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Review Article

CRISPR–Cas9 Technology and its Utilization in Crop Advancement

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ABSTRACT

A technique for genome editing, which has transformed the genetic fields and agricultural development, is the regularly interspaced short palindrome recalls/CRISPR-linked protein 9. CRISPR-Cas9 is a multiskilled technology that uses gRNA (guided RNA) and a Cas9 endonucleases activity. CRISPR technology provides a way to generate novel plants by removing negative genetic elements or by introducing dominant resistant genes. Targeted editing can be used to substitute single-base of a gene of interest, tissue-specific knockouts using CRISPR technology, and induce mutation in the cell or tissue via specific promotors. In this review, the overview of CRISPR-Cas9 technology and crop improvement using CRISPR technology are being discussed.

Keywords: *Protein, Endonucleases, Resistant, Knockout, Mutation, Promotors.*

INTRODUCTION

For the improvement in crop plants, the creation of variation in the current gene pool is necessary. Genetic erosion can be seen at both the gene level and the species level through continuous breeding practices. This genetic erosion makes modern plants more vulnerable to biotic and abiotic stresses when compared to their wild relatives. Using gene-editing tools researchers can produce new varieties and create variability. Genome editing is a technique for either introducing an insert or deleting knock-off target genes that interferes with a certain gene function. Sequencing technologies and genomic information have become available for several plant species and gene-editing technology is proving new opportunities to improve crop quality and yield. Three technologies were initially developed in gene editing and the designed nuclease ZFN (zinc finger nuclease) described. Second, a more flexible designed nuclease was created for transcript activator such as effectors nuclease (TALEN) (Joung & Sander, 2012; & Baltes et al., 2014). The updated technique has been created as a simplified and more flexible, engineered nuclease, clustered with a regularly spaced short palindrome repeat (CRISPR)/CRISPR-associated protein-9 (Cas9).

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This technique avoids heterozygosity, sterility and lengthy cycles of life (Boch et al., 2009). CRISPR-Cas9 exploits interaction between DNA-RNA and includes two components: Cas9 and gRNA (guided RNA). Protein Cas9 is an RNA-dependent DNA endonuclease that creates a gRNA complex. In addition to the targeted sequence, gRNA contains 20 nucleotides (short RNA). This is essential for enrolling the protein Cas9 in its specific location. CRISPR, gene editing, or genome editing is the most frequent usage, whereas DNA-protein-based linkage, design, and expression of two distinct DNA-binding domains (500-700 amino acid for TALENs) are needed by the Target Site for ZFN and TALENs (Table 1). This is a tough procedure. for agricultural crop enhancement (Joung & Sander, 2012).

The usage of ZNF and TALEN in their specific genome editing is extensively utilized as a new technique and restricted. This prokaryotic method is immediately accepted in eukaryotic host cells for the production of genomes (Jinek et al., 2012; & Nakayama et al., 2013). CRISPR targets endogenous genes that cannot be targeted by RNAi. Endogenous microRNAs control RNAi genes (miRNAs). miRNAs can induce hylomorphic mutations and untargeted phenotypes when displaced from exogenous miRNAs (Khan et al., 2019). CRISPR-Cas9 is a multiple technique extensively utilized in the production of GMOs. This technique is programmable as a genome software, Cas9 protein, and scientists utilize this technology to detect the specific DNA sequence, to chop down the DNA sequence and to introduce new DNA of interest, called gene knock or gene knock out. Based on the Watson and crick base pairing technology CRISPR-Cas9 makes the tool more powerful and applicable (Doudna & Charpentier, 2014). CRISPR-Cas9 applications provide potential in designing microbial consortia to disclose the genetic function and bio intelligence (Bittihn et al., 2018).

CRISPR-Cas9 edited variants of rice at the 30- end of *OsLOGL50* coding sequence increased grain yield under low nitrogen, watered, and normal nitrogen conditions and grain yield (Cui et al., 2020). Knock-out genes *gs3* and *dep1* resulted in enhanced grain size and number of panicles (Wang et al., 2020). This suggested that genome editing through CRISPR-Cas9 is a novel approach for précised breeding. This novel technique allows scientists to develop foods that meet the demand of the growing population. This system can also be used for the improvement of crops against changing climatic and tolerance to harsh conditions, disease and improved quality and quantity of grain crops. In this review, CRISPR-Cas9 technology and crop improvement using CRISPR technology are being discussed.

