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Genetically Modified Organisms (Control of Release) Ordinance Cap. 607 Expert Group

Review of the Exemption of Genetically Modified Papayas in Hong Kong

Purpose

This paper briefs members on a review of the exemption of genetically modified (GM) papayas in Hong Kong.

Background

2. The Genetically Modified Organisms (Control of Release) Expert Group (Expert Group) in its first meeting held on 5 July 2011 discussed the risk assessment and disposal of GM papayas in Hong Kong. The risk assessment undertaken in 2011 for GM papayas indicated that they were highly unlikely to pose any risk to the biodiversity of the local environment and the possible biosafety effect of GM papayas was deemed acceptable. Besides, in view of the widespread and scattered presence of home-grown papayas in the territory of which some were GM papayas, it was considered impractical and highly undesirable for the authority to undertake enforcement against the maintenance of GM papayas in Hong Kong. In this connection, the Expert Group recommended that GM papayas should be exempted from the application of section 5 (restrictions on release into environment and maintenance of lives of GMOs) and section 7 (restrictions on import of GMOs intended for release into environment) of the Genetically Modified Organisms (Control of Release) Ordinance Cap. 607 (the Ordinance).

3. The Expert Group also advised the Agriculture, Fisheries and Conservation Department (AFCD) continue to monitor the latest progress and development of GM

papayas and carry out a review of the exemption of GM papayas in three years' time for reporting to the Expert Group. Furthermore, AFCD was also advised to carry out a survey on the distribution profile of GM papayas in the territory. The Expert Group also recommended AFCD and other relevant bodies to step up publicity on GM crops and organic farming to both the general public and the stakeholders.

4. Subsequently, the Genetically Modified Organisms (Control of Release) (Exemption) Notice (which took effect on 23 June 2012) exempts all varieties of GM papayas from the application of section 5 of the Ordinance and two commercialised lines of GM papayas (GM papaya with the unique identifier code of CUH-CP551-8 and GM papaya with the transformation event code of Huanong-1) from the application of section 7 of the Ordinance.

5. AFCD has conducted a review of the exemption of GM papayas and the results of the review are presented in the ensuing paragraphs.

GM Papayas

6. Papaya (*Carica papaya*) is a soft-wooded perennial plant of the Caricaceae family. It is believed to be originated from eastern Central America and is now cultivated in all tropical countries as well as many sub-tropical regions of the world.

7. Viral infection is one of the major limiting factors for commercial production of papaya in many parts of the world. The most important viral pathogen of papaya is the Papaya Ringspot Virus (PRSV). In order to cope with the widespread and devastating infection of papaya by PRSV, papaya had been genetically modified to enhance its resistance to PRSV. This was achieved by inserting the PRSV genes into the papaya genome. An antibiotic resistance gene was also inserted as an expression reporter gene. At present, several lines of GM PRSV-resistant papayas were developed and some of which were commercialised in the international food market.

8. The 55-1 line (CUH-CP551-8) was developed and approved for commercial production in Hawaii since 1998 and now contributes to over 90% of papaya fruit production in Hawaii. This line has been hybridised with other non-GM papayas and developed into other GM varieties, for example "SunUp", "Rainbow", and "Laie Gold", carrying the same transgene. "Rainbow" and "SunUp" are widely available in the international food market but "Laie Gold" is grown for local market in

Hawaii only. Two other GM papaya lines (i.e. "CUH-CP631-7", "UFL-X17CP-6") were also deregulated by the United States Department of Agriculture, but they have not been commercialised.

9. Three lines of PRSV-resistant papayas (i.e. Huanong-1, ZS-1, ZS-2) were developed in China but only Huanong-1 was approved for commercial production. Several virus-resistant GM papaya lines have also been developed in Taiwan. As there are no laws or regulations governing the environmental release of GMOs in Taiwan, their commercial production is not regulated and the two Taiwan lines of PRSV-resistant GM papaya, 16-0-1 and 18-2-4, ("TW-lines") have been found among imported and locally grown papayas (see paragraphs below).

GM Papayas in Hong Kong

10. Papaya is commonly used as a fruit for direct consumption or as an ingredient for cooking locally. Samples of papayas were collected by AFCD for GM testing. Among the 117 imported papayas collected from the local markets in 2011-2014, up to 61 % were found to be genetically modified. These imported GM papayas included the 55-1 line, the Huanong-1 line and the TW-lines. The TW-lines were the dominant GM lines found, which contributed up to 73% of the imported GM papayas in the study period. It was followed by the Huanong-1 and 55-1 which made up 19% and 8%, respectively, of the imported GM papayas (Table 1). GM papayas imported from Mainland China were derived mainly from the TW-lines, whereas the Huanong-1 line was less common. On the other hand, the GM papayas imported from other countries belong to the 55-1 line, the Huanong-1 line, and the TW-lines.

