Frequently Asked Questions About GENE EDITED PLANTS

BCIL

Biotech Consortium India Limited

New Delhi

2022

1 What are gene edited plants ?

Gene editing (also called genome editing) is a group of technologies used to make precise and targeted changes in an organism's Deoxy-Ribonucleic Acid (DNA). Gene editing is done using site directed nucleases (SDNs) such as Zinc-Finger nucleases, TALENs, CRISPR/Cas9 and advancements such as base editing, prime editing etc.

The plants modified using gene editing techniques are referred to as gene edited plants.

2 How is gene editing possible ?

All living organisms have DNA within their cells, which is like an instructional manual to build and maintain an organism. Sequences of DNA, referred to as genes contain information to determine a particular characteristic/trait.

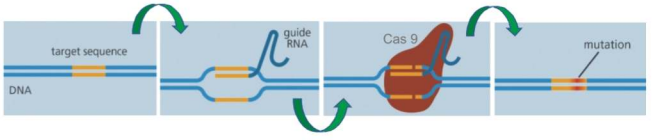
Gene editing is built on scientific advances and a better understanding of natural processes. It has been possible due to advances in knowledge of plant breeders or scientists about function and sequences of genes and available tools to add, remove, or change a particular location of a gene or several genes This is similar to editing in a book with the tools available to identify the exact page, the exact paragraph and even the exact word to be changed. Just like editing a book, where you need to be able to read the book in order to edit, genome editing requires precise knowledge of the plant genome and the DNA sequence of the gene you wish to edit.

3 What is CRISPR and how does it work ?

CRISPR has been the most revolutionary of editing tools because of its simplicity and efficiency of editing. Its discoverers, Drs. Emmanuelle Charpentier and Jennifer Doudna,were awarded the Nobel prize for Chemistry in 2020.

CRISPR stands for clustered regularly interspaced short palindromic repeats. These are repeated DNA sequences that occur in the genomes of many bacteria and play an important role in bacterial defense system. CRISPR along with Cas9 (CRISPR associated protein 9) can be used to make gene edits.

This technology is comprised of a guide RNA and a Cas protein. The guide RNA helps direct the Cas protein to a particular region of the genome and the Cas protein then makes a cut in the DNA. After the DNA is cut, normal cellular processes repair the break resulting in different types of modifications depending on the presence or absence of a DNA template.



4 What are various types of gene editing ?

Gene editing uses site directed nucleases (SDNs) to make changes that may either be a small deletion, a substitution or the addition of a number of nucleotides. Such targeted edits result in a new and desired characteristic.

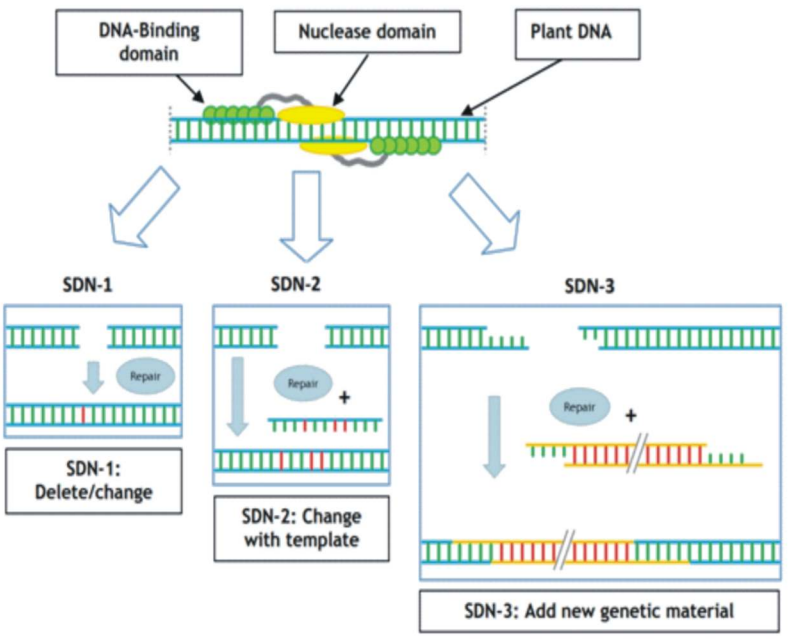
Gene editing applications are divided into three techniques: SDN-1, SDN-2, and SDN-3:

SDN-1: Produces a double-stranded break in the genome of a plant without the addition of foreign DNA. The spontaneous repair of this break can lead to a modification or deletion, causing gene silencing, gene knock-out or a change in the activity of a gene. The mutations produced in this way are of the same type that occur naturally or as a result of chemically or radiation induced mutations.

