In India, genome edited plants under categories SDN-1 and SDN-2 have been exempted from the provisions (Rules 7 to 11) under Rule 20 of the Rules for the Manufacture, Use/Import/Export and Storage of Hazardous Micro Organisms/Genetically Engineered Organisms or Cells, 1989 (Rules, 1989) of the Environment Protection Act, 1986, as per the Ministry of Environment, Forest and Climate Change (MoEF&CC) Office Memorandum F. No. C-12013/3/2020-CS-III, issued on March 30, 2022. Subsequently, the Department of Biotechnology (DBT) issued “Guidelines for the Safety Assessment of Genome Edited Plants, 2022” dated October 4, 2022. These SOPs are targeted to meet the threshold for exemption from the provisions of the Rules, 1989, i.e., the genome edited plant(s) must fall within SDN-1 and SDN-2 categories and must be free of exogenous introduced DNA.

The SOPs provide a step-wise guidance covering the following aspects:

- Initiating research and development of genome edited plants
- Suggested procedure for handling genome edited plants
- Seeking exemption from Rules, 1989
- Import of SDN-1 and SDN-2 genome edited plants/seeds/propagules for research, testing, and product development
- Record keeping

These SOPs are targeted to meet the threshold for exemption from the provisions of the Rules, 1989, i.e., the genome edited plant(s) must fall within SDN-1 and SDN-2 categories and must be free of exogenous introduced DNA.
A flowchart indicating the steps in the process and the associated information requirements to be submitted to regulatory authorities viz. Institutional Biosafety Committee (IBSC) and Review Committee on Genetic Manipulation (RCGM), have been provided. Protocols to show that the gene edited plants are free from exogenous introduced DNA include demonstrating the absence of selection/scorable marker and absence of vector DNA using overlapping Polymerase Chain Reaction (PCR)/nested PCR. Other methods can be used only if these have the same level of stringency and with permission from IBSC. To ensure consistency among investigators, a method of nomenclature for genome edited lines that are free from exogenous introduced DNA has also been described.

A set of formats to ensure reporting to the regulatory authorities has been included:

- Format for information and review on SDN-1 and/or SDN-2 genome edited plants to IBSC.
- Checklist for information on SDN-1 and/or SDN-2 genome edited plants to IBSCs.
- Format for seeking permission to import SDN-1 and/or SDN-2 genome edited plants for research, testing, and development purposes.
- Format for communicating confirmation of the absence of exogenous introduced DNA from SDN-1 and/or SDN-2 genome edited plants by IBSCs.

An addendum clarifying certain requirements listed in the “Guidelines for the Safety Assessment of Genome Edited Plants, 2022” but not found necessary by the expert committee for SDN-1 and SDN-2 categories of genome edited plants has also been included. These SOPs are expected to support research and development of genome edited plants in the country.

A copy of the SOPs can be accessed at: https://dbtindia.gov.in/sops-for-regulatory-review and https://ibkp.dbtindia.gov.in/Content/Rules

Genome Editing: Evolving Global Landscape
Hridi Prova Saha, Brac University

According to the Cartagena Protocol on Biosafety, “living modified organism” means any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology. Since the beginning of the use of genome editing in research and product development, a broad public discourse on the approach to be taken for genome edited organisms for commercial release has been going on. The discussion has revolved around whether a genome edited organism should be treated as genetically modified organism (GMO) or similar to an organism produced through natural or induced mutagenesis. To answer this, it is reasonable to understand the techniques used in the production of genome-edited organisms. Moreover, it is imperative for scientists, policymakers, and the public to understand genome editing in the context of traditional breeding so that an informed decision may be made, keeping in view the need or not for a pre-market safety assessment, which is a vital prerequisite for all LMOs/GMOs.
Let us first understand what happens in nature. In nature, sometimes the chromosome breaks and through an inherent repair mechanism they join back. However, while doing so, changes in the genome sequence may happen. Such changes are known as point mutations. When scientists make similar changes in an organism’s genome in a targeted manner, then it is known as genome editing. Genome editing can be achieved by a few techniques, including Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector-based Nucleases (TALEN), and the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas) system. The most well-known system in the genome editing toolbox is CRISPR/Cas9, for which Emmanuelle Charpentier and Jennifer Doudna received the Nobel Prize in 2020.