Table 1. Percentage of GM papayas and the major GM lines sampled in the market from 2011 to 2014.

Year	Papaya sampled in market	Percentage of GM papaya	Percentage of the major GM lines among the sampled GM papaya			
			55-1	Huanong-1	TW-lines	hybrids
2011-12	16	69%	n/a	n/a	n/a	n/a
2012-13	59	68%	0%	21%	79%	0%
2013-14	42	48%	25%	15%	60%	0%
Total	117	61%	8%	19%	73%	0%

11. While the seeds of GM papayas are not readily available for sale in the international seed markets, it is quite common for people living in the rural areas to grow papayas in their backyards or along the edges of farmlands from the seeds obtained from the consumed GM papaya fruits, which may be a more popular choice for planting because of their usually better appearance than the non-GM fruits. Since they show better growth performance due to their enhanced resistance to viral infection, these GM papaya plants are also more common in the territory.

12. In 2011-March 2015, AFCD's survey for GMOs has collected 1,386 samples of papayas from locally-grown papaya plants and amongst which 54% were found to be genetically modified (Table 2). GM papaya plants were found to be very widespread in the rural areas of Hong Kong, and they were grown by local farmers and residents in their farmlands and backyards (Figure 1). Data from 2011 to March 2015 show that the TW-lines were the most common, making up 83% of the locally grown GM papaya plants, followed by the Huanong-1 (14%), 55-1 (1%) and hybrids between them. Geographically, GM papaya plants have been found to be very widespread over the rural areas of Hong Kong (Figure 2). With the exception of Hong Kong Island where the percentage of GM papayas was 13%, GM papayas made up to 50% or more in other parts of Hong Kong.

Table 2. Percentage of GM papaya and the major GM lines sampled among the locally grown papaya (from farms and rural areas) from 2011 to March 2015.

Year	Locally grown papaya sampled	Percentage of GM papaya	Percentage of the major GM lines among the sampled GM papaya			
			55-1	Huanong-1	TW-lines	hybrids
2011-12	188	60%	n/a	n/a	n/a	n/a
2012-13	290	50%	1%	9%	87%	3%
2013-14	503	49%	1%	9%	87%	3%
2014-Mar 15	405	60%	1%	24%	73%	3%
Total	1386	54%	1%	14%	83%	3%

Figure 1. Geographical distribution of locally grown non-GM (green) and GM (red) papayas sampled from 2011 to March 2015.

