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Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Review Article

CRISPR-Cas9: A GENOME EDITING TOOL AND ITS APPLICATIONS IN PLANT BIOLOGY

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ABSTRACT

After the discovery of molecular structure of DNA by Watson and Crick in 1953, many Scientists have developed different gene editing methodologies that can influence the genetic material of cells and organisms for well-being of mankind. Among the different findings, one of the great findings is CRISPR-Cas 9 system. It is one of an easy and effective technique for genome manipulation. Over the years, it is one of the popular methods of genome editing as it is easy to manipulate, efficient and have wide applications in gene mutation and transcriptional regulation in plants. In this review, we are discussing about CRISPR-Cas9 and its applications in plants biology and its future prospects.

Keywords: CRISPR- Cas9, Gene modification, Plants, Gene editing technology.

1. INTRODUCTION

CRISPR-Cas9 is the novel findings among the gene editing tools. While working with the prokaryotic genome, scientists found some unusual repeated sequences in *E. coli,* "it confined five highly homologous sequences of 29 base pairs including a dyad symmetry of 14 bp that were combined by variable spacer sequences of 32 bp" [1]. Few years later, in halophilic Archaea *Haloferax mediterranei,* similar repeated sequences were observed at regular intervals [2].

Later on, the bioinformatics analysis revealed that such type of repetition of sequences are common phenomenon in prokaryotes and it bears identified features as: they are short sequences, partially clustered palindromic sequence separated by unique intervening sequences (spacers) of definite length which are originated from mobile genetic elements (MGEs) such as bacteriophages, transposons or plasmids [3], signifying an inherited origin and high biological importance [4]. Thus, the term CRISPR was abbreviated from Clustered Regularly Interspaced Short Palindromic Repeats [5]. CRISPR sequences are preceded by an AT-rich leader sequences and are generally ended by set of *cas* genes which encoding Cas proteins [6-9]. Prokaryotes which carrying these repeated sequences seems to be un-affected from the infection, the reason behind it, is that, plasmids or viruses containing a repeated sequence are matching with spacer (protospacer) sequences, were generally absent in the prokaryotes carrying the spacer. The corresponding findings suggested a role of CRISPR as an adaptive immune response in bacteria towards viruses and the spacer sequences are designated as a 'memory of past' "genetic aggressions" [10].

It is well known fact that, the CRISPRs are transcribed into long RNA molecules (i.e., pre-crRNA), which are then processed and cleaved to yield small CRISPR-RNA (crRNAs) [2, 11]. Some scientists assumed that, Cas proteins are also involved in this process [9] form complex with Cas proteins and infect invading genome. Extensive research from several years leads to the identification of different CRISPR-Cas system, and these are divided into two major classes [12]. "In the Class 1 systems, specialized Cas proteins assemble into a large CRISPR-associated complex for antiviral defence (Cascade). The Class 2 systems are simpler and contain a single multidomain crRNA-binding protein (e.g. Cas9) that contains all the activities necessary for interference" [13].

2. ADAPTIVE DEFENCE SYSTEM IN CRISPR

Adaptive Defence system in CRISPR was demonstrated by using different strains of bacteria having different CRISPR-Cas systems i.e., Class I system and Class II system.

In Class II type, a strain of *Streptococcus thermophilus* was used for study. In this experiment, virulent bacteriophage was used to infect bacterial strain, and it was observed that, new spacer sequences were found in bacterial genome which shows resistant against phage. These sequences are matched with the protospacer sequences in phage. As these sequences (spacer in bacteria) were deleted, it loses the resistant against

bacteriophage. In addition to this, deletion of cas-5 gene leads to the loss of phage resistance in prokaryotes. This experiment thus suggested the role of products of *cas* gene in CRISPR-Cas-mediated immunity and its specificity on the spacer sequences [14].

Class I type CRISPR-Cas system was well explained while studying the genome of *E coli*. It contains about eight different Cas proteins. Out of eight, five proteins were purified as a multiprotein complex and named as Cascade (i.e., CRISPR- associated complex for antiviral defence). The function of Cascade is to cleave the long transcripts in the repeated regions and produce small cr-RNA molecules, which contain virus-derived sequences [15]. This short cr-RNA was taken by Cascade and together with cas-encoded helicase, i.e., Cas3, they interfere with the further proliferation of phage.