Let us take a closer look into the CRISPR system. There are two components–a guide RNA (gRNA) targeting the gene of interest and an enzyme, CRISPR associated protein also known as Cas. The gRNA contains one portion called crRNA to target the sequence and another component, tracer RNA, which helps to anchor with Cas. The Cas nuclease is directed to a specific region of the genome by the gRNA, where it creates a DNA double-strand break (DSB) upon target site recognition. Genome editing through site-directed nucleases (SDNs) encompasses different types, which include SDN-1, -2, and -3, etc. SDNs that induce small-sized, undirected alterations at the target site are called site-directed nuclease-1 (SDN-1). SDN-2 uses template-guided repair by homologous recombination to introduce a specific DNA sequence replacement in the genome, while SDN-3 inserts larger genetic elements (e.g., full genes) in a similar manner as transgenic research.

Originating from the bacterial immune system, the CRISPR/Cas9 system is a very precise and efficient tool for small base pair editing, thus introducing point mutations in desired genes. As this technique depends on precise editing of the desired gene sequence, it is necessary to ensure that the target sequence is efficiently recognized and cut accordingly. Scientists take the help of bioinformatics, thus genome sequencing the organism to identify the desired sequence for editing.

To cut the target sequence at that precise site, CRISPR nuclease plays an important role. Scientists have discovered Cas9 and Cas13, which cuts DNA and RNA, respectively, to achieve site-specific editing. Thus, the chance of off target genome editing is tackled. Still, for further confirmation, screening of the resulting product for the mutation is done. At this stage, whether editing has occurred or not, and whether it has occurred with precision or not, is checked. However, before these products are actually used, their traits (functional effect) are assessed and confirmed. Through these evaluations, finally the target point mutation products are selected for human benefit, answering all the concerns over the technology and the product.

Now, let us compare various processes of genetic/genome modification. Conventional breeding relies primarily on crossing and selecting offspring with the desired characteristics. Mutation breeding is the process of exposing seeds to mutagens to generate mutation in their genomes, producing desirable traits to be bred with other cultivars. Both of these, after selection, readily go for commercial cultivation. In transgenic organisms, the desired gene(s) is introduced from an unrelated organism through recombinant DNA technology or modern biotechnology. In contrast, genome editing is a target-specific mutation resulting in changes in the genome, including point mutations, small insertions or deletions, allele replacement, and gene insertions.

While conventional breeding products are readily accepted, there is often major hindrances to the approval of products of modern biotechnology, especially GMOs. The major issue raised against GMOs is the insertion of foreign DNA (DNA from other organisms) that may have deleterious effects on the environment and human health. The insertion of foreign genes is avoided in most types of CRISPR/Cas9 edits as through this technique, mostly the endogenous genes are edited. No foreign genes would be introduced (in SDN-1 and SDN-2).

The insertion of foreign genes is avoided in most types of CRISPR/Cas9 edits as through this technique, mostly the endogenous genes are edited. No foreign genes would be introduced (in SDN-1 and SDN-2).
natural variants where such mutations might occur naturally. Therefore, compared to GMOs, the genome edited organisms using the CRISPR/Cas9 system show genome modification, which is like naturally and conventionally bred organisms. Moreover, the CRISPR edited plants can produce transgene-free offspring, which should not have any issues for health safety, as traditionally mutated organisms are considered as safe.

