Genome Editing in Plants

Introduction

Crop improvement has always been central to agriculture, food security, and economic development. Historically, plant breeding relied on natural genetic variability and conventional crossing techniques, but these approaches are time-consuming and imprecise. The Green Revolution in the mid-20th century demonstrated the power of genetics in agriculture, but the pace of conventional breeding cannot keep up with emerging challenges such as climate change, declining soil fertility, pest outbreaks, and rising global food demand.

Genome editing is a next-generation breeding approach that allows scientists to introduce precise changes in the DNA sequence of plants. Unlike genetic engineering (GM crops), which inserts foreign DNA, genome editing relies on site-directed nucleases (SDNs) to modify native genes at targeted sites. This enables the creation of crops with enhanced yield, resilience, and nutritional quality, while minimizing regulatory hurdles and public concerns.

India, with its vast agricultural landscape and diverse cropping systems, has invested significantly in genome editing research. Through initiatives led by the Department of Biotechnology (DBT), Indian Council of Agricultural Research (ICAR), and State Agricultural Universities (SAUs), the country has begun deploying genome editing tools for crops such as rice, wheat, maize, mustard, tomato, and banana. The release of two genome-edited rice varieties in May 2025 marks a landmark achievement in India's biotechnology journey.

Evolution of Plant Genome Manipulation

Conventional Plant Breeding

- Based on cross-pollination and selection.
- Requires 6–10 years to develop stable varieties.
- Alters thousands of genes simultaneously, making precision control impossible.

Mutation Breeding

- Introduces random mutations using chemicals or radiation.
- Has produced over 3,000 crop varieties worldwide (e.g., rice, groundnut).
- Lacks predictability and precision.

Genetic Engineering (GM Crops)

Genetic engineering (GE) in plants, commonly referred to as **Genetically Modified (GM) crops**, involves the **direct manipulation of an organism's genome** using molecular techniques to introduce, delete, or modify specific genes. Unlike conventional breeding, which relies on crossing and recombination, genetic engineering allows **transgenes (genes from other species)** to be incorporated in a controlled manner, enabling precise modification of plant traits.

- GM crops in India are regulated under the **Rules for Manufacture, Use, Import, Export & Storage of Hazardous Microorganisms / Genetically Engineered Organisms or Cells, 1989** (Rules, 1989) of the Environment (Protection) Act, 1986.

- **Key regulatory bodies:**

    - **GEAC (Genetic Engineering Appraisal Committee)**: Primary authority for approval of GM field trials and commercial release.
    - **RCGM (Review Committee on Genetic Manipulation)**: Technical oversight and monitoring of laboratory and contained greenhouse experiments.
    - **IBSC (Institutional Biosafety Committee)**: Ensures compliance with biosafety practices within institutions.

- **Mandatory requirements for GM crops:**

    - Risk assessment (environmental, food/feed safety)
    - Contained trials → confined field trials → multi-location testing
    - Biosafety compliance, labeling, and post-release monitoring
    - Introduces foreign DNA (transgenes) into plants.
    - Examples: Bt cotton (India, 2002), herbicide-tolerant soybean (USA).
    - Subject to stringent GMO regulations and public debate.

Genome Editing

Genome editing refers to a set of molecular techniques that allow **precise, targeted modifications** of an organism's DNA. Unlike conventional breeding, which relies on natural recombination, or GM crops, which introduce foreign genes, genome editing can modify **specific genes within the plant's own genome** without introducing exogenous DNA (particularly in SDN-1 and SDN-2 approaches).

- Employs molecular scissors (nucleases) to cut DNA at precise locations.
- Allows small insertions, deletions, or substitutions (SDN-1 and SDN-2).
- Avoids introduction of foreign genes in many cases, making it more socially acceptable.