Mechanism of the CRISPR/Cas9

Cas9 gene, the most widely utilized gene for which G-rich (5′-NG-3′) adjacent motif (PAM) is required is obtained from Streptococcus pyrogens (SpCas9). Specific Cas9 encoding genes tailored for plants are necessary to enhance genome editing.

The CRISPR-Cas9 immunity mechanism includes three-stage adjustment, expression, and interference (Figure 1). Adaptation includes the intrusion of DNA from plasmids or viruses which are chopped into tiny parts and integrated into CRISPR loci. CRISPR locus is transcribing and processing for the generation of RNA (crRNA), which guides the effector endonucleases through basic complementarity to the viral material (Yosef et al., 2012). CRISPR/Cas Type II Interference DNA needs just one Cas9 protein. Cas9 proteins include two functional fields consisting of a large-scale (REC) field and a smaller (NUC) domain. Moreover, the small nuclear domain includes two preserved endonuclease sites, RuvC and HNH, and a site interacting with Protospacer's adjacent motif (PAM). HNH supplements the sgRNA sequence, whereas RuvC acts as the uncomplimentary strand. The gRNA and RNP complex interact with Cas9. RNP complex undergoes successive modifications such as targeting, binding, rearrangement, enabling and endonuclease

activity at the target location (Jackson et al., 2014; & Mulepati et al., 2014).

In addition to the target site, the single guided RNA (sgRNA) is a key component of CRISPR. SgRNA has 20 nuclear sites (5 pour-NGG-3) on three ends, followed by a partly complementary sequence called "RNA scaffold" SGRNA is 20 nucleotides long and has three nucleotide PAM sites. RNA scaffold in the natural CRISPR-Cas9 system, while sgRNA is known as crRNA. The structure is formed to lead the division to the PAM location. Transcription of the genes sgRNA and Cas9 depends mostly on the type of promoter. Guanine(G) nucleotide, as an initial base prior to sgRNA, is favored for the effective activity of wheat u6 promoters, for example, by RNAIII polymerase promoters such as U3 or U6. G should thus be included in the sgRNA at 5 by the end. The targets of the CRISPR-Cas9 only are specific loci genomes and this system includes the RNA (gRNA) guide Cas9 nuclease from bacteria to generate targeted double beaches repaired by the non-homologous end joints (NHEj)/homologous genome editing (HDR) to produce genomic changes, gene knockouts, and insertions of genes CRISPR-Cas9 (Qi et al., 2013).

Description	ZFNs	TALENs	CRISPR-Cas9
Components	ZF-Folk fusion protein	TALE-folk fusion protein	Cas9 protein and guided RNA
Site of recognition	9-18 bp per ZNF monomer	14-20 bp per TALENs monomer	20 bp per
Mechanism of action	ZF domain recognizes target DNA sequences and dimerization of FokI nucleases generate DSBs in DNA	TALE domain recognizes target DNA sequences and dimerization of FokI nucleases create DSBs in DNA	Guide RNA recognizes target DNA sequence next to NGG motif and Cas9 nuclease induces DSBs in DNA
Of-target effects	Frequent	Limited	Negligible
Execution time	Time-consuming	Time-consuming	Quick

Table 1 Comparison of nuclease-mediated genome editing

CRISPR-Cas9 application in crop improvement

Since agricultural domestication, producers have tried to modify plant characteristics to increase the quality and production of crops and to react to environmental influences, including diseases and abiotic conditions, such as high temperatures and drought. CRISPR-Cas9 technology was initially proven in plants as an effective method for genome editing in 2012. This technique may be used to introduce desired genes such as single knockouts. The initial CRISPR-Cas9 work focused on rice genome editing (Oryza sativa), the world's top staple food crop (Feng et al., 2013). In the initial trial, the system was feasible and additional research was encouraged for the use of this technology. In this work, the sgRNA and Cas9 genes were developed and effective alteration of the rice genome was achieved (CaMV 35S and OsU6-2). In various plants including the tobacco of Triticum (*Nicotiana benthamiana*), rice (*Oryza sativa*), or tobacco of Triticum maize, the CRISPR-Cas9 system has been effectively employed (Bortesi & Fischer, 2015). Different biallelic mutations in Arabidopsis and ZmHKT1 genes have been generated and their potential is testing for multiplex genome editing by verifying biallelic mutations of the TRY and CPC genes (Cong et al., 2013).