CRISPR can be used for an array of benefits to agriculture, medicine, and human beings. To take advantage of the technique, before the edited lines can be introduced into breeding programs and especially, be used as a product, the country that intends to use the lines has to look into its regulatory framework. Different countries have adopted different regulation strategies based on their perception of whether they consider genome-edited organisms as equivalent to products of conventional breeding or GMOs. The USA, Canada, and four South American countries have implemented regulations that categorize genome-edited crops (SDN-1 and SDN-2) as equivalent to products of conventional breeding. Russia has also categorized transgene-free edited crops as equivalent to those generated by conventional breeding. In the African continent, so far, two countries, Nigeria and Kenya, have implemented regulations for a case-by-case review of genome-edited crops. Of these, Nigeria is the first country to adopt decision making through biosafety regulations on genome editing. If edited lines do not contain a new combination of genetic material, they can be considered as conventional varieties or products. However, the European Court of Justice categorized genome-edited plants with GMOs. The genome-edited organisms are regulated under the full provisions of Directive 2001/18/EC for the deliberate release of GMOs in 2018. Thus, in the EU, all genetically engineered organisms, including plants altered by SDNs, need to undergo an environmental, as well as food and feed risk assessment. But, this opinion is being revisited, with 20 out of 26 member states in favor of exempting genome edited plants from biosafety, as the technology has far greater precision and there is no need for marker genes. The Commission is currently working on rewriting the legal framework. The UK is also in favor of genome editing technology and is in the process of making rules regulating research on genetically engineered crops. In 2019, Japan became the first Asian country that did not distinguish between traditional breeding methods and genome editing in terms of safety. On March 30, 2022, the Indian Government signed the Office Memorandum “Exemption of the Genome Edited plants falling under the categories of SDN1 and SDN2.” The memorandum states that work with genome-edited plants must be carried out under strict safety precautions, until it can be ensured that exogenous introduced DNA is no longer present. The guidelines cover genome-edited plants produced by SDN-1 and SDN-2. If validated to be free of transgenes, they are exempt from the current GMO regulations and can be released as a new variety and used for further development and evaluation. In May 2022, the Department of Biotechnology, Government of India, released “Guidelines for the Safety Assessment of Genome Edited Plants, 2022,” which provides detailed guidance on the regulatory requirements. Notably, Bangladesh, Nepal, Sri Lanka, and Cambodia have “seeds without borders” agreements in place that will likely lead to harmonization of genome editing guidelines.

One report says that 52 percent of the world’s population lives in countries with a positive or partially positive outlook to exempt genome editing from rigorous biosafety assessment. In most of the other countries, details of the procedure for classifying genome edited organisms are still being worked out. The CRISPR/Cas gene editing tool is undoubtedly very easy to use and fast, the gene editing tool at present having an enormous potential to bring another revolution in science, especially in sustainable agriculture and food security. The most important task for the scientific community is to convey the potential advantages and precision of the tool so that its products are realized in a timely manner to ensure harvesting the benefit of the technology.

References:


The USA, Canada, and four South American countries have implemented regulations that categorize genome-edited crops (SDN-1 and SDN-2) as equivalent to products of conventional breeding. Russia has also categorized transgene-free edited crops as equivalent to those generated by conventional breeding.
Arctic Fuji Apple: A Special Apple with Prolonged Freshness

Joya Prottasha Das, Brac University

Apple is considered a very nutritious fruit as it contains high fiber and antioxidants. It has several health advantages, including a reduced risk of many chronic diseases such as diabetes, heart disease, and cancer. Because of these health benefits and its exquisite flavor, apple is a popular and common snack all over the world and hence, apple trees are grown worldwide. However, due to unappealing swelling by the browning of completely edible apples, this delicious fruit ends up as food waste. Almost 50% of the apples grown in the United States is discarded due to this browning or to unappealing swelling. Addressing this natural phenomenon of browning of apple, an agrarian biotechnology organization situated in Summerland, British Columbia, Canada named Okanagan Specialty Fruits Inc. (OSF) has genetically modified a series of apple varieties, which they named Arctic Apples. In 2016, Arctic Fuji Apple, made by modifying the Fuji apple cultivar, was OSF’s third non-browning apple, following Arctic Golden Apple and Arctic Granny Apple of the Arctic Apple series. Fuji apple was chosen for this trait improvement because of its popularity, due to its sweetness and crispiness. To keep the Fuji apple fresh and non-browning for longer, even with bruising and cutting, OSF inserted a chimeric Polyphenol Oxidase (PPO) suppression element derived from apple PPO sequences.

