भारत सरकार
विज्ञान और प्रौद्योगिकी मंत्रालय
जैव प्रौद्योगिकी विभाग
GOVERNMENT OF INDIA
MINISTRY OF SCIENCE & TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

सत्यमेव जयते

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File No. PID-15011/1/2022-PPB-DBT

Date: 04.10.2022

OFFICE MEMORANDUM

Sub.: Standard Operating Procedures for Regulatory Review of Genome Edited Plants under SDN-1 and SDN-2 Categories, 2022

1. In India, all activities related to Genetically Engineered organisms (GE organisms) or cells and hazardous microorganisms and products thereof are regulated as per the "Manufacture, Use/Import/Export and Storage of Hazardous Microorganisms/ Genetically Engineered Organisms or Cells, Rules, 1989" (Rules, 1989) notified by the Ministry of Environment, Forest and Climate Change (MoEF&CC), Government of India under the Environment (Protection) Act, 1986 (EPA 1986).

2. MoEF&CC vide Office Memorandum (F. No. C-12013/3/2020-CS-III) dated 30.03.2022 Notified Exemption of the genome edited plants falling under thecategories of SDN-1 and SDN-2 from the provisions of the rules 7 -11 (both inclusive) under the Rule 20 of the Rules, 1989 of the EPA, 1986. Subsequently, Department of Biotechnology (DBT), Ministry of Science and Technology notified the 'Guidelines for the Safety Assessment of Genome Edited Plants, 2022' on 17.05.2022 for research and development of genome edited plants in India. Pursuant to the guidelines, drafting of Standard Operating Procedures (SOPs) was initiated by the Department of Biotechnology under the power conferred to Review Committee on Genetic Manipulation (RCGM) through the Rules, 1989 of the EPA, 1986 to provide guidanceto stakeholders and Institutional Biosafety Committee (IBSCs) to carry out research and development under containment.

3. The "Standard Operating Procedures for Regulatory Review of Genome Edited Plants under SDN-1 and SDN-2 Categories, 2022" have been prepared after extensive deliberations by the expert committee constituted for this purpose. RCGM in its 240th meeting, held on 07.09.2022, approved and recommended to notify the same.

4. The Department of Biotechnology hereby notifies the "Standard Operating Procedures for Regulatory Review of Genome Edited Plants under SDN-1 and SDN-2 Categories, 2022".

5. The SOPs provide regulatory road map and requirements for research, development and to meet the threshold for exemption of genome edited plant(s) under the categories of SDN-1 or SDN-2 as per MoEF&CC O.M. No C-12013/3/2020-CS-III) dated 30.03.2022.

6. These SOPs shall be applicable for all organizations involved in research, development and handling of the genome edited plants under SDN-1 and SDN-2 categories from the date of notification.

7. The "Standard Operating Procedures for Regulatory Review of Genome Edited Plants under SDN-1 and SDN-2 Categories, 2022" can be accessed from www.dbtindia.nic.in and https://ibkp.dbtindia.gov.in/.

(Nitin Kumar Jain)
Member Secretary, RCGM
Scientist F, DBT

To,
All IBSCs

DEPARTMENT OF BIOTECHNOLOGY
Ministry of Science and Technology
Government of India

सत्यमेव जयते

Standard Operating Procedures for Regulatory Review of Genome Edited Plants under SDN-1 and SDN-2 Categories

2022

Standard Operating Procedures for Regulatory Review of Genome Edited Plants under SDN-1 and SDN-2 Categories

Department of Biotechnology
Ministry of Science & Technology,
Government of India

2022

Contents

ABBREVIATIONS

CRISPR	-	Clustered Regularly Interspaced Short Palindromic Repeats
DNA	-	Deoxyribonucleic Acid
DBT	-	Department of Biotechnology
GE	-	Genetic Engineering
EPA	-	Environment (Protection) Act
IBSC	-	Institutional Biosafety Committee
IBKP	-	Indian Biosafety Knowledge Portal
MoEF&CC	-	Ministry of Environment, Forest and Climate Change
NBPGR	-	National Bureau of Plant Genetic Resources
RCGM	-	Review Committee on Genetic Manipulation
R&D	-	Research and Development
RNP	-	Ribonucleoprotein
RULES, 1989	-	Rules for the manufacture, use, import, export & storage of hazardous microorganisms/genetically engineered organisms or cells, 1989
SDN	-	Site Directed Nuclease
SOPs	-	Standard Operating Procedures
TALEN	-	Transcription activator-like effector nucleases
ZFN	-	Zinc-Finger Nucleases

1. Preamble

Ministry of Environment, Forest and Climate Change (MoEF&CC) has issued an Office Memorandum (O.M.) regarding the exemption of specified categories of genome edited plants from the provisions of Rules 7 to 11 (both inclusive) of the "Rules for the manufacture, use, import, export & storage of hazardous microorganisms/genetically engineered organisms or cells,1989"[1] (commonly referred as Rules, 1989) of the Environment (Protection) Act, 1986. This exemption applies to site-directed nuclease (SDN)-1 and SDN-2 categories of genome edited plants, which are free from exogenous introduced DNA. Copy of the O.M. F. No. C -12013/3/2020-CS-III dated 30th March 2022 is placed as Annexure-I.