Thus, there are two steps in CRISPR behavior, first: CRISPR expression and cr-RNA maturation and second, interference step which require Cas3 protein.

There are some sequences which prevent the splicing of own CRISPR spacers called PAM (protospacer adjacent motifs). These sequences are short sequences and are present upstream i.e., some nucleotides away from protospacer sequences [16, 17].

The repeated presence of PAMs in many viruses and eukaryotes, including human being and plants, is the main reason why CRISPR-Cas systems are enormously useful and applicable in multiple areas within biology.

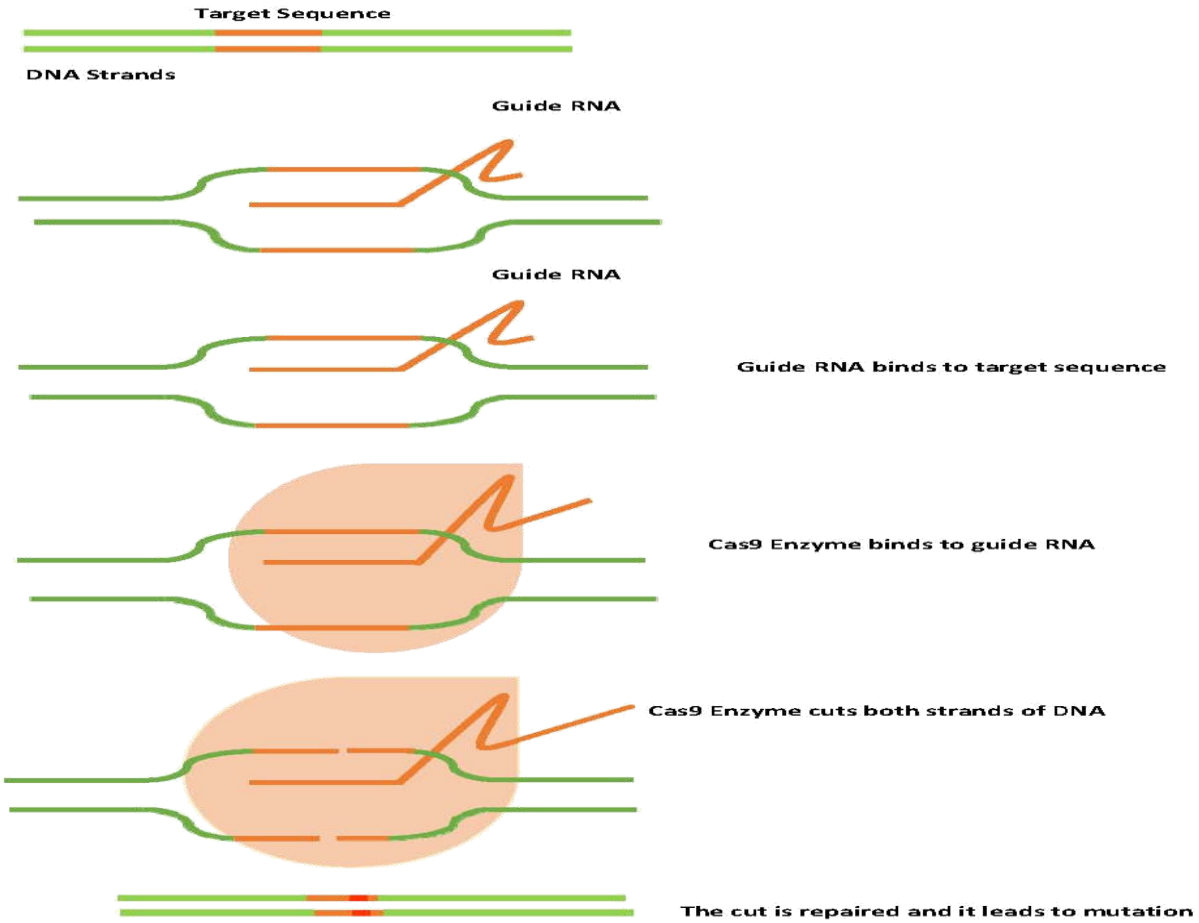

Fig. 1: Diagram showing how the CRISPR-Cas9 editing tool works

Image Credit: Genome Research Limited

3. APPLICATIONS OF CRISPR-CAS9 SYSTEM IN PLANTS

To improve the desired characters in plants, plant breeding, radiations and chemicals are some of the conventional methods. Furthermore, it does not guarantee the improvement in desired characters. In case of plants, it is usually hard to transfect cells due to its rigid cell wall and even more difficult to edit due to genetic complexity i.e., two copies of chromosomes,

and it is necessary that, each chromosome get mutated in order to achieve a specific trait [18].

But with the emergence of this new technique of CRISPR-Cas9 we have assured the required improvements in crops. Following are some of the applications of CRISPR-Cas9 to improve the traits in plants. CRISPR aided gene editing has been tested on rice [19], wheat [20], maize [21], soybeans [22], potatoes [23], tomatoes, apples and citrus species [24] with promising results in crops such as resistant against wind, drought, cold, heat or humid conditions which results in increase in yield.

It has been observed by World Health Organization (WHO) that, 90 million pre-school children in developing countries suffer from Vitamin-A deficiency which leads to blindness and increased mortality [25]. To combat this problem, CRISPR-Cas9 technology has been used to improve beta carotene in rice which internally converts into vitamin-A [26].

A deadly disease found in Citrus plants known as Huanglongbing (HLB), also known as citrus greening or yellow dragon disease. It is caused due to bacterium *Candidatus*. This disease is widespread in Africa, Asia, and from 2005 in U.S. [27]. It leads to high economic losses in citrus industry. The technique of CRISPR has been used to make citrus plants less susceptible to the disease HLB.

