

## CRISPR Cas9 in Crop Genome Editing: Application of CRISPR for Precise Trait Improvement and Recent Success Stories

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CRISPR-Cas9 is a revolutionary gene editing technology whose name stands for *Clustered Regularly Interspaced Short Palindromic Repeats*. Developed in 2012 by Jennifer Doudna and Emmanuelle Charpentier, who were later awarded the 2020 Noble price in chemistry- it has transformed the way scientist can improve crops. Traditional crop breeding often takes decades to produce new, improved varieties while earlier Genetic Modification (GMO) techniques involved introducing foreign DNA, which sparked public concerns. CRISPR-Cas9 changes the landscape by enabling researchers to make precise, targeted changes directly to a plant's own genetic code – enhancing traits without adding genes from other species. The Technology draws inspiration from a natural defense system found in bacteria and archaea, which use CRISPR sequences and cas9 protein to recognize and cut the DNA of invading viruses, such as Bacteriophages. By adopting this system, plant scientist can now develop crop varieties that are high yielding, more nutritious, and better able to withstand pests, diseases, and climate stresses faster and more accurately than ever before.

### How CRISPR -Cas9 works

CRISPR-Cas9 is like a programmable “molecular scissors” system that can cut DNA at a specific location chosen by scientists. It has two main parts:

**1. Guide RNA (gRNA)-** a short RNA sequence designed to match the DNA sequence scientists want to change. It leads to the correct spot in the genome.

**2. Cas9-** An enzyme that cuts both strands of DNA at the target location identified by the guide RNA.

The plant's natural DNA repair systems fix the break in one or two ways:

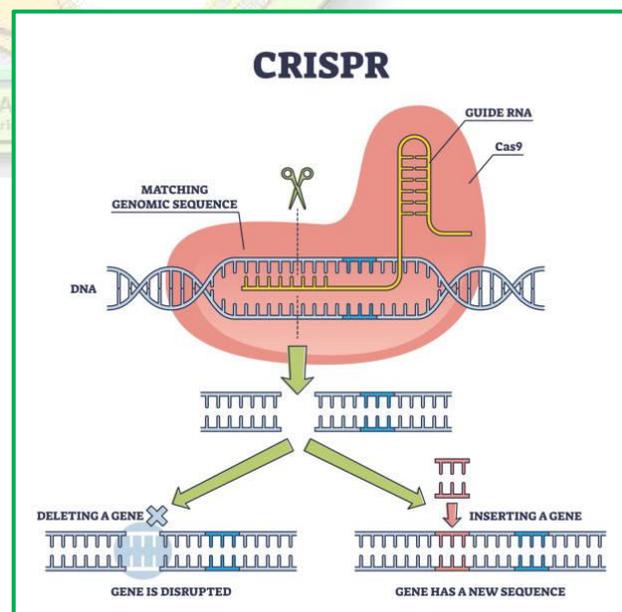
**1. Non-Homologous End Joining (NHEJ)-** often introduces small insertions or deletions, which can disrupt (knock out) the target gene.

**2. Homology-Directed Repair (HDR)-** uses a supplied DNA template to insert or replace specific genetic material, adding new traits.

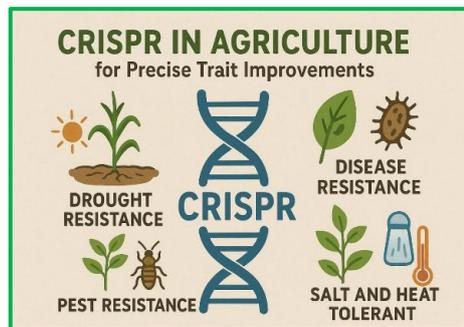
### CRISPR in agriculture for precise trait improvement

It can edit multiple genes at once , to enable the development of crops with improved traits to meet the challenges of climate change, food security, and sustainable farming. Key application includes,

✓ Drought tolerance



- ✓ Disease resistance
- ✓ Pest resistance
- ✓ Herbicide tolerance
- ✓ Higher yield
- ✓ Better quality
- ✓ Salt tolerance
- ✓ Heat and Cold tolerance
- ✓ Reducing anti nutritional factors
- ✓ Enhancement of shelf life in perishables.



### Recent Success Stories

India has launched its first genome edited rice varieties – **DRR Dhan 100 (kamala)** and **Pusa DST Rice 1** designated to boost yield, enhance climate adaptability, and improve resource efficiency, without incorporating foreign DNA. Developed by ICAR using CRISPR-Cas9 technology.

