannot be induced by XOO due to mutation in UPT PthXo1 box thus providing resistance against bacterial blight disease. However, suppression of xa13 allele in transgenic rice plants has been observed to result into male sterility indicating its role in pollen development (Bart, Ronald, and Hake 2006; Chu, Yuan, et al., 2006). On the other hand, tissue-specific knock-down of dominant allele Xa13 using artificial miRNA technology has resulted in resistance against bacterial blight without affecting pollen development (Li et al., 2012).

In this regard, present study reports *in silico* designing of TALENs for introducing mutation at effectors binding element site inXa13 locus locus-specific.) Disruption of effector binding element will abolish the effector binding and hence provide resistance against bacterial blight in indica rice cultivar IR64, while not affecting the gene expression in other tissues and developmental stages.

MATERIALS AND METHODS

Xa13 sequences

Nucleotide sequences of Xa13 allele were identified by BLASTN against *oryza sativa* genome sub-database (taxid: 4530) using 4454 bp long complete sequence of Xa13 locus (GenBank ID: DQ421395.1) of indica rice IR64 as query sequence(NCBI 2015). E-value cut-off <0.001 was applied to get highly similar sequences.

Comparison of PthXo1 binding sequences of Xa13 allele

All sequences of xa13 allele across rice cultivars are summarized in table S1. Multiple sequence alignment of sequences of Xa13 allele in various rice cultivars was performed using CLUSTALW program in BioEdit tool (Hall 2011; Thompson, Higgins & Gibson 1994). Identity among various PthXo1 binding sequences was plotted by star indicating similarity. Deletions in sequences were plotted as a dash sign. A WebLogo of PthXo1 effector binding sequences in all susceptible rice cultivars was generated using web-based tool WebLogo3 (Crooks et al., 2004).

Designing of TALENs against Xa13 allele

Indica rice cultivar IR64 Xa13 locus specific TALENs were designed using TALEN Targeter web-tool (Doyle et al., 2012). TALENs were designed against 400 bp sequence upstream to coding sequence possessing PthXo1 effector binding site at position 151–174. Default parameters of TALEN designing were used except spacer and RVD lengths. TALENs were designed with repeat length ranging from 15–25 and

spacer length from 15–30. Pre-loaded genome sequence of *Oryza sativa* (GenBank assembly accession: GCA_002151415.1) was selected to count putative target sites of predicted TALENs. All predicted TALEN pairs were screened to select best TALENs.

Screening of predicted TALENs

TALENs predicted using TALEN targeter web-tool were screened at multiple levels. Total predicted TALENs were first screened to follow Streubel's guidelines of TALE RVD specificities and efficiencies (Streubel et al., 2012). Further, TALENs were screened for their target having more than 40% GC content. Thus obtained TALENs were screened to have predicted cut position within 20 bp range of PthXo1 effector binding site at Xa13 locus. At final stage of screening, TALENs were screened to have zero off-target sites in indica rice genome. TALENs obtained after these screenings were further analysed for their score.

Analysis of screened TALENs

TALENs obtained after four levels of screening, were further analysed to score below a cut-off of four using paired target finder tool of TAL effector nucleotide targeter 2.0 interface (Doyle et al., 2012). For analysis of TALENs score, scoring matrix by Doyle et al.,, available at web-tool interface was used.

Designing of rice codon optimized TALEN coding genes

Best scoring TALEN pair was selected for designing of gene sequence to obtain complete functional TALEN in rice. The TALEN coding gene sequence was codon-optimized for rice for its optimum expression in host (Zhoua et al., 2016).Amino acid sequences of N-terminal, C-terminal and FokI nuclease domain were retrieved from NCBI database and repeat sequences were designed by TALEN targeter. Truncated N-terminal and C-terminal sequences were taken from natural TALE proteins in XOO and FokI nuclease domain sequence was taken from *Flavobacterium okenokoites*. The TALEN coding DNA sequence of complete functional TALEN was designed for its optimum expression in rice through synonymous mutations using gene designer tool(Villalobos et al., 2006). Major parameters considered for synthetic DNA sequence designing include codon usage, GC content, GC3 content and removal of splicing sites, mRNA destabilizing sequences and polyadenylation sites.

RESULTS AND DISCUSSION

This study aims at designing and analysis of Xa13 locus-specific TALENs for their use in development of bacterial blight disease resistant rice cultivar IR64, through genome editing. However, Xa13 locus mediated disease resistance has been observed to occur naturally in some rice cultivars through mutation in PthXo1 effector binding sequence in promoter region of sugar transporter gene Os8N3(T. Yuan et al., 2011). Although, classical breeding programs have successfully imparted BB disease resistance in a few rice cultivars which have been commercialized in different regions of world (Aruna kumari et al., 2016; Kameswara Rao Kottapalli, Narasu, and Jena 2010; Shanti et al., 2010; Sundaram et al., 2008). However, need of speedy development of as many possible rice cultivars drives the need of implementation of improved genome editing strategies.

Identification/retrieval of Xa13 locus sequences

Thirty-eight sequences across all BB susceptible and resistant rice cultivars were identified using BLASTN search in NCBI *oryza sativa* sub-database (4530) for identification of sequences similar to indica rice IR64 Xa13 locus sequence (Figure S1). Out of total thirty-eight sequences, twelve sequences from different rice cultivars belonging to chromosome 8 were retained for further analyses (Table S1).

Comparison of PthXo1 binding sequences of Xa13 allele

Analysis of twelve sequences of Xa13 allele revealed presence of PthXo1 effector binding site only in susceptible rice cultivars. Further analysis of promoter region sequence showed deletion of PthXo1 effector binding site in all BB resistant cultivars (Figure 2A). Comparison of twelve sequences showed

conservation of PthXo1 effector binding sequence in susceptible rice cultivars (Figure 2B).

Designing of TALENs against Xa13 allele

A 400 bp long sequence upstream to Os8N3 gene coding sequence in IR64 rice cultivar was used for designing of TALENs. TALEN designing using parameters described in materials and methods section resulted into 333 pairs of TALENs. To obtain specific, efficient and active TALENs against Xa13 locus in indica rice IR64, all 333 TALEN pairs designed using TALEN targeter were screened at four different stages.

