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Biotechnological Advancements in Managing Bacterial Plant Diseases: From Transgenics to CRISPR-CAS9-based Genome Editing

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SUMMARY

Bacterial diseases in crops, such as bacterial blight in rice and bacterial wilt in solanaceous vegetables, pose significant threats to global agricultural productivity. Conventional management strategies, such as cultural practices, copper-based chemical sprays, antibiotics, and resistance breeding, are frequently insufficient due to unpredictable weather and the rapid evolution of bacterial pathogens. To secure stable future harvests, modern plant pathology is increasingly focusing on molecular interventions. This article analyses the transition from traditional chemical controls to advanced biotechnological approaches. This article reviews transgenic techniques that strengthen plants' natural defenses by introducing antibacterial proteins and activating resistance (R) gene expression, as well as the two RNA interference (RNAi) approaches: Host-Induced Gene Silencing (HIGS) and environment- friendly Spray-Induced Gene Silencing (SIGS). Furthermore, the article discusses how CRISPR-Cas9 genome editing enables precise, non-transgenic modification of host susceptibility (S) genes. By focusing on the critical molecular interactions between the pathogen and host, these innovative, advanced genetic tools present a promising and sustainable pathway to achieve durable, broad-spectrum disease resistance in major crops.

INTRODUCTION

Bacterial plant diseases represent significant obstacles to modern agricultural productivity, persistently threatening global food security and destabilizing agricultural economies. Pathogens such as *Xanthomonas oryzae* pv. *oryzae*, which causes bacterial leaf blight in rice, and soil-borne pathogens like *Ralstonia solanacearum*, responsible for bacterial wilt in solanaceous crops, cause severe yield losses annually across various agro-climatic zones (Tripathi et al., 2024; Shirisha et al., 2025). For generations, the management of these bacterial plant pathogens has relied heavily on traditional crop protection approaches: cultural practices, biological control agents, and chemical interventions. Although these conventional strategies form the backbone of traditional integrated pest management (IPM) systems, recent field studies indicate their short-term efficacy. Cultural practices reduce initial inoculum, but their effectiveness in the field is inconsistent. Biological control agents offer an eco-friendly option, yet their performance is often compromised by variable environmental conditions. often falter under unpredictable environmental conditions. On the other hand, excessive application of copper-based sprays and chemical antibiotics triggers rapid resistance development in bacterial populations and poses serious environmental risks. Furthermore, resistance conferred by the conventionally bred crop varieties is often short-lived, as continuous mutations in bacterial populations quickly break down single-gene resistance in plants (Rai et al., 2025).

To overcome these limitations, modern plant pathology increasingly emphasizes internal genetic reinforcement. Advancements in molecular biotechnology have enabled the development of durable, broad-spectrum resistance through precise manipulation of host and pathogen genomes. Genetic engineering represents a paradigm shift by facilitating the targeted introduction, silencing, or editing of specific genes associated with plant immunity.

The Molecular Battlefield: Understanding Pathogen Attack

Plant pathogenic bacteria frequently deliver effector proteins into plant cells through a type III secretion system (TTSS) to alter host cell functions. A special class of type III effectors, known as transcription activator-like effectors (TALEs) in *Xanthomonas*, are transported into the plant nucleus and bind to effector binding elements (EBEs) in the promoter of the susceptibility (S) genes to facilitate infection. These TAL effectors can interact with the EBEs of susceptibility (S) or resistance (R) genes in the host, resulting in either susceptibility or resistance by activating the transcription of downstream genes. Altering EBE promoter sequences hampers TALEs' binding and subsequent activation of susceptibility genes. Some plants have evolved mechanisms to modify the EBE promoter sequences to evade the recognition by TAL effectors. Susceptible plant varieties possess susceptibility (S) genes that facilitate bacterial growth, proliferation, and disease development. Loss or modification of these S genes leads to resistance. In contrast, executor genes become transcriptionally active upon recognition of pathogen TALEs that bind to EBEs in their promoters. It induces a hypersensitive response that causes localized cell death and inhibits pathogen proliferation. By altering TALE recognition sites or host gene targets, modern biotechnology can ensure durable, broad-spectrum disease resistance through two distinct pathways:

Active Resistance: Engineering molecular alarms

Addition of TAL effector target sites, such as different effector binding elements (EBEs), to the promoters of TAL effector-dependent R genes or executor genes actively enhances resistance through genetic engineering approaches (transgenic Approach).