SDN-2: Produces a double-stranded break, and while the break is repaired by the cell, a small nucleotide template that is complementary to the target region is supplied, which is used by the cell to repair the break. The template contains one or several small sequence changes in the genomic code, that are copied into the plant's genetic material resulting in a modification of the target gene.

SDN-3: Also induces a double-stranded break in the DNA, but is accompanied by a template containing a gene or other sequence of genetic material. The cell's natural repair process then utilizes this template to repair the break; resulting in the introduction of the genetic material.

SDN-1 and SDN-2 plants do not contain inserted foreign DNA, while SDN-3 plants may contain foreign DNA.



Simplified overview of SDN-1, SDN-2 and SDN-3 techniques

5 How do gene edited plants compare with those developed using traditional plant breeding techniques ?

Plants developed using traditional plant breeding techniques and gene edited plants, both aim to incorporate desirable characteristics for crop improvement. The plants altered for particular changes by the two techniques may be similar to each other, but the process of development is different particularly in terms of precision and time to achieve final outcome.

Traditional breeding process uses cross breeding or mutagenesis triggered by chemicals or irradiation that results in random transfer of several genes with not enough clarity on number and location of these in the genome. Process of selection takes years and multiple generations to achieve desired outcome.

Gene editing uses genetic and molecular knowledge to introduce precise and targeted changes and the time taken to achieve the same outcome is typically shorter. For example, it may take 10-12 years in conventional breeding to achieve a desired result in a plant while gene editing takes 2-3 years to achieve the same result.

6 Why do we need to use gene editing in agriculture ?

Agriculture faces a variety of challenges from drought, floods, heat, diseases and pests. At the same time, demand for nutritious food is increasing and consumer preferences are changing.

Gene edited plants have the potential to make a positive impact in agriculture by incorporating wide range of traits for higher crop yields, lower use of chemical fertilizers and pesticides, better crop resilience to climate stress, reduced postharvest losses, and more nutritious foods.

7 Are gene edited plants and derived products approved for commercialisation ?

The first gene edited plant to be commercialised was a soybean variety followed by high GABA tomato.

High oleic soybean: As two genes involved in fatty acids synthesis have been turned off, resulting soybean oil has 80% higher oleic acid, 20% less saturated fatty acids and 0 grams trans fat per serving. It is claimed to have three times the fry-life and a longer shelf-life compared to the current soybean oil being sold in the market.

High GABA tomato: High GABA tomato contains around five to six times the normal level of a type of amino acid called gamma-aminobutyric acid, or GABA. This was achieved by clipping out one of the tomato's genes that inhibits the synthesis of GABA. It is expected to help in lowering blood pressure.

Some other products that are approved for use include varieties of mushroom, canola, rice etc.

Non-browning mushroom: White button mushroom (Agaricus bisporus) has been modified with small deletions in a specific polyphenol oxidase gene with no foreign DNA integration into the mushroom genome. The anti-browning trait reduces the formation of brown pigment (melanin), improving the appearance and shelf life of mushroom, and facilitating automated mechanical harvesting.

High oil containing canola: Canola with increased oil content in seeds has been developed by activating a negative regulator of the enzyme acetyl-CoA carboxylase (ACCase), the key enzyme for producing fatty acids for oil biosynthesis. Reducing activity of the regulator protein has resulted in significantly increased oil content in seeds.

B**acterial blight resistant rice:** Gene edited rice has been developed by disrupting the function of promoters for sugar transport genes critical for plants susceptibility to infection by *Xanthomonas sp.* resulting in resistance to bacterial blight.

Many other improved crops are in various stages of development.