Screening of designed TALENs

Of total 333 TALEN pairs designed, 153 pairs of TALENs following Streubel's guidelines were retained for further analyses whereas remaining 180 pairs were excluded from the study. This screening was done to achieve high specificity and efficiency of TALENs (Streubel et al., 2012).

Assessment of target specificity

Filtration of TALENs on the basis of Streubel et al. (2012) guidelines selects/screens highly active TALENs containing properly spaced strong RVDs. Further, these guidelines filter TALENs to have strong RVDs: HD against C nucleotide and NH against G nucleotide. NH RVD is supposed to bind with highest strength with G nucleotide in comparison to NN RVD targeting this nucleotide. Selection of strong RVDs (HD and NH) in TALENs is desirable to obtain highly efficient TALENs owing to hydrogen bonds between these RVDs and DNA bases. On the other hand, incorporation of weak RVDs (NI and NG targeting A and T nucleotide, respectively) in TALENs reduces efficiency and specificity due to van der Waals interactions between these RVDs and DNA bases.

Assessment of target site composition

Further, screening of obtained 153 pairs of TALENs, on the basis of GC content >40% of their target site, resulted into 139 TALEN pairs. GC content of target site was used as a parameter of screening to achieve high strength of binding between DNA and designed TALENs. Since, G and C nucleotides are targeted by strong RVDs NH and HD respectively, screening of TALENs on basis of GC content of target locus yields TALENs with high binding strength and specificity of TALENs to target locus (Streubel et al., 2012).

Cleavage near to EBE sequence in Xa13 promoter

Next stage of TALENs screening, on basis of predicted cut position in immediate vicinity of PthXo1 effector binding site of Xa13 locus in IR64, resulted into 11 TALEN pairs. Since, proximity of the TALEN mediated cleavage site enhances the probability of mutations at the target locus, this parameter of TALENs screening improves greatly the mutations/deletions events at the PthXo1 effector binding site.

Off-target analysis

Selected 11 TALEN pairs were further analysed for their putative off-target sites in indica rice genome. All these 11 TALEN pairs showed unique target site in indica rice genome. Off-target sites of selected TALEN pairs were analysed to avoid any detrimental effects due to undesirable manipulations in the host genome owing to multiple target sites of homo-dimeric or hetero-dimeric TALENs.

Scoring of screened TALENs

TALEN pairs obtained after four levels of screening were analysed for TAL score. Predicted scores of individual TAL and average scores of all eleven TALEN pairs are listed in table 1. Out of eleven, three TALEN pairs showed average TAL score below four. Low score observed indicates specific activity of these three TALEN pairs in host genome.

Designing of TALEN coding genes

Amino acid sequences of complete functional TALEN (TAL1 and TAL2) are shown in figure 4. Designing of synthetic DNA sequences for functional TALEN resulted into DNA sequence having codon usage pattern of rice, and devoid of putative splicing sites, mRNA destabilizing sequences and polyadenylation sequences. Synthetic DNA sequences of TAL1 and TAL2 are shown in figure S2 and figure S3 respectively. Nucleotide analysis of synthetic sequences revealed that GC content of TAL1 and TAL2

was 57.58% and 57.89% respectivelyandGC3 content of TAL1 and TAL2 was 60.49% and 59.71% respectively. Nucleotide content of DNA sequences affects gene expression level in species and gene specific manner (Elhaik, Pellegrini & Tatarinova 2014).

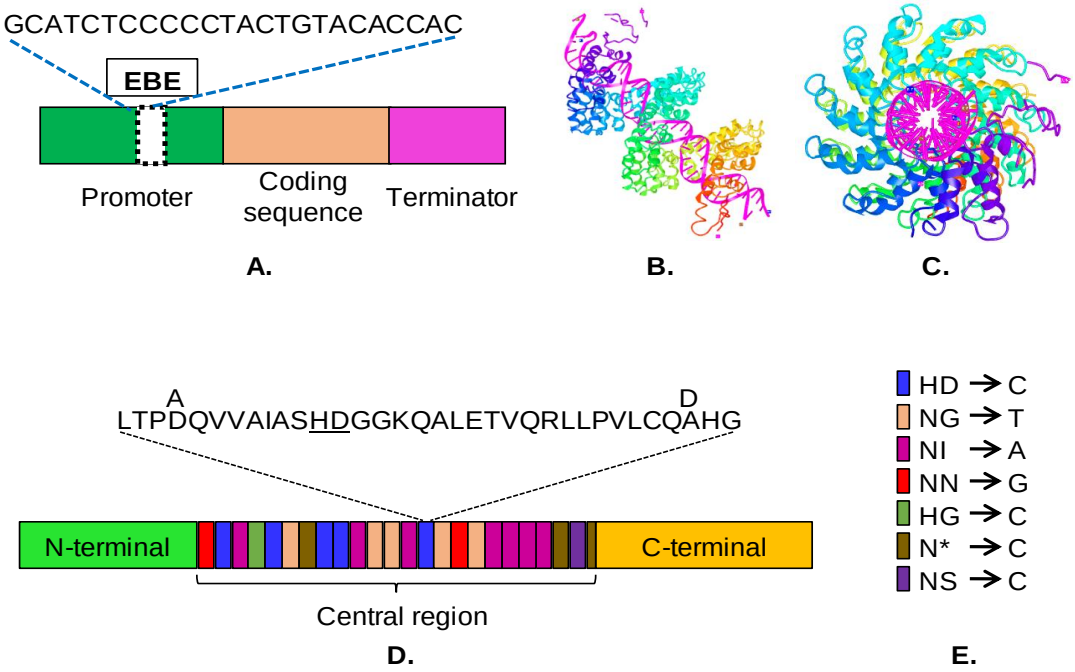

Figure 1: Schematic representation of Xa13 allele and PthXo1 TALE protein. A. Schematic representation showing details of Xa13 allele. Nucleotide sequence marked as EBE in promoter region represents PthXo1 effector binding site; B & C. 3D structure of PthXo1 effector bound to its target DNA showing side view and top view respectively; D. Diagrammatic representation of structure of PthXo1 with complete amino acid sequence of one repeat. Underlined amino acids at 12th and 13th positions represent repeat variable di-residues (RVDs). Extra amino acids at 4th and 32nd position represent uncommon variable amino acids in repeat region; E. RVDs in coding region of PthXo1 and their preferably interacting nucleotides.