The O.M. indicates that,

- *The development of genome edited plants will be carried out under containment, until free from exogenous introduced DNA. This process is to be regulated by Institutional Biosafety Committees (IBSCs) following guidelines issued by the Central Government.*
- *For such genome edited plants to be released as a new variety, further development and evaluation will be as per other applicable Laws/Acts/Rules.*

Pursuant to the O.M., Department of Biotechnology (DBT), Ministry of Science and Technology notified the '*Guidelines for the Safety Assessment of Genome Edited Plants, 2022*'[2] on 17th May 2022 for research and development of genome edited plants in India.

The guidelines provide overarching guidance for all categories of genome edited plants viz., SDN-1, SDN-2 and SDN-3. The guidelines provide pointers for regulatory pathways and information/data requirements for the safety assessment of genome edited plants.

[1] Available at https://geacindia.gov.in/resource-documents/biosafety-regulations/acts-and-rules/Rules-for-the-manufacture-use-import-export-and-storage-1989.pdf

[2] Available at https://dbtindia.gov.in/sites/default/files/Final_%2011052022_Annexure-I%2C%20Genome_Edited_Plants_2022_Hyperlink.pdf

The "Standard Operating Procedures (SOPs) for regulatory review of genome edited plants under SDN-1 and SDN-2 categories" have been prepared by the expert committee, constituted by DBT for finalization of the SOPs and checklist on Genome Edited Plants, to provide guidance to applicants and IBSCs for activities under containment and until free from exogenous introduced DNA. The data requirements in the SOPs are targeted to meet the threshold for exemption, which is that the genome edited plant(s) must fall within the categories of SDN-1 or SDN-2 and must be free of exogenous introduced DNA.

The expert committee for the preparation of SOPs decided that certain requirements from the guidelines were not necessary for meeting the threshold for the exemption as per the MoEF&CC O.M. - F. No. C -12013/3/2020-CS-III dated 30th March 2022. Details are provided in the **Addendum**.

The definition of SDN-1 and SDN-2 genome edited plants are provided in the box below.

Definition of SDN-1 and SDN-2 genome edited plants[3]

SDN-1 *Involves the unguided repair of a targeted DNA break by the natural endogenous DNA repair mechanism of the host organism such as non-homologous end joining. The spontaneous repair of this break can lead to a mutation causing gene silencing, gene knock-out, or a change in the activity of a gene. The SDN-1 genome edited plants produced will be free from exogenous/foreign DNA. These mutations can be base substitution/indels/deletions including large deletions or structural changes. These resultant mutations are comparable to those occurring in nature, obtained through conventional mutagenic treatments or natural variation found in the primary/secondary gene pool.*

SDN-2 *Involves a template-guided repair of a targeted DNA break using an externally supplied template sequence. The donor carries one or several small mutations flanked by two sequences matching both ends of the DNA break, and is thus recognized as a repair template, allowing the introduction of the mutation(s) at the target site. The resultant mutant carries a modified sequence, leading to an altered expression profile of the gene and/or altered activity of the encoded protein/RNA. Thus, the edited version could be regarded as an allelic form comparable to those available in the primary/secondary gene pool.*

[3] Source: *Guidelines of the Safety Assessment of Genome Edited Plants, 2022*

2. Objective

To facilitate regulatory review for research and development of genome edited plants falling under the categories of SDN-1 and/or SDN-2 until free from exogenous introduced DNA.

These SOPs are applicable only for research and development under contained conditions.

3. SOPs for regulation of genome editing in plants under SDN-1 and SDN-2 categories

3.1 Initiating research and development of genome edited plants

3.1.1 Research and development on genome edited plants must be conducted with authorization from IBSCs and subsequent information to RCGM in form D1.

3.1.2 The research and development of genome edited plants is to be conducted under containment as per "*Regulations & Guidelines for Recombinant DNA Research and Biocontainment, 2017*"[4].

3.1.3 Applicants and IBSCs shall also refer to the "*Handbook for Institutional Biosafety Committees (IBSCs)*"[5] by DBT which *is* updated periodically.

3.1.4 The IBSC shall consist of members as per the "*Handbook for IBSCs".* IBSCs may co-opt one or more additional experienced subject matter expert(s) on plant genome editing or related discipline.

3.1.5 The applicant shall fill Form D1 and submit to the IBSC.

3.1.6 The IBSC shall review and recommend the D1 application as per existing guidelines and forward it to RCGM for information through Indian Biosafety Knowledge Portal (IBKP) along with minutes of the IBSC meeting.

[4] Available at https://ibkp.dbtindia.gov.in/Content/Rules

[5]Third Revised Edition, September 2020' (https://ibkp.dbtindia.gov.in/Content/Rules)

3.1.7 RCGM shall communicate its observations to the applicant/IBSC.

3.2 Suggested procedure for handling genome edited plants

3.2.1 Progenies of individual T0 events should be maintained separately. Selfed seeds of individual T1 plants should be raised in a single plant to progeny row.

3.2.2 Investigators are recommended to provide the following information to the IBSC, if available:

i. Numbers of T0 transformants generated.

ii. The generation in which mutation in the target site was detected. Whether in that generation the mutation was in homozygous or heterozygous state?

iii. Whether the mutation will be maintained in heterozygous or homozygous condition?

iv. If homozygosity was attained, at which generation did this occur?

v. Generation at which genome edited lines were found to be free of exogenous introduced DNA?

