GMO regulatory frameworks and detection methods in Japan

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National Agriculture and Food Research Organization
# GMO testing and its legal basis

<table>
<thead>
<tr>
<th>Purpose [Legal basis]</th>
<th>Concerned ministries &amp; agencies</th>
<th>Testing institution</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food labeling authenticity <a href="#">Food Labeling Act</a> (newly enforced in 2015)</td>
<td><strong>Consumer Affairs Agency (CAA) (established in 2009)</strong></td>
<td><strong>MHLW (Ministry of Health, Labour and Welfare)</strong> Pharmaceutical &amp; Food Safety Bureau Dept. of Food Safety</td>
<td>Quarantine</td>
</tr>
<tr>
<td>Food safety [Food Sanitation Act]</td>
<td><strong>MHLW</strong> Pharmaceutical &amp; Food Safety Bureau Dept. of Food Safety</td>
<td><strong>MHLW</strong> Pharmaceutical &amp; Food Safety Bureau Dept. of Food Safety</td>
<td>Quarantine</td>
</tr>
<tr>
<td>Feed safety [Law concerning safety assurance and quality improvement of feeds]</td>
<td><strong>MAFF</strong> Food Safety &amp; Consumer Affairs Bureau Animal Products Safety Division</td>
<td>FAMIC</td>
<td>Unauthorized GM feeds</td>
</tr>
<tr>
<td>Biodiversity [Cartagena Protocol Domestic Law]</td>
<td><strong>MAFF</strong> Food Safety &amp; Consumer Affairs Bureau Plant Products Safety Division also <strong>ME (Ministry of the Environment)</strong></td>
<td>Plant Protection Station</td>
<td>Unauthorized GM plants (imported items)</td>
</tr>
<tr>
<td>Seed authenticity [Plant Variety Protection &amp; Seed Act]</td>
<td><strong>MAFF</strong> Food Industry Affairs Bureau New Business &amp; Intellectual Property Division</td>
<td>National Center for Seeds and Seedlings</td>
<td>Unauthorized GM plants (commercially available items)</td>
</tr>
</tbody>
</table>

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1) Because CAA has no testing institutes under its direct jurisdiction, practical operation is performed by MHLW & MAFF.

2) If necessary, NCSS also conducts GMO testing based on the Cartagena Protocol Domestic Law.
GMO regulatory frameworks and GMO testing

Topics:

- Food labeling authenticity
- Food safety
- Feed safety
- Biodiversity
Food Labeling Authenticity
GMO regulatory framework and GMO testing - labeling

**Legal basis:**
- Food Labeling Act (Law number: Act No. 70 of 2013) (enforced in 2015)
- Act on Standardization and Proper Labeling of Agricultural and Forest Products (JAS Law)
- Food Sanitation Act
- Food Sanitation Act (Law number: Act No. 233 of 1947)

**Inspection objective:**
- Authorized GM foods

**Inspection body:**
- Quarantines (MHLW), FAMIC (MAFF), municipal institutes of public health etc.
- Food companies, commercial testing labs
**Authorized GM foods**

Authorization of transgenic plants in Japan under the Food Sanitation Act (As of Jun., 2015)

<table>
<thead>
<tr>
<th>GM crops (302)</th>
<th>number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato</td>
<td>8</td>
</tr>
<tr>
<td>Soybean</td>
<td>20</td>
</tr>
<tr>
<td>Beet</td>
<td>3</td>
</tr>
<tr>
<td>Maize*</td>
<td>201</td>
</tr>
<tr>
<td>Canola</td>
<td>20</td>
</tr>
<tr>
<td>Cotton</td>
<td>45</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>4</td>
</tr>
<tr>
<td>Papaya</td>
<td>1</td>
</tr>
</tbody>
</table>

*Because of combined trait products (stacked varieties), the number of approved maize is quite high.

