Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.5566

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (4):** 916-919 (2017)

Review Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Applications of CRISPR-Cas system in Plant Pathology: A Review

Afsha Tabassum[1], Shweta[2] and Vishnutej Ellur[3*]

[1&3]Ph.D. student, Department of Plant Pathology, Washington State University, Pullman, Washington, USA

[2]Ph.D. student, Dept. of Genetics & Plant breeding, University of Agricultural Sciences, Raichur, Karnataka, India – 584104

*Corresponding Author E-mail: afshatabassum1@gmail.com

Received: 8.08.2017 | Revised: 16.08.2017 | Accepted: 17.08.2017

ABSTRACT

Gene editing of living organisms has been tired by scientists since the discovery of DNA. Earlier approaches of gene editing was using oligonucleotides, self-splicing introns etc. Zinc Finger Nucleases (ZFN's) and TALENs are effective but not widely adapted. The CRISPR (Clustered Regularly Interspaced Palindromic Repeat) – Cas (CRISPR- associated) is hundreds of millions of years old found in bacteria as survival mechanism against infection of viruses. The mechanism of CRISPR-Cas was understood only in early 2000s. This review presents overview of CRISPR technology and its applications against Plant Pathogens including Fungi, Bacteria and Viruses.

Key words: *Gene editing, Zinc Finger Nucleases, TALENs, CRISPR-Cas, Plant Pathogens*

INTRODUCTION

Scientists have been trying to alter the genome of plants, animals and humans since the discovery of DNA double helix by James Watson and Francis Crick in 1953. Bacteria and other prokaryotic organisms defend themselves against bacteriophage[8]. Bacteria and yeast have been shown to carry out precise editing of DNA through the phenomenon called 'Restriction and modification (RM) system', which subsequently ushered in the new era of using restriction enzymes and the subsequent birth of recombinant DNA technology[4]. The discovery of the RM system and restriction enzymes coupled with advances in our understanding of DNA repair pathways in bacteria and yeast have contributed to increased efforts in developing strategies for targeted genome engineering[5]. Early approaches to targeted DNA cleavage were through use of oligonucleotides, small molecules or self-splicing introns for site-specific recognition of DNA sequences. Oligonucleotides coupled to chemical cleavage/cross linking reagents such as bleomycin and psoralen. These methods were not robust for site specific genome modification. Although Zinc Finger Nucleases (ZFN's) and TALENs are effective genome editing reagents they are not widely adapted because of the difficulty and validating such proteins for a specific DNA locus of interest[4].

Cite this article: Tabassum, A., Shweta and Ellur, V., Applications of CRISPR-Cas system in Plant Pathology: A Review, *Int. J. Pure App. Biosci.* **5(4):** 916-919 (2017). doi: http://dx.doi.org/10.18782/2320-7051.5566

The CRISPR (Clustered Regularly Interspaced Palindromic Repeat) - Cas (CRISPR-associated) technology is at least hundreds of millions of years old. Bacteria originally used CRISPR, as a survival mechanism to fight infection of viruses. It was not until early 2000s, the CRISPR- Cas mechanism was understood[4]. This adaptive immunity occurs in three stages: 1. Short sequence of invading DNA insertion as a spacer into CRISPR array; 2. Precursor CRISPR RNAs (crRNAs) crRNA (pre-crRNA) transcription to generate individual crRNAs; and 3. Cleavage of foreign nucleic acid using crRNA and cas proteins[4].

Two different groups are credited with having developed the CRISPR-Cas system: Jennifer Doudna, University of California, Berkeley together with Emmanuelle Charpentier (Now at Max Planck Institute in Berlin), and Feng Zhang, at Broad Institute of Harvard and MIT. There is litigation underway over the ownership of this technology[8].

CRISPR-Cas9 approach has already been applied to modify, regulate or mark genomic loci in a wide variety of cells and organisms from all three domains of life: Archaea, Bacteria and Eukarya[8]. Using CRISPR, the genome of mosquitoes were edited to produce more males. Only female mosquitoes bite and the edited mosquitoes are resistant to malarial parasite. CRISPR was used to splice out HIV from the genome of cells. Cooking of potatoes at high temperature produces cancer-causing acrylamide. CRISPR was used to edit genome of potato to prevent browning. CRISPR can be used to delete the genes in the human embryo and know their function. CRISPR was used to create superdogs by editing the muscle-inhibiting gene myostatin[8].

Dr. Yinong Yang, a plant pathologist at Pennsylvania State University, engineered the common white button (*Agaricus bisporus*) mushroom that resist browning using CRISPR-cas. The injury caused to the mushrooms during picking, handling and storage causes the discoloration reaction in which phenolic compounds are oxidized by enzymes to quinones and brown colored melanins are formed. Polyphenol oxidase (PPO) is the well studied enzyme from the melanin pathway involved in conversion of phenols to quinones[11]. By targeting one of the six-polyphenol oxidase enzyme in common white button mushrooms (*Agaricus bisporus*) browning in mushroom was reduced by 30%. The USDA issues a guidance that it will not regulate this CRISPR-engineered mushroom[10].

Xanthomonas oryzae causes blast disease in rice. Bacterial immune system is comprised of CRISPRs and cas genes that is directed against extrachromosomal genetic elements through RNAi-like mechanism. Uptake of phage sequence called as protospacer as a leader-proximal CRISPR spacer by *Streptococcus thermophilus* led to phage resistance. *X.oryzae* strain Xo604 can serve as host for bacteriophage Xp10 (in spite of harboring spacer identical to a protospacer in the phage) but not for bacteriophage Xop411. Similarly, *X.oryzae* strain Xo21 can serve as host for bacteriophage Xop411 but not for Xp10. CRISPR machineries function differently in different bacteria. Protospacer from *X.oryzae* phage is in different orientation than that of *S. thermophilus* phage that is causing difference in the resistance mechanism in the bacteria[9].