Scientists found that the browning of apple flesh occurs following damage, cutting, or bruising due to an enzymatic reaction catalyzed by polyphenol oxidase (PPO). In the cell, phenolic substrates and PPO are separately compartmentalized, with phenolic substrates located in...
the vacuole and PPO in plastids. When cells are damaged, this compartmentalization is lost, and browning happens due to their interaction. However, if there is little to no presence of PPO in the cell, then the cell disruption can be avoided, keeping the apple fresh. Considering this hypothesis, OSF employed the technique of reducing the activities of PPO by inserting a PPO suppression cassette. Transcription of the PPO transgene suppression construct resulted in suppression of endogenous genes, which form dsRNA and trigger an RNA interference response. As a result of the destruction of endogenous mRNAs, reduced levels of the PPO protein accumulate in the plastids, leading to decreased PPO activity in the transformed apples.

Now, to determine the level of enzymatic activity in Arctic Fuji in comparison to unmodified Fuji, the PPO levels and enzymatic browning in response to mechanical bruising of mature fruit were studied. Compositional and nutritional evaluations were also performed to determine whether Arctic Apples are equivalent to untransformed apples. According to the published data provided by the United States Department of Agriculture (USDA) on nutrient values for apples, the composition of Arctic Apples and their natural counterpart are approximately close in terms of fat, protein, moisture, ash, carbohydrates, calories, and sugar profile and nutritionally equivalent for dietary fiber. However, Arctic Apples were found to have significantly higher levels of potassium, phenolics, and vitamin C than conventional Fuji apples, though the difference for phenolics was not statistically significant.

OSF did an FDA consultation regarding Arctic Fuji Apples since accumulated evidence showed substantial equivalence, i.e., no difference from their traditional counterparts in terms of safety and nutritional characteristics. FDA evaluated OSF’s submission to determine if Arctic Fuji Apples raise any safety or regulatory issues with respect to the intended modifications or with respect to their use in human or animal food. FDA did not identify any safety or regulatory issues under the United States Federal Food, Drug and Cosmetic Act that would require further evaluation based on the information provided by the company and other information available to the agency. The USDA Animal and Plant Health Inspection Service (USDA APHIS) granted approval to Arctic Fuji on September 23, 2016, and the Canadian Food Inspection Agency and Health Canada gave approval on January 30, 2018 for food, feed, and cultivation.

USDA APHIS evaluated the plant pest risk of Arctic Fuji Apples by assessing their similarity to the deregulated OSF ancestor apple events. From the similarity assessment, they concluded that from the transformation process, the insertion or expression of new genetic material, or from changes in metabolism, there is no plant pest risk in modified apples. They also concluded that there are no plant pest effects expected on these or other agricultural products and no impacts are expected to APHIS pest control programs. From the similarity studies, Arctic Fuji Apples are unlikely to adversely impact non-target organisms and unlikely to become weedy than the antecedents, which are not weedy. Additionally, these were found not to pose any significant changes to agricultural or cultivation practices. Thus, modified Arctic Fuji Apples can be consumed as a great nutritious snack option that can be kept fresh without any further processing.

As Arctic Apple is found to be similar to the natural apple, it has successfully entered supermarkets in the USA and Canada. The improvement of apple is not restricted to the use of genetic engineering technology. Recently, researchers have used genome editing to demonstrate its application in apple to introduce beneficial characteristics, such as virus resistance, overall quality improvement, and reduced fire blight susceptibility. Gala Apple is one such success story.

Globally, food and nutritional security is a huge challenge. Food waste makes this more challenging. Improvement of food through modern biotechnology can contribute to addressing food security.