Passive Resistance: Editing out vulnerabilities

Passive resistance is achieved by removing TAL effector target sites through modification or deletion of binding domains in the promoters of major host susceptibility genes, thereby manipulating host resistance via genome editing technologies such as the CRISPR-Cas9 non-transgenic approach.

Transgenic approaches: Upgrading the plant's natural armory

The transgenic approach involves integrating a specific gene from another variety or species into the crop's genome to provide permanent immunity. To generate transgenic plants, the isolated gene of interest is cloned into an expression vector containing a promoter and a selectable marker, which is then introduced into a delivery vehicle like *Agrobacterium tumefaciens* or coated onto gold microparticles for biolistic bombardment. Target plant explants are inoculated with this vehicle to allow stable genomic integration, after which they are transferred to a selective medium that allows only successfully transformed cells to grow, followed by regeneration into whole plantlets through tissue culture organogenesis. Finally, the regenerated plantlets undergo greenhouse hardening and acclimatization before molecular verification and pathogenicity assays confirm stable transgene expression.

Scientists have successfully engineered transgenic crops using multiple robust strategies (Mourgues et al., 1998):

Production of antibacterial proteins: Expressing lytic peptides from insects (which puncture bacterial membranes) or lysozymes (which dissolve bacterial cell walls) protects plants from physical infection.

Inactivation of bacterial toxins: In *Pseudomonas syringae* pv. *tabaci*, the gene ttr (tabtoxin resistance) encodes an enzyme that inactivates the tabtoxin, a glutamine synthetase inhibitor, providing complete resistance to the bacteria.

Enhancement of natural plant defenses: Injecting new life into a plant's innate immune system has yielded phenomenal field results-

Enhanced production of elicitors: - Transgenic tubers producing a pectinolytic enzyme from *Erwinia carotovora* were much less susceptible to the bacteria than untransformed tubers.

Expression of cloned resistance genes: - Introducing the *Xa21* R gene from a resistant line into a susceptible rice cultivar made the plants highly resistant to various *Xanthomonas oryzae* pv. *oryzae* isolates.

Enhanced production of reactive oxygen species: Hydrogen peroxide and related ROS play several roles in plant defense, including cell death, pathogen inhibition, cell wall reinforcement, and defense signalling.

Expression of plant defense genes: Transgenic plants use heterologous defense genes to produce phytoalexins, PR proteins, or antimicrobial peptides for fungal resistance, while thionins are widely used for bacterial resistance due to their membrane-disrupting toxicity.

Successful case studies related to the Manipulation of Host Resistance through Genetic Engineering (Transgenic Approach):-

Exploitation of Different TAL Effector-Dependent Executor R Genes:

Xa10 and *Xa27* are executor R gene that confer narrow-spectrum race-specific resistance to a few races of *Xanthomonas oryzae* pv. *oryzae* (Xoo). Hummel et al. (2012) added 6 EBEs to the rice Xa27 gene that confers broad-spectrum resistance to both bacterial blight and bacterial leaf streak disease in rice. Zeng et al. (2015) genetically engineered the *Xa10* promoter to achieve broad-spectrum and durable resistance to Xoo. They accomplished this by adding five effector binding elements (EBEs) to the *Xa10* promoter, creating binding sites for both virulent TAL effectors (PthXo1, PthXo6, and PthXo7) and avirulent TAL effectors (AvrXa10 and AvrXa27). So, executor gene specificity can be broadened by adding EBEs through the Genetic Engineering Transgenic Approach.

Overexpression of different transcription factors involved in the regulation of defense genes:

Qiu et al. (2008) showed that overexpression of *OsWRKY13* in rice reprograms plant defense by binding promoters of defense genes, activating the SA pathway, and suppressing the JA pathway. So, OsWRKY13 provides a transgenic tool for engineering broad-spectrum and whole-growth-stage resistance in rice breeding programs.