In the case of a quadruple stack variety, such as ABCD, not only ABCD but also ABC, BCD, CDA, DAB, AB, BC, CD, DA, AC, DB, A, B, C, and D are assessed and approved.
Labeling policy on GM products (1)

Products covered:

• Foods and food ingredients derived from eight GM crops (soy, maize, potato, canola, cotton, alfalfa, sugar beet and papaya) approved by MHLW shall be labeled.

• There are 33 processed foods subject to the labeling with the exception of:
  1. Processed foods in which DNA and protein are removed or highly degraded.
  2. It is not one of the three major ingredients in terms of weight or its proportion not 5% or more by weight.
### Labeling categories and requirements:

<table>
<thead>
<tr>
<th>Classification</th>
<th>Labeling example</th>
<th>Labeling</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ⅰ</strong> Produce whose composition or nutritional value is markedly different from</td>
<td>High oleic acid soybeans</td>
<td>Mandatory</td>
</tr>
<tr>
<td>that of conventional produce</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Ⅱ</strong> Produce whose composition and nutritional value are similar to that of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>conventional produce</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Processed foods containing genetically modified DNA or protein even after</td>
<td></td>
<td></td>
</tr>
<tr>
<td>processing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i If made from GM crops</td>
<td>Genetically modified</td>
<td>Mandatory</td>
</tr>
<tr>
<td>ii If made from agricultural products not segregated from GM products</td>
<td>Not segregated from GM products</td>
<td>Mandatory</td>
</tr>
<tr>
<td>iii If made from non-GM crops segregated throughout the production and</td>
<td>(Labeling unnecessary, and only name of crop</td>
<td>Voluntary</td>
</tr>
<tr>
<td>distribution stages*</td>
<td>required) soybeans (non-GM)</td>
<td></td>
</tr>
<tr>
<td>2 Processed foods not containing modified DNA or protein after processing</td>
<td>(Labeling unnecessary, and only name of crop</td>
<td>Voluntary</td>
</tr>
<tr>
<td>(e.g., soy source, corn oil, isomerized liquid sugar)</td>
<td>required) soybeans (non-GM)</td>
<td></td>
</tr>
</tbody>
</table>

*The labeling is authorized only for foods verified to have been handled according to identity preserved (IP)-handling. IP-handling is a management method in which GM products and non-GM products are not commingled each other at each stage of production, distribution and processing from farms to food manufacturers. It shall be verified by documents indicating that the management has been conducted.

If the IP-handling is properly conducted, the adventitious presence of GMO is accepted up to 5%.
GMO regulatory framework and GMO testing - labeling

**Legal basis:**

- Food Labeling Act (Law number: Act No. 70 of 2013) (enforced in 2015)
- Act on Standardization and Proper Labeling of Agricultural and Forest Products (JAS Law)
- Food Sanitation Act
- Food Sanitation Act (Law number: Act No. 233 of 1947)

**Inspection objective:**

- Authorized GM foods

**Inspection body:**

- Quarantines (MHLW), Food and Agricultural Materials Inspection Center (FAMIC) (MAFF), municipal institutes of public health etc.
- Food companies, commercial testing labs
How to quantify GMO %

- Japanese quantitative method is based on the quantification of targets’ “copy numbers” by PCR.
- The copy numbers of “GM specific target sequence” and “taxon specific sequence” are quantified individually.
- The GM amount in a bulk sample is calculated.
- At this time, the copy number ratio is converted into wt/wt-base ratio using a conversion factor (Cf).

\[
\text{GM amount(\%)} \quad \text{(experimentally defined)} = \frac{\text{Target seq.}}{\text{Taxonic seq.}} \times \frac{1}{\text{Cf}} \times 100
\]
Plasmids including PCR amplicons as “calibrant”

Target regions amplified by tailed primers
For the quantification of copy number for each target, a precise calibration curve is essential.

In order to draw a calibration curve, GM seeds accurately mixed into non-GM seeds are prepared.

Integrated fragments
Since seeds are agricultural products, it is difficult to obtain seeds in same quality all the time.

