The recent regulatory framework of genome editing organisms and foods in Japan

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Overview

1. R&D for genome editing (GE) for agriculture field in Japan
   • 1st stage SIP (Cross-Ministerial Strategic Innovation Promotion Program) project
   • Example of development

2. Regulatory framework for genome editing in Japan
   • Current framework for GMO
   • The regulation framework for bio-diversity influence of genome editing organisms
   • The regulation framework for food safety of genome editing foods and food additives

3. Next challenge
   • 2nd stage SIP genome editing and public understanding of genome editing
Cross-ministerial program: SIP*
“Establishment of NPBT”

*SIP: Strategic Innovation Promotion Program
Examples under development

- High yield rice
- Long shelfed tomato
- No solanine potato
- Skin color modified grape
- Self compatible broccoli
- Gentle Tuna
- Thick red sea bream

From SIP and MHLW
Regulatory framework of GMO in Japan

<table>
<thead>
<tr>
<th>Safety Category</th>
<th>Legislation</th>
</tr>
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<tbody>
<tr>
<td>Bio-Safety</td>
<td>Cartagena Act*</td>
</tr>
<tr>
<td>Food Safety</td>
<td>Food Sanitation Act</td>
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- Handling of genome editing (GE) organism are discussed if genome editing products are applicable to GMO regulation.
- So far, the guidance under Cartagena Act and Food Sanitation Act were established.

* Law concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms
In this Act, “genetically modified organism and others" refers to an organism having a nucleic acid obtained by use of the following technology or a copy thereof.

(i) Technology to process nucleic acid outside cells, which is specified by the ordinance of the competent ministry

(ii) Technology to fuse cells of organisms belonging to different taxonomical families, as specified by the relevant ministry ordinance

GMO is defined as “the organism containing extracellularly processed nucleic acid or its replicate”
Food Sanitation Act, Article 2(excerpt).

GM Food is defined as
“the food including the organism which was obtained by recombinant DNA technique; the technique to generate recombinant DNA by cleavage/ligation, insert the DNA into living cell and multiply”.
**Category of Genome editing**

1. **Targeted mutagenesis**
   - **SDN-1** (Site-Directed Nuclease -1)
     - (deletion, insertion, substitution)
   - Non-homologous end-joining (NHEJ)

2. **Gene targeting**
   - **SDN-2**
     - (substitution of several bases)
   - **SDN-3**
     - (foreign gene integration)

**Cleavage of target sequence**

<table>
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<tr>
<th>5'</th>
<th>3'</th>
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Non-homologous end-joining (NHEJ)

Deletion: 

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__________
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Substitution:

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________________________
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Insertion:

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__________
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Homologous Recombination (HR) using template

5' 3' 3' 5'
Cabinet Decision: 2018, June 15

Clarify each handling policy for GE organism and GE Food under respective competent law by the end of Mar 2019 and promote actions toward international harmonization.

Above decision accelerated the policy development.

Bio Strategy 2019 (draft)
Proposal from the Cabinet Office: 2019, June 11

Operation of the handling system for non-regulated GE organism/food should be discussed and finalized by March, 2021 in order to encourage appropriate use of GE technology.
Handling of GE under Cartagena Act
Externally processed nucleic acid was inserted

The inserted DNA is present in the final product

Regulated as GMO

Out of the scope of GMO regulation

- Direct delivery of protein or RNP
- Transient expression

SDN-1

SDN-1 and null segregant
Incorporation of artificial nuclease expression gene (e.g. CRISPR/cas-9) into a host genome

- However, SDN-1 will be out of regulation if notification is accepted.
  *Note: genome editing organisms categorized as SDN-1 should be regulated until the notification is accepted.

Genome editing organism contains extracellularly processed nucleic acid or its replicate (SDN-2, 3.)

- Because SDN-2 and SND-3 is integrated extracellularly processed nucleic acid into the genome.
- Self-cloning and natural occurrences are excluded from regulation of genetically modified organisms
Handling of non-regulated GE organism

Considering the purpose of the Convention on Biological Diversity and Cartagena Protocol on Biosafety,

- Request developers to provide notification including the information such as development processes and no impact on biodiversity in order to accumulate knowledge regarding genome editing organisms.
- Publish a part of the notified information on Japan Biosafety Clearing House website.
- Notification is not mandatory.