Overview of Genome Editing Technologies

Evolution of Plant Genome Manipulation

- **Conventional Breeding**: Relies on crossing and selection; imprecise and time-consuming.
- **Genetic Engineering (GM crops)**: Involves insertion of foreign DNA; regulated as GMOs.
- **Genome Editing**: Uses nucleases like CRISPR to make precise DNA cuts for targeted edits.

Genome Editing Tools

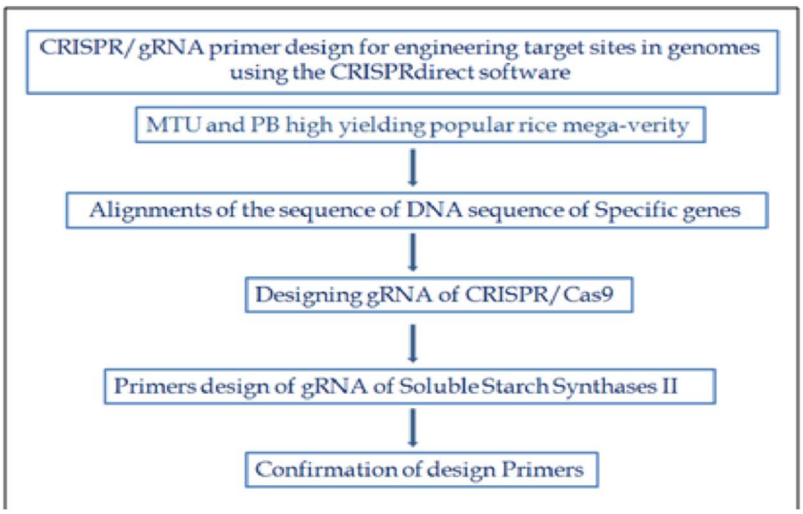
- **Zinc Finger Nucleases (ZFNs)**: Early tool, complex protein engineering.
- **TALENs (Transcription Activator-Like Effector Nucleases)**: Improved precision, but costly.
- **CRISPR-Cas9**: Simple, programmable, widely adopted in plant research.

CRISPR-Cas Mechanism

- **Cas9 protein** acts as "molecular scissors."
- **Guide RNA (gRNA)** directs Cas9 to the target DNA site.
- **Repair Pathways**:
  - **SDN-1**: Error-prone repair (insertions/deletions).
  - **SDN-2**: Template-directed precise changes.
  - **SDN-3**: Insertion of foreign DNA (regulated as GMO).

Mechanism of CRISPR-Cas9

1. **Guide RNA (gRNA)** identifies target DNA sequence through base-pairing.
2. **Cas9 protein** binds the gRNA and cuts DNA at the target site.
3. The cell's repair machinery fixes the cut using either:
   - **Non-Homologous End Joining (NHEJ)** → SDN-1 edits.
   - **Homology-Directed Repair (HDR)** → SDN-2 edits.



Laboratory and Field Workflows

Workflow for SDN-1 Edits

- **Gene Target Selection**: Identify functional genes linked to trait.
- **gRNA Design**: Use bioinformatics software (e.g., CRISPR-P, CHOPCHOP).
- **Vector Construction**: Clone gRNA and Cas9 gene into a plant expression vector.

- **Delivery into Plant Cells**:Agrobacterium-mediated transformation (preferred in rice, tomato). Biolistics (gene gun).

- **DNA Repair**: Double-strand break repaired by NHEJ, creating insertions/deletions.
- **Plant Regeneration**: Edited cells induced to regenerate whole plants via tissue culture.
- **Molecular Screening**: PCR, sequencing, and restriction enzyme assays confirm edits.
- **Phenotypic Analysis**: Edited plants evaluated for desired traits.