RNA interference (RNAi): Nature’s silencer for harmful genes

RNAi is evolutionarily conserved in eukaryotes (a natural defense mechanism in plants) and regulates all aspects of biological processes. Double-stranded RNA (dsRNA) triggers gene silencing, which protects plants from pathogen infections by suppressing the expression of specific pathogen genes (Mello, 2007; Sundaresha et al., 2022). RNAi causes both transcriptional gene silencing (TGS) [involves decreased RNA synthesis due to DNA methylation] and post-transcriptional gene silencing (PTGS) [involves sequence-specific mRNA degradation].

Mechanism:

The RNAi mechanism (refer to Figure 1) initiates when long double-stranded RNA is cleaved by the enzyme Dicer into short small interfering RNA (siRNA) pieces. This signal can be amplified by RDR (RNA-dependent RNA polymerase) to strengthen the cellular response. These siRNAs are incorporated into the RNA-induced silencing complex (RISC), where the core protein AGO (Argonaute) unwinds the duplex, discards the passenger strand, and retains the guide strand. Guided by this sequence, the AGO/RISC complex locates and precisely cleaves the complementary target mRNA, thereby preventing its translation into protein and effectively silencing the gene.

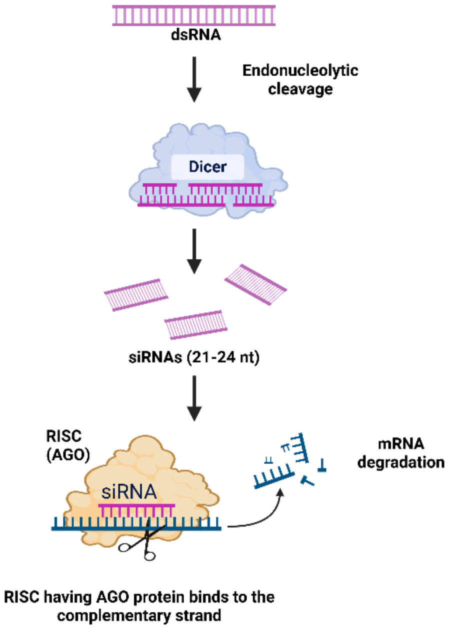

Figure 1: Mechanism of RNAi (created in biorender.com)

Overview of key RNAi-Based Strategies for Pathogen Control: -

Host-induced gene silencing (HIGS) and spray-induced gene silencing (SIGS) are key RNAi-based strategies for pest and pathogen control.

Host-induced gene silencing (HIGS): It involves genetically engineered plants that produce dsRNA targeting pathogen virulence genes, thereby reducing pathogenicity upon infection. Genetic transformation is commonly achieved using *Agrobacterium tumefaciens*, which transfers modified T-DNA into the plant genome and enables stable RNAi-based resistance across generations. These engineered plants produce dsRNA or hairpin RNAs to trigger RNAi. However, commercial adoption of HIGS remains limited due to intellectual property restrictions, high regulatory costs, lengthy approval processes, and public concerns regarding genetically modified organisms (GMOs).

Spray-induced gene silencing (SIGS): It is a non-transgenic approach that utilizes exogenous dsRNA applications, which offers a cost-effective and flexible alternative for disease management. It can be delivered via foliar sprays, trunk or stem injections, root absorption, or seed treatments. Following application, dsRNA is absorbed by plant cells, and initiates RNA silencing. However, the plant cell wall acts as a physical barrier, affecting the dsRNA uptake. Therefore, the effective delivery of exogenous dsRNAs, siRNAs, or hpRNAs into plant cells is a critical step in triggering the RNAi machinery. dsRNAs can be conjugated with nanoparticles like clay nanosheets, carbon nanotubes, and cationic nanoparticles, for more efficient delivery. These carriers enhance dsRNA stability, uptake, and gradual release, thereby ensuring prolonged protection.

RNAi in bacterial resistance:

Plant-induced bacterial gene silencing: a novel control method for bacterial wilt disease:

Jang et al. (2024) engineered plants using HIGS, via a viral vector (Tobacco rattle virus, TRV) in the plant *Nicotiana benthamiana*, to produce siRNAs aimed at virulence genes of the pathogen *Ralstonia solanacearum*. This silenced the bacterial genes and dramatically reduced wilt disease, showing for the first time that plants can be designed to directly silence bacterial genes. Thus, this “plant-induced bacterial gene silencing (PIBGS)” could be a promising, gene-based, specific way to control soil-borne bacterial diseases like bacterial wilt.