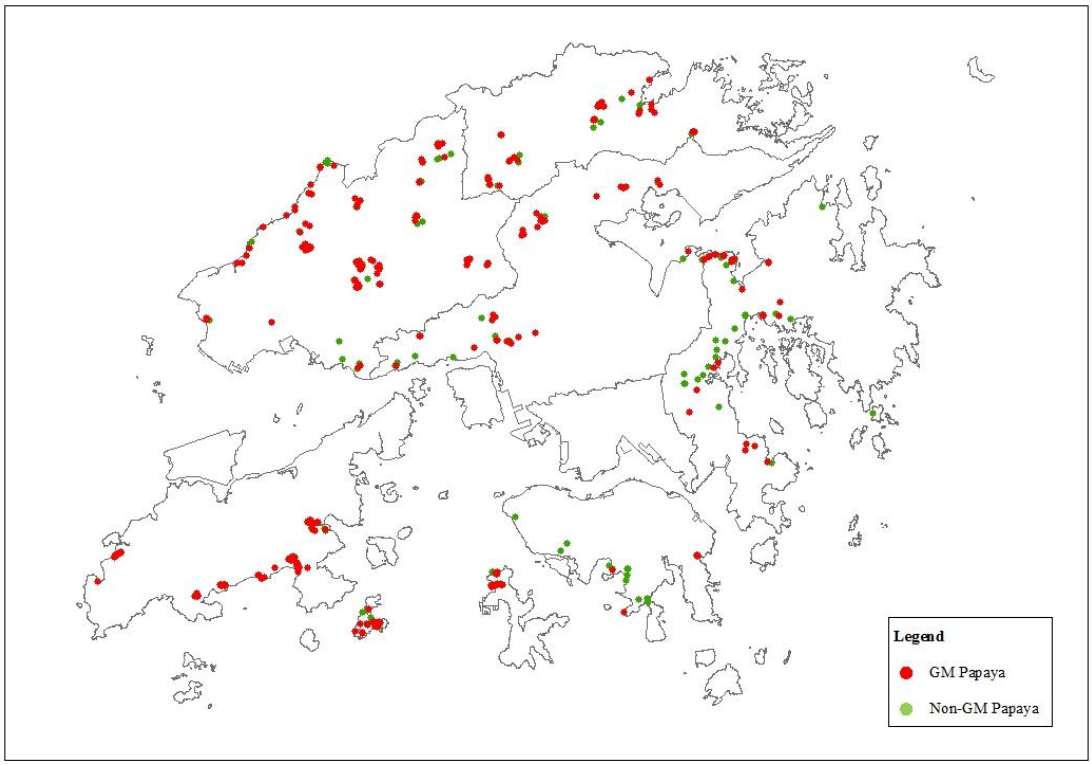
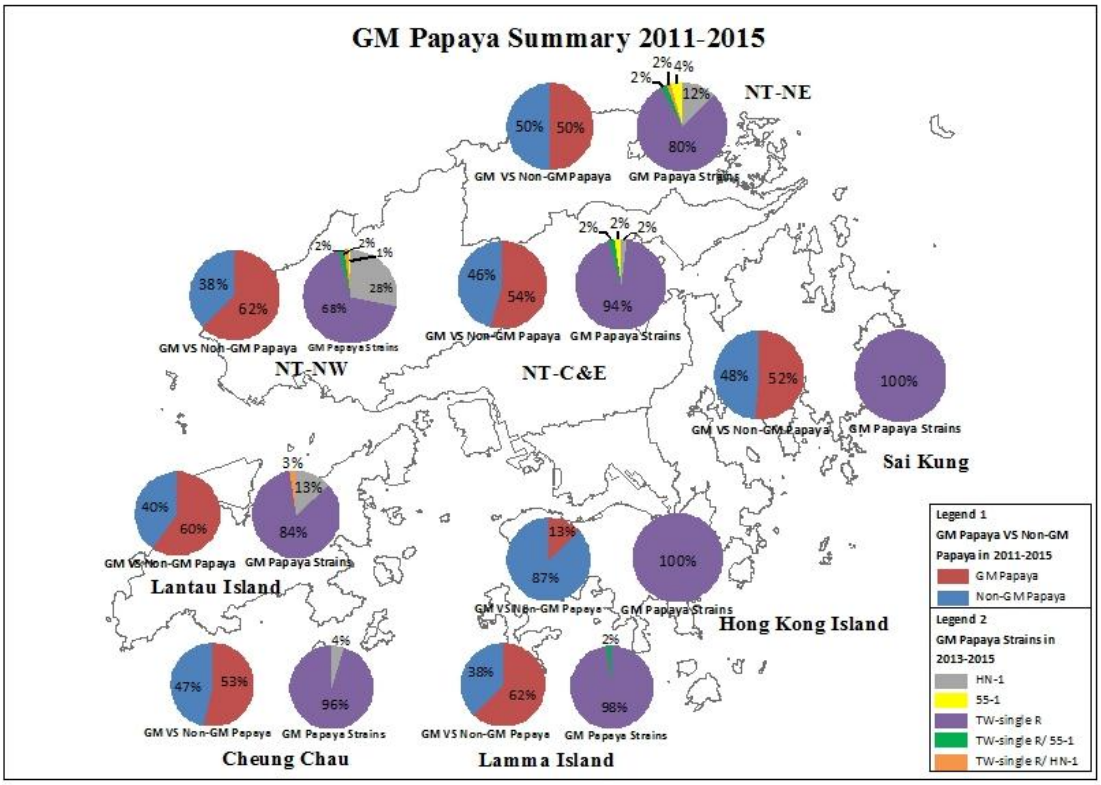


Figure 2. Percentage of GM papayas (left pie charts in red/blue) and the major GM lines (right pie charts in purple/grey/yellow) in different regions of Hong Kong from 2011 to March 2015.



Risk Assessment

13. A risk assessment was undertaken to assess the possible adverse effects of GM papayas on the conservation and sustainable use of biological diversity in the local environment in 2015 and an updated risk assessment was conducted in relation to the present review. The potential adverse biosafety effects of the cultivation of GM papayas in Hong Kong would remain to be the:

a) potential gene flow to wild relatives of papaya,
b) potential to become a weed,
c) production of harmful substances,
d) horizontal gene transfer, and
e) impact on soil microbial diversity.