3.2.3 The IBSC to regularly monitor the activities as per the Handbook for IBSCs and extant guidelines.

3.3 Seeking exemption from Rules, 1989

3.3.1 Once investigators have developed genome edited plant(s) free from exogenous introduced DNA and intend to take SDN-1 and/or SDN2 plants out of containment conditions, they shall submit data in the prescribed format to IBSCs (Annexure-II) and the Checklist (Annexure-III) for seeking exemption from the provisions of Rules 7 to 11 (both inclusive) of the Rules, 1989.

3.3.2 IBSC to review data for the following:

i. Category(ies) of genome editing (SDN-1 and/or SDN-2).

ii. Confirmation of genome editing at target locus/loci using DNA sequencing.

iii. Whether selfing and/or backcrossing has been carried out to segregate the exogenous introduced DNA?

iv. Whether any DNA-free method such as RNA-protein complex was used for genome editing?

v. Evidence to confirm that the genome edited plant is free from exogenous introduced DNA.

vi. Depending on the type of modification and trait expression under containment conditions, phenotypic data to be provided.

vii. In case, any unintended phenotypic changes were observed on the genome edited plant whether it was selected/ segregated out.

viii. In the case of nutrition-related traits for food and feed, data on the targeted nutritional trait to be provided in comparison to the parental line.

ix. Whether information on identical allele(s) is already documented? If yes, information to be provided.

3.3.3 Applicant shall use protocols/methods prescribed in section 5 of this document to show that the genome edited plants are free from exogenous introduced DNA.

3.3.4 After the information provided in Annexure-II and as per the checklist in Annexure-III is examined and found to be satisfactory, the IBSC shall submit the minutes of the meeting (along with Annexure-II and Annexure-III) through IBKP to RCGM for information. Following this, the IBSC shall convey the decision to the applicant confirming the exemption in the format placed at Annexure-V.

3.3.5 In case methods other than specified in the SOPs have been used to demonstrate the absence of exogenous introduced DNA, the same may be verified and highlighted in the minutes of the IBSC meeting.

3.3.6 Further development, evaluation, and release of a genome edited plant as a new variety/hybrid shall be governed as per other applicable Laws/Acts/Rules.

3.3.7 In cases, where the genome edited plant does not fall under the categories of SDN-1 and SDN-2 and/or is not free from exogenous introduced DNA, it would be subjected to the existing regulation as per the Rules 1989 and other applicable guidelines.

Submission of minutes by IBSC to RCGM

- The IBSC will submit minutes of the meeting along with Annexure II and Annexure III on IBKP for RCGM information.
- The minutes must include:
  - *Scientific considerations based on which the proposal by the applicant for the absence of exogenous introduced DNA has been approved.*
  - *In case methods other than specified in the SOPs have been used to demonstrate the absence of exogenous introduced DNA, the same may be verified and highlighted in the minutes of the IBSC meeting.*
  - *Confirmation that the IBSC has at least one experienced subject matter expert on plant genome editing or related disciplines.*
  - *Duly signed attendance sheet of the IBSC members.*
- Once the minutes of IBSC meeting (along with Annexure II & Annexure III) are accepted for completeness pertaining to documentation on IBKP, the IBSC shall communicate the decision to the applicant confirming the exemption in the format placed at Annexure-V.

3.4 Import of SDN-1 and SDN-2 genome edited plants/seeds/ propagules for research, testing, and product development

3.4.1 The applicant who wishes to import SDN-1 and SDN-2 genome edited plants/seeds/propagules, which are free from exogenous introduced DNA, would apply to the IBSC in the prescribed format at Annexure IV.

3.4.2 The application shall include the status of use in the country of origin.

3.4.3 In case methods other than specified in the SOPs have been used to demonstrate the absence of exogenous introduced DNA, the same may be highlighted in the minutes of the IBSC meeting.

3.4.4 Upon review, the IBSC shall submit the minutes of the meeting along with

Annexure – IV to RCGM. Upon receiving RCGM acceptance for IBSC minutes, a letter will be issued to the applicant (importer) by the IBSC confirming that the subject material is exempted from regulation under the Rules, 1989 in the format placed at Annexure-V.

3.4.5 Applicant to submit the Plant Quarantine application to National Bureau of Plant Genetic Resources (NBPGR), New Delhi for the import of germplasm/research breeding materials (PQ Form-08). Acceptance of IBSC minutes of meeting by RCGM should be appended to the Plant Quarantine import permit application.

3.4.6 If the SDN-1 and/or SDN-2 genome edited plants are not free of exogenous introduced DNA, their import for research purposes will be governed by procedures that are applicable to genetically engineered/ transgenic material.