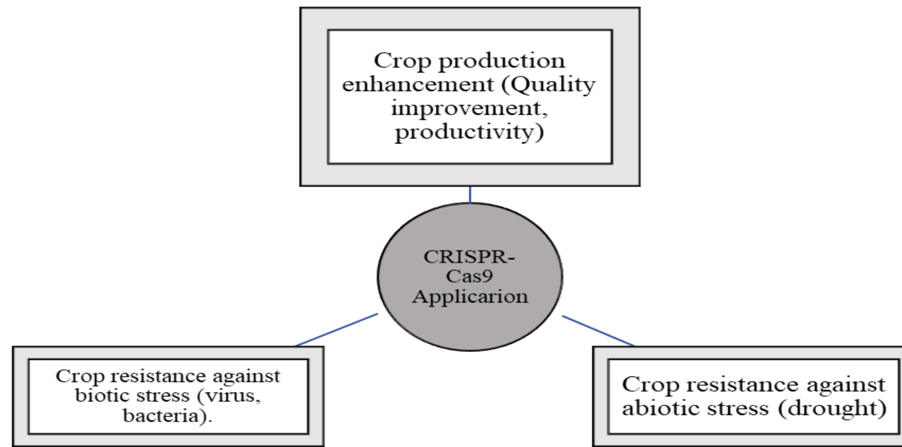

Figure 2 Applications of CRISPR/Cas9 system in plants

Foreign DNA Target DNA PAM

Acquisition

Bacterial Genome

CRISPR loci

TracrRNA Cas9 Cas genes crRNA crRNA crRNA

TracrRNA

Primary transcription

crRNA biogenesis

tracrRN-Cas9-crRNA complex

Cas9

Interference

Inactivation of foreign DNA

Cleavage

Crop production enhancement

The yield of a crop is a quantitative feature and time-consuming and hard to enhance yield and associated features through conventional breeding. The parents must be picked, the design of the crossover selected and the selection of suitable plants for all generations must be followed by hybridization. Much work has been done for the editing of genes that connect to improved quality via the use of CRISPR-Cas9.

The technology CRISPR-Cas9 is fast and circumvents the arduous hybridization procedure. Mediated mutagenesis CRISPR-Cas9 was performed for four rice gene(s): Ideal plant architecture 1 (IPA1), grain sizes 3 (GS3) (Gn1a). Editing of these genes included 27.5 to 67.5 percent mutation rate, tillers, grain sizes, grain number and panicle architecture. All the previously listed features showed improvement and better output from the mutant plants (Li et al., 2016). Four genes editing negative weight regulators in rice, i.e. grain widths 2, 5, and 6 (GW2, GW5 and GW6) and GS3; grain weight and weight also improved fast (Xu et al., 2013). Grain filling helps to produce rice grains, OsSweet11 (sugar transporter gene) gene knock-out using the CRISPR-Cas9 leads to a reduction in sucrose, grain weight and mutant rice seed, which shows the importance of this gene for grain fillings and saccharose and sucrose transfer. Overexpressing OsSWEET11 can be a good technique to improve rice output. Increased rice plant length and output were also achieved by IsPPKL1 gene editing (Huang et al., 2013). In rice, the OsPRX2 gene has evolved potassium deficiency tolerance (Mao et al., 2018). The SBEI IIb gene was also increased in amylase concentration (Sun et al., 2016). The aim of the waxy gene was to lower the amylose concentration and to convert rice into gluten without changing agronomic characteristics using CRISPR-Cas9. This research has shown the high potential of CRISPR-Cas9 to market elite crops (Zhang et al., 2018).

