CRISPR Based Technology and Applications

- **Bhavsar S. P. ***
Assistant Professor, Department of Microbiology,
H. P. T. Arts and R.Y.K. Science College, Nasik

The CRISPR-Cas system is a revolutionary gene-editing technology derived from a natural defense mechanism found in bacteria and archaea. It allows scientists to precisely target and modify DNA sequences, enabling breakthroughs in genetic research, medicine, and agriculture. At its core, the system uses a guide RNA to direct the Cas enzyme to specific DNA sites for editing, deletion, or replacement. The CRISPR-Cas system plays a pivotal role in gene manipulation by enabling precise editing, silencing, or insertion of specific genetic sequences within the genome. Natural crRNA (crispr RNA) mechanism in fighting against diseases remains an endless area of research in biotechnology and agrotechnology[1,6]

Key Words : CRISPR, Agro-industries

Lactobacilli, a chassis for user-friendly CRISPR variants:

CRISPR is reshaping the role of probiotics in microbiome manipulation. Lactic Acid Bacteria (LAB) is well matrixed in the domain of food supply from 1000s of years. It's the most potential nominee in the pool of gut flora. In probiotic and recombineered *Lactobacillus* spp. CRISPR-Cas 9 is employed for genome editing. CRISPR technology is used to architecture the probiotic bacteria for enhancing their potency and eliminating the unwanted bacterial strains and viruses via CRISPR-Cas 9 system (delivered through the bacteriophages). CRISPR-Cas 9 induced nucleases target the chromosome leading to destruction and elimination of unwanted genes (by modulation) or the whole cell bacteria. It is the CRISPR locus which yields the crRNA guiding the nuclease towards complementary target gene sequence. The estate of CRISPR Cas system in lactobacilli provides a platform to use the native cas enzymes with the user-defined CRISPR arrays for selecting the recombinant genotypes. Recombineered CRISPR-Cas is recruited via *Lactobacillus plantarum* to overgrow the wild type cells. CRISPR-Cas directs the adaptive immune system of bacteria to shield the human body against invasive phages and extra chromosomal genetic elements[6].

CRISPR-Cas system is also deployed to control the viral infectious particles by targeting the viral mRNA. It can be another way of suppressing the genes responsible for replication of viruses. Cas 13's tailorable RNA targeting efficiency was assessed by researchers from Broad Institute of Massachusetts Institute of Technology (MIT) and Harvard, Cambridge, United States. Cas13 efficiently controlled the replicating and the evolving viral RNA. Researchers screened a panel of crRNAs targeting the conserved viral regions in coronoviruses (with 90% efficiency), lymphocytic choriomeningitis cirs

(LCMV), influenza A virus (IAV) and Vesicular somatitis virus (VSV)[2,3].

CRISPR in Agrotechnology:

Agro-industries have expertise in crafting the CRSIPR box for technological solutions in agriculture. The CRISPR box is utilised to get disease free, stress-tolerant (salt, drought, metals, heat, nutrient limitation etc.) and variety of crop with increased yield. To explore the metabolic engineering and synthetic biology study, CRISPR Cas-9 combined with SSDR (Single Strand DNA Recombineering) is employed in the superbug, *Pseudomonas putida*. Cold Spring Harbor Laboratory in collaboration with various research institutes manipulated the photoperiod response gene box in tomato using CRISPR-Cas9. Resultantly, flower process was fastened with enhanced growth and early yield of tomatoes[4,5].

Table 1: Timeline of events in clustered regularly interspaces palindromic repeats (CRISPR) discovery and use

Year	CRISPR discovery and Use
1987-1995	Unusual repetitive DNA sequences, later identified as CRISPRs, were first observedin E. coli and archaea
2002	The term CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) was proposed
2005	Mojica and colleagues, along with other research groups, independently discovered that CRISPR spacer sequences matched sequences from bacteriophages and plasmids, suggesting a role in adaptive immunity.
2007-2008	Philippe Horvath's team experimentally demonstrated that CRISPRs act as an immune system in Streptococcus thermophilus, showing that resistance to phage attack could be altered by manipulating spacer DNA.
2010	Scientists coin the term 'protospacer' to denote viral sequence that corresponds to a 'spacer' in the CRISPR-Cas9 system. Scientists characterised the RNA processing pathway in CRISPR system
2012	A landmark paper by Emmanuelle Charpentier and Jennifer Doudna described CRISPR-Cas9 as a gene-editing tool.
2013	Human Genome edited by CRISPR CAS system
2014	Routine application of CRSIPR CAS for crop improvement
2015	Gene editiing tool, CRISPR, successfully used to improve muscle function in mouse model of Duchenne muscular dystrophy
2017	Possibility of editing gene defect in pre-implanted human embryos for preventing inherited heart disease