- Organisms obtained by newly developed GE technology in future will be applied to above policy as far as possible.
Handling of GE under Food Sanitation Act
Foreign DNA is absent from the final product

The change induced by genome editing is within the range of naturally occurring sequence repair (nucleotide deletion/insertion, substitution, naturally occurring gene deletion, and one to several bases insertion)

Out of the scope of GM Regulation
...deletions or substitutions of bases or deletions of genes that are likely to occur in nature, and the consequent insertion of mutations of one to several bases due to repair of the cleavage site of an artificial restriction enzyme do not correspond to recombinant DNA techniques on the Food Sanitation Act,.....
Need to confirm that safety of genome editing food is equivalent to that of conventional variety. Thus

- Request developers to provide notification including the information such as development processes, in order to accumulate knowledge regarding genome editing food.

- Publish a part of the notified information, with attention to confidential information.

- Notification is not mandatory.
About difference in handling of SDN2 between MOE and MHLW

In MOE,
- GMO is defined as “the organism containing extracellularly processed nucleic acid or its replicate” (Cartagena Act)
- Although some product by SDN2 can not be distinguished from the product from SDN1 or the product by mutation, product by SDN-2 is regulated as GMO. This is because template used for SDN-2 is regarded as extracellularly processed nucleic acid

In MHLW,
- Complete product-based judgment
- As a product-based evaluation, same regulation of genetically modified foods under the Food Sanitation Act will not be applied to genome editing foods that modified DNA sequences are indistinguishable from natural mutation or conventional artificial mutagenesis.

How to handle this difference specifically is a future task.
**Summary: handling of genome editing in Japan (in terms of SDN class)**

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<tr>
<th>Class</th>
<th>MOE (Environment)</th>
<th>MHLW (Food)</th>
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<tr>
<td>SDN1</td>
<td>Non-GMO</td>
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</tr>
<tr>
<td>SDN2</td>
<td>GMO*</td>
<td>Case by case</td>
</tr>
<tr>
<td>SDN3</td>
<td>GMO</td>
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*Self-cloning and natural occurrences are excluded from regulation of genetically modified organisms under the Cartagena Act.

If the template used in SDN-2 is recognized as self-cloning, SDN-2 in the Cartagena Act becomes non-GMO.

Under the Food Sanitation Act, only microorganism obtained by self-cloning and natural occurrence can be exempted from GMO regulation.
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Consultation with agencies prior to the notification must be important.
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How do we confirm null segregant

Null Segregant is a major premise that genome editing organisms/food are non-regulated
Genomic DNA sequences can be determined by a massive sequencing technology, such as Illumina HiSeq.

The NGS technologies are powerful, but the mapping of NGS data is difficult. The results of different programs can vary.

We need a simple method that can produce consistent and stable data.

In light of the regulations of the use of GMO, we do not need to detect all the nucleotide changes in the genome.

Here we focus on the detection of external DNA sequences that remain in the genome after genome editing.
Detecting External DNA Sequences by Small Fragment Pattern Matching

- Obtain short reads of NGS from a sample treated by genome editing, GM, etc.
- Extract k-mers, the sequence of k nucleotides in length, from the reads.
- If vector sequence remains in the progeny of genome editing, there should be some k-mers that match the vector sequence.

[Diagram showing short read, k-mers, and vector sequence with hits indicated]
Simulation: K-mer method
20 bp insertion at the position 4,865

The inserted DNA is hardly distinguishable from rice sequences when 10-mers and 15-mers were used.

Relatively clear using 20-mer analysis.

Model rice which integrated 20-bp sequence located at 4865 bp of transformation vector.
Social Implementation of GE products

Social Implementation

Regulation
Cartagena Act
Food Sanitation Act

Development cost
(intellectual property etc.)

Public understanding

Development of superior GE crops/animals
The 2nd period: SIP approach for public understanding

Objective
Research on appropriate information and their distribution.

Measures
- Establishment of one-stop website for the information
- Distribution of the information to media and academia
- Research on AI for appropriate information and their distribution.
Future perspective

- Detail contents of the notification for non-regulated GE organism/food will be announced by end of August, 2019.
- Prior to the announcement of notification, public comments about notification by MHLW and MAFF were started from 27th June and 28th June, respectively.
- GE's labeling policy also will be announced by end of August, 2019.
Thank you for your attention