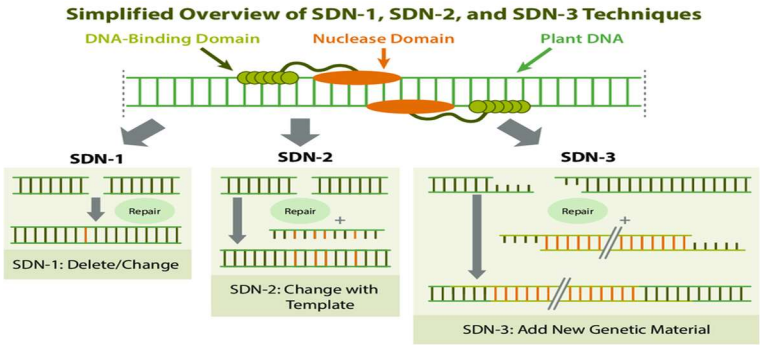
Workflow for SDN-2 Edits

1. Same initial steps as SDN-1.
2. **Repair Template**: Short DNA fragment carrying desired change supplied.
3. **HDR Pathway** integrates the precise change.
4. Regeneration, molecular screening, and trait evaluation follow.

4.3 Field Testing

- **Contained Greenhouse Trials** for preliminary validation.

- **Confined Field Trials (CFTs)** for agronomic performance and biosafety.



India's Regulatory Framework

1. Legal & policy foundation

- MoEF&CC Office Memorandum (OM), 30 March 2022: The Ministry of Environment, Forest & Climate Change issued an OM stating that genome-edited plants without exogenous/foreign DNA (i.e., falling within SDN-1 and SDN-2 categories when free of introduced DNA) are exempt from the requirement to follow the provisions of the Rules, 1989 under the Environment (Protection) Act, 1986. The OM frames the statutory basis for treating DNA-free SDN-1/SDN-2 products differently from transgenic (SDN-3) products.
- DBT Guidelines for Safety Assessment of Genome Edited Plants, 2022 (issued May 2022): Following the OM, the Department of Biotechnology (DBT), Ministry of Science & Technology, issued comprehensive Guidelines that (a) define SDN-1, SDN-2 and SDN-3 categories, (b) describe the biosafety data and information to be generated during R&D, and (c) outline high-level pathways for regulatory review and risk assessment appropriate to each category. These Guidelines provide the central scientific/regulatory guidance for institutions and developers.

2. SOPs for Regulatory Review of SDN-1 & SDN-2 (Oct 2022): To operationalize the Guidelines and the OM, DBT notified Standard Operating Procedures (SOPs) and a checklist specifically for SDN-1 and SDN-2 projects. The SOPs clarify how Institutional Biosafety Committees (IBSCs) should review projects, what documentation is required to claim the SDN-1/SDN-2 exemption threshold, and the steps required to validate that final plants are free of exogenous DNA. The SOPs were notified publicly in Oct 2022

Institutional roles and who does what

- **Institutional Biosafety Committee (IBSC):** Primary oversight body at the institution where R&D is done. IBSCs are responsible for reviewing and

approving contained laboratory and greenhouse work, ensuring compliance with the DBT guidelines/SOPs, and maintaining records. For SDN-1 and SDN-2 projects that meet the exemption criteria, the IBSC is the first and main authority to certify that the work meets containment and data requirements. The SOPs give IBSCs specific checklists and procedures to follow

- **RCGM (Review Committee on Genetic Manipulation):** RCGM, an expert committee under DBT, historically reviews higher-level technical issues and can issue technical recommendations. SOPs and the Guidelines were prepared with inputs from RCGM and the RCGM continues to play an advisory role, especially when there is uncertainty or a need for technical review beyond IBSC capacity.
- **GEAC (Genetic Engineering Appraisal Committee) and MoEF&CC:** GEAC traditionally handled approvals and regulation of genetically engineered organisms under Rules, 1989. The OM (Mar 30, 2022) made clear that **SDN-3** (foreign DNA insertions) continue to be regulated as GMOs and remain under GEAC/MoEF&CC oversight. For SDN-1/SDN-2 that are DNA-free, GEAC is typically **not** required for approval unless the product does not meet the SOP threshold or involves SDN-3 features.
- **Other agencies / downstream regulators:** Varietal release and commercialization involve other statutory mechanisms (e.g., central/state seed certification, PPV&FR Act processes, Food Safety authorities for food/feed safety assessment, and agricultural extension for deployment). The DBT guidelines explicitly note that exemption from Rules, 1989 does **not** remove a product from other applicable laws governing seed release, commercialization, import/export, or market authorization.