2018	Researchers identify pre-existing antibodies targeting CAS9 proteins raising possibility of immune responses undermining utility of CRISPR-Cas9 for gene therapy and First CRISPR-Cas9 clinical trial launched
2019	Chinese scientist convicted for using CRISPR-Cas9 in human babies
2020	Charpentier and Doudna were awarded the Nobel Prize in Chemistry for their development of CRISPR-Cas9 as a gene-editing tool.
2021	CRISPR technology has advanced rapidly, with ongoing research and clinical trials exploring its potential in various applications, including treating genetic diseases and developing new therapies.
2022	FDA gives Vertex green light to submit rolling application for review of CRISPR based therapy to treat sickle cell disease and beta thalassemia
2023	World's first CRISPR–Cas9 gene editing therapy given conditional approval in UK for two blood disorders
2024	CRISPR gene therapy reported to be promising treatment for inherited vision loss in phase 1/2 trial
2025	Clinical trials for cystic fibrosis using CRISPR to correct CFTR mutations

(Source: Bhatta BP and Malla S, Review, Plants, MDPI)

Table 2: Examples of horticultural crops where CRISPR/Cas9 technology was used ot meet research objectives.

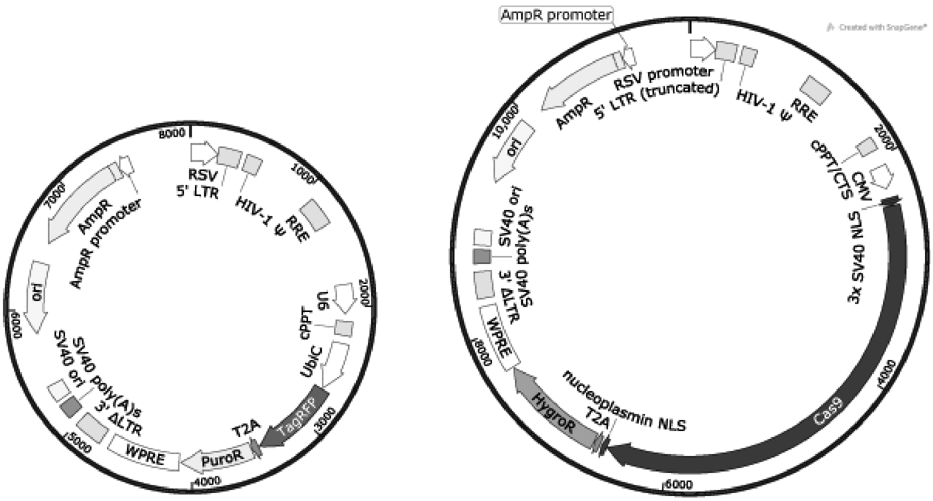
Crop	Research Objective Met Using CRISPR/Cas9
Tomato Capsicum Carrot Potato	• Understand the role of a photoreceptor in seedling development/ stress tolerance • Bacterial speck resistance • Combine desired traits with useful traits present in wild type Confirm function of a gene involved in Fusarium wilt tolerance Improve lycopene content • Develop Tomato Yellow Leaf Curl Virus resistance Long shelf life, generate parthenocarpy Transgene-free powdery mildew resistant plants Achieve ideotype • Develop day-neutral and early yielding plants • Understand the role of a transcription factor in chloroplast development and fruit color • Generate haploid plants Reduce enzymatic browning Overcome self-incompatibility Reduce steroidal glycoalkaloids Develop amylopectin starch cultivars Enhance Fusarium wilt resistance • Validate function of vacuolar sugar transporter gene Obtain gynoecious genotypes

Sweet potato Watermelon	• Resistance to Fusarium oxysporum f. sp. niveum Race 1 • Functional characterization of a gene in fruit flesh sugar accumulation Herbicide resistance

(Source: Bhatta BP and Malla S, Review, Plants, MDPI)

CRISPR tools:

CRISPR-Cas9 allows researchers to perform gene knock out, DNA-free gene editing, gene ‘Knock-in’ and gene silencing. Gene screening, targeting and subsequent manipulation is achieved using CRISPR products. Sigma Aldrich (Merck) recruits genetically engineered sgRNA (single guide RNA), crRNA, CRISPRi and CRISPRa for gene editing with desirable changes in gene expression. Stunning gene architecture has been achieved using CRISPR-cas9, a suite of genetic engineering in Lentivirus, as available through Thermo Fischer Scientific and Genscript genetic stores[4,5].



pRSG16-U6-sg-UbiC-TagRFP-2A-Puro
8.0 kb

pR-CMV-Cas9-2A-Hygro
11.6 kb

Figure 1: CRISPR-Cas9 Lentiviral Guide RNA Cloning Vectors and Control Constructs (2-vector System)

(Source: Biocat Genomics System Website)

All-in-all, the new advances are improving our understanding of biological processes and are paddling CRISPR-Cas based tools towards clinical, biotechnological and agricultural use in gene and cell therapies.

Conclusion:

Overall, advances in CRISPR technology are deepening our understanding of biological systems and driving the application of CRISPR-Cas tools in clinical, biotechnological, and agricultural domains, particularly in gene and cell therapies.

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