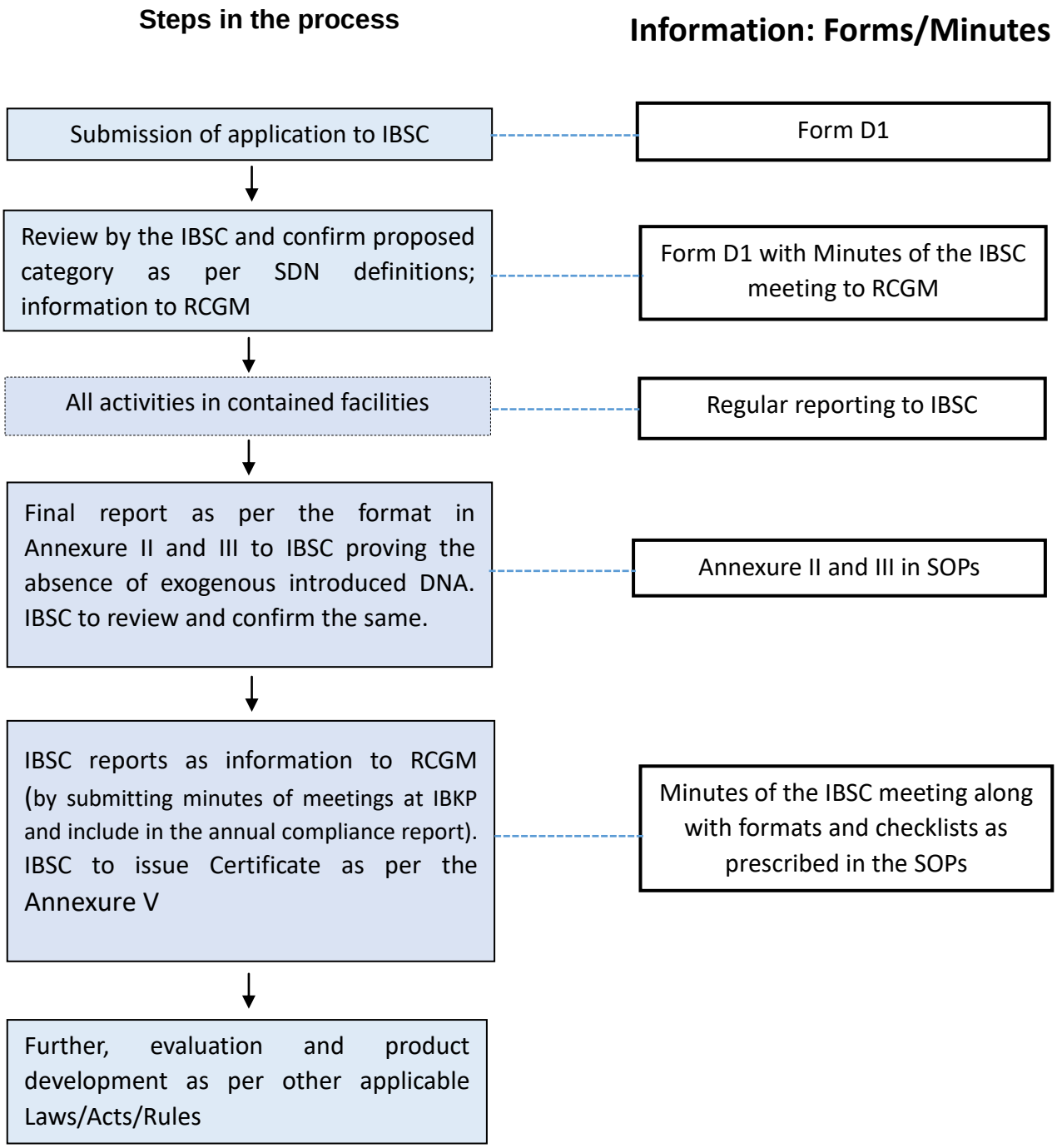
3.4.7 These SOPs are applicable for the import of genome edited plants/ seeds/propagules and other products for research, testing, and product development only, and not applicable for trade/commerce.

3.5 Record keeping

3.5.1 All records associated with research and development and granting exemption from Rules 7-11 (both inclusive) of the Rules, 1989 must be maintained by the IBSCs and made available to RCGM or designated regulatory authorities upon request.

3.5.2 The IBSC shall archive copies of the records for all applications submitted for exemptions for a minimum of ten (10) years, whether or not the regulated material is granted the exemption.

4. Flow chart for seeking exemption for SDN-1 and SDN-2 genome edited plants



5. Protocols to show that the genome edited plants are free from exogenous introduced DNA

The two steps described below are the recommended protocol to show that the genome edited plants of SDN-1 and/or SDN-2 categories are free from exogenous introduced DNA.

5.1 Absence of selection/scorable marker

The final edited plant lines must be sensitive to the selection reagents (antibiotics/herbicide/ any other) at the concentration used for selecting the plants having exogenous introduced DNA. In the case of a scorable marker, the final edited plant line should be phenotypically negative for the same. Seeds of a segregant line harboring the exogenous introduced DNA should be used as the positive control, and the parental genotype used for genome editing as the negative control.