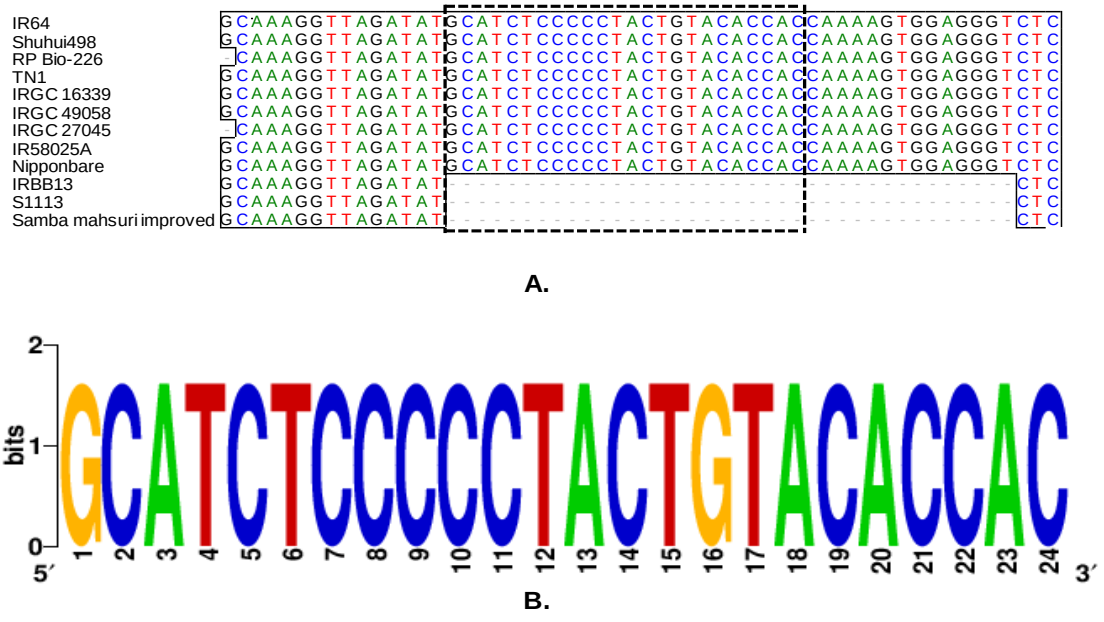

Figure 2: Nucleotide sequence analysis of PthXo1 effector binding site. A. Multiple sequence alignment of promoter region of Xa13 locus from BB susceptible and resistant rice cultivars. Sequence titles represent rice cultivar of corresponding sequence. Nucleotide sequence inside the dashed box represents PthXo1 effector binding site in promoter; B. WebLogo showing conservation of PthXo1 effector binding site in promoter region of BB susceptible rice cultivars.