RNAi-mediated gene silencing can be used for inducing crown gall resistance in plum rootstocks:

Alburquerque et al. (2017) created crown gall-resistant plum rootstocks by silencing key *Agrobacterium* genes (oncogenes *ipt* and *iaaM*) with RNA interference.

Foliar spray of the nanocarrier-dsRNA complex to control Bacterial Leaf Blight:

Pal et al. (2024) achieved durable resistance to bacterial blight in rice by spraying nanocarrier-delivered dsRNA to silence key plant susceptibility genes-*SDIR1* (a gene involved in stress and defense signaling) and *SWEET14* (a susceptibility gene exploited by Xoo's TAL effector). This non-transgenic approach using the CPP6 polymer offers an effective new tool for crop protection.

CRISPR-Cas9: The Molecular Scissors

CRISPR-Cas9 is an efficient, reliable system that is less labour-intensive and time-consuming than any other method. As no foreign DNA is present, this procedure may allow the plants to avoid GMO legislation and be classified as non-GMOs. In 2012, Jennifer Doudna and Emmanuelle Charpentier demonstrated that the CRISPR-Cas9 system could be programmed to cut DNA at specific sites using a synthetic guide RNA (gRNA). Feng Zhang and George Church independently showed in 2013 that CRISPR-Cas9 could be used for genome editing in eukaryotic cells.

Components of the CRISPR-Cas9 System:-

CRISPR Array: Short, repeated DNA sequences (repeats) alternate with unique fragments of captured viral DNA (spacers).

Cas9: It is a bacterial endonuclease that acts like a molecular scissor. Guided by a sequence, it locates, binds to, and cuts double-stranded DNA at a specific target location. Its two domains, HNH and RuvC, cleave the complementary and non-complementary DNA strands, respectively.

Guide RNA (gRNA): A synthetic RNA molecule directs Cas9 to its target. It combines two parts: crRNA, which matches the target, and tracrRNA, which serves as a scaffold to hold the Cas9 protein. These two components are combined into a sgRNA (single guide RNA) that helps Cas9 find its target, along with help from the Rec-lobe and the PAM-interacting (PI) domain.

PAM (Protospacer Adjacent Motif): A short DNA sequence (usually 5'-NGG-3' for Cas9) found next to the target DNA site. Cas9 can only bind and cut when this sequence is present.

Mechanism:

A target-specific single guide RNA (sgRNA) is designed to form a complex with the Cas9 protein. The CRISPR/Cas9 complex binds to the target DNA and induces a double-stranded break at specific sequences. The cell’s endogenous repair mechanisms, such as Non-Homologous End Joining (NHEJ) or Homology-Directed Repair (HDR), can introduce genetic modifications. NHEJ often results in gene knockout or small insertions and deletions, whereas HDR enables precise gene insertion or correction of specific mutations (Figure 2).

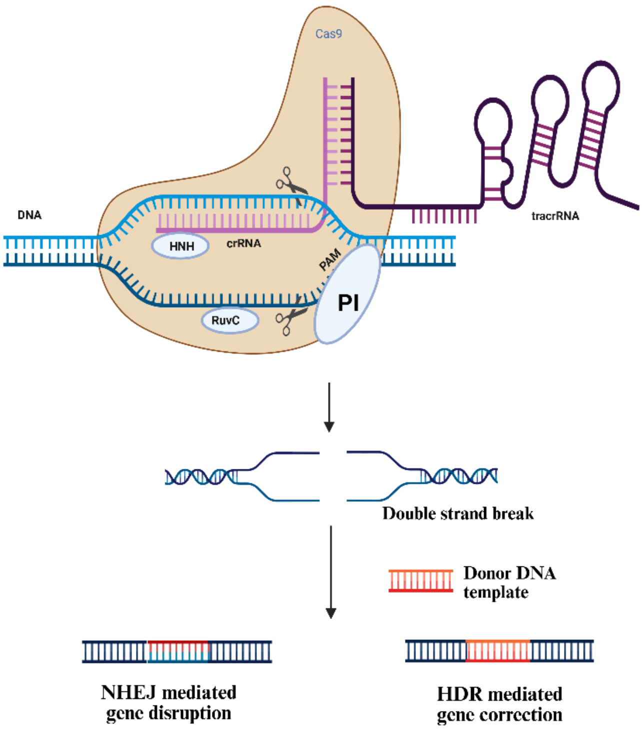

Figure 2: Mechanism of CRISPR-Cas9-based genome editing (created in biorender.com)