8 What is the status of gene edited plants in India ?

The potential of gene editing has been recognized as a focus area under the National Biotechnology Development Strategy 2021-2025. Extensive research efforts are underway with support from Department of Biotechnology, Govt of India, Indian Council of Agriculture Research and other funding agencies.

Some of the products in advanced stages include high vitamin A banana, high yielding rice, improved mustard etc.

9 How are gene edited plants different from genetically engineered (GE) plants ?

Genetically engineered (GE) plants, also referred to as genetically modified (GM) plants, typically contain newly inserted DNA introduced using genetic engineering techniques. The newly inserted gene could be from the same or a related plant species, or a different organism.

Although genetic engineering techniques may be used in the early stages of their development, SDN-1 and SDN-2 gene edited plants do not contain introduced new genes, but rather contain small changes in the existing genome of the plant. Gene editing mostly focuses on making improvements that could occur in nature but makes them in a more precise way.

10 Are gene edited plants safe to eat ?

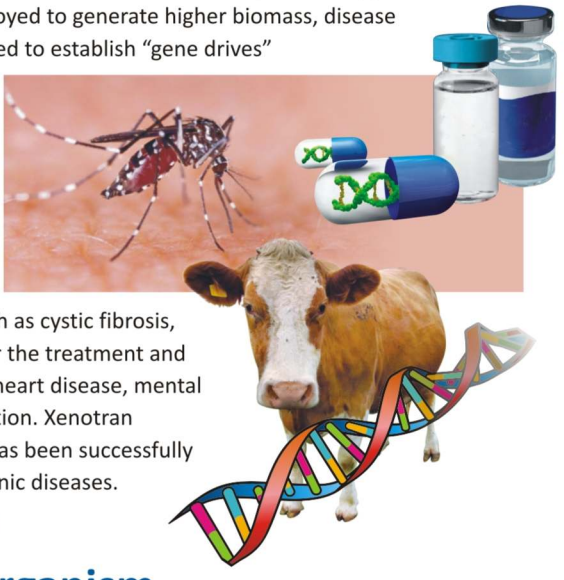
Most applications of gene editing, particularly SDN-1 and SDN-2 include changes within the plant's own genetic code, these are considered as safe as traditional breeding. In fact, with the use of genome editing, plant breeding has become much more knowledge based. Genome editing reduces the amount of uncertainty, thereby contributing to safety.

11 Does gene edited plants affect environment and biodiversity ?

No. In fact, there is no evidence of any crop plants, whether produced by conventional breeding, genetic engineering, or genome editing, causing environmental damage or adversely affecting biodiversity.
The greatest impacts on the environment and biodiversity are human activities, including agricultural practices, land clearing, industrialization and urban development to mention just a few. Crop improvement techniques such as genome editing are being used to make changes that will improve agricultural sustainability and resilience to climate change, thus helping protect the environment and safeguarding biodiversity.

12 Is gene editing used in other organisms, medicine etc. ?

Gene editing is being successfully used in understanding biological processes and for applications ranging from healthcare to industrial processes, in addition to agriculture. It is being used to breed disease resistant animals, improve animal welfare and high productivity of livestock. Gene editing is already being employed to generate animal models for human studies, gene therapy and xenotransplantation. In aquatic animals its being employed to generate higher biomass, disease resistance and sterility. The use of gene editing extended to establish "gene drives" to control the spread of infectious diseases such as Zika, and develop cleaner energy sources such as algae-based biofuels.



Gene editing has been used in developing diagnostic kits for disease screening, including developing diagnostic kits and vaccines for COVID 19.It is being explored in research and clinical trials for a wide variety of diseases, including single-gene disorders such as cystic fibrosis, hemophilia, and sickle cell disease. It holds promise for the treatment and prevention of more complex diseases, such as cancer, heart disease, mental illness, and human immunodeficiency virus (HIV) infection. Xenotran splantation of heart and kidney from gene edited pig has been successfully demonstrated and will go a long way in managing chronic diseases.