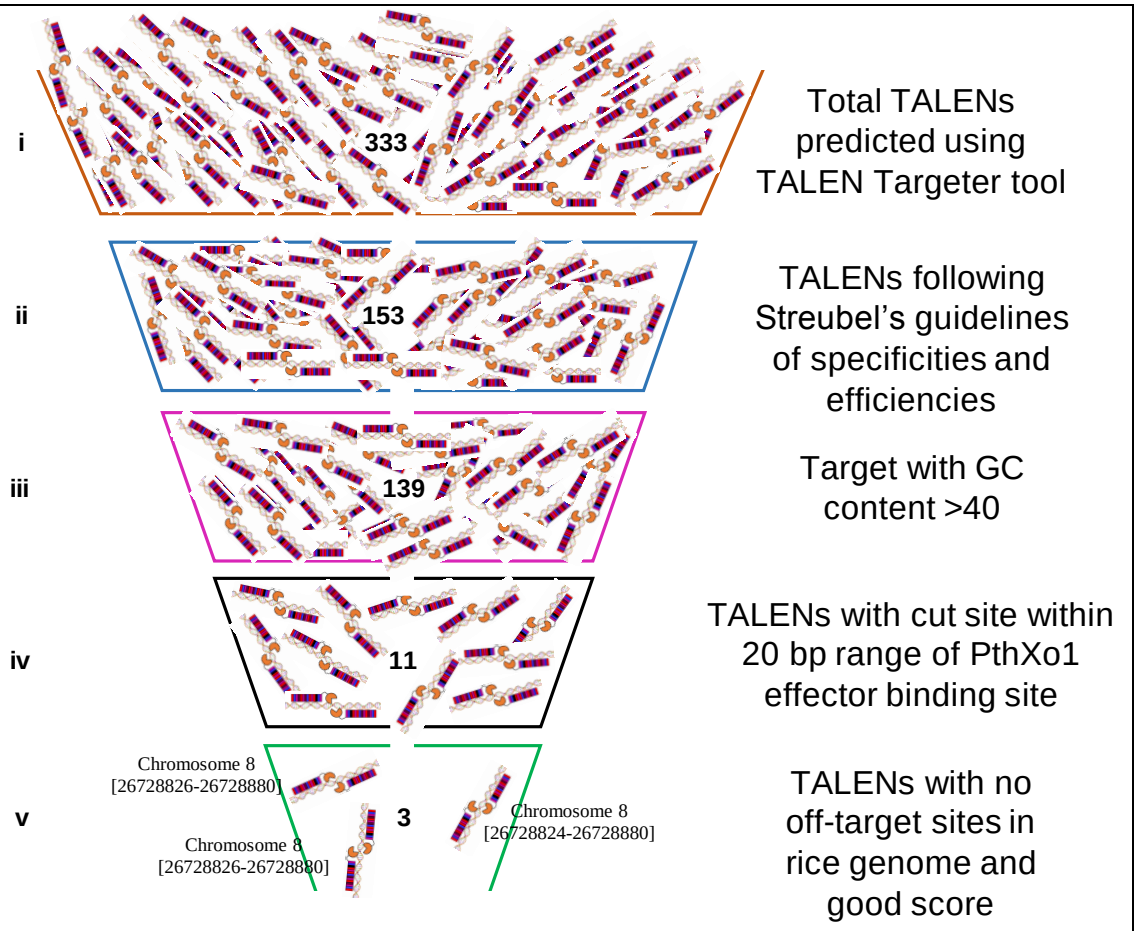

Figure 3: Designing and screening of TALENs against promoter region of Xa13 locus. i. Total number of TALENs predicted against 400 bp long sequence of promoter region of Xa13 locus in indica rice IR64; ii – v. Screening of predicted TALENs on the basis of Streubel's guidelines of TALE RVD specificities and efficiencies, GC content of target locus, distance of cleavage site from PthXo1 effector binding site at Xa13 locus and putative off-target sites & score of predicted TALENs in rice genome, respectively. Finally the potential TALEN pairs were identified.

PKKKRKVMVDLRTLGYSQQQQEKIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQDM
IAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQLDTGQLLKIAKRGGVTAVEAVHAWRNALT
GAPLNLTPAQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGKQALETVQRLLPVLCQA
HGLTPAQVVAIASNHGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGKQALETVQRLLPVLCQAHG
LTPAQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGKQALETVQRLLPVLCQAHGLTP
AQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNHGGKQALETVQRLLPVLCQAHGLTPAQ
VVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPAQVV
AIASNGGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAI
ASNGGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGRPALESIVAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLPHAPALIKRTNRRIPERTSHRVA
QLVKSELEEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYT
VGSPIDYGVIVDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKG
NYKAQLTRLNHITNCNGAVLSVEELLIGGEMIKAGTL TLEEVRRKFNNGEIN

A.

PKKKRKVMVDLRTLGYSQQQQEKIKPKVRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTVAVKYQDM
IAALPEATHEAIVGVGKQWSGARALEALLTVAGELRGPPLQLDTGQLLKIAKRGGVTAVEAVHAWRNALT
GAPLNLTPAQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNGGGKQALETVQRLLPVLCQA
HGLTPAQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNGGGKQALETVQRLLPVLCQAHG
LTPAQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNHGGKQALETVQRLLPVLCQAHGLTP
AQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQ
VVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPAQVV
AIASNGGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAI
ASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIAS
NGGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHD
GGKQALETVQRLLPVLCQAHGLTPAQVVAIASNIGGKQALETVQRLLPVLCQAHGLTPAQVVAIASHDGG
KQALETVQRLLPVLCQAHGLTPAQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNGGGKQ
ALETVQRLLPVLCQAHGLTPAQVVAIASNGGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNGGGKQAL
ETVQRLLPVLCQAHGLTPAQVVAIASNHGGKQALETVQRLLPVLCQAHGLTPAQVVAIASNHGGRPALESI
VAQLSRPDPALAALTNDHLVALACLGGRPALDAVKKGLPHAPALIKRTNRRIPERTSHRVAQLVKSELEEK
KSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVI
VDTKAYSGGYNLPIGQADEMQRYVEENQTRNKHINPNEWWKVYPSSVTEFKFLFVSGHFKGNYKAQLTRL
NHITNCNGAVLSVEELLIGGEMIKAGTL TLEEVRRKFNNGEIN

B.

Figure 4: A & B. Amino acid sequence of TAL1 and TAL2 respectively. Sequences highlighted in orange, blue, green, black and pink colour represent SV40 NLS, N-terminal, repeat region, C-terminal and FokI nuclease domain respectively.

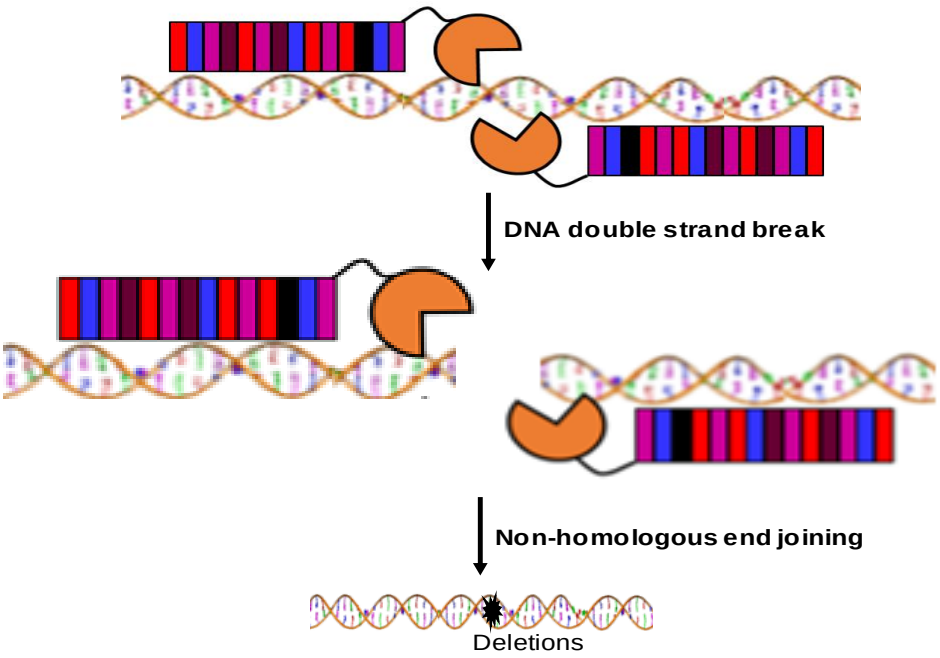

Figure 5: TALEN mediated locus specific editing *in vivo*. FokI nuclease domains dimerize to generate DNA double strand break in spacer region. Non-homologous end joining mechanism of DNA repair re-joins the broken ends with some deletions before ligation of ends.

Table1: List of selected TALENs designed against Xa13 locus and their properties. Data highlighted in bold represents three pairs of TALENs selected after four levels of screening.

