AgriCos e-Newsletter
ISSN: 2582-7049
Volume: 02 Issue: 06 June 2021 Article No: 24

CRISPR - CAS9 and its Application in Pest Management

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SUMMARY

CRISPR/Cas9 has been considered one of the most powerful tools for genome editing of various important pest and crops because of its high efficiency, relatively low cost, and ease of use. The flexibility, ease and efficiency of this editing system have open possibilities in genome manipulation. The ability of CRISPR/Cas9 with the help of donor DNA provide a good strategy to enable a corrective genomic DNA modification in parasitic nematodes and other pathogens. It enables us to enhance genome engineering via two major DNA double-strand break repair pathways: non-homologous end joining and homologous recombination. It is a novel approach to validate predictions from genomic and transcriptomic studies of the important pathogens allowing identification of molecules that are essential to the functions of sensory neurons which can manipulate their behaviours.

INTRODUCTION

In recent years, various Genome editing (GE) tools have been explored for editing simple and complex genomes. GE is a technique which introduces DNA mutations in the form of insertions and/or deletions (indels) or base substitutions in target sequences. GE comprises various techniques, such as the use of zinc finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs), and the most recently developed clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated nuclease 9 (Cas9) system. CRISPR/Cas9 is a unique tool for genome editing that enables to edit parts of the genome by removing, adding or altering sections of the DNA sequence. CRISPR loci occur on prokaryotic chromosomal DNA and are characterized by having repeated identical palindromic segments (repeats) that are 21-40 base pairs (bp) in length.

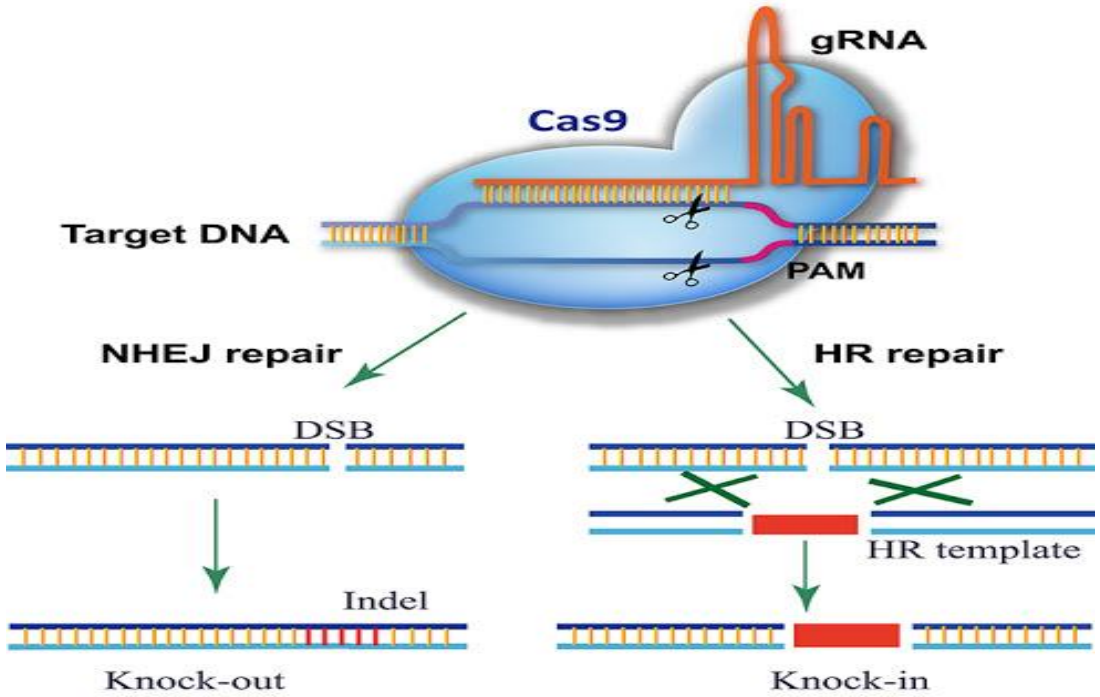

FIG: Genome editing by CRISPR/Cas9.
Source- http://crispr.hzau.edu.cn/CRISPR2/images/CRISPR2.jpg

The repeat sequences are not directly adjacent: spacer sequences (protospacers) in length sequences (protospacers) ranging from 20-58 bp in length separate each repeat sequence on a CRISPR locus. Each spacer occurring only once at a given CRISPR locus. The spacers for a given species do not match spacers found in other species. *Streptococcus pyrogenes*, which has multiple CRISPR loci each with different repeat units. (Mojica *et al.* 2000; Jansen et al. 2002). CRISPR loci along with the CRISPR associated Cas endonuclease denote prokaryotic molecular immune system. When foreign DNA enters a cell, RNA molecules transcribed from the CRISPR loci along with the Cas endonuclease recognize and bind this foreign DNA, while Cas protein cuts it.