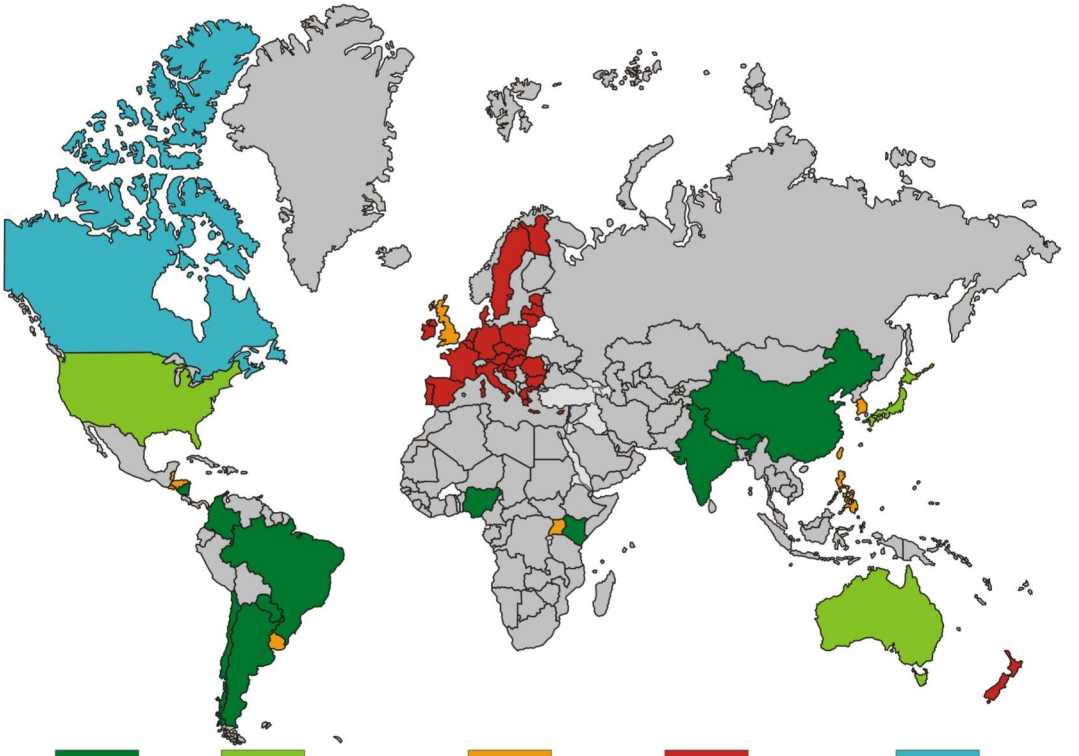
13 Are any gene edited organism other than plants approved so far ?

Gene edited fish developed by Japanese researchers viz. Tiger puffer and red sea bream with enhanced muscle mass and short body length have already been approved for use in Japan.

Gene edited fish approved in Japan:
Tiger Puffer and Red Sea Bream

14 Are gene edited plants regulated ?

The regulations for gene edited plants are evolving globally. There is a growing consensus that gene edited plants that do not contain exogenous foreign DNA should not be regulated under the same rules that apply to genetically engineered or transgenic plants.These kinds of gene edited plants are typically exempt from biosafety assessment and are regulated in the same way as products of conventional plant breeding. As of now, 52 percent of the world's population live in countries with positive or partial positive decisions on exempting SDN-1 and SDN-2 gene edited plants from biosafety regulation.

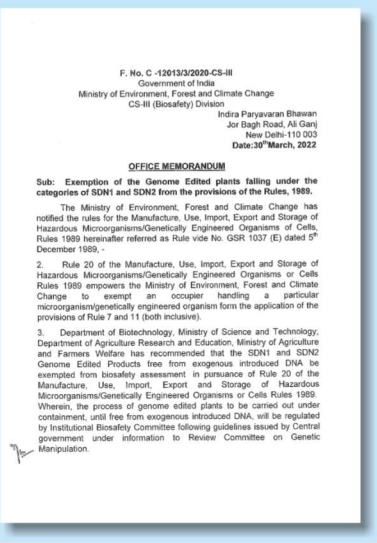


15 What are the existing regulations in place for gene editing in India ?

In India, the manufacture, import, use, research and release of genetically engineered (GE) organisms and derived products are governed by the rules notified by Ministry of Environment, Forest and Climate Change (MoEFCC), Government of India, on December 5, 1989, under the Environment (Protection) Act 1986, commonly referred to as the Rules, 1989, The definition of gene technology included all new techniques for introducing genetic changes.