**DRR Dhan 100 (kamala):** Derived from Samba Mahsuri variety, modified through Site Directed Nuclease 1 (**SDN 1**) targeting the Cytokinin Oxidase 2 (**CKX2**) gene (Gn1a) to increase grain number. Features include early maturity (15-20 days earlier), drought tolerance and better nitrogen use efficiency.

**Pusa DST Rice 1:** Based on Maruteru 1010 variety, genome edited via **SDN 1** targeting the DST gene to improve drought and salinity tolerance. It delivers 30.4% higher yield in coastal saline areas, 14.66% in alkaline soils, and 9.47% in inland saline conditions.

**Site Directed Nuclease Technology:** It is a genome editing technique that introduces precise changes in DNA using enzymes called nucleases. **SDN 1** introduces small insertion / deletion without using foreign DNA, while **SDN 2** uses a template DNA (similar to host) to introduce specific desired changes.

Indian scientists have created country's first **low pungent mustard variety** that is pest and disease resistant developed using CRISPR-Cas9

technology. Scientists targeted glucosinolate transporter (**GTR**) genes in the **Varuna** mustard variety. By deactivating 10 of 12 GTR genes using CRISPR-Cas9 glucosinolate levels in seeds dropped below 30 ppm, while levels in leaves and pods increased. So the edited plants retained strong pest and disease resistance while producing healthier less pungent seeds suitable for edible oil and animal feed.

Non-browning bananas were released by Tropic Biosciences of Philippines using the CRISPR-Cas9 technology. These have potential to reduce food waste and carbon-di-oxide emissions equivalent to removing 2 million cars from the road per annum.

### INDIA'S FIRST GENOME-EDITED RICE VARIETIES

Developed by ICAR using advanced CRISPR-Cas9 genome-editing technology.

<p><b>DRR DHAN 100 (KAMALA)</b></p> <ul style="list-style-type: none"> <li>• Early maturity (15–20 days earlier)</li> <li>• Drought-tolerant</li> <li>• High nitrogen-use efficiency</li> </ul>	<p><b>PUSA DST RICE 1</b></p> <ul style="list-style-type: none"> <li>• 30.4% higher yield in coastal salinity</li> <li>• 14.66% higher in alkaline soils</li> <li>• 9.67% higher in inland salinity</li> </ul>
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**SITE-DIRECTED NUCLEASE (SDN) TECHNOLOGY**

Uses specific enzymes to make precise DNA changes



## Advanced CRISPR-dCas9

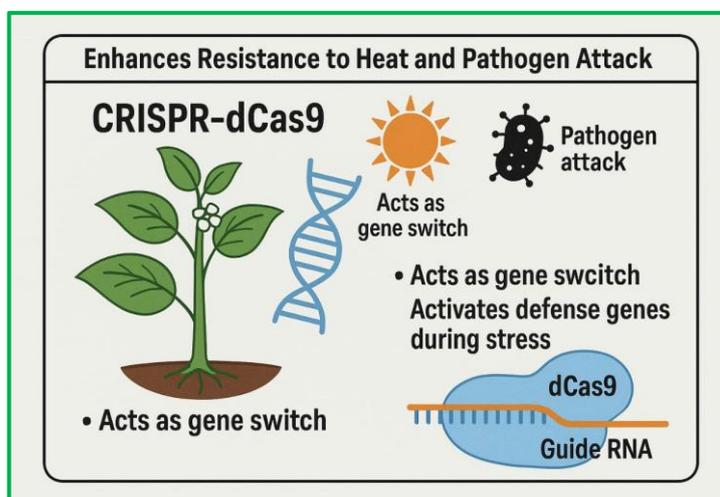
Scientists from the Bose Institute under the Department of Science and Technology have developed a CRISPR-dCas9 based molecular tool to boost plant resistance against heat stress and pathogen attack. Unlike regular CRISPR-Cas9, dCas9 doesn't cut DNA but acts as a gene switch, activating defense genes only during stress.

**Mechanism:** A tomato protein domain keeps dCas9 outside the nucleus under normal conditions; during stress, it enters the nucleus to trigger defense genes.

**Pathogen attack:** Activates CBP60g and SARD1 (immune response).

**Heat stress:** Activates NAC2 and HSFA6b (improves water retention, leaf health, thermotolerance).

**Tested on:** Tomato, potato, tobacco- most effective in tomato.



## Conclusion

CRISPR-Cas9 is not just a tool – it's agriculture's genetic game-changer. From boosting yields to building climate-ready crops, this precision-editing marvel is powering a biotech-driven revolution in our fields. With each success story, it brings us closer to sustainability. With CRISPR in our hands, we are not just growing crops, we are cultivating a smarter, greener, and food secure tomorrow.

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