A list of plant genome editing through CRISPR-Cas9 for bacterial resistance is given below in **Table 1**:

Plant Species	**Pathogen**	**Targeted gene**	**Reference**
Oryzae sativa	*Xanthomonas oryzae* pv. *oryzae*	*OsSWEET13* *OsXa5* *OsXa13* *OsSWEET14*	Zhou et al. (2015) Tao et al. (2021) Li et al. (2019) Zafar et al. (2020)
Citrus sinensis	*Xanthomonas citri subsp. citri*	*CsWRKY22*	Wang et al. (2019)
Solanum lycopersicum	*Pseudomonas syringae* pv. tomato	*SlJAZ2*	Ortigosa et al. (2019)
Malus domestica	*Erwinia amylovora*	*DIPM-1* *DIPM-2* *DIPM-4*	Malnoy et al. (2016)
Manihut esculenta	*Xanthomonas phaseoli* pv. *manihotis*	*SWEET10a*	Wang et al. (2024)
Gossypium hirsutum	*Xanthomonas citri* pv. *malvacearum*	*GhSWEET10* *GhSWEET14a* *GhSWEET1ab*	Mormile et al. (2025)
Musa spp.	*Xanthomonas spp.*	*DMR6*	Tripathi et al. (2021)

CONCLUSION:

Traditional methods of combating bacterial plant diseases are no longer sufficient to protect our crops from rapidly evolving pathogens. Modern biotechnology offers an effective internal defense for plants through genetic engineering. While transgenic methods successfully enhance a plant's natural defense, recent advances in non-transgenic technologies, such as SIGS and CRISPR-Cas9 genome editing, represent significant progress. These tools precisely silence pathogenicity genes or knock out host plants' susceptibility genes, resulting in durable, broad-spectrum disease resistance. Notably, CRISPR and sprayable dsRNA do not leave any trace of foreign DNA, and they can bypass stringent GMO regulations. Adoption of these highly specific molecular tools is essential for developing climate-resilient, disease-resistant crops that support global food security.

REFERENCES:

Alburquerque, N., Faize, L., & Burgos, L. (2017). Silencing of Agrobacterium tumefaciens oncogenes ipt and iaaM induces resistance to crown gall disease in plum but not in apricot. *Pest Management Science*, 73(10), 2163-2173.

Hummel, A. W., Doyle, E. L., & 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 Phytologist*, 195(4), 883-893.

Jang, S., Kim, D., Lee, S., & Ryu, C. M. (2024). Plant-induced bacterial gene silencing: a novel control method for bacterial wilt disease. *Frontiers in Plant Science*, 15, 1411837.

Li, C., Li, W., Zhou, Z., Chen, H., Xie, C., & Lin, Y. (2019). A new rice breeding method: CRISPR/Cas9 system editing of the Xa13 promoter to cultivate transgene-free bacterial blight-resistant rice. *Plant Biotechnology Journal*, 18(2), 313.

Li, C., Liu, B., Dong, H., & Yang, B. (2025). Enhancing resistance to bacterial blight in rice using CRISPR-based base editing technology. *The Crop Journal*, 13(1), 115-124.

Malnoy, M., Viola, R., Jung, M. H., Koo, O. J., Kim, S., Kim, J. S., ... & Nagamangala Kanchiswamy, C. (2016). DNA-free genetically edited grapevine and apple protoplast using CRISPR/Cas9 ribonucleoproteins. *Frontiers in plant science*, 7, 1904.

Mello, C. C. (2007). Return to the RNAi world: rethinking gene expression and evolution. *Cell Death & Differentiation*, 14(12), 2013-2020.

Mormile, B. W., Yan, Y., Bauer, T., Wang, L., Rivero, R. C., Carpenter, S. C., ... & Shan, L. (2025). Activation of three targets by a TAL effector confers susceptibility to bacterial blight of cotton. *Nature communications*, 16(1), 644.

Mourgues, F., Brisset, M. N., & Chevreau, E. (1998). Strategies to improve plant resistance to bacterial diseases through genetic engineering. *Trends in Biotechnology*, 16(5), 203-210.

