NEW ERA AGRICULTURE MAGAZINE

# A NOVEL TECHNOLOGY FOR CROP IMPROVEMENT: CRISPR/CAS9

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**Abstract: -**

CRISPR/Cas9 has emerged as a transformative genome-editing tool in agriculture, offering precise and efficient modifications to enhance crop resilience against biotic (pests, pathogens) and abiotic (drought, salinity, heat) stresses. CRISPR allows targeted gene editing without introducing foreign DNA, making it a powerful tool for developing crops with enhanced resistance.

**Keywords:** CRISPR/Cas9, crop resilience against biotic and abiotic stress and gene editing etc.

**Introduction:**

CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats associated protein 9) is a revolutionary genome-editing tool that allows precise modifications in the DNA of plants and other organisms. Derived from a bacterial immune defense mechanism, this technology has become a cornerstone in modern crop improvement programs due to its efficiency, accuracy, and cost-effectiveness. Unlike traditional breeding or transgenic approaches, CRISPR is a revolutionary genome-editing tool that allows precise modifications in the DNA of organisms and plants with enhanced resistance to biotic stresses (pests, diseases) and abiotic stresses (drought, salinity, heat, etc.).

The main aim of this review is to explore the use of CRISPR to develop crops that can adapt to changing climatic conditions and maintain their productivity under stress, thereby ensuring food security and agricultural sustainability.

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**Basic Gene Editing Strategy/ Steps**

Gene editing with site-specific nucleases introduces double-stranded DNA breaks (DSBs) at a specific site of genomic sequence with the help of complementary base pairing of a single-guide RNA (sgRNA) to evoke DNA repair mechanisms and converted into genetic modifications such as gene replacement, gene insertion, and targeted mutagenesis. Non-homologous end joining (NHEJ) is the most widely used DSB repair mechanism in crop plants.

**Synthesis of Target-Specific Guide RNAs**

CRISPR/Cas9 is a complexed, two-component system using a short guide RNA (gRNA) sequence to direct the Cas9 endonuclease to the target site. Modifying the gRNA independent of the Cas9 protein confers ease and flexibility to improve the CRISPR/Cas9 system as a genome-editing tool.

CRISPR/Cas systems can be designed by inserting the DNA target protospacer sequence into the crRNAs or sgRNAs. The editing potential of these tools has increased as a result of the discovery of several PAM (protospacer adjacent motif) specific Cas orthologs & polymorphisms (Anzalone *et al.* 2020). With this technique, foreign nucleic acids are specifically interfered with based on the sequence of short guide RNAs. Target locus needs alteration of genome *via* CRISPR/Cas9. DSBs, which happen when two repair processes alter the

