**Experience of Developing of Detection Methods for Unapproved GM papaya**

 **(for seeds and seedlings)**

In December 2010, the Ministry of Agriculture, Forestry and Fisheries (MAFF) received information about the possibility of existence of unapproved genetically modified (GM ) papaya which might have been cultivated in Japan.

The following shows an experience of developing of detection methods for the unapproved GM papaya, imported for commercial seeds and seedlings.

**Introductory remarks**

* The unapproved GM papaya which turned out to be labeled “Tainung No.5” has not been approved for the commercial usage in any country. The unapproved GM papaya has only been grown in the confined area of a Taiwan University.
* An approval of GM papaya called “Rainbow” had shortly been obtained. A detection method of the GM papaya (Rainbow) had been in the process of development.
* After finding the unapproved GM papaya fruits, the detection method for fresh fruits and processed food products of the unapproved GM papaya had been developed by the National Institute of Health Sciences. The developed detection method needed to be modified to detect unapproved GM papaya for seeds or seedlings in the following conditions.
	+ There was no information about grinding method for papaya seeds or papaya raw leaves.
	+ There was no certified reference material of the unapproved GM papaya.
* In order to prevent further importation of the unapproved GM papaya, the development of monitoring methods of imported papaya seed and seedlings was requested although there was no practical information about the contamination rate per lot of the unapproved GM papaya seed.

**Background**

In Japan, GM crops are under regulations in the light of food safety, feed safety, biosafety and labelling.

In 2010, in the light of food safety, feed safety and biosafety, the approved process of a GM papaya whose name is “Rainbow” had been finalized. The food labeling system for Rainbow was under consideration by the responsible authority named the Consumer Affairs Agency in the Cabinet. At the same time, the National Institute of Health Sciences was trying to prepare a real-time PCR detection method\*, for fresh papaya fruits and processed papaya products in order to distinguish the approved GM papaya from unapproved one.

Papaya is not a major plant in Japan, and also is not an indigenous plant of Japan. On the other hand, in some prefectures, un-ripened papaya fruits are eaten locally as an important vegetable source of vitamin in summer. Especially, in Okinawa Prefecture, papayas are commonly cultivated and sold in the local market. Considerable amounts of papaya seeds are being imported to Japan annually for cultivation.

\*Identification and Detection Method for Genetically Modified Papaya Resistant to Papaya Ringspot Virus YK Strain

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（[www.researchgate.net/publication/51685579\_Identification\_and\_detection\_method\_for\_genetically\_modified\_papaya\_resistant\_to\_papaya\_ringspot\_virus\_YK\_strain/file/60b7d515b78ebb1556.pdf](http://www.researchgate.net/publication/51685579_Identification_and_detection_method_for_genetically_modified_papaya_resistant_to_papaya_ringspot_virus_YK_strain/file/60b7d515b78ebb1556.pdf).）

**First report of the incident**

In December 2010, we received from the National Institute of Health Sciences that one of papaya fruits which were sold in a local market resulted positive in the trial test of their developing detection method.

Because papaya fruits in a local market are usually produced in the local area, it meant that the unapproved GM papaya\*2 might have been cultivated in the area.

In order to prevent further importation or cultivation of the unapproved GM papaya, the MAFF had to develop detection methods for seeds and seedlings, immediately.

The National Institute of Health Sciences also informed the possible two primers/probe-sets both of which were arranged to detect the specific inserted region of the unapproved GM papaya. The unapproved GM papaya has the same sequence to the specific DNA region of GM papaya which is under research at a University of Taiwan.

 \*2 The information about the features of the unapproved GM papaya, please see the following URL; http://bch.cbd.int/database/record.shtml?documentid=103897

**Task and Challenges**

The informed detection method was a detection method for fresh papaya fruits and processed papaya products. The MAFF need to develop practical grinding methods for seeds or leaves and to confirm the utility of the two primers/probe-sets in DNA extract from seeds or leaves.

There was no certified reference material of the unapproved GM papaya seeds and leaves.

However, it was an urgent situation because unapproved GM papaya might have already been cultivated.

**Development of grinding methods (for seeds)**

The fruit which resulted positive in a trial test was ripened and had seeds in it. The MAFF started to develop grinding methods using the seeds of the fruit as a positive control material.