Ortigosa, A., Gimenez-Ibanez, S., Leonhardt, N., & Solano, R. (2019). Design of a bacterial speck resistant tomato by CRISPR/Cas9-mediated editing of Sl JAZ 2. *Plant biotechnology journal*, 17(3), 665-673.

Pal, G., Ingole, K. D., Yavvari, P. S., Verma, P., Kumari, A., Chauhan, C., ... & Vemanna, R. S. (2024). Exogenous application of nanocarrier-mediated double-stranded RNA manipulates physiological traits and defence response against bacterial diseases. *Molecular Plant Pathology*, *25*(1), e13417.

Qiu, D., Xiao, J., Xie, W., Liu, H., Li, X., Xiong, L., & Wang, S. (2008). Rice gene network inferred from expression profiling of plants overexpressing OsWRKY13, a positive regulator of disease resistance. *Molecular Plant*, 1(3), 538-551.

Rai, R., & Rai, M. N. (2025). Tackling bacterial diseases in crops: current and emerging management strategies. *Phytopathology Research*, 7(1), 58.

Shirisha, P. S., Raju, M. R., Madhusudhan, P., Rani, C. S., & Srinivas, T. (2025). Assessment of bacterial leaf blight severity in rice caused by *Xanthomonas oryzae* pv. *oryzae* in the Godavari zone of Andhra Pradesh. *The Andhra Agric. J*, 72 (2), 151-155

Sundaresha, S., Jeevalatha, A., Kumar, R., Sood, S., Sharma, S., Bhardwaj, V., ... & Chakrabarti, S. K. (2022). RNA interference: A versatile tool to augment plant protection strategies in potato. In *Sustainable management of potato pests and diseases* (pp. 369-388). Singapore: Springer Singapore.

Tao, H., Shi, X., He, F., Wang, D., Xiao, N., Fang, H., ... & Ning, Y. (2021). Engineering broad-spectrum disease-resistant rice by editing multiple susceptibility genes. *Journal of Integrative Plant Biology*, 63(9), 1639-1648.

Tripathi, A. N., Tiwari, S. K., Sharma, S. K., Sharma, P. K., & Behera, T. K. (2024). Current status of bacterial diseases of vegetable crops. *Vegetable Science*, 51, 106-117.

Tripathi, J. N., Ntui, V. O., Shah, T., & Tripathi, L. (2021). CRISPR/Cas9-mediated editing of DMR6 orthologue in banana (Musa spp.) confers enhanced resistance to bacterial disease. *Plant Biotechnology Journal*, 19(7), 1291.

Wang, L., Chen, S., Peng, A., Xie, Z., He, Y., & Zou, X. (2019). CRISPR/Cas9-mediated editing of CsWRKY22 reduces susceptibility to Xanthomonas citri subsp. citri in Wanjincheng orange (Citrus sinensis (L.) Osbeck). *Plant Biotechnology Reports*, 13(5), 501-510.

Wang, Y., Geng, M., Pan, R., Zhang, T., Lu, X., Zhen, X., ... & Yao, Y. (2024). Editing of the MeSWEET10a promoter yields bacterial blight resistance in cassava cultivar SC8. *Molecular Plant Pathology*, 25(10), e70010.

Zafar, K., Khan, M. Z., Amin, I., Mukhtar, Z., Yasmin, S., Arif, M., ... & Mansoor, S. (2020). Precise CRISPR-Cas9 mediated genome editing in super basmati rice for resistance against bacterial blight by targeting the major susceptibility gene. *Frontiers in plant science*, 11, 575.

Zeng, X., Tian, D., Gu, K., Zhou, Z., Yang, X., Luo, Y., ... & Yin, Z. (2015). Genetic engineering of the Xa10 promoter for broad-spectrum and durable resistance to *Xanthomonas oryzae* pv. *oryzae*. *Plant biotechnology journal*, 13(7), 993-1001.

Zhou, J., Peng, Z., Long, J., Sosso, D., Liu, B. O., Eom, J. S., ... & Yang, B. (2015). Gene targeting by the TAL effector PthXo2 reveals cryptic resistance gene for bacterial blight of rice. *The Plant Journal*, 82(4), 632-643.