S. No.	TALEN	Cut site	TAL1 start	TAL2 start	Spacer range	TAL1 RVDs	TAL2 RVDs	Target plus strand sequence	TAL1 Score	TAL2 Score	Average TAL score
1	**TALEN pair 1**	**165**	**143**	**197**	**158-172**	**NG NI NH NI NG NI NG NH HD NI NG HD NG HD HD**	**NI NG NI NH NG NG NH NH NI NH NI HD HD HD NG HD HD NI HD NG NG NG NG NG NH NH**	**T TAGATATGCATCTCC ccctactgtacacca CCAAAAGTGGAGGGTCTCCAACTAT A**	**4.47**	**2.85**	**3.66**
2	**TALEN pair 2**	**166**	**143**	**197**	**159-173**	**NG NI NH NI NG NI NG NH HD NI NG HD NG HD HD HD**	**NI NG NI NH NG NG NH NH NI NH NI HD HD HD NG HD HD NI HD NG NG NG NG NG NH**	**T TAGATATGCATCTCCC cctactgtacaccac CAAAAGTGGAGGGTCTCCAACTAT A**	**4.4**	**3**	**3.7**
3	**TALEN pair 3**	**167**	**143**	**199**	**160-174**	**NG NI NH NI NG NI NG NH HD NI NG HD NG HD HD HD HD**	**NI NG NI NG NI NH NG NG NH NH NI NH NI HD HD HD NG HD HD NI HD NG NG NG NG**	**T TAGATATGCATCTCCCC ctactgtacaccacc AAAAGTGGAGGGTCTCCAACTATAT A**	**4.77**	**3.16**	**3.965**
4	TALEN pair 4	187	150	223	175-198	NH HD NI NG HD NG HD HD HD HD HD NG NI HD NG NH NG NI HD NI HD HD NI HD HD	NH NH HD HD NG NG NH NH HD HD NI NG NH NH HD NG HD NI NH NG NH NG NG NG NI	T GCATCTCCCCCTACTGTACACCACC aaaagtggagggtctccaactata TAAACACTGAGCCATGGCCAAGGCC A	4.28	4.41	4.345
5	TALEN pair 5	188	150	225	175-200	NH HD NI NG HD NG HD HD HD HD HD NG NI HD NG NH NG NI HD NI HD HD NI HD HD	NG NG NH NH HD HD NG NG NH NH HD HD NI NG NH NH HD NG HD NI NH NG NH NG NG	T GCATCTCCCCCTACTGTACACCACC aaaagtggagggtctccaactatata AACACTGAGCCATGGCCAAGGCCAA A	4.39	4.41	4.4
6	TALEN pair 6	189	154	223	179-198	HD NG HD HD HD HD HD NG NI HD NG NH NG NI HD NI HD HD NI HD HD NI NI NI NI	NH NH HD HD NG NG NH NH HD HD NI NG NH NH HD NG HD NI NH NG NH NG NG NG NI	T CTCCCCCTACTGTACACCACCAAAA gtggagggtctccaactata TAAACACTGAGCCATGGCCAAGGCC A	4.28	4.42	4.35
7	TALEN pair 7	190	156	223	181-198	HD HD HD HD HD NG NI HD NG NH NG NI HD NI HD HD NI HD HD NI NI NI NI NH NG	NH NH HD HD NG NG NH NH HD HD NI NG NH NH HD NG HD NI NH NG NH NG NG NG NI	T CCCCCTACTGTACACCACCAAAAGT ggagggtctccaactata TAAACACTGAGCCATGGCCAAGGCC A	4.28	4.34	4.31
8	TALEN pair 8	191	156	225	181-200	HD HD HD HD HD NG NI HD NG NH NG NI HD NI HD HD NI HD HD NI NI NI NI NH NG	NG NG NH NH HD HD NG NG NH NH HD HD NI NG NH NH HD NG HD NI NH NG NH NG NG	T CCCCCTACTGTACACCACCAAAAGT ggagggtctccaactatata AACACTGAGCCATGGCCAAGGCCAA A	4.39	4.34	4.365
9	TALEN pair 9	192	156	228	181-203	HD HD HD HD HD NG NI HD NG NH NG NI HD NI HD HD NI HD HD NI NI NI NI NH NG	NH NH NG NG NG NH NH HD HD NG NG NH NH HD HD NI NG NH NH HD NG HD NI NH NG	T CCCCCTACTGTACACCACCAAAAGT ggagggtctccaactatataaac ACTGAGCCATGGCCAAGGCCAAACC A	4.19	4.34	4.265
10	TALEN pair 10	193	156	230	181-205	HD HD HD HD HD NG NI HD NG NH NG NI HD NI HD HD NI HD HD NI NI NI NI NH NG	NH NG NH NH NG NG NG NH NH HD HD NG NG NH NH HD HD NI NG NH NH HD NG HD NI	T CCCCCTACTGTACACCACCAAAAGT ggagggtctccaactatataaacac TGAGCCATGGCCAAGGCCAAACCAC A	4.19	4.34	4.265
11	TALEN pair 11	194	156	232	181-207	HD HD HD HD HD NG NI HD NG NH NG NI HD NI HD HD NI HD HD NI NI NI NI NH NG	NH NG NH NG NH NH NG NG NG NH NH HD HD NG NG NH NH HD HD NI NG NH NH HD NG	T CCCCCTACTGTACACCACCAAAAGT ggagggtctccaactatataaacactg AGCCATGGCCAAGGCCAAACCACAC A	4.22	4.34	4.28

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Supplementary data

AAGTTATGCATTCATATGACCACATATTCAGAGTAGTGGAGAGAGGGACAGATCTAGAGGTAGAAAAAGAAAATTCAAA
TCATATAAATGATATATCAGAGTGAAAAAGAAATATCAAGCACAAGAAAAAAAAAAGCAAAGGTTAGATATGCATCTCCC
CCTACTGTACACCACCAAAAGTGGAGGGTCTCCAACTATATAAACACTGAGCCATGGCCAAGGCCAAACCACACATGCAG
TTGTAGTAGCACTTAAGCCTTCCTCTCTAGCTAGCATCTCTTGTGTCAGGAAGTTGGAAGGGATTTCTGGCTAGTTTCTAGC
TGGTGTCTCCTCTCCTCTTCCTAACCTTCTCACTGATTAACACCTTAGAGTTAGTTAATAACCTTCATCACCAGTAGCA

Figure S1: DNA sequence of promoter region of Xa13 locus in IR64 rice cultivar used for TALEN designing. Underlined sequence represents PthXo1 effector binding element.