References:
### BANGLADESH

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<td>Conference on Genome Editing in Plants: Harnessing the Benefits for Bangladesh</td>
<td>Bangladesh Academy of Sciences (BAS), Bangladesh Agricultural Research Council (BARC), South Asia Biosafety Program (SABP), Agriculture &amp; Food Systems Institute (AFSI), and Biotech Consortium India Ltd. (BCIL)</td>
<td>October 18-19, 2022 Dhaka</td>
<td><a href="https://foodsystems.org/event/ge-ag-bangladesh-2022-conference/">https://foodsystems.org/event/ge-ag-bangladesh-2022-conference/</a></td>
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<td>International Conference on Advances in Biotechnology: Research, Innovations, and Entrepreneurial Venues - An Outlook</td>
<td>PG Department of Biotechnology, Dwaraka Doss Goverdhan Doss Vaishnav College</td>
<td>October 19-20, 2022 Online</td>
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<td>Hands On Training: Basics of DNA Fingerprinting</td>
<td>Tamil Nadu Agricultural University (TNAU)</td>
<td>November 14-18, 2022 Coimbatore</td>
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<td>19th Biennial International Conference on Nutritional Technologies to Augment Livestock, Poultry, Canine and Fish Production for Global Competitiveness</td>
<td>Animal Nutrition Society of India (ANSI) and Guru Angad Dev Veterinary and Animal Sciences University (GADVASU)</td>
<td>November 16-18, 2022 Ludhiana</td>
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<td>1st National Conference on Plant Genetic Resource Management (NCPGRM 2022)</td>
<td>Indian Society of Plant Genetic Resources (ISPGR), ICAR-IARI, ICAR-National Bureau of Plant Genetic Resources (NBPR), Alliance of Bioversity International, and CIAT-India Office</td>
<td>November 22-24, 2022 New Delhi</td>
<td><a href="http://www.nbpgr.ernet.in/">http://www.nbpgr.ernet.in/</a></td>
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<td>National Conference on Biotechnology for Sustainable Development and Human Welfare</td>
<td>Department of Biotechnology, School of Chemical and Life Sciences, Jamia Hamdard</td>
<td>November 23-24, 2022 New Delhi</td>
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<td>International Conference on Biotechnology, Sustainable Bioresources, and Bioeconomy</td>
<td>Indian Institute of Technology Guwahati</td>
<td>December 7-11, 2022, Guwahati</td>
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<td>International Conference on Food and Nutritional Security (iFANS-2023)</td>
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<td>January 6-9, 2023 Mohali</td>
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The South Asia Biosafety Program (SABP) is an international development program implemented in India and Bangladesh with support from the United States Agency for International Development (USAID). SABP aims to work with national governmental agencies and other public sector partners to facilitate the implementation of transparent, efficient, and responsive regulatory frameworks for products of modern biotechnology that meet national goals as regards the safety of novel foods and feeds, and environmental protection.

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<td>International Seminar and Workshop on CRISPR/Cas Based Plant Functional Genomics and Computational Modeling (ISWCPC-2023)</td>
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<td>International Conference on Pulses: Smart Crops for Agricultural Sustainability and Nutritional Security</td>
<td>Indian Society of Pulses Research and Development (ISPDR), ICAR-Indian Institute of Pulses Research (IIPR), and Indian Council of Agricultural Research (ICAR)</td>
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<td>2nd Indian Rice Congress - Transforming Rice Research: Learning from Recent Scientific Developments and Global Food Crisis</td>
<td>The Association of Rice Research Workers (ARRW) and ICAR-National Rice Research Institute (NRRI)</td>
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<td>Fifteenth Meeting of the Conference of the Parties to the Convention on Biological Diversity (Part Two) Tenth Meeting of the Conference of the Parties serving as the Meeting of the Parties to the Cartagena Protocol on Biosafety (Part Two) Fourth Meeting of the Conference of the Parties Serving as the Meeting of the Parties to the Nagoya Protocol on Access and Benefit-Sharing (Part Two)</td>
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