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CRISPR/Cas9 Genome Editing Technology (^{*}Khushbu Chittora) Rajasthan College of Agriculture, MPUA&T, Udaipur-313001 <u>chittorakhushbu23@gmail.com</u>

CRISPR stands for clustered regularly interspaced short palindromic repeats. The CRISPR system is a gene editing mechanism present in many prokaryotes, encoded by CRISPR loci and the accompanying CRISPR-associated (*cas*) genes to provide immunity against bacteriophage infection and plasmid transfer. The bacteria capture snippets of DNA from viruses that have previously infected them and use these snippets to create DNA segments known as CRISPR arrays. The CRISPR arrays allow the bacteria to remember the viruses or closely related ones.

How CRISPR Works

When viruses attack again, the bacteria produce RNA segments from the CRISPR arrays to target the viruses DNA. The bacteria then use Cas9 or a similar enzyme to cut the DNA apart, which disables the virus. Hence these sequences play a key role in the immune system of prokaryotes. This CRISPR-Cas9 mechanism works similarly in the lab. A guide RNA sequence is created which is able to binds a target sequence of DNA in a genome. The RNA also binds to the Cas9 enzyme. As in bacteria, the modified RNA is used to recognize the DNA sequence, and the Cas9 enzyme cuts the DNA at the targeted location. CRISPR can make precise mutations by substituting existing DNA sequences with desired ones. It also can disable whole genes by snipping them out or via imprecise repairs that knock out gene function. The Cas9 enzyme itself can be manipulated to enhance or suppress gene expression. The plant CRISPR/Cas9 and its derived system have shown various genome-editing ability, such as gene knock-in, knockout, knockdown, and expression activation as well.

Uses of CRISPR Tools for a crop improvement

CRISPR/Cas9 method of gene editing has been adopted for various traits including yield improvement, quality improvements resistance against biotic and abiotic stress management. It is also used in improving the crop physical appearance by modification of shape and size of the crops according to consumer preferences. In enhancing shelf life of crops as in of tomatoes and bananas.

Advantages of CRISPR and Their Applications

Applications of CRISPR/Cas9 in genome editing of plants is one of the most rapidly emerging technologies in biosciences. More importantly, the CRISPR/Cas9 is becoming a user-friendly tool for development of non-transgenic genome edited crop plants to cope up with changing climate and ensure future food security. CRISPR combine the advantages of design simplicity, with high specificity, directly control endogenous gene expression at the transcriptional level, and can act on both coding and noncoding sequences. CRISPR have been applied in large-scale genomic screening, showing minimal off-target effects in different systems.

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