Crop resistance against biotic stress

CRISPR-Cas9 technology is utilized to produce plants with virus, bacterium and fungal resistance. Plant diseases (viruses, bacteria and mushrooms) naturally occur in the environment and commonly infect the plant that affects plant growth and development. For breeding disease resistance a number of traditional techniques have been explored. The induction of resistance in cotton by transfer of the *G. anomalum* and *G. arboreum* genes to *G. hirsutum* was done through interspecific hybrids (Miah et al., 2013). Traditional reproduction resistance methods, however, take time and are occasionally connected to undesired genes that may influence plant growth, which is often difficult and resistant. (Spanish version, 2011). OsSWEET13 mutagenesis in rice improves bacterial bone resistance through CRISPR-Cas9 (Zhou et al., 2015). Also utilized in Japonica rice for CRISPR-Cas9, targeted translation from OsERF922 has been exploited to create resistance to blight disease. Over the expression of the OsERF22 gene, seed production, thousands of seed weigher and viable tillers may be increased (Zhou et al., 2015). The spread of illnesses is caused by several microorganisms. Bacterial bacteria, for example, are devastating diseases produced by *Xanthomonas oryzae* in rice (Oryza sativa). Oryceans (Xoo) require effectors, TAL, for bine host diseases that are vulnerable to plant infection. Xoo also requires an activator. OsSweet11(also known as Os8n3) is a gene that susceptibles Xoo TAL to infection and causes bacterial distress (Yang et al., 2006). CRISPR-Cas9 application to the deletion Os8n3 gene has shown bacterial Xoo resistance susceptible (Kim et al., 2019).

Crop resistance against abiotic stress

Temperature and drought are also growing with climate change. High warmth, dryness and ground salinity lead to reduced agricultural output. The creation of climate-intelligent crops or abiotic stress-tolerant plants is a significant application of CRISPR-Cas9.

In comparison to wild-type crops, RNAi CRISPR-Cas9 generated double mutants ugt79b2/b3, which produced less anthocyanin and were more vulnerable to stress and therefore confirmed their function.

OsSAPK2 was described functionally by examination of rice loss-of-function mutants generated by CRISPR/Cas9. In addition to abscisic acid (ABA) insensitivity at germination/seedling stage in comparison with wilderness plants, the Sapk2 mutants displayed an enhanced sensitivity towards reactive oxygen species and dryness, showing the participation of OsSAPK2 in ABA-mediated drought signal/tolerance. SAPK2 provides plant drought resistance through stomatal shutting, suitable solution buildup and gene overexpression coding for reactive oxygen spraying antioxidant enzymes (Li et al., 2017).

Limitation of Cas9 system

Although, CRISPR-Cas9 provides a major breakthrough in plant research but limitations need to be mentioned in terms of efficiency and fidelity (Peng et al., 2016). Cas9 system is an excellent tool for editing genome but important aspects related to CRISPR technology remain uncertain. The mutation that is caused because of off-targets and cleavage efficiency requires good knowledge. The need for PAM adjacent to the targeted site is another limitation of this approach. A repairing mechanism for the desired modifications needs to be screened carefully.

With gene editing, identifying a crop whether a CRISPR has been developed or traditionally altered is challenging for farmers and regulators. CRISPR/Cas9 modified plants are altered in the genome itself and no other species officially introduces a gene. The USDA has nevertheless failed to control the cultivation and marketing, with the CRISPR/Cas9 non-browning of the common white button mushroom (*Agaricus bisporus*), thereby clearing a discussion on the CRISPR-modified agricultural regulation status (Waltz, 2016).

Conclusion and future prospects

CRISPR-Cas has many flairs and is a natural tool that has already shown itself in the past several years and has been extensively accepted with a large number of applications in agriculture and crop development. In comparison to other conventional approaches, it is easy, rapid and accurate to use CRISPR-Cas9 in crop modification. The results of CRISPR-Cas9-based genome editing research and investigations have shown that sgRNA is dependent on sgRNA with about 20 basis sequences for targeted DNA. CRISPR-Cas9 technological developments provide an efficient approach to cropping, including food shortages and climate change, under tough situations. The basic goal of ko agriculture is genome editing to create variety in crops. As an alternative to traditional breeding methods, CRISPR-Cas9 is emerging. This technique should be utilized to improve crops under changing climate circumstances, in order to improve quality, production and sustainability.