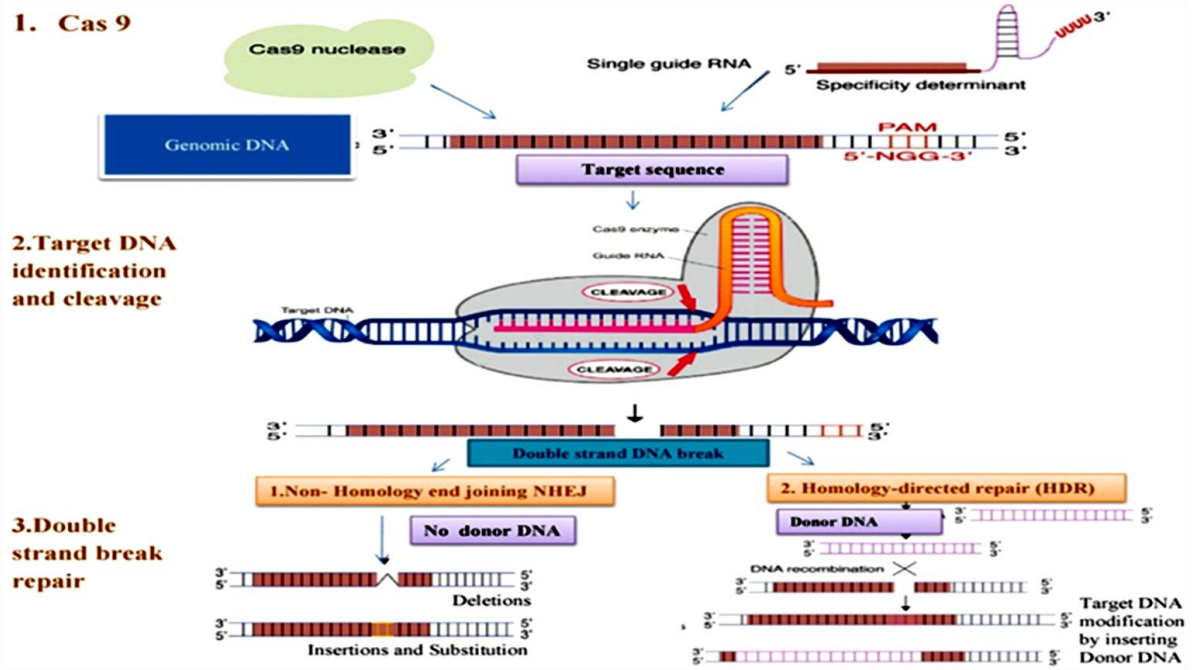


**Fig**: Overview of CRISPR/Cas9 technology for plant genome editing (i) two mechanisms for gene alteration include homology-directed repair (HDR) as well as non-homologous end joining (NHEJ) (ii) targeted DNA is cleaved and then repaired by NHEJ or HDR

same gene, are brought on by the site-specific nucleases. Genes are deleted or fused using NHEJ, or non-homologous ending combining, is carried out without donor DNA. By using homologous portions as its foundation, homology-directed repair (HDR) adjusts gene sequences in response to even the smallest changes in either DNA strand

The fundamental component of the RNP compound is the ribonucleoprotein (CRISPR/Cas9) complex, which is composed of the gRNA and Cas9 nuclease. On the 3'ends of DNA targets, 5'-NGG-3' sequences bearing the PAM motif are necessary.

The targeting sequence (crRNA), which is situated 20 nucleotides before the PAM sequence, will be divided into roughly three bases by the Cas9 nuclease. The target region's gRNA can only attach to the genomic DNA if it has a particular protospacer neighboring motif (PAM). Later, the Cas9 nuclease separates the DNA into two strands (denoted by the scissors). A customized sgRNA with a Cas9 nuclease-recruiting domain and an aiming sequence (crRNA sequence) is required by the CRISPR/Cas9 system (tracrRNA).

**Selection of the Target Gene**

Mostly in CRISPR/Cas9 system, the combination produced by the tracrRNA and crRNA attracts Cas9 and gives instructions to cleave the DNA sequence at a particular genomic region. When the complex of tracrRNA and crRNA breaks down, sgRNA, which has just one strand of RNA, produces. The Cas protein is guided by the sgRNA and recognizes a conserved sequence. It precisely recognizes and binds to the protospacer neighboring motif after unraveling the DNA with two strands (PAM). The desired sequence and the matching crRNA sequence are linked upstream of the PAM.

**CRISPR-Cas9 allows researchers to perform the following:**

⇨ **Gene knock-out**

The CRISPR-induced double-strand break can also be used to create a gene "knock-ins" by exploiting the cells' homology-directed repair. The precise insertion of a donor template can alter the coding region of a gene. Previous studies have demonstrated that single-stranded DNA can be used to create precise insertions using CRISPR/Cas9 system.

⇨ **DNA-Free Gene Editing**

CRISPR can be used for DNA-free gene editing without the use of DNA vectors, requiring only RNA or protein components. A DNA-free gene editing system can be a good choice to avoid the possibility of undesirable genetic alterations due to the plasmid DNA integrating at the cut site or random vector integrations.

⇨ **Gene Insertions or ''Knock-Ins''**

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➪ **Transient Gene Silencing**

By modifying the Cas9 protein so it cannot cut DNA, transient. The CRISPR-induced double-strand break can also be used to create a gene "knock-ins" by exploiting the cells' homology-directed repair. The precise insertion of a donor template can alter the coding region of a gene. Previous studies have demonstrated that single-stranded DNA can be used to create precise insertions using CRISPR-Cas9 system gene silencing or transcriptional repression can also be done.

**CRISPR/Cas gene editing improves abiotic stress tolerance of crops**

- Abiotic stresses such as salinity, drought, extreme temperature and heavy metals are important factors affecting plant growth and development, which can lead to 50% crop yield reduction (Liu et al., 2022). It is essential to generate crop types with greater adaptability for growth under a variety of environmental conditions in such circumstances.
- Salt stress induces osmotic stress, ion stress and secondary stress in plants, which reduces yield and quality of crops (Yang and Guo, 2018). In tomato plants, the exact deletion of SlHyPRP1 negative-response domain(s) significantly enhanced the salinity tolerance at both of the germination and vegetative stages (Tran et al., 2021).
- Drought stress is the main cause of serious loss of yield and productivity of major crops and poses the greatest threat to global food security. Using CRISPR/Cas system, the natural ARGOS8 promoter sequence of maize was replaced by GOS2 promoter to improve the yield of maize under drought stress in field (Shi et al., 2017). CRISPR/Cas9-mediated mutagenesis of OsERA1 resulted in great drought stress tolerance in rice (Ogata et al., 2020).
- Cold stress, which includes chilling (<20°C) and freezing (<0°C) temperatures, inhibited growth and development of plants, and seriously restricts plant spatial distribution and agricultural productivity (Ding et al., 2020). Low temperature directly inhibits plant metabolic response and induces osmotic stress, oxidative stress

and other stress. Zeng et al. showed that the ospin5b mutant, gs3 mutant and osmyb30 mutant created by CRISPR/Cas9 increased spike length, grain size and cold tolerance. High temperature affects the whole growth cycle of crops, especially in the heat sensitive period such as early establishment, flowering and gametophytogenesis.

