JUST AGRICULTURE
multidisciplinary e-Newsletter

CRISPR-Cas9 for Plant Disease Management: A Promising Approach of Genome Editing

[1]Satyajit Balasaheb Korade, [2]R.V. Sundarrajan, [3]Patel Harsh Jitendrakumar and [4]Manoharmayum Dolpriya Devi

[1]Ph.D. Scholar, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra
[2]Research Scholar, Horticultural College, and Research Institute, Periyakulam, Tamil Nadu
[3]Ph.D. Scholar, Junagarh Agricultural University, Junagarh, Gujrat
[4]Assistant Professor, KL College of Agriculture, KLEF, Vaddeswaram, Andhra Pradesh

ARTICLE ID: 08

Abstract

The development of CRISPR-Cas9 technology has transformed genome editing by providing previously unheard-of levels of efficiency and precision in targeted genetic alterations. CRISPR-Cas9 has shown promise in the field of plant disease management as a means of boosting pathogen resistance. An overview of the use of CRISPR-Cas9 to modify plant genomes to impart resistance against different diseases is given in this article. By carefully altering important genes linked to vulnerability, the technique strengthens the plant's defences against disease. Recent developments in CRISPR-Cas9-mediated genome editing for plant disease resistance, encompassing a variety of crops and pathogens, are highlighted in the abstract. It also addresses possible difficulties and moral issues related to the application of technology in agriculture. The integration of CRISPR-Cas9 into crop breeding programs holds great promise for sustainable and efficient management of plant diseases as the technology continues to advance.

Keywords: CRISPER-Cas9, Disease management, Genome editing, Vulnerability

Introduction

Despite their taxonomic differences, fungi and oomycetes are highly devastating plant diseases because they share filamentous growth and host infection structures (Dong et al., 2015). The dire need for disease-resistant plants is highlighted by instances of catastrophic crop losses, such as the Bengal famine brought on by the rice brown spot fungal pathogen Cochliobolus miyabeanus (Chakrabarti, 2001) and the Irish potato famine brought on by the oomycete Phytophthora infestans (Turner, 2005). In response to this difficulty, CRISPR/Cas9 technology has become an effective instrument in pathology and plant breeding, providing ease

JUST AGRICULTURE
multidisciplinary e-Newsletter

of use and accuracy in the control of both genetic and nongenetic plant features. Since its discovery in Haloferax mediterranei in 1993 and subsequent identification in several bacterial and archaeal genomes, the CRISPR/Cas system—which involves Clustered Interspaced Palindromic Repeats (CRISPR) and CRISPR-associated proteins (Cas)—has attracted significant attention (Guo et al., 2010). Early in the new millennium, sequence similarity between CRISPR spacer sections and those of bacteriophages, archaeal viruses, and plasmids was discovered, supporting CRISPR's role as an immune system. The iap gene in E. coli was first linked to CRISPR/Cas9 systems, which were first discovered as bacterial cell immunity responses against viral invasion using proportionate genomic analysis. The word "CRISPR" was first used in 2002 by Jansen et al. and gained traction in the scientific community as more research was done on these sequences. The potential for creating plants resistant to harmful infections through the application of CRISPR technology presents a focused and effective means of improving agricultural sustainability. When considering the implementation of CRISPR/Cas9 as a strategy for controlling plant disease outbreaks, it is important to acknowledge the inherent complexity of these systems, since their disassembly presents hurdles that have developed over millions of years (Dort et al., 2020).

Fungal Resistance Via CRISPR/Cas9 Technology:

By selectively targeting susceptibility (S) genes, such as rice Ethylene Response Factor 922 (OsERF922) and mildew resistance locus O (MLO), CRISPR/Cas9 technology has proven important in providing fungal resistance. The 1942 discovery of the MLO gene (Jørgensen, 1992) is crucial for barley's vulnerability to powdery mildew. Wang et al. (2014) used TALEN and the TaMLO-A1 allele of exon 2 through CRISPR/Cas9 to successfully target all three homoeoalleles (MLO-A1, MLOB1, and MLO-D1) in wheat, resulting in heritable resistance against Blumeria graminis f. sp. tritici. Tomelo is a non-transgenic tomato cultivar created by CRISPR/Cas9; in tomatoes, powdery mildew is caused by the Ascomycete fungus Oidium neolycopersici (Jones et al., 2001). When two sgRNAs were used to target the SlMlo1 locus, a 48 bp loss resulted in resistance. In addition to MLO, other S genes associated with powdery mildew have been found, such as PMR4, which is connected to the disease in Arabidopsis. Koseoglou (2017) used CRISPR/Cas9 to target the ortholog SlPMR4 in tomatoes. She saw uncommon inversion changes as well as deletions in the targeted exon-2, which gave the T2 progenies partial resistance against O. neolycopersici. Using CRISPR/Cas9 technology, the

JUST AGRICULTURE
multidisciplinary e-Newsletter

OsERF922 gene in rice was targeted. This gene encodes an APETELA2/ethylene response factor (AP2/ERF) type transcription factor that is significantly activated by Magnaporthe oryzae (Liu et al., 2012). This strategy produced resistance to blast disease, and the T2 mutant lines showed various agronomic features that were like those of wild-type rice plants.