CCAAAGAAAAAGAGGAAGGTAATGGTTGACCTGCGGACACTCGGCTATTCTCAACAACAACAGGAAAAAATTAAACCCAAGGTCCGCA
GCACGGTTGCGCAGCATCACGAGGCATTGGTGGGTCATGGTTTCACACACGCTCATATCGTGGCCCTCTCGCAACATCCCGCAGCGCTG
GGCACTGTCGCGGTCAAATATCAAGATATGATCGCGGCGTTGCCCGAGGCAACGCACGAAGCTATTGTGGGGGTCGGAAAGCAATGGT
CCGGAGCGCGGGCCCTTGAGGCTTTGCTCACAGTGGCCGGCGAGCTGAGGGGGCCACCCCTCCAACTCGATACCGGTCAGCTTTTGAA
GATCGCCAAAAGGGGGGGGGTCACTGCTGTCGAGGCTGTCCACGCTTGGCGGAATGCACTCACTGGAGCTCCACTGAACCTTACGCCA
GCCCAAGTGGTTGCCATCGCCTCGAACGGCGGGGGTAAGCAGGCGTTGGAAACTGTCCAGAGGCTCCTGCCCGTGCTCTGTCAGGCGC
ACGGACTGACACCCGCACAGGTGGTTGCTATAGCGTCTAATATCGGCGGCAAACAGGCACTTGAAACCGTTCAGCGTCTCCTCCCGGTT
CTCTGTCAGGCACATGGCCTCACCCCTGCGCAAGTTGTCGCAATCGCCTCTAATCACGGTGGCAAACAGGCTCTCGAAACGGTCCAAAG
ACTCCTGCCTGTTCTCTGTCAAGCACATGGTTTGACTCCAGCCCAAGTAGTGGCTATTGCATCGAACATTGGGGGGAAACAAGCGCTGG
AAACTGTGCAAAGGCTCTTGCCGGTGCTCTGCCAAGCACATGGACTCACGCCCGCCCAAGTTGTTGCCATTGCCAGCAACGGGGGCGGA
AAGCAAGCACTTGAGACTGTTCAGCGCCTGCTGCCTGTCCTTTGCCAGGCACACGGCCTTACTCCCGCCCAAGTCGTCGCGATTGCGTCT
AATATTGGGGGAAAGCAGGCTCTCGAGACAGTGCAGCGGCTTTTGCCGGTATTGTGCCAGGCCCACGGACTCACGCCCGCTCAGGTTG
TCGCCATTGCGTCAAACGGGGGGGGCAAGCAGGCGCTGGAGACCGTGCAGAGGCTTCTCCCAGTTCTGTGCCAGGCTCATGGCCTTAC
ACCGGCCCAGGTCGTTGCGATAGCGTCAAATCATGGTGGAAAGCAGGCACTGGAAACCGTACAAAGACTCCTTCCTGTTCTGTGTCAGG
CACACGGCTTGACCCCCGCGCAAGTCGTCGCCATCGCCAGTCATGATGGCGGCAAGCAAGCTCTTGAAACAGTTCAACGTCTTCTGCCG
GTCCTTTGTCAGGCGCATGGGCTTACACCTGCTCAAGTGGTGGCTATTGCCTCCAATATCGGAGGCAAGCAAGCGCTCGAAACCGTCCA
AAGACTTCTCCCAGTCTTGTGCCAGGCACATGGACTGACCCCTGCCCAAGTCGTGGCGATTGCATCTAACGGTGGGGGGAAGCAAGCCC
TGGAAACCGTGCAACGGCTGCTCCCGGTCCTGTGTCAGGCTCACGGCTTGACGCCAGCCCAGGTGGTCGCCATAGCCTCTCATGACGGA
GGGAAGCAGGCCTTGGAGACGGTGCAGCGCCTTCTCCCCGTGTTGTGCCAAGCTCATGGCCTCACTCCTGCGCAAGTGGTCGCCATCGC
GAGTAATGGTGGAGGCAAACAAGCTCTGGAGACAGTTCAACGCCTCTTGCCCGTCCTGTGCCAAGCGCACGGTCTCACACCTGCGCAG
GTGGTGGCGATTGCGTCCCACGATGGCGGTAAGCAGGCCCTGGAAACAGTCCAACGGTTGCTGCCCGTGCTTTGCCAGGCGCACGGTC
TTACCCCGGCCCAGGTAGTTGCCATAGCATCCCATGACGGCGGGAGACCAGCACTTGAGTCGATCGTGGCTCAGCTTTCACGGCCGGAT
CCCGCGCTCGCCGCCCTCACTAATGACCATCTCGTGGCACTGGCGTGCCTGGGCGGCCGGCCCGCACTCGATGCTGTGAAGAAGGGGCT
GCCCCATGCTCCCGCGCTCATCAAGCGCACGAATAGGAGAATTCCTGAGCGTACCAGTCACCGGGTTGCACAGCTTGTGAAGAGCGAG
CTGGAGGAGAAGAAGTCGGAACTTCGTCACAAGCTCAAATACGTCCCACATGAGTACATTGAACTTATAGAAATCGCTCGGAACTCTAC
CCAGGACAGGATTTTGGAGATGAAGGTCATGGAATTTTTTATGAAGGTGTATGGCTATAGAGGTAAGCATCTGGGTGGTAGCCGCAAA
CCGGATGGCGCGATCTATACGGTTGGCAGCCCGATAGATTATGGAGTCATCGTGGATACCAAAGCATACTCAGGAGGCTATAATCTTCC
AATAGGGCAGGCGGACGAAATGCAGAGGTACGTCGAAGAGAATCAGACGAGGAACAAGCATATCAATCCCAACGAGTGGTGGAAGG
TCTACCCGTCCTCCGTTACTGAGTTCAAGTTCCTGTTCGTTTCGGGACACTTTAAGGGCAATTACAAGGCCCAACTGACCAGACTGAACC
ATATTACGAACTGCAATGGGGCAGTCCTGTCTGTGGAGGAGCTTCTTATTGGTGGTGAAATGATCAAAGCGGGCACACTCACCCTCGAA
GAGGTGAGGAGAAAGTTTAACAACGGCGAGATCAAC

Figure S2: Synthetic DNA sequence of TAL1 codon-optimized for optimum expression in rice. Sequences highlighted in orange, blue, green, black and pink colour represent SV40 NLS, N-terminal, repeat region, C-terminal and FokI nuclease domain.