**CRISPR/Cas gene editing improves biotic stress tolerance of crops**

Biotic stresses, such as viral, fungal, and bacterial infections, account for 20–40% of global agricultural output losses (Walker, 1984). In order to address the food crisis, conferring pathogen resistance to host plants can lessen the impact of disease on crop productivity. So far, scientists have obtained plants that are highly resistant to fungal, bacterial and viral diseases, as well as insects, through CRISPR/Cas9 knockout

- CRISPR/Cas9 was used to knock out all three TaMLO alleles in wheat, and wheat plants with enhanced powdery mildew resistance were obtained (Wang et al., 2014). Similarly, CRISPR/Cas9-mediated knockdown of SlMLO and VvMOL3 made tomato (Nekrasov et al., 2017) and grape (Wan et al., 2020) resistant to powdery mildew. In addition, CRISPR/Cas9-

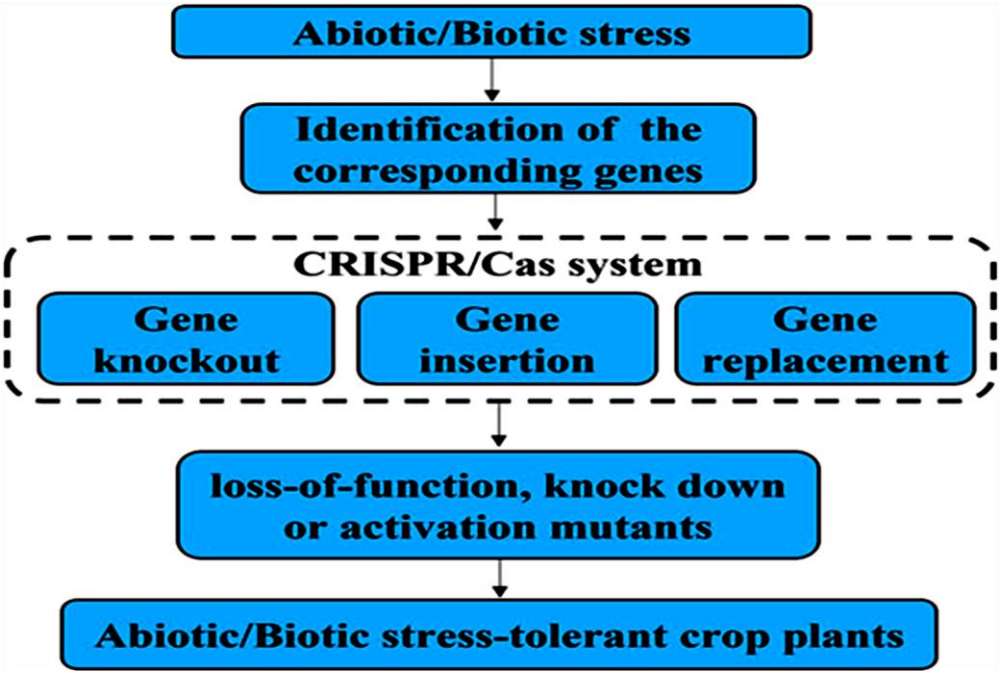


**Fig:** Review of the applications of CRISPR/Cas systems in improving biotic and abiotic stress tolerance of crop. CRISPR-Cas9 system can be used for gene knockout, gene insertion and gene replacement, resulting in loss-of-function, knock down or activation mutants, which can lead to generation of abiotic/biotic stress-tolerant crop plants.

mediated SlPMR4 mutation also significantly increased tomato powdery mildew resistance, but could not completely immune (Santillán Martínez et al., 2020).

- The level of salicylic acid levels was raised when the serotonin biosynthesis was prevented by disrupting OsCYP71A1, which results in greater resistance to plant hoppers and stem borers in rice (Lu et al., 2018). Li et al. (2022) showed that the gmcdpk38 mutant with Hap3 knockout using CRISPR/Cas9 showed high resistance to common cutworm (Li et al., 2022).

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