5.2 Overlapping PCR/ nested PCR

i. Total genomic DNA is to be used as the template for PCR amplification.

ii. The primers must be chosen such that the amplicons cover the full length of the exogenous introduced DNA (full length of the vector DNA).

iii. The intended amplicons should be at the maximum of 500 bp in size and the overlap between consecutive amplicons should be at least 50 bp.

iv. For each amplification reaction, three PCR positive controls must be used:

- **a.** A well-known endogenous low copy gene (actin/tubulin, etc.) of the same species using the same amount of the same genomic DNA sample of the final edited lines as mentioned above.
- **b.** The genomic DNA of a segregant line harboring full or part of the exogenous introduced DNA (using the same primer pairs and the same amount of genomic DNA used for overlapping PCR).

c. Genomic DNA of the parental genotype spiked with at the most 1/1000th (*w*/*w*) of the full-length purified vector DNA used to develop the genome edited line (using the same primer set used for overlapping PCR).

v. The amount of template DNA and the PCR conditions should be such that a clear band of the expected size is visible in all the positive controls.

vi. No amplification should be detected in any of the reactions with primers directed against exogenous introduced DNA for the final genome edited line, while clear amplification should be detected in all the positive controls by ethidium bromide-stained agarose gel electrophoresis.

5.3 Use of other methods

Evidence of the absence of exogenous introduced DNA with alternative methodologies/ technologies may be considered provided IBSC is convinced that it is at the same level of stringency as the method described above.

6. Nomenclature for the genome edited lines that are free from exogenous introduced DNA

- Information for genome edited lines that are free from exogenous introduced DNA must include a unique number for each line
- To ensure consistency, all investigators shall use the following system for nomenclature
    - The first two letters of the genus followed by first two letters of the species/variety name or accession number; UAC number of D1 form; event number.
    - Examples are given below:

i. For a mutation of chickpea (*Cicer arietinum*) accession no. ICC4958, UAC no. of D1 form INDTONAA3235, event no. 1: The name will be: Ciar/ICC4958/ INDTONAA3235/1

ii. For Rice variety IR64 mutation, UAC no. of D1 form INDTONAA3235, event no. 2: The name will be: Orsa/IR64/ INDTONAA3235/2

7. Review of the SOPs

7.1 These SOPs will be reviewed periodically by RCGM/DBT in line with technological advancements, particularly the methods to be used to show that the genome edited plants are free from exogenous introduced DNA.

7.2 The revised SOPs will be posted on the DBT website for Compliance.

Annexure-I

MoEF&CC Office Memorandum F. No. C -12013/3/2020-CS-III dated 30.03.2022 regarding Exemption of the genome edited plants falling under the categories of SDN-1 and SDN-2 from the provisions of the Rules, 1989

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.

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.

30/3/2022

(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

Annexure-II

Format for information and Review on SDN-1 and/or SDN-2 genome edited plants to IBSC

A: Details about the investigator(s) and the institution		
Name of the Principal Investigator		
Details of the IBSC/ RCGM approval (UAC No. of the Form D1)		
Name of the Institution		
Name of the Chairperson and Member Secretary, IBSC		
Partners/collaborators details		
B: Details about the genome edited plant(s)		
S. No.	**Item**	**Information**
1.	Name of the plant species and genotype	
2.	Targeted trait(s)	
3.	Name of the genome edited line(s) as indicated in Section 6 of the SOPs	
4.	Category of genome editing: SDN-1 and/or SDN-2	
5.	Targeted genomic region 5a. Name of the gene(s) or locus/loci 5b. Specific region of the gene or locus (promoter, terminator, other regulatory elements, coding region, intron, etc.) 5c. Nucleotide sequence of the parental allele 5d. Specific site(s) chosen for editing (in case of SDN-2)	
6.	Genome editing method used including CRISPR-Cas, TALEN, ZFN, RNPs, Base editing, prime editing, etc.	
7.	Details of vector, gene construct, editing reagents (molecular tools) including maps and nucleotide sequences	
8.	The method used for delivery of gene editing reagents (Agrobacterium-mediated, Biolistic, etc.)	

9.	Current Generation of the genome edited plant(s) (T0, T1, T2, etc.)	
10.	Data on phenotypic expression of the target trait(s) as assessed under containment conditions, if applicable	
11.	Molecular data for confirmation of the targeted editing 11a. By sequencing of the parental and the modified allele(s) using Sanger or other Sequencing technologies with a minimum 10X coverage of the edited region(s) and -base quality of minimum Phred score 30 11b. Sequence difference between parental and modified/edited allele through sequence alignment	
12.	Whether the mutation is homozygous or heterozygous. Provide evidence for inheritance of the mutation through two generations using sequencing.	
13.	Whether selfing and/or backcrossing has been carried out to segregate the exogenous introduced DNA? Provide information.	
14.	Whether any DNA-free method such as RNA-protein complex was used for genome editing? If yes, provide information.	
15.	Provide evidence to confirm the absence of exogenous introduced DNA in genome edited plant by phenotypic selection (sensitivity to herbicide/ antibiotic, or absence of scorable marker) and using overlapping PCR/ Nested PCR/other appropriate methodology (as described in Section 5).	
16.	In case, any unintended phenotypic changes were observed on the genome edited plant whether it was selected/ segregated out. If selected out, provide information.	
17.	In the case of nutrition-related traits, provide data on the targeted nutritional trait in comparison to the parental line.	