Using CRISPR technology, some researchers managed to increase the yield 10 times, increase the fruit size three folds and enhance the lycopene content of fruit five folds which are economically and neutraceutically beneficial [28]. A most common disease in tomato is powdery mildew,it was controlled by the CRISPR technology [29].

Genome editing in plants helps to carried out regulation and enhancement of beneficial traits like shelf life of fruits, high salt tolerance, resistance against pest and diseases, herbicide tolerance, etc. [30] and gluten [31].

To improve the traits of the plants, CRISPR/Cas9 is efficiently used, the traits can be regulating by negative regulatory genes, it can be improved by knockout or weakening of the genes. Simultaneous knockout of the three *TaMLO* homologs in common wheat produced resistance to powdery mildew [32]. Mutation of the ERF transcription factor gene OsERF922 [33] in rice increase resistance to rice blast fungal pathogen [34].

Some of the classic work in plants are: production of acrylamide free potatoes [35], after cutting, it resist browning of apples, mushrooms and potatoes by mutating Polyphenol oxidase (PPO) genes [35-37] and by inducing low phytic acid in maize [38].

Some of the notable examples of CRISPR-Cas9 are thermosensitive male sterility in maize [39] and wheat [40], enhancement of nutritional properties in sorghum and wheat [41 -42], resistance to pathogens [43, 44], and resistance to herbicides [45, 46]. In Cucumber, the eukaryotic translation initiation factor gene *elF4E*, responsible for yellowing of vein was inactivated through CRISPR-Cas9 and also developed resistance to the potyviruses Zucchini yellowmosaic virus in Papaya which is responsible for ring spot mosaic virus [47]. Some scientists develop or engineered artificial resistance in maize and tomato through CRISPR-Cas9 such as maize lethal necrosis disease and tomato brown rugose fruit virus (48-50) which was originally not found in crops.

4. CONCLUSIONS

CRISPR-Cas9, a widely used gene editing tool is a new revolution in conventional gene editing technology. It can be seen as a potential tool to combat with the agricultural problems, it enhance the yield, disease resistant potential, environmental stresses, moreover, it can develop artificial resistance in crops which was originally absent in crops. These characters are necessary to increase the production of food to fulfill the need of overgrowing population. For the proper use of any technology it should be use wisely and properly for human benefit only.

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