To overcome this situation, we developed the quantitative analytical methods utilizing plasmid DNA as a calibrator.
Standardization of quantitative methods with plasmid calibrant

- Development of quantitative methods for GM maize and soy
- Method validation (collaborative study) Designed based on internationally harmonized protocols, IUPAC, AOAC
- Standardization in Japan & Korea
- Published in ISO21570 Annex
  - Bt11 maize, Event176 maize, MON810 maize, T25 maize, GA21 maize, and Roundup Ready soy
  - MON876 maize, NK603 maize, T25 maize, TC1507 maize, LY038 maize, MIR604 maize, MIR 602 maize, MON89788 soy, and A2704-12 soy

Shindo et al., J. AOAC Intl., 85(5), 1119-26, 2002
Kodama et al., J. AOAC Intl., 92(1), 223-233, 2009
Takabatake et al., J. Food Hyg. Soc. Japan, 51(5), 242-6, 2010
Takabatake et al., J. Food Hyg. Soc. Japan, 52(2), 100-7, 2011
Testing scheme in practice

Raw material (maize) → DNA extraction → Quantitative screening

- > 4.5 % ≤ 4.5 % → O.K.

- Individual event-specific quantification
  - > 5 % ≤ 5 % → O.K.

Single kernel-based test → Inspection of documentations
### Emerging problems
- combined trait products (stacked varieties) -

#### Stacked varieties of GM maize:

<table>
<thead>
<tr>
<th>Maize single event: 29</th>
<th>Events (incl. stacked varieties): 201 (As of Jun. 1st, 2015)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bt11</strong> (+sweet), Event 176, MON810, T25, DLL25, DBT418, GA21 (+sweet), NK603, T14, MON863, 1507, DAS-59122-7, MON88017 (+sweet), LY038, 6275, MIR604, MON89034 (+sweet), MIR162 (+sweet), 3272, MON87460, 40278, Event5307, MON87427, DP-004114-3</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Maize stacked variety: 172</th>
</tr>
</thead>
<tbody>
<tr>
<td>MON863xNK603, GA21xMON810, NK603xMON810, T25xMON810, 1507xNK603, MON810xMON863, MON863xMON810xNK603, MON88017xMON810, 1507xDAS-59122-7, DAS-59122-7xNK603, DAS-59122-7x1507xNK603, Bt11xGA21, Bt11xMIR604, MIR604xGA21, Bt11xMIR604xGA21, LY038xMON810, MON89034xMON88017, MON89034xNK603, MON89034x1507, MON89034xDAS-59122-7, 1507xMON88017, DAS-59122-7xMON88017, MON89034x1507xMON88017, MON89034x1507xDAS-59122-7, MON89034xDAS-59122-7xMON88017, 1507xDAS-59122-7xMON88017, MON89034x1507xDAS-59122-7xMON88017, NK603xT25, Bt11xMIR162, MIR162xMIR604, MIR162xGA21, Bt11xMIR162xMIR604, Bt11xMIR162xGA21, MIR162xMIR604xGA21, Bt11xMIR162xMIR604xGA21, ....</td>
</tr>
</tbody>
</table>
How to calculate GM presence level

Grind a bulk

Extract DNA

Quantification

$5 + 0 = 5\%$

$5 + 5 = 10\%$
How about stacked varieties of GM maize?

2/20 = 10%

1/20 = 5%

5% + 5% = 10%

Overestimate!
Concept of single-kernel based analysis

Grind
- DNA Extraction
- Qualitative Analysis

\[
\frac{\text{# of positive kernels}}{\text{# of total kernels}} = \frac{2}{20} = 10\%
\]

Grind
- DNA Extraction
- Qualitative Analysis

\[
\frac{\text{# of positive kernels}}{\text{# of total kernels}} = \frac{1}{20} = 5\%
\]
Raw material (maize)

DNA extraction
Quantitative screening

\[ > 4.5\% \quad \leq 4.5\% \rightarrow \text{O.K.} \]

Individual event-specific quantification

\[ > 5\% \quad \leq 5\% \rightarrow \text{O.K.} \]