Recognizing that SDN 1 and SDN 2 categories of plants, are free from any transgenes, Ministry of Environment, Forest and Climate Change has issued a notification on March 30, 2022 to exempt products of SDN1 and SDN2 (free from transgenes) from the provisions of Rules 7 and 11 (both inclusive) of Rules, 1989, whereas products of SDN 3 (with transgenes) will be treated in the same way as GE organisms under Rules, 1989, This exemption has been issued under Rule 20 of Rules, 1989 that allows the implementing ministry to provide exemptions for specific organisms from its provisions. The process of genome edited plants is to be carried out under containment, until free from exogenous introduced DNA, will be regulated by Institutional Biosafety Committee following stipulated guidelines under information to Review committee on Genetic Manipulation.

For their release as new variety, development and evaluation will be as per other applicable Laws/Acts/ Rules. The decision has been taken based on recommendations by Department of Biotechnology, Ministry of Science and Technology; Department of Agriculture Research and Education, Ministry of Agriculture and Farmers welfare.



F. No. C -12013/3/2020-CS-III
Government of India
Ministry of Environment, Forest and Climate Change
CS-III (Biosafety) Division
Indira Paryavaran Bhawan
Jor Bagh Road, Ali Ganj
New Delhi-110 003
Date:30th March, 2022

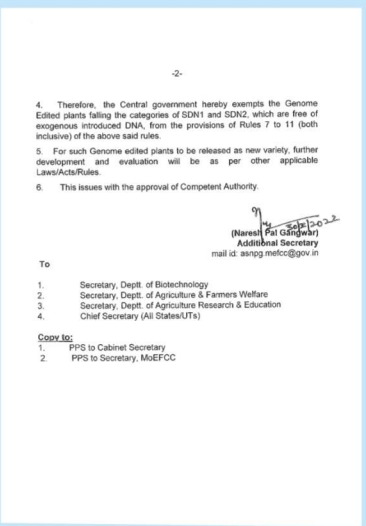
OFFICE MEMORANDUM

Sub: Exemption of the Genome Edited plants falling under the categories of SDN1 and SDN2 from the provisions of the Rules, 1989.

The Ministry of Environment, Forest and Climate Change has notified the rules for the Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms/Genetically Engineered Organisms of Cells, Rules 1989 hereinafter referred as Rule vide No. GSR 1037 (E) dated 5th December 1989, -

2. Rule 20 of the Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms/Genetically Engineered Organisms or Cells Rules 1989 empowers the Ministry of Environment, Forest and Climate Change to exempt an occupier handling a particular microorganism/genetically engineered organism form the application of the provisions of Rule 7 and 11 (both inclusive).

3. Department of Biotechnology, Ministry of Science and Technology; Department of Agriculture Research and Education, Ministry of Agriculture and Farmers Welfare has recommended that the SDN1 and SDN2 Genome Edited Products free from exogenous introduced DNA be exempted from biosafety assessment in pursuance of Rule 20 of the Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms/Genetically Engineered Organisms or Cells Rules 1989. Wherein, the process of genome edited plants to be carried out under containment, until free from exogenous introduced DNA, will be regulated by Institutional Biosafety Committee following guidelines issued by Central government under information to Review Committee on Genetic Manipulation.



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4. Therefore, the Central government hereby exempts the Genome Edited plants falling the categories of SDN1 and SDN2, which are free of exogenous introduced DNA, from the provisions of Rules 7 to 11 (both inclusive) of the above said rules.

5. For such Genome edited plants to be released as new variety, further development and evaluation will be as per other applicable Laws/Acts/Rules.