Many of the research now focuses on using CRISPR-Cas9 crop feature enhancement machinery. Case enzymes can be developed more precisely for different applications. Research using Cas13 showed that this mechanism may be made more precise and efficient. CRISPR-Cas9 technology can modify the strength of a gene. The biology of agricultural stress will contribute to the design of optimal or superior plants.

CRISPR-Cas9 is an excellent approach to tackle illnesses specific sequence nuclease editing (wang et al., 2016). Targeted for the OsERF922 rice gene, 21 CRISPR-ERF922 mutants from 50 T0 transgenic plants have been discovered (Wang et al., 2016). In addition, great efficiency of emendation of target codons in rice may be attained using combining cytidine deaminase enzyme Cas9 (Li et al., 2016). The technologies of CRISPR-Cas9 have a future potential for creating desirable mutations, as they may be used to change a crop as desired.

REFERENCES

Baltes, N. J., Gil-Humanes, J., Cermak, T., Atkins, P. A., & Voytas, D. F. (2014). DNA replicons for plant genome engineering. *The Plant Cell, 26*(1), 151-163.

Bittihn, P., Din, M. O., Tsimring, L. S., & Hasty, J. (2018). Rational engineering of synthetic microbial systems: from

single cells to consortia. *Current opinion in microbiology, 45*, 92-99.

Boch, J., Scholze, H., Schornack, S., Landgraf, A., Hahn, S., Kay, S., & Bonas, U. (2009). Breaking the code of DNA binding specificity of TAL-type III effectors. *Science, 326*(5959), 1509-1512.

Bortesi, L., & Fischer, R. (2015). The CRISPR/Cas9 system for plant genome editing and beyond. *Biotechnol. Adv. 33*, 41–52. doi: 10.1016/j.biotechadv.2014. 12.006.

Cong, Á., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., & Marraffini, L. A. (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science, 339*(6121), 819-823.

Cui, Y., Hu, X., Liang, G., Feng, A., Wang, F., Ruan, S., & Qian, Q. (2020). Production of novel beneficial alleles of a rice yield-related QTL by CRISPR/Cas9. *Plant biotechnology journal, 18*(10), 1987-1989.

Cui, Y., Jiang, N., Xu, Z., & Xu, Q. (2020). Heterotrimeric G protein are involved in the regulation of multiple agronomic traits and stress tolerance in rice. *BMC plant biology, 20*(1), 1-13.

Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science, 346*(6213).

Feng, Z., Zhang, B., Ding, W., Liu, X., Yang, D. L., Wei, P., & Zhu, J. K. (2013). Efficient genome editing in plants using a CRISPR/Cas system. *Cell research, 23*(10), 1229-1232.

Huang, L., Zhang, R., Huang, G., Li, Y., Melaku, G., Zhang, S., & Hu, F. (2018). Developing superior alleles of yield genes in rice by artificial mutagenesis using the CRISPR/Cas9 system. *The Crop Journal, 6*(5), 475-481.

Jackson, R. N., Golden, S. M., van Erp, P. B., Carter, J., Westra, E. R., Brouns, S. J., & Wiedenheft, B. (2014). Crystal structure of the CRISPR RNA–guided surveillance complex from Escherichia coli. *Science, 345*(6203), 1473-1479.

Jinek, M., Krzysztof; Chylinski, Ines; Fonfara, M; Hauer, Jennifer; Doudna, and Emmanuelle Charpentier. (2012). “A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity.” *Science, (337)*, 816-21.

Joung, J. K., & Sander, J. D. (2013). TALENs: a widely applicable technology for targeted genome editing. *Nature reviews Molecular cell biology, 14*(1), 49-55.

Khan, A. A., Betel, D., Miller, M. L., Sander, C., Leslie, C. S., & Marks, D. S. (2009). Transfection of small RNAs globally perturbs gene regulation by endogenous microRNAs. *Nature biotechnology, 27*(6), 549-555.

Kim, Y. A., Moon, H., & Park, C. J. (2019). CRISPR/Cas9-targeted mutagenesis of Os8N3 in rice to confer resistance to Xanthomonas oryzae pv. oryzae. *Rice, 12*(1), 1-13.