Single kernel-based test
Food Safety
GMO regulatory framework and GMO testing - food safety

**Legal basis:**
- Food Sanitation Act (Law number: Act No. 233 of 1947)

**Inspection objective:**
- Unauthorized GM foods

**Inspection body:**
- Quarantines (MHLW), municipal institutes of public health etc.
- Commercial testing labs
Detection methods of unauthorized GM foods:

- Flax: FP967
- Wheat: MON71800
- Rice: 63Bt, NNBt, CpTl
- Rice: LL601
- Maize: Bt10
- Maize: CBH351
- Maize: DAS59132
- Canola: RT73 B. rapa
- Papaya: PRSV-YK, PRSV-SC, PRSV-HN

Zero-tolerance policy
Qualitative detection methods
Case 1: Unauthorized GM papaya

GM papaya: PRSV-YK, PRSV-SC, and PRSV-HN

- In 2011: MAFF, MHLW and ME announced that unknown GM papaya was commercially distributed in Japan.
- Authorization of GM papaya 55-1 was predicted in 2011.
- In the course of the development of a detection method for 55-1, GM papaya different from 55-1 was found.
- Sequence analysis revealed that the transgenic vector construct of the unknown GM papaya was identical to that of GM papaya resistant to the infection of PRSV-YK strain isolated in Taiwan.

- In 2013: Another GM papaya event, PRSV-SC was found in imported papaya processed products from Thailand.

- In 2015: Another GM papaya event, PRSV-HN was found in imported papaya processed products from Vietnam.

- Monitoring for PRSV-YK, -SC, -HN has been conducted.
The Ministry of Health, Labour and Welfare (MHLW) develops and issues the “imported foods monitoring and guidance plan” every fiscal year.


- Monitoring inspection: the inspections that are systematically implemented every fiscal year for the purpose of monitoring safety conditions of various foods based on the provision of paragraph 1, Article 28 of “the Food Sanitation Act (the Act)” and of taking measures including reinforced inspections when violations of the Act are identified.

- Inspection orders: the orders requiring importers to have imported foods with a high possibility of violating the Act inspected each time that the goods are imported, based on the provision of paragraph 2 or 3, Article 26 of the Act.

<table>
<thead>
<tr>
<th>Category of inspiration items</th>
<th>Food type</th>
<th>No. of inspection specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMOs</td>
<td>Agricultural foods (vegetables, fruit, wheat, barley, corn, beans, peanuts, nuts, seeds, and other products)</td>
<td>450</td>
</tr>
<tr>
<td></td>
<td>Processed agricultural foods (frozen products (processed vegetables), processed vegetable products, processed fruit products, spices, instant noodles, and other products)</td>
<td>200</td>
</tr>
</tbody>
</table>

will be conducted using event-specific detection methods.
Feed Safety
**Legal basis:**
- Law concerning safety assurance and quality improvement of feeds (Law number: Act No. 35 of 1953)

**Inspection objective:**
- Unauthorized GM foods

**Inspection body:**
- FAMIC (MAFF)

Outline of regulation on GM feed

In order to ensure animal health and safety of animal product, Japan allows to import and distribution of approved GMO only.

Bio-tech firms (GMO developers)

Bio-tech firms sell the seed approved in Japan.

Production in exporting countries.

Application for approval

Ministry of Agriculture, Forestry, and Fisheries

MAFF approves the GMO based on the evaluation of the Agricultural Material Council (consultative body of the MAFF) and the Food Safety Committee.

If there is a possibility of non-approved GMO contaminating, MAFF instructs the FAMIC to conduct on-site inspection.

Only approved GMO is to import.

Development of an analysis method.