Target DNA cleavage occurs at a specific site upstream of the protospacer associated motif (PAM) that represents a trinucleotide sequence (NGG for the Cas9 protein) recognized by the Cas protein and required for its binding to the target DNA. Recently, the CRISPR/Cas9 technology has emerged as a breakthrough among the approaches of targeted eukaryotic genome alternation. The use of recombinant Cas9 endonuclease with synthetic sgRNAs (can be developed using bioinformatics tools) complementary to the target DNA region and functionally similar to the spacers makes it likely to introduce mutations in any gene. CRISPR/Cas9 provides efficient genome editing using directed breaks in both DNA strands that initiate repair mechanisms by either homologous recombination or nonhomologous end joining (NHEJ), leading to the generation of the indels (insertions or deletion of nucleotides) and the loss of function of the target gene.

Components of CRISPR CAS9

- **Crisper Array:** It is the sequence of palindromic repeats with spacers
- **Pre cr RNA:** Precursor of crRNA, long primary transcript of CRISPR array
- **sgRNA**: Single-guide RNA is the combination of tracrRNA and crRNA. **crRNA** is processed from pre crRNA, it have single spacer flanked by repeats fragments and it contains two main parts, the spacer sequence that directs the complex to the target DNA and a region that binds to tracrRNA. When tracrRNA binds to crRNA, a functional guide RNA is formed for Cas9 to recognize.
- **PAM** (Protospacer adjacent motif): This is a 2-6 nucleotide sequence which follows the DNA sequence to be cleaved. For Cas9 5′-NGG-3′ is PAM sequence. (5′-NNNNNNNNNNNNNNNNNNNN-NGG-3′, N can be A, G, C, or T)
- **CAS 9** (Cas proteins): It is an endonuclease enzyme which functions as a pair of molecular scissors to cut the target DNA sequence with help of sgRNA.

Applications of CRISPR/CAS9 System in Pest Management

Select Your Target Sequence and Design Your gRNA.

- Know your cell line/specise and genomic sequence.
- Select gene and genetic element to be manipulated.
- Select gRNAs based on predicted on-target and off-target activity .
- Synthesize and clone desired gRNAs.
- Deliver Cas9 and gRNA.

Validate genetic modification through-

- Mismatch-cleavage assay (for NHEJ repaired DSBs)
- PCR and restriction digest (for HDR repaired DSBs)
- PCR amplification and gel electrophoresis (for HDR or NHEJ)
- PCR amplification, subcloning and Sanger sequencing (for HDR or NHEJ)
- PCR amplification and next-generation sequencing (for HDR or NHEJ)

Advantages of CRISPR/CAS9 System

Gene editing- CRISPR/CAS9 allows to destroy target DNA or knock out or knock in any gene or silence any of this gene for that we can use this specific cr RNA that we design invitro and we provide it in the cell and cleave the target DNA.

Allow Selective Transcription- here cas9 nuclease activity is totally shut down and CRISPR RNA allow this total CRISPR CAS9 complex to be associated and attached with the target DNA but as cas 9 activity is deactivated so additional factors can be put from outside like transcriptional activator and molecular enhancer, then they can intiate transcription of this specific gene in that particular location.so, hence just by providing our own design target RNA we can transcribe our target DNA region inside of the cell and we can amplify the number of RNA from that stretch of target DNA as many as we can.

Inhibition of Transcription- we can add some inhibitory component to prevent the transcription of specific region of the DNA, that is in a stretch of specific genom we can block the transcription of targeted genes.

Paint Genome- CRISPR CAS9 system can be tagged with GFP (Green fluorescent protein) or any short of fluorescent tag to the protein to mark specific location of the genes in the genome, that means we can paint the genome. Also to find out the regional location of specific locations in the DNA we can mark them with the help of different types and colours of fluorescent tag with the help of CRISPR/Cas9 system.
CRISPR allows us to perform targeted alteration in the genome.
The susceptibility genes can be targeted for deletion through CRISPR/cas9 by genetically modifying the host.

Limitation of CRISPR/CAS9 System

- Off target effects are variable and can alter the function of a gene and may result in genomic instability.
- CRISPR/Cas9 introduces mutations at nonspecific loci which are similar, but not identical, in homology to target sites.
- The requirement of a PAM site at the target sequence limits the application of Cas9 because Cas9 it only recognizes the NGG PAM site once every 8–16 bp.
- It takes a long time to select and characterize mutants.
- The size of the CRISPR/Cas9 system is relatively large, and so it is not suitable for packing into viral vectors.
- Low Homology directed repair efficiency.
- Unintended consequences in future.

CONCLUSION

In the past few years we have seen a spurt of CRISPR/Cas9 genome editing application and it has been rapidly developed and applied in numerous pest to mediate their behaviour. CRISPR/Cas9 has been considered one of the most powerful tools for genome editing of various nematodes and important pest because of its high efficiency, relatively low cost, and ease of use compared with other genome editing techniques, such as ZFNs and TALENs. It can be used to add desirable and remove undesirable alleles simultaneously in a single event. CRISPR/Cas9 has begun to revolutionize biological research, as the method of choice for targeting specific genome sequences in simple or complex organisms. It is necessary to avoid off-target effects by CRISPR/Cas9, and new and improved strategies are required, not only to avoid, but also to detect more easily, off-target effects. Although some limitations of the CRISPR/Cas9 system limit its widespread use, different strategies are being developed to improve its effectiveness for editing human, animal, and plant cells. Although significant progress has been made to increase its efficiency and target specificity, more work remains to be done to improve it.

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