Annexure-III

Checklist for information on SDN-1 and/or SDN-2 genome edited plants to IBSCs

S. No.	Item	Information provided	Remarks, if any
1.	Name of the plant species and genotype	Yes/No	
2.	Targeted trait(s)	Yes/No	
3.	Name of the Genome edited line(s) as indicated in Section 6 of the SOPs	Yes/No	
4.	Category of genome editing: SDN-1 and/or SDN-2	Yes/No	
5.	Targeted genomic region 5a. Name of the gene(s) or locus/loci 5b. specific region of the gene or locus (promoter, terminator, other regulatory elements, coding region, intron, etc.) 5c. Nucleotide sequence of the parental allele 5d. Specific site(s) chosen for editing (in case of SDN-2)	Yes/No	
6.	Genome editing method(s) used (CRISPR- Cas, TALEN, ZFN, RNPs, etc.)	Yes/No	
7.	Details of vector, gene construct, editing reagents (molecular tools) including maps and nucleotide sequences	Yes/No	
8.	The method used for delivery of gene editing reagents (Agrobacterium-mediated, Biolistic, etc.)	Yes/No	
9.	Current Generation of the genome edited plant(s) (T0, T1, T2, etc.)	Yes/No	
10.	Data on Phenotypic expression of the target trait(s) as assessed under containment conditions, if applicable	Yes/No	
11.	Molecular data for confirmation of the targeted editing 11a. By sequencing of the parental and the modified allele(s) using Sanger or other Sequencing technologies with a minimum 10Xcoverage of the edited region(s) and base quality of minimum Phred score 30	Yes/No	

	11b. Sequence difference between parental and modified/edited allele through sequence alignment		
12.	Whether the mutation is homozygous or heterozygous. Provide evidence for inheritance of the mutation through two generations using sequencing.	Yes/No	
13.	Whether selfing and/or backcrossing has been carried out to segregate the exogenous introduced DNA.	Yes/No	
14.	Whether any DNA free method such as RNA-protein complex was used for genome editing.	Yes/No	
15.	Whether the evidence is provided to confirm the absence of exogenous introduced DNA in genome edited plant by phenotypic selection (sensitivity to herbicide/ antibiotic, or absence of scorable marker) and using overlapping PCR/ Nested PCR/ other appropriate methodology (as described in Section 5).	Yes/No	
16.	In case, any unintended phenotypic changes were observed on the genome edited plant whether it was selected/ segregated out. If selected out, whether the information is provided.	Yes/No	
17.	In the case of nutrition-related traits, whether data on the targeted nutritional trait is provided in comparison to the parental line	Yes/No	

Annexure-IV

Format for seeking permission to import SDN-1 and/or SDN-2 genome edited plants for research, testing, and development purposes

A: Details about the Importer		
Applicant information: • Name and position • Organization • Contact details • Collaborators, if any		
Source of import • Name and position • Organization • Contact details		
Quantities to be imported		
Status of use in the country of origin and export • Research • Commercial use • "Not regulated as GMO" or equivalent status under biosafety rules (please attach relevant documents)		
Status of use in countries other than the country of origin and export		
Name of the Chairperson, IBSC of the importing organization		
B: Details about the genome edited plant(s)		
S. No.	**Item**	**Information**
1.	Name of the plant species and genotype	
2.	Targeted trait(s)	
3.	Name of the genome edited line(s) • In the country of origin • as indicated in Section 6 of the SOPs	
4.	Targeted genomic region 4a. Name of the gene(s) or locus/loci 4b. specific region of the gene or locus (promoter, terminator, other regulatory elements, coding region, intron, etc.) 4c. Nucleotide sequence of the parental allele 4d. Specific site(s) chosen for editing (in case of SDN-2)	
5.	Category of genome editing: SDN-1 and/or SDN-2	
6.	Genome editing method used including CRISPR- Cas, TALEN, ZFN, RNPs, Base editing, prime editing, etc.	
7.	Details of vector, gene construct, editing reagents	

	(molecular tools) including maps and nucleotide sequences	
8.	The method used for delivery of gene editing reagents (Agrobacterium-mediated, Biolistic, etc.)	
9.	Current Generation of the genome edited plant(s) (T0, T1, T2, etc.)	
10.	Data on Phenotypic expression of the target trait(s) as assessed under containment conditions, if applicable	
11.	Molecular data for confirmation of the targeted editing a. By sequencing of the parental and the modified allele(s) using Sanger or other Sequencing technologies with a minimum 10X coverage of the edited region(s) and base quality of a minimum Phred score 30 b. Sequence difference between parental and modified/edited allele through sequence alignment	
12.	Whether the mutation is homozygous or heterozygous. Provide evidence for inheritance of the mutation through two generations using sequencing.	
13.	Whether selfing and/or backcrossing has been carried out to segregate the exogenous introduced DNA? Provide information.	
14.	Whether any DNA-free method such as RNA-protein complex was used for genome editing? If yes, provide information.	
15.	Provide evidence to confirm the absence of exogenous introduced DNA in genome edited plant by phenotypic selection (sensitivity to herbicide/ antibiotic, or absence of scorable marker) and using overlapping PCR/ Nested PCR/other appropriate methodology (as per Section 5).	
16.	In case, any unintended phenotypic changes were observed on the genome edited plant whether it was selected/ segregated out. If selected, provide information.	
17.	In the case of nutrition-related traits, provide data on the targeted nutritional trait in comparison to the parental line.	