Inspection

Japanese port

Export to Japan

Food factory

Testing targets

Detection methods of unauthorized GM feeds:

Notification 8598(Director-General of Agricultural Production Bureau, and Director-General of Fisheries Agency)(2003, Apr. 1st)
https://www.famic.go.jp/ffis/feed/tuti/21_8776_1.html

- Maize: CBH351
- Maize: Bt10
- Maize: DAS59132
- Flax: FP967
- Wheat: MON71800
Low-level presence of unauthorized GM feeds

• As a consequence of an asymmetric authorization, low levels of GM plant materials that have passed a feed safety assessment in one or more countries may on occasion be present in feed in importing countries in which the feed safety of the relevant GM plants has not been determined.

• If the competent authority of a foreign government who conducted the safety assessment of the relevant GM plants has a safety assessment system with either equaling or surpassing safety level as Japan has, up to 1% of the relevant GM plants’ commingling in feed is permissible.

• Therefore, not only qualitative, but also quantitative detection methods may be needed to ensure the commingling levels of the relevant GM material not exceeding 1%.
Biodiversity
GMO regulatory framework and GMO testing - biodiversity

**Legal basis:**

- Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms (Cartagena Law) (Law number: Act No. 97 of 2003)
- Plant Variety Protection and Seed Act (Law number: Act No.83 of 1998)

**Inspection objective:**

- Unauthorized GM seeds and seedlings

**Inspection body:**

- Plant protection stations (MAFF), and National Center for Seeds and Seedlings (MAFF)
Testing targets

Inspection plan for unauthorized GM seeds and seedlings for FY2015:


- Flax: FP967
- Papaya: PRSV-YK
- Papaya: PRSV-SC
- Cotton: GM cotton
Case 1: Unauthorized GM papaya

**GM papaya: PRSV-YK**

- In 2011: MAFF, MHLW and ME announced that unknown GM papaya was commercially distributed in Japan.
- Sequence analysis revealed that the transgenic vector construct of the unknown GM papaya was identical to that of GM papaya resistant to the infection of PRSV-YK strain isolated in Taiwan.

- The GM papaya was first found in domestically produced processed foods.
- The results of monitoring revealed that nearly 20% of commercially planted papaya in Okinawa prefecture was GM papaya, PRSV-YK.
- All of the GM papaya trees were cut down.

- This caused tremendous economic losses for papaya produces in Japan.
- This case may have been prevented, if an appropriate GM monitoring was conducted at the border.
Case 2: Unauthorized GM cotton

GM cotton: MON531, MON1445

- In 2014: GM cotton were found in imported seeds by a surveillance in the process of preparation for the introduction of GM cotton testing.
- Eventually nine samples of imported cotton seeds out of 10 samples tested were found to contain GM cotton, such as MON531 and MON1445 at the plant quarantine.
- No GM cotton event has been approved for commercial planting in Japan, while there are many GM cotton events planted globally.
Limitations of Japanese monitoring system

- In the case of GM papaya, commingling of GM papaya was found in a research institute.
- In the case of GM wheat, the information of possible GM wheat commingling was provided by the US government.
- In the case of GM cotton, commingling of GM cotton was found in preliminary surveillance.
- Regarding unauthorized GM monitoring, official testing laboratories conduct mainly event-specific detection tests.
- Without information from outside sources, Japanese monitoring system at the border has not worked to control on unauthorized GM foods entry.
Case 2’:
Unauthorized GM cotton

- Various kinds of GM plants have been developed around the world.
- A country whose food self-sufficiency ratio is less than 40%, such as Japan, cannot avoid international food trade.
- MAFF decided to partially introduce non-event specific methods, such as real-time PCR array, for the monitoring of unauthorized GM cotton in 2014.

A set of primer-probe is pre-added into each well.

Real-time PCR array allows to detect different DNA segments on one plate.
Conclusions

• With respect to each law, different national competent authorities play different roles.
  ✓ Information sharing is required.

• The number of authorized and unauthorized/unknown GMOs has been increasing.
  ✓ New measures are required.
  ✓ Especially, non-event specific detection will be essential.

  Toward that end, ...  

• To apply non-event specific GM detection methods to other crops, an event identification scheme should be established.

• In addition, in preparation for the commingling of unknown GM events, a scheme to elucidate GM-related segments and/or their sequences will be require.