We developed two grinding methods for seeds; one is for three-seed scale, the other is for 100-seed scale.\*3

Considering of the practical and efficient implementation of monitoring, it was desirable to have a grinding method that secures homogeneity of the sample consisting of many seeds including one positive seed. As a result of preliminary test, taking into account the uncertainty of homogeneity, we decided to carry out three times of DNA extraction from a ground sample consisting of 100 seeds including one positive seed, tentatively.

\*3 100-seed scale means that a sample consists of 99 non-GM seeds and one GM seed.

**Development of grinding methods (for leaves)**

Regarding positive control materials of leaves, the MAFF asked the Taiwanese University which developed the unapproved GM papaya to provide us with positive leaves. Fortunately, it understood our situation and sent the materials (leaves), which had the same DNA sequence to the unapproved GM papaya, to us.

Because of the procedures to import the leaves from Taiwan to Japan, the study of grinding methods for leaves began 4 months after start of seeds grinding test.

We developed two grinding methods for leaves; one is for one-leaf scale, the other is for 100-leaf scale.\*4

Much like with seeds, we decided to carry out three times of DNA extraction from a ground sample consisting of 100 leaf sections including one positive leaf section, tentatively.

\*4 One-leaf scale means a 50 mg section of a leaf. 100-leaf scale means that a sample consists of 99 non-GM leaf sections and one GM leaf section.

**Confirmation of the utility of possible primers/probe-sets in DNA extract from seeds/leaves**

The MAFF conducted preliminary tests and found out the efficiency of each primers/probe-sets in the DNA extract form seeds or leaves. When we used one of primers/probe-set for DNA extracts derived from papaya raw leaves, sometimes, a non-specific amplification curve was recognized. On the other hand, when we used the other primers/probe-set, the amplification signals were always weak.

These results meant, if we use only one of them, we may have false test results.

Therefore, we decided to use both of the two primers/probe-sets.

**The summary of developed methods**

The MAFF modified the detection method which was developed for papaya fruits, in order to apply it to papaya seeds and seedlings, as follows;

* increased number of DNA extraction, considering uncertainty of homogeneity of the ground sample tentatively
* configured two primers/probe-sets in order to prevent a false result, considering the efficiency of the detection reagents

**The summary of collaborative studies**

Because of the lack of sufficient positive control materials, we could not conduct the program of collaborative study in an ideal situation. The conducted collaborative studies, with participation of five testing laboratories, were as follows;

* Four blind materials for each laboratory, in three-seed scale
* Three blind materials for each laboratory, in 100-seed scale, one-leaf scale and 100-leaf scale

In these collaborative studies, there was no false result. We finalized the detection methods for papaya seeds and seedlings with usage of two primers/probe-sets. And for seedlings, three times of DNA extraction from a ground sample of 100-leaf scale was needed.

[P] \*5 Information about the details of the developed methods, please see the following URL.

**Start of Monitoring**

Using the developed methods, we started analyzing samples from all papaya seeds and seedlings, available in the Japanese seeds/seedlings market. At the start of the monitoring, we needed to determine a contamination rate of GM seeds/seedlings per lot. There was no reliable information about the contamination rate.

We provisionally set the contamination rate of GM seeds/seedlings per lot as approximately 5 %, because, as a general recognition of the market, papaya seeds are usually produced under severe quality control with purity rate of more than 95 %.

We sampled 9 g of seeds (equal to 300~500 seeds) per lot and prepared a test sample of 100 seed/leaf scale. Test 100 seeds/leaves per lot enable us to detect a 5 % contamination rate per lot at 99 % confidence approximately.

**Result of Monitoring**

From February to June, 2012, we analyzed 29 seed lots and 4 seedling lots. As a result, one seed lot was positive. This lot had been labeled “Tainung no.5”

All 10 seeds picked up in a random manner from the positive lot turned out to be the unapproved GM papaya seeds. From this fact, we qualitatively estimated that the contamination rate of the lot would be very high. Rather, we thought the lot would be the GM papaya itself, not contaminated one.

**Current Situation of Japan**

We have been continuing the monitoring of imported seeds and seedlings.

After identified the unapproved GM papaya to be labeled as “Tainung no.5”, we started analyzing of cultivated papaya plant in farms in Okinawa Prefecture. Until the end of 2011, all commercially cultivated “Tainung no.5” had been cut down. From February 2011, we started monitoring of papayas which are growing on a vacant plot or along roadside.