CCAAAGAAAAAGAGGAAGGTAATGGTTGACCTGCGGACACTCGGCTATTCTCAACAACAACAGGAAAAAATTAAACCCAAGGTCCGCA
GCACGGTTGCGCAGCATCACGAGGCATTGGTGGGTCATGGTTTCACACACGCTCATATCGTGGCCCTCTCGCAACATCCCGCAGCGCTG
GGCACTGTCGCGGTCAAATATCAAGATATGATCGCGGCGTTGCCCGAGGCAACGCACGAAGCTATTGTGGGGGTCGGAAAGCAATGGT
CCGGAGCGCGGGCCCTTGAGGCTTTGCTCACAGTGGCCGGCGAGCTGAGGGGGCCACCCCTCCAACTCGATACCGGTCAGCTTTTGAA
GATCGCCAAAAGGGGGGGGGTCACTGCTGTCGAGGCTGTCCACGCTTGGCGGAATGCACTCACTGGAGCTCCACTGAACTTGACTCCC
GCGCAGGTTGTGGCCATTGCGTCGAACATCGGCGGTAAGCAGGCCCTCGAGACCGTACAGAGGCTGCTGCCTGTTTTGTGCCAGGCCC
ACGGACTCACTCCGGCCCAAGTGGTGGCCATCGCTTCCAATGGTGGCGGCAAACAGGCCCTGGAGACTGTGCAGCGCCTTCTGCCGGT
GCTCTGTCAAGCACACGGCCTCACACCAGCCCAGGTGGTGGCCATTGCATCTAACATTGGGGGCAAGCAGGCGCTTGAAACCGTTCAAA
GGTTGCTGCCAGTGCTCTGCCAGGCCCATGGTCTGACACCTGCTCAGGTGGTTGCGATAGCATCAAACCACGGTGGCAAACAAGCCCTC
GAAACAGTGCAGCGCCTCTTGCCTGTACTGTGTCAGGCGCATGGTCTCACGCCCGCTCAAGTCGTTGCGATCGCATCCAACGGTGGGGG
CAAACAAGCATTGGAAACTGTGCAAAGGCTGCTCCCTGTCTTGTGTCAGGCCCACGGCCTCACTCCAGCTCAAGTCGTAGCTATTGCCTC
GAACGGAGGTGGCAAGCAGGCCCTGGAAACAGTTCAGAGGCTCCTCCCTGTGCTCTGTCAAGCGCATGGACTTACGCCAGCCCAGGTC
GTGGCCATTGCTTCGAACCATGGTGGGAAACAAGCGTTGGAAACTGTCCAGAGGCTGTTGCCGGTGTTGTGCCAGGCTCATGGCTTGA
CGCCGGCCCAGGTCGTCGCTATAGCCTCAAACCATGGCGGAAAGCAAGCGCTGGAAACCGTGCAGCGGTTGTTGCCTGTGCTTTGTCAA
GCGCACGGTCTCACCCCGGCACAGGTTGTGGCAATCGCTTCCAACATTGGTGGGAAGCAGGCGCTCGAGACTGTCCAACGCCTTCTTCC
CGTGCTGTGTCAGGCCCACGGCCTGACCCCGGCCCAAGTTGTCGCGATTGCGTCCAATCATGGGGGGAAGCAAGCATTGGAAACAGTT
CAGCGCCTGCTCCCTGTGCTGTGCCAAGCACACGGCCTGACTCCAGCGCAGGTCGTCGCAATTGCCTCTAACATCGGCGGCAAACAAGC
GCTTGAGACGGTTCAAAGACTCCTTCCGGTTCTCTGCCAGGCCCATGGATTGACCCCTGCTCAAGTTGTGGCTATAGCCTCCCACGATGG
AGGTAAGCAAGCTCTGGAGACAGTTCAAAGGCTTCTCCCGGTATTGTGTCAGGCTCACGGGCTTACCCCCGCTCAGGTGGTCGCAATTG
CCTCTCATGATGGTGGCAAGCAGGCCCTTGAGACGGTCCAGCGGCTTCTCCCTGTACTTTGCCAGGCGCACGGGTTGACGCCCGCCCAA
GTCGTTGCAATTGCCTCGCACGATGGCGGTAAACAAGCTCTGGAAACAGTCCAAAGGCTTTTGCCGGTGCTTTGCCAAGCCCATGGTCTT
ACACCCGCTCAGGTAGTCGCCATCGCGTCAAATGGAGGAGGCAAACAGGCCCTTGAAACTGTGCAGAGACTCCTGCCCGTACTCTGCCA
AGCGCACGGACTCACCCCTGCGCAGGTTGTTGCTATCGCGTCTCATGATGGTGGAAAGCAAGCTTTGGAAACCGTGCAGAGGCTCTTGC
CTGTCCTTTGCCAGGCACACGGCCTTACTCCTGCCCAGGTGGTCGCGATCGCGTCACACGACGGAGGTAAGCAGGCACTCGAGACCGTG
CAAAGACTTTTGCCAGTCCTCTGCCAAGCTCATGGATTGACTCCCGCTCAGGTCGTCGCCATCGCATCTAATATTGGTGGAAAGCAGGCC
TTGGAAACGGTTCAAAGGCTCCTGCCGGTGCTGTGTCAAGCCCACGGGCTGACGCCAGCGCAGGTGGTCGCAATCGCCTCACATGATG
GGGGTAAACAGGCATTGGAGACTGTGCAACGGCTGCTCCCCGTGCTCTGCCAAGCCCACGGTCTCACACCCGCGCAAGTTGTTGCAATT
GCGTCAAATGGTGGAGGCAAACAAGCCCTTGAAACCGTGCAACGCCTCTTGCCAGTCCTTTGTCAAGCCCATGGCCTCACACCTGCGCA
AGTCGTCGCAATAGCAAGTAATGGGGGAGGCAAACAAGCGCTGGAGACAGTCCAACGTCTGCTTCCCGTACTTTGCCAAGCGCATGGC
CTTACGCCGGCTCAGGTTGTGGCTATCGCATCTAACGGTGGCGGTAAGCAAGCGCTCGAAACGGTCCAACGCTTGCTCCCGGTCTTGTG
TCAAGCGCACGGCCTTACCCCTGCGCAAGTAGTTGCCATAGCATCTAACGGCGGTGGTAAGCAAGCCCTGGAGACAGTGCAGCGGCTC
CTCCCCGTACTTTGCCAGGCTCACGGTCTTACGCCCGCGCAAGTTGTTGCTATAGCTTCGAATCACGGCGGAAAACAGGCCTTGGAGACC
GTGCAGCGTCTGCTCCCGGTTCTGTGCCAAGCACATGGGCTCACGCCCGCACAGGTCGTGGCAATCGCAAGTAACCACGGCGGCCGGC
CCGCCTTGGAATCGATCGTGGCTCAGCTTTCACGGCCGGATCCCGCGCTCGCCGCCCTCACTAATGACCATCTCGTGGCACTGGCGTGCC
TGGGCGGCCGGCCCGCACTCGATGCTGTGAAGAAGGGGCTGCCCCATGCTCCCGCGCTCATCAAGCGCACGAATAGGAGAATTCCTGA
GCGTACCAGTCACCGGGTTGCACAGCTTGTGAAGAGCGAGCTGGAGGAGAAGAAGTCGGAACTTCGTCACAAGCTCAAATACGTCCCA
CATGAGTACATTGAACTTATAGAAATCGCTCGGAACTCTACCCAGGACAGGATTTTGGAGATGAAGGTCATGGAATTTTTTATGAAGGT
GTATGGCTATAGAGGTAAGCATCTGGGTGGTAGCCGCAAACCGGATGGCGCGATCTATACGGTTGGCAGCCCGATAGATTATGGAGTC
ATCGTGGATACCAAAGCATACTCAGGAGGCTATAATCTTCCAATAGGGCAGGCGGACGAAATGCAGAGGTACGTCGAAGAGAATCAGA
CGAGGAACAAGCATATCAATCCCAACGAGTGGTGGAAGGTCTACCCGTCCTCCGTTACTGAGTTCAAGTTCCTGTTCGTTTCGGGACACT
TTAAGGGCAATTACAAGGCCCAACTGACCAGACTGAACCATATTACGAACTGCAATGGGGCAGTCCTGTCTGTGGAGGAGCTTCTTATT
GGTGGTGAAATGATCAAAGCGGGCACACTCACCCTCGAAGAGGTGAGGAGAAAGTTTAACAACGGCGAGATCAAC