Li, M., Li, X., Zhou, Z., Wu, P., Fang, M., Pan, X., & Li, H. (2016). Reassessment of the four yield-related genes Gn1a, DEP1, GS3, and IPA1 in rice using a CRISPR/Cas9 system. *Frontiers in plant science, 7*, 377.

Li, P., Li, Y. J., Zhang, F. J., Zhang, G. Z., Jiang, X. Y., Yu, H. M., & Hou, B. K. (2017). The Arabidopsis UDP - glycosyltransferases UGT79B2 and UGT79B3, contribute to cold, salt and drought stress tolerance via modulating anthocyanin accumulation. *The Plant Journal, 89*(1), 85-103.

Mao, X., Zheng, Y., Xiao, K., Wei, Y., Zhu, Y., Cai, Q., & Zhang, J. (2018). OsPRX2 contributes to stomatal closure and improves potassium deficiency tolerance in rice.

Biochemical and biophysical research communications, 495(1), 461-467.

Miah, G., Rafii, M. Y., Ismail, M. R., Puteh, A. B., Rahim, H. A., Asfaliza, R., & Latif, M. A. (2013). Blast resistance in rice: a review of conventional breeding to molecular approaches. *Molecular biology reports, 40*(3), 2369-2388.

Mulepati, S., Héroux, A., & Bailey, S. (2014). Crystal structure of a CRISPR RNA–guided surveillance complex bound to a ssDNA target. *Science, 345*(6203), 1479-1484.

Nakayama, T., Fish, M. B., Fisher, M., Oomen - Hajagos, J., Thomsen, G. H., & Grainger, R. M. (2013). Simple and efficient CRISPR/Cas9 - mediated targeted mutagenesis in Xenopus tropicalis. *genesis, 51*(12), 835-843.

Qi, L. S., Larson, M. H., Gilbert, L. A., Doudna, J. A., Weissman, J. S., Arkin, A. P., & Lim, W. A. (2013). Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell, 152*(5), 1173-1183.

Sun, Y., Zhang, X., Wu, C., He, Y., Ma, Y., Hou, H., & Xia, L. (2016). Engineering herbicide-resistant rice plants through CRISPR/Cas9-mediated homologous recombination of acetolactate synthase. *Molecular plant, 9*(4), 628-631.

Waltz, E. (2016). Gene-edited CRISPR mushroom escapes US regulation. *Nature News, 532*(7599), 293.

Wang, C., Wang, G., Gao, Y., Lu, G., Habben, J. E., Mao, G., & Greene, T. W. (2020). A cytokinin-activation enzyme-like gene improves grain yield under various field conditions in rice. *Plant molecular biology, 102*(4), 373-388.

Wang, F., Wang, C., Liu, P., Lei, C., Hao, W., Gao, Y., & Zhao, K. (2016). Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene OsERF922. *PloS one, 11*(4), e0154027.

Wang, M., Xu, Z., Gosavi, G., Ren, B., Cao, Y., Kuang, Y., & Zhou, H. (2020). Targeted base editing in rice with CRISPR/ScCas9 system. *Plant biotechnology journal, 18*(8), 1645.

Xu, R., Yang, Y., Qin, R., Li, H., Qiu, C., Li, L., & Yang, J. (2016). Rapid improvement of grain weight via highly efficient CRISPR/Cas9-mediated multiplex genome editing in rice. *Journal of Genetics and Genomics= Yi chuan xue bao, 43*(8), 529-532.

Yang, B., Sugio, A., & White, F. F. (2006). Os8N3 is a host disease-susceptibility gene for bacterial blight of rice. *Proceedings of the National Academy of Sciences, 103*(27), 10503-10508.

Yosef, I., Goren, M. G., & Qimron, U. (2012). Proteins and DNA elements essential for the CRISPR adaptation process in Escherichia coli. *Nucleic acids research, 40*(12), 5569-5576.

Zhang, J., Zhang, H., Botella, J. R., & Zhu, J. K. (2018). Generation of new glutinous rice by CRISPR/Cas9-targeted mutagenesis of the Waxy gene in elite rice varieties. *Journal of integrative plant biology, 60*(5), 369-375.

Zhou, J., Peng, Z., Long, J., Sosso, D., Liu, B., 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.