Viruses belonging to *Potyviridae* requires Eukaryotic translation initiation factor (*elf4E*) to maintain their life cycle. Mutations in *elf4E* can lead to resistance to potyviruses. Chandrasekran *et al*. targeted the two different sites in cucumber *elf4E* gene by designing two single guide RNAs (sgRNAs – sgRNA1 and sgRNA2)[3]. Broad virus resistance was found against *Cucumber vein yellowing virus (CVYV), Zucchini yellow mosaic virus (ZYMV) and Papaya ringspot mosaic virus – W(PRSV-W).*

Geminiviruses are plant DNA viruses with circular ssDNA genome and twin icosahedral capsid. Geminiviruses replicate by rolling cycle amplification (RCA) mechanism or by recombination mediated replication. Ali

et al. 2015 delivered sgRNAs specific to *Tomato yellow leaf curl virus* (TYLCV) into the *Nicotiana benthamiana* plants overexpressing the Cas9 endonuclease. Challenge inoculation of these plants with TYLCV showed reduced viral DNA accumulation. Baltes *et al.* targeted the *Bean yellow dwarf virus* (BeYDV) genome by transiently expressing BeYDV genome in *N. benthamiana* plants expressing CRISPR-Cas9 system[2]. Challenge inoculation of these plants with BeYDV showed reduced viral load of BeYDV in *N. benthamina*.

Phytophthora sojae causes damping off of soybean seedlings/stem and root rot of established plants. Functional Genomic studies are hampered due to lack of efficient strategies for genome engineering. Fang and Tyler (2016) targeted RXLR effector gene Avr4/6 of *P.sojae* by CRISPR/Cas 9 system. Non-homologous end-joining (NHEJ) and homology-directed repair (HDR) mutants generated by CRISPR-Cas9 system helped to validate the contribution of Avr4/6 to recognition by soybean R gene loci *Rps4* and *Rps6*.

Citrus greening/Huanglongbing (HLB) is the devastating disease affecting citrus crop worldwide. Florida's citrus industry incurred a loss of $9 billion due to this disease alone. Control options are inadequate to manage the disease. Dr. Luo, F from Clemson University, has proposed a project to engineer Citrus greening/Huanglongbing (HLB) tolerant/ resistant citrus using CRISPR genome editing technology[7].

CRISPR-Cas9 applications in plants against plant pathogens promise to change the pace and course of agricultural research. Future research directions to improve the technology will include engineering/identifying smaller Cas9 variants with distinct specificity that maybe more amenable to delivery in cells. Understanding the homology directed repair mechanisms that follow Cas9-mediated DNA cleavage will enhance insertion of new/corrected sequences into genomes. The development of specific methods for efficient and safe delivery of cas9 and its guide RNAs to cells and tissues will also be critical for application of the technology in human gene therapy[4].

Regulatory oversight is less clear in the application of CRISPR-Cas to edit food crops. Based on areas where scientists are going to apply this technology, commercialization of CRISPR based crop traits ultimately depends on regulatory oversight and consumer acceptance. There is worldwide race to push the limits of CRISPR's capabilities but scientists call for patience and extreme caution[8].

REFERENCES

1. Ali, Z., Abulfaraj, A., Idris, A., Ali, S., Tashkandi, M and Mahfouz, M.M. CRISPR/Cas9 – mediated viral interference in plants. *Genome Biology*. **16:** 238 (2015).
2. Baltes, N.J., Hummel, A.W., Konecna, E., Cegan, R., Bruns, A.N., Bisaro, D.M and Voytas, D.F., Conferring resistance to geminiviruses with the CRISPR-Cas prokaryotic immune system. *Nature plants*. **1:** 1-4 (2015).
3. Chandrasekaran, J., Brumin, M., Wolf, D., Leibman, D., Klap, C., Pearlsman, M., Sherman, A., Arazi, T and Gal-On, A., Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology. *Molecular Plant Pathology*. **17(7):** 1140-1153 (2016).
4. Doudna, J.A and Charpentier, E., The new frontier of genome engineering with CRISPR-Cas 9. *Science*. **346 (6213):** (2014).
5. Dupuis, M. E., Villion, M., Magadan, A.H and Moineau, S., CRISPR-Cas systems are compatible and increase phage resistance. *Nature communications*. 1-7 (2013).
6. Fang, Y. and Tyler, B.M., Efficient disruption and replacement of an effector gene in the oomycete *Phytophthora sojae* using CRISPR/Cas9. *Molecular Plant Pathology*. **17(1):** 127-139 (2016).

7. Luo, F., Selection, Molecular and Genetic analysis of HLB tolerant/resistant variant citrus plants (2017).
8. Park, A. 2016. The CRISPR Pioneers. *Time (Magazine)*. December 116-122.
9. Semenova, E., Nagornykh, M., Pyatnitskiy, M., Artamonova, I.I and Severinov, K., Analysis of CRISPR system function in plant pathogen *Xanthomonas oryzae*. *FEMS Microbiology letters*. **296:** 110-116 (2009).
10. Waltz, E., *Gene edited C*RISPR mushroom escapes US regulation. *Nature.* 532-293 (2016).
11. Weijn, A., Bastiaan-Net, S., Wichers, H.J and Mes, J.J., Melanin biosynthesis pathway in *Agaricus bisporus* mushrooms. *Fungal Genetics and Biology*. **55:** 42-53 (2013).