Resistance Against Bacteria Using CRISPR/Cas9:

Technology Although CRISPR/Cas9 has been widely used in crops to confer fungal and viral resistance, very few studies have shown its use in combating bacterial infections. The γproteobacterium Xanthomonas oryzae pv. oryzae uses type III transcription-activator-like effectors (TALEs) to increase host gene expression, which makes the host susceptible. It has been determined that the sucrose transporter gene OsSWEET13 confers sensitivity to the X. oryzae pv. oryzae effector protein PthXo2. OsSWEET13 allele alterations generated by CRISPR/Cas9 gave resistance against bacterial blight, indicating its potential to fight bacterial illnesses. Plant defence regulation has been linked to DMR6, or Downy Mildew Resistance 6, a negative regulator of plant defence. When the DMR6 ortholog SlDMR6–1 was targeted in tomato, mutant plants with a shortened form of SlDMR6 were produced. These plants exhibited broad-spectrum resistance against Pseudomonas syringae, Xanthomonas gardneri, X. perforans, and Phytophthora capsici. Apples and other commercially valuable Rosaceae plants are susceptible to the fire blight disease caused by the enterobacterium Erwinia amylovora. Four leucine-rich-repeat, receptor-like serine/threonine kinases, called DIPM 1, 2, 3, and 4, are generated by the DspE-interacting proteins of Malus (DIPM) genes and interact with the pathogenicity effector DspE of E. amylovora. used the CRISPR/Cas9 system to target the DIPM 1, 2, and 4 genes in apple protoplasts in an effort to give resistance against the fire blight disease.

Achievements in Plant Virus Resistance Through CRISPR/Cas9:

Technology Viral resistance has been successfully achieved by using the CRISPR/Cas9 system to target the host susceptibility factor or the viral genome. The genes found in jeneminiviruses, especially those with genomes made of single-stranded DNA (ssDNA), are essential for the mobility, replication, and inhibition of host defensive systems. The majority of studies on CRISPR/Cas9-mediated viral resistance use temporary experiments or the creation of transgenic models, mainly in plants such as Arabidopsis thaliana and Nicotiana benthamiana. Ji et al. (2015) presented the groundbreaking study that used CRISPR/Cas9 for

JUST AGRICULTURE
multidisciplinary e-Newsletter

geminivirus resistance. They used the CRISPR/Cas9 system to overexpress sgRNA-Cas9 in Arabidopsis and N. benthamiana plants to give resistance against the beet severe curled top virus (BSCTV). Ali et al. (2015) used the tobacco rattle virus (TRV) vector to introduce guide RNAs that target the viral capsid protein (CP), the replication protein (Rep)'s RCRII motif, and the tomato yellow leaf curl virus's (TYLCV) intergenic region (IR) into Cas9-expressing N. benthamiana. The most successful strategy was to target the stem-loop region inside the origin of replication in the IR, which resulted in resistance to TYLCV as well as other geminiviruses including the bipartite Merremia mosaic virus (MeMV) and the monopartite Beet Curly Top Virus (BCTV). This method worked well for creating resistance to the Cotton leaf curl Kokhran virus (CLCuKoV), demonstrating the CRISPR/Cas9 system's potential for long-term viral resistance. Sequence-directed endonucleolysis of the target single-stranded RNA is carried out by the RNA endonuclease Cas13a, which also processes pre-CRISPR RNAs (crRNAs). By targeting the HC-Pro and GFP sequences rather than the coat protein (CP) sequence, Aman et al. (2018) were able to increase resistance to the turnip mosaic virus (TuMV) by interfering with its RNA genome with the CRISPR/Cas13a system in N. benthamiana. Pyott et al. (2016) used a similar genome editing strategy with CRISPR/Cas9 technology, focusing on the Arabidopsis thaliana eIF(iso)4E gene. Turnip mosaic virus resistance was demonstrated by the resultant genome-edited plants (TuMV). The T2 generation showed evidence of CRISPR/Cas9 transgene segregation, and the resulting homozygous lines, known as T3, had a phenotype that was morphologically normal. In different research, Macovei et al. (2018) targeted the translation initiation factor 4 gamma (eIF4G) gene to confer resistance to tungro disease (caused by rice tungro spherical virus, RTSV) in the vulnerable rice cultivar IR64.

