

# COTTON Innovate



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## CRISPR-Cas9 Technology for Targeted Genome Editing in Cotton

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CRISPR (Clustered regularly interspaced short palindromic repeats)/Cas (CRISPR-associated protein; Cas9) system has revolutionized genomic research in general and genome editing in particular across the kingdoms of living organisms. Basically, it is an adaptive immune mechanism existing in bacteria and Archaea where it protects the host cell from the invading plasmids and phages. Insights about this mechanism have created an excitement among the biologists for its application in eukaryotic genome editing. CRISPR-Cas9 is the latest among genome editing technologies discovered to date such as Zinc finger nucleases (ZFNs), Transcription activation like effector nucleases (TALENs) and meganucleases. CRISPR-Cas9 is relatively simpler, efficient, versatile, flexible and less time-consuming than the former technologies to induce user defined targeted mutations within the genomes of plants, animals and other eukaryotes. CRISPR-Cas9 technology mainly comprises of two components; one is Cas9 protein for endonuclease activity and the other is sgRNA (tracrRNA+crRNA) for target recognition and interaction with Cas9 to induce conformational changes in Cas9 to position it at the place of target site to induce double strand breaks upstream of NGG the PAM (Protospacer adjacent motif) sequence. CRISPR/Cas9 technology has been successfully employed and evaluated for its efficacy in Arabidopsis, tobacco, rice, sorghum and tomato. Further its application has been extended for the targeted modifications to establish beneficial traits in crop plants such as powdery mildew resistance in wheat, tomato fruit ripening, drought tolerant maize and herbicide tolerance in soybean. Genome sequence information available for *G. raimondii*, *G. arboreum*, *G. hirsutum* and *G. barbadense* can be readily utilized for successful application of CRISPR/Cas9 technology. Very few reports in cotton are available which have highlighted the possible exploitation of CRISPR-Cas9 technology for precise genome editing. Cotton is world's widely cultivated commercial crop and known to be damaged by number of pests and diseases offers a large number of traits that could be improved through the use of precise, targeted mutation or genome editing.

In the past ZFNs and TALENs were not employed in cotton due to unavailability of cotton genome sequence, large genome size, its ploidy level and recalcitrant response of cotton genotypes for transformation. However, recent availability of cotton genome sequence, information regarding its transcriptome and discovery of CRISPR-Cas9 genome editing technology facilitated the cotton researchers for the preliminary studies to edit cotton genome to induce targeted mutations. Janga and co-workers demonstrated the use of CRISPR/Cas system for targeted knocking out of a *GFP* gene. They have also studied different types of indels obtained by Cas9-mediated cleavage of the *GFP* gene, guided by three independent sgRNAs. Chen and co-workers targeted *GhCLA1* and *GhVP* genes using two guide RNAs. Mutations from CRISPR/Cas9 were confirmed with efficiency of mutations varying from 47.6% to 81.8% in cotton transformants with no off-target effects. Wang *et al.* (2017) generated site directed mutations in *GhCLA1* gene and reported that 75% of regenerated plants exhibited albino phenotype with obvious nucleotides and DNA fragments deletion. Gao *et al.* (2017) enumerated that not all sgRNAs are equally efficient and it is difficult to predict their efficiency by bioinformatics. Therefore, they have developed transient transformation assay to study the efficiency of designed sgRNA in short duration before going for stable transformation and regeneration. These preliminary studies in cotton have paved the way for the development of next generation cotton using CRISPR-Cas9 technology. During last two decades, the cotton crop cultivation in India has undergone dramatic changes with commercial cultivation of insect resistance *Bt* cotton (Bollgard and Bollgard II). However, now there are reports of development of insect resistance in

*Bt*- cotton. In addition, there are other concerns like cotton leaf curl virus, fibre quality improvement, tolerance to biotic and abiotic stress factors, etc. With great potential and encouraging recent reports, CRISPR-Cas9 technology can be a potential tool for genetic engineers to develop next generation cotton to address emerging concerns.

**Suggested reading:**

- Chen *et al.*, (2017). Targeted mutagenesis in cotton (*Gossypium hirsutum* L.) using the CRISPR/Cas9 system. *Sci. Reps.* 7:44304. doi: 10.1038/srep44304.
- Gao *et al.*, 2017. Genome Editing in Cotton with the CRISPR/Cas9 System. *Front. Plant Sci.* doi: 10.3389/fpls.2017.01364.
- Janga *et al.*, (2017). CRISPR/Cas9-mediated targeted mutagenesis in upland cotton (*Gossypium hirsutum* L.). *Plant Mol. Biol.* 94:349-360. doi: 10.1007/s11103-017-0599-3.
- Wang *et al.*, (2017). High efficient multisites genome editing in allotetraploid cotton (*Gossypium hirsutum*) using CRISPR/Cas9 system. *Plant Biotechnol. J.* doi:10.1111/pbi.12755.
- Wang *et al.*, 2016. CRISPR/Cas9 in genome editing and beyond, *Annu. Rev. Biochem.*, 85:227–264.

**Precautions while handling pesticides demonstrated to farmers/ farm laborers**

A team comprised of Dr V. N. Waghmare Director, Dr A. R. Raju, Principal Scientist (Agronomy) and Dr Vishlesh Nagrare, Principal Scientist (Entomology) visited Savargaon village, Kalamb Taluka in Yavatmal district and participated in the farmers meet organized by IFFCO on demonstration while handling pesticides and precautions to be taken by the farmer/ farm laborers. Precautionary aids/ safety kits and a booklet on pink bollworm management recently published by ICAR-CICR in local language were distributed to farmers and farm laborers.



## Meetings attended/Nominated

Dr S. M. Wasnik, Principal Scientist (Extension) have been appointed as an External Examiner by the Hon. Vice Chancellor, Dr Panjabrao Krishi Vidyapeeth, Akola for conducting qualifying Viva Voce Examination of four Ph. D students Sh NP Jangwad, Miss Jyoti M. Patil, Miss Trupti P. Rathod, Miss P.S.Telange of Extension Education on 10-11 October 2017



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