14. The updated risk assessment has the same findings as the one done in 2011, indicating that gene flow of GM papayas to other wild relatives of the Caricaceae family does not exist as no native species of the Caricaceae family is known to occur in Hong Kong. As the inserted genes would very unlikely be transferred to other plant species in Hong Kong due to species barrier, cross-contamination of other plant species would not occur. As regards the potential for GM papayas to become a weed, it is deemed to be very low as papaya is a domesticated plant and is easily outgrown by vines and other plants.

15. Besides, no harmful substance is known to be produced as a result of the genetic modifications of GM papaya plants. On the other hand, as shown in a number of studies, it was very unlikely that horizontal gene transfer from GM papayas to other organisms would occur, and there is no evidence to suggest that soil microbial diversity would be adversely affected as a result of growing GM papayas.

16. Based on the updated risk assessment, it is concluded that it is highly unlikely for GM papayas to pose any adverse biosafety effect on the biodiversity of the local environment. The detailed risk assessment report is at the Annex.

Public Awareness and Education

17. To support the implementation of the Ordinance, publicity and education programme, including roving exhibition, production of promotional pamphlets, advertisement, and distribution of circular letters, was carried out in 2013-15 to raise

the public awareness on the local control of GMOs. In 2013-15, over 18,000 copies of pamphlets were distributed to the public. The pamphlets included specific topics on GM aquarium fish, seeds of GM crops, GMOs for food, feed or processing, etc. Two bilingual advertisement videos were also broadcasted in the Hong Kong International Airport to remind visitors on the control of GMOs. In addition, we have maintained the online GMOs Register (www.afcd.gov.hk/gmo) which allows the public to readily access to information about the Ordinance as well as updated results of the AFCD's GMO surveys.

18. Despite the exemption mentioned in paragraph 4 above, import and export of GM papayas are still under the control of Section 23 of the Ordinance. It requires the shipment of GMO intended for direct consumption as food or feed, or for processing (GMO-FFP) to be accompanied by prescribed documents to enable easy identification of the GMOs and to provide the contact points for further information. Therefore, circular letters were issued to local traders (retailers, wholesalers and importers) of papaya and its seeds to remind them of the documentation requirements for GMO-FFP of the Ordinance.

Advice Sought

19. In the light of the findings of the latest review, it is proposed that the current control and exemption under the Ordinance shall be maintained, subject to further review in three years' time.

20. Members are invited to note and provide their views on the review on the exemption of GM papayas.

Agriculture, Fisheries and Conservation Department
April 2015

Annex

Risk Assessment Report

2015

Genetically Modified Papayas

1. Introduction

1.1 The Genetically Modified Organism (GMO) surveys conducted by the Agriculture, Fisheries and Conservation Department during the period from 2011 to March 2015 found that genetically modified (GM) papayas were available for sale as food in the local markets and grown in the local environment. About 61% of imported papaya fruits in the local markets and about 54% of the home-grown papayas were found to be GM. Due to the prevalence of GM papayas in the local environment, a risk assessment was undertaken to assess the possible adverse biosafety effects of GM papayas on the local environment. This risk assessment report was prepared in accordance with Schedule 3 to the Genetically Modified Organisms (Control of Release) Ordinance Cap. 607 with respect to the requirements on risk assessment on possible adverse biosafety effects of GMOs on the local environment.

2. Identities of the GMOs

2.1 At present, eight GM lines of papayas were developed (3 in the United States, 3 in China, and 2 in Taiwan) for resistance to the Papaya Ringspot Virus (PRSV). The GM papaya line with the transformation event code "55-1" is the first approved GM papaya line. Its progenies and hybrids with other non-GM papayas have given rise to three varieties, i.e. "SunUp", "Rainbow" and "Laie Gold", which are now being grown in Hawaii and a few other places in the United States for commercial production (1, 2, 3, 4, 5). The remaining two non-commercialised lines (i.e. transformation event code "63-1" and "X17-2") were deregulated by the United States Department of Agriculture (USDA) and they could be planted without regulatory oversight by the USDA's Animal and Plant Health Inspection Service (APHIS) in the United States (2, 6).

2.2 In China, the GM papaya lines, ZS1 and ZS2, were approved for field trials in 2000 (7) while Huanong-1 was approved for commercial production in Guangdong in 2010 (8). Several virus-resistant GM papaya lines have also been developed in Taiwan. As there are no laws or regulations governing the environmental release of GMOs in Taiwan, their commercial