6. This issues with the approval of Competent Authority.

(Naresh Pal Gangwar)
Additional Secretary
mail id: asnpg.mefcc@gov.in

To

1. Secretary, Deptt. of Biotechnology
2. Secretary, Deptt. of Agriculture & Farmers Welfare
3. Secretary, Deptt. of Agriculture Research & Education
4. Chief Secretary (All States/UTs)

Copy to:
1. PPS to Cabinet Secretary
2. PPS to Secretary, MoEFCC

16 Who can make gene edited plants ?

Gene edited plants are developed by researchers to meet emerging needs in agriculture by making targeted changes using gene editing techniques. These may include scientists at universities, research institutions in public sector, private industry and any other organization with capabilities in molecular biology and plant tissue culture.

In view of ease of using gene editing techniques, increasing number of organizations are involved in development of gene edited plants in India and globally.

17 What are the global practices for gene edited crops while submitting applications to regulatory authorities ?

In most countries, the developers are required to submit data/information to demonstrate the absence of exogenous/foreign DNA from the gene edited plants, in order to seek exemption from further regulations and to be treated in the same way as conventionally bred plants. The techniques used for demonstrating the absence may include any of the available techniques such as southern hybridization analysis, Polymerase chain reaction (PCR) analysis or sequence comparison.In general, the dossier contains information on the absence of transgene in final product, intended trait and phenotype and modification method used.

18 Do farmers have to purchase seeds of gene edited plants every year or these can be replanted ?

Whether the farmers have to purchase seeds every year or can replant, it depends on whether they are growing varieties or hybrids. As in the conventional seed production, farmers can save seeds in case of varieties and in case of hybrids they will have to purchase it every year. The same practice has to be followed in gene edited plants.

The hybrids are produced by crossing two different varieties of the same crop plant and thereby incorporate certain desirable characteristics of both plants. The reason why farmers have to purchase seeds in case of hybrid cultivation, is because only F1 (First Generation) seeds are recommended as there is a possibility of segregation of the parental traits during the F2 seeds thereby reducing the optimum productivity. This is applicable for both conventional and gene edited hybrids.

19 How can gene edited plants be detected ?

As SDN-1 and SDN-2 gene edited plants do not carry any foreign DNA, these are indistinguishable from naturally occurring or conventionally bred counterparts and it is impossible to detect in most cases with established detection tools. In case a genome sequence different from two plants is detected, it is challenging to decide whether this difference was naturally occurring, chemical induced or introduced using genome editing techniques.

SDN 3 gene edited plants can be detected using protein or DNA based detection methods in the same way as GE plants

20 How do gene editing help dealing with challenges of climate change and contribute to sustainable agriculture ?

Gene editing permits desired changes in the DNA much more efficiently as compared to the conventional tools of plant breeding, and thus facilitates developing new varieties with complex traits such as resistance to multiple abiotic stresses like drought, heat and flooding. Traits like climate resilience, resource-use efficiency and higher nutrition can be combined in a single crop variety. Therefore, an unprecedented revolution in the use of genome editing technologies in agriculture is being witnessed.

Use of gene editing and other modern biotechnologies is crucial for dealing with climate change and other challenges. Increasing crop yields is expected to help higher income for farmers and healthier products for consumers, which would contribute to achieving Sustainable Development Goals (SDGs), particularly the first three viz. 1) end poverty in all its forms, everywhere; 2) end hunger, achieve food security and improved nutrition and promote sustainable agriculture; and 3) ensure healthy lives and promote well-being for all at all ages.

Genome editing has emerged as a novel tool for crop improvement, as it enables both precise and efficient modification in a plant's genome. The Nobel prize in chemistry in 2020 has also been awarded to inventors of the CRISPR/Cas9 genome editing technology i.e., Dr. Emmanuelle Charpentier of France and Dr. Jennifer Doudna of USA. CRISPR/Cas9 and other genome editing techniques are currently being used extensively all over the world to incorporate desirable traits in different crops including cereals, pulses, oilseeds, fruits and vegetables. In some countries such products have been approved for commercial cultivation. Ttranslating the research initiatives into products requires enabling policies and understanding among key stakeholders.

This brochure provides information on frequently asked questions about gene edited plants in simple language with a view to facilitate easy understanding about this novel technology.

Prepared by

BCIL

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