Annexure-V

Format for communicating confirmation of the absence of exogenous introduced DNA from SDN-1 and/or SDN-2 Genome Edited plants by IBSCs

To

The Applicant
Name of the Institute (Affiliation)
Address

The IBSC in its meeting held on ___ has reviewed the application titled _____ for genome edited plant having unique identification number(s)_____under the category of SDN-1/SDN-2 for seeking exemption from Rules, 1989 based on the Office Memorandum F. No. C/12013/3/2020/CS-III dated 30.03.2022 issued by Ministry of Environment, Forest and Climate Change, Government of India.

The details of the genome edited plant(s) are as follows:

Name of genome edited line(s):

Name of the genus and species:

SDN-1 or SDN-2:

Name/ID of Edited locus:

Trait(s), if applicable:

After a detailed examination of data and methods as specified in the "*Standard Operating Procedures for Regulatory Review of Genome Edited Plants under SDN-1 and SDN-2 categories*", the IBSC confirms the absence of exogenous introduced DNA in the above-mentioned genome edited line(s).

Further development and evaluation of these line(s) are exempted from the Rules, 1989, and will be carried out as per other applicable Laws/Acts/Rules.

Signature of the Chair, IBSC

Copy for information to:-

1. *Member Secretary, RCGM* (rcgm.dbt@nic.in)
2. *Member Secretary, GEAC*
3. *Food Safety and Standards Authority of India (FSSAI)*
4. *Dept. of Animal Husbandry & Dairying*
5. *Department of Agriculture Research and Education, MoA&FW*

GLOSSARY

Backcrossing	Crossing of an F1 individual with one of its parents or with the genetically equivalent organism. The offspring of such a cross are referred to as the backcross generation or backcross progeny.
Base editing	A genome editing method to convert one or more target base(s) into another base(s) using site directed nucleases and deaminases without requiring the creation and repair of double stranded break(s). Since it is an unguided repair of the target locus, base editing can be classified under the SDN-1 category.
Containment	Safe methods (Combination of facilities, practices, and procedures) for managing hazardous microorganisms, genetically engineered organisms or cells in the laboratory environment where they are being handled or maintained.
CRISPR	Clusters of regularly interspaced short palindromic repeats, a component of a bacterial immunity used to recognise and protect against viruses.
Exogenous introduced DNA	DNA introduced into cell/tissue for genome editing including entire plasmid vector construct sequences. For the purposes of this document, exogenous introduced DNA includes the entire coding sequences for genome editing reagents, selectable markers, intact gene regulatory elements (e.g., promoters, transcriptional activators or modifiers, termination signals), and vector backbone. In SDN-2 genome edited plants, the sequences integrated at the ectopic locus to serve as homologous DNA template are considered as exogenous DNA, whereas any changes introduced at the target locus through genome editing would not come under the definition of exogenous introduced DNA.
Genome Editing	Genome editing refers to a technique for targeted alterations that modify, insert, replace, or delete DNA sequences from a genome.
Gene pool	The entire set of genes and alleles of a defined population of organisms at a given time.
Germplasm	An individual, group of individuals, or a clone representing a genotype, variety, species or culture, held in an *in situ* or *ex situ* collection.
Locus	A site on a chromosome.
Non Homologous End Joining (NHEJ)	A means for repair of DNA double-strand breaks (DSBs) without the use of a homologous repair sequence. An error-prone process that often causes small substitutions, insertions, and/or deletions

	at the DSB site resulting in mutations.
Off-target mutations	Mutations in the genome made by programmable nuclease(s) at sites different from the target site in the genome.
Phenotype	The visible and/ or measurable characteristics of an individual (with respect to one or more traits) which reflects the reaction of a given genotype in a given environment.
Prime editing	A genome editing method for targeted changes in the DNA sequence such as small insertions, deletions, and base swapping in a precise way using RNA repair template without requiring the creation and repair of DSBs. In prime editing, a RNA template is used to guide the repair of the target locus through activity of a reverse transcriptase. The prime editing is classified under SDN-2 category.
Rules, 1989	The rules for the manufacture, use/import/export and storage of hazardous microorganisms/ genetically engineered organisms or cells, 1989 notified under the Environment (Protection) Act, 1986.
Segregation	For genes, the separation of allele pairs from one another and their resulting assortment into different cells at meiosis. For chromosomes, the separation and re-assortment of the two homologues in anaphase of the first meiotic division. For individuals, the occurrence of different genotypes and/or phenotypes among offspring, resulting from chromosome or allele separation in their heterozygous parents.
Selfing	The process of fertilization of egg cells with pollen from the same plant.
Site Directed Nuclease (SDN)	Engineered DNA nucleases that are programmed to specific sites within the genome of an organism where they break a DNA chain by separating nucleotides.
Unintended effect	An effect that is considered to be a consistent difference between the genome edited plant and its parental line, which goes beyond the primary intended effect(s) of the genome editing.