DBT 2022 Guidelines

In 2022, the **Department of Biotechnology (DBT), Ministry of Science & Technology** issued guidelines clarifying the regulation of genome-edited crops. Key provisions:

- **SDN-1 and SDN-2**: Not treated as GMOs if no foreign DNA remains.
- **SDN-3**: Regulated as GMOs.
- **Institutional Biosafety Committees (IBSCs)**: First-level review.
- **Review Committee on Genetic Manipulation (RCGM)**: Central approval for SDN-1/SDN-2 projects.
- Streamlined **Standard Operating Procedures (SOPs)** for proposal submission.

Implementation

- **Institutional Level**: IBSCs in universities and ICAR institutes.
- **National Level**: RCGM evaluates edits and decides exemptions.
- **Field Trials**: Managed under existing biosafety protocols.

Practical stepwise pathway for a developer

1. **Pre-project preparation**
    - Convene IBSC; register project; prepare project dossier with methods and risk mitigation.
2. **Containment R&D**
    - Conduct editing (DNA-free methods preferred), regeneration, and molecular screening under IBSC oversight.
3. **Demonstrate DNA-free status**
    - Use PCR/qPCR/Southern/WGS and segregation evidence per SOP; document thoroughly.
4. **IBSC certification**
    - IBSC vets the data; if satisfied that the product is SDN-1/SDN-2 and DNA-free, IBSC records and certifies compliance; no mandatory GEAC review is required unless criteria unmet.
5. **Contained field trials / confined field evaluation**
    - If field trials are necessary, follow SOPs for confinement, monitoring and reporting—IBSC coordinates and maintains trial records; RCGM may be consulted for technical review.
6. **Multi-location trials & varietal release**
    - For commercialization, follow normal varietal release pathways (trial networks, seed certification, registration), and provide any additional safety/compositional data requested by relevant authorities.
7. **Post-release monitoring & stewardship**
    - Implement stewardship: monitor for trait stability, non-target effects and gene flow; maintain records for traceability and stewardship.

Recent National Progress

On **May 4, 2025**, India released its **first genome-edited rice varieties**:

1. **DRR Dhan 100 'Kamala'** (developed at ICAR-Indian Institute of Rice Research, Hyderabad)
    - Trait: Improved grain quality, climate resilience.
2. **Pusa DST Rice 1** (developed at ICAR-Indian Agricultural Research Institute, New Delhi)
    - Trait: Drought and salinity tolerance.

Significance:

- First SDN-1/SDN-2 edited crops formally released under DBT 2022 guidelines.
- Represents a shift toward faster, precise crop improvement.

Applications of Genome Editing in India

- **Abiotic stress tolerance** (drought, salinity, heat).

- **Biotic stress resistance** (pests, pathogens).
- **Nutritional enhancement** (biofortification of rice, wheat).
- **Yield improvement**.
- **Quality traits** (grain size, aroma, oil content).

Biosafety and Socio-Economic Considerations

- **Biosafety**: Even though SDN-1/SDN-2 edits are not GMOs, rigorous validation ensures off-target edits are absent.
- **Public Perception**: Genome editing seen as safer and more acceptable compared to GM crops.
- **Economic Impact**: Faster breeding cycles, cost savings for farmers, reduced dependency on chemical inputs.
- **Global Trade**: Acceptance of genome-edited crops varies across countries, which may affect exports.

Future Directions

- Expansion of genome editing beyond rice to wheat, maize, pulses, and horticultural crops.
- Integration with **AI-driven gRNA design** and **speed breeding**.
- Public-private partnerships to ensure farmer adoption.
- Development of a transparent communication strategy for public trust.