Figure S3: Synthetic DNA sequence of TAL2 codon-optimized for optimum expression in rice. Sequences highlighted in orange, blue, green, black and pink colour represent SV40 NLS, N-terminal, repeat region, C-terminal and FokI nuclease domain.

Table S1: List of sequences selected for analysis of PthXo1 effector binding site

S. No.	Rice cultivars	Descriptions	Accession number
1	IR64	Oryza sativa (indica) cultivar IR64 disease resistant allele XA13 (Xa13) gene, complete cds	DQ421395.1
2	Shuhui498	Oryza sativa Indica Group cultivar Shuhui498 chromosome 8 sequence	CP018164.1
3	Nipponbare	Oryza sativa Japonica Group DNA, chromosome 8, cultivar: Nipponbare, complete sequence	AP014964.1
4	RP Bio-226	Oryza sativa Indica Group cultivar RP Bio-226 chromosome 8 sequence	CP012616.1
5	IRBB13	Oryza sativa (indica) cultivar IRBB13 disease resistant allele xa13 (xa13) gene, complete cds	DQ421394.1
6	TN1	Oryza sativa Indica cultivarTN1 xa13 (xa13) gene, promoter region and partial cds	JN564605.1
7	IRGC 16339	Oryza sativa Indica cultivar IRGC 16339 xa13 (xa13) gene, promoter region and partial cds	JN564607.1
8	IRGC 49058	Oryza sativa Indica cultivar IRGC 49058 xa13 (xa13) gene, promoter region and partial cds	JN564606.1
9	IRGC 27045	Oryza sativa Indica cultivar IRGC 27045 xa13 (xa13) gene, promoter region and partial cds	JN564608.1
10	IR58025A	Oryza sativa Indica cultivar IR58025A xa13 (xa13) gene, promoter region and partial cds	JN564604.1
11	S1113	Oryza sativa Indica cultivar S1113 xa13 (xa13) gene, promoter region and partial cds	JN564603.1
12	Improved samba mahsuri	Oryza sativa Indica improved samba mahsuri xa13 (xa13) gene, promoter region and partial cds	JN564609.1

CONCLUSION

Functional TALEN designed against dominant allele Xa13, in this study, was best scoring TALEN pair. Moreover, screening on the basis of Streubel's guidelines of TALE RVD specificities and efficiencies, GC content of target locus, its predicted cleavage site near PthXo1 effector binding site and unique target site in rice genome further validates its use for editing at Xa13 locus in rice. Codon-optimization of synthetic DNA sequence of functional TALEN further warrants its optimum expression and activity in rice. These designed TALEN encoding genes can be expressed under constitutive promoter and used for introducing xa13 mediated bacterial blight resistance in various rice cultivars.

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