Sources: *The definitions of key terms, as in the glossary, have been derived from national rules and guidelines, as well as documents by international agencies such as Food and Agriculture Organization (FAO)*

Addendum

Applicability of information and data requirements, as listed in the "Guidelines for the safety assessment of genome edited plants, 2022" by DBT for SDN-1 and SDN-2 categories of genome edited plants

1. Subsequent to the MoEF&CC Office Memorandum F. No. C-12013/3/2020-CS-III of 30th March 2022 regarding exemption of the genome edited plants falling in the categories of SDN-1 and SDN-2 from the provisions of Rules, 1989, Department of Biotechnology has issued "Guidelines for the Safety Assessment of Genome Edited Plants, 2022" (hereinafter DBT guidelines).
2. The guidelines have been prepared to provide overarching guidance for all categories of genome edited plants viz. SDN-1, SDN-2, and SDN-3. The guidelines provide for regulatory pathways and information/data requirements for the safety assessment of genome edited plants.
3. As indicated in the OM, SDN-1 and SDN-2 genome edited plants free from exogenous introduced DNA, are exempted from the provisions of Rules 7 to 11 (both inclusive) of the "Rules for the manufacture, use, import, export & storage of hazardous microorganisms/genetically engineered organisms or cells,1989 (commonly referred as Rules, 1989) of the Environment (Protection) Act, 1986.
4. In line with the above, the committee for the preparation of SOPs has reviewed the data requirements and prepared SOPs for seeking exemption of the SDN-1 and SDN-2 plants. The data requirements in the SOPs are targeted to meet the threshold for exemption, which is that the genome edited plant(s) must fall within the categories of SDN-1 or SDN-2 and must be free of exogenous introduced DNA.
5. All issues related to potential undesirable phenotypes or inferior agronomic performance will be addressed through the normal varietal evaluation and selection processes for products of traditional breeding, including induced mutation breeding.
6. Data requirements that are not applicable to demonstrate the absence of exogenous introduced DNA are listed below.

S. No.	**Data requirement in *Guidelines of the Safety Assessment of Genome Edited Plants, 2022*** **(Section 4.B; Page 14-17)**	**Applicability to demonstrate the absence of exogenous introduced DNA**
a.	Existing trait value range in the species being targeted for change	The researchers/developers would be considering the trait value range in the species as part of the varietal development process.
b.	Un-targeted alterations, edited	The number of untargeted alterations (generally

	loci and its direct or indirect effects	referred to as off-target mutations) generated in genome editing process would be very few as compared with that of conventional mutagenesis procedures. If any of the off-target mutations would be affecting the plant phenotype, the developer would segregate and remove phenotypically odd plants that might arise during the genome editing process. Further, the mutant lines developed will also be evaluated in field trials and if any undesirable trait is identified, they will be discarded at that stage. Hence, identification of off-target mutations is not required.
c.	Comparative molecular expression profile of target gene and/or product before and after editing.	The SOPs require the provision of detailed information regarding the mutation that has occurred. This information is considered to be sufficient and would be in-line with the procedures followed during the varietal development process and mutagenesis procedures. Therefore, the molecular expression profiling of the target gene is considered to be not required.
d.	Methods followed to identify and/or remove off-target changes. Backcross breeding for sufficient number of generations to remove any possible off-target changes in case of genome edited plants. In case of perennials or plants that reproduce mainly through vegetative propagation, additional molecular data may be required on a case-by-case basis. In case of any off-target changes, information to show that they are not different from any variant for that off-target trait found in the species or among segregating progenies in conventional breeding materials.	The number of off-target mutations generated in genome editing process would be very few as compared with that of conventional mutagenesis procedures. If any of the off-target mutations would be affecting the plant phenotype, a developer would segregate and remove phenotypically odd plants that might arise during the genome editing process. Further, the mutant lines developed will also be evaluated in field trials and if any undesirable trait is identified, they will be discarded at that stage. Hence, backcrossing to remove off-target mutations is not required.
e.	When the intended change is the	The SOPs indicate that in the case of nutrition-

	introduction of novel food/feed trait by altering the composition in the edible parts beyond the existing normal range present in the food/feed crop and has no history of safe use, applications may be referred to Food Safety and Standards Authority of India (FSSAI) if meant for human consumption or Department Of Animal Husbandry, Dairying and Fisheries (DoAHDF) if meant for animal consumption for their approval.	related traits, data on the targeted nutritional trait needs to be provided in comparison to the parental line. The SDN-1 and/or SDN-2 genome edited plants free from exogenous introduced DNA would be dealt with in the same way as it would in the case of a product of conventional mutation breeding.

ACKNOWLEDGEMENTS

- ✓ Review Committee of Genetic Manipulation (RCGM)

- ✓ Expert Committee under the Chairmanship of Prof. Ramesh V. Sonti, Professor & Chair, Biology and Dean Faculty, IISER, Tirupati; constituted by DBT for finalization of the SOPs and checklist on Genome Edited Plants. Committee members include:
  - Dr. A.K. Singh, Director, ICAR-IARI, New Delhi
  - Prof. K.C. Bansal, Former Director, ICAR-NBPGR, New Delhi & Secretary, National Academy of Agricultural Sciences, New Delhi
  - Dr. Pradyumna Kumar Singh, CSIR-NBRI, Lucknow
  - Dr. Swarup K. Parida, NIPGR, New Delhi
  - Dr. Debasis Chattopadhyay, NIPGR, New Delhi
  - Dr. A.K. Pradhan, Professor, University of Delhi South Campus, New Delhi
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  - Dr. Lalitha R. Gowda, Representative – FSSAI
  - Dr. Viswanathan Chinnusamy, Principal Scientist & Head, Division of Plant Physiology, Representative – ICAR
  - Dr. Nitin K. Jain, Scientist 'F', DBT, New Delhi
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