Conclusion

The multifaceted CRISPR-Cas9 method has greatly expanded our knowledge of genome engineering by revealing the complex molecular dynamics of biological systems. Although there are still difficulties, especially with the technology's refinement, using CRISPR to build plant disease resistance offers a viable way around breeding obstacles. Using several 'Omics' techniques, such as transcriptomics, proteomics, and metabolomics, is essential to fully comprehend plant defense mechanisms in the context of plant–pathogen interactions. These comprehensive investigations may identify a wide range of cellular targets, opening the door for the development of plant cultivars with greater resistance.

JUST AGRICULTURE
multidisciplinary e-Newsletter

References

Aman, R., Ali, Z., Butt, H., Mahas, A., Aljedaani, F., Khan, M. Z., et al. (2018). RNA virus interference via CRISPR/Cas13a system in plants. Genome Biol. 19:1. doi: 10.1186/s13059-017-1381-1.

Corredor-Moreno, P., and Saunders, D. G. O. (2020). Expecting the unexpected: factors influencing the emergence of fungal and oomycete plant pathogens. New Phytol. 225, 118–125. doi: 10.1111/nph.16007.

Dong, S., Raffaele, S., and Kamoun, S. (2015). The two-speed genomes of filamentous pathogens: waltz with plants. Curr. Opin. Genet. Dev. 35, 57–65. doi: 10.1016/j.gde.2015.09.001.

Dort E.N., Tanguay P., Hamelin R.C. 2020. CRISPR/Cas9 gene editing: an unexplored frontier for forest pathology. Frontiers in Plant Science 11: 1126. DOI: https://doi.org/10.3389/fpls.2020.01126.

Gao, H., Smith, J., Yang, M., Jones, S., Djukanovic, V., Nicholson, M. G., et al. (2010). Heritable targeted mutagenesis in maize using a designed endonuclease. Plant J. 61, 176–187. doi: 10.1111/j.1365-313X.2009.04041.x.

Jones, H., Whipps, J. M., and Gurr, S. J. (2001). The tomato powdery mildew fungus Oidium neolycopersici. Mol. Plant Pathol. 2, 303–309. doi: 10.1046/j.1464-6722.2001.00084.x.

Jørgensen, I. H. (1992). Discovery, characterization and exploitation of Mlo powdery mildew resistance in barley. Euphytica 63, 141–152. doi: 10.1007/BF00023919.

Koseoglou, E. (2017). The Study of SlPMR4 CRISPR/Cas9-Mediated Tomato Allelic Series for Resistance Against Powdery Mildew. Master's thesis, Wageningen University and Research, Wageningen.

Langner, T., Kamoun, S., and Belhaj, K. (2018). CRISPR Crops: plant genome editing toward disease resistance. Annu. Rev. Phytopathol. 56, 479–512. doi: 10.1146/annurev-phyto-080417-050158.

Macovei, A., Sevilla, N. R., Cantos, C., Jonson, G. B., Slamet-Loedin, I., Èermák, T., et al. (2018). Novel alleles of rice eIF4G generated by CRISPR/Cas9-targeted mutagenesis confer resistance to Rice tungro spherical virus. Plant Biotechnol. J. 16, 1918–1927. doi: 10.1111/pbi.12927.

Malnoy, M., Viola, R., Jung, M. H., Koo, O. J., Kim, S., Kim, J. S., et al. (2016). DNA free genetically edited grapevine and apple protoplast using CRISPR/Cas9 ribonucleoproteins. Front. Plant Sci. 7:1904. doi: 10.3389/fpls.2016.01904.

Pyott, D. E., Sheehan, E., and Molnar, A. (2016). Engineering of CRISPR/Cas9-mediated potyvirus resistance in transgenefree Arabidopsis plants. Mol. Plant Pathol. 17, 1276–1288. doi: 10.1111/mpp.12417.

Turner, R. S. (2005). After the famine: plant pathology, Phytophthora infestans, and the late blight of potatoes, 1845–1960. Hist. Stud. Phys. Biol. Sci. 35, 341–370. doi: 10.1525/hsps.2005.35.2.341.

Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C., et al. (2014). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat. Biotechnol. 32, 947–951. doi: 10.1038/nbt.2969.

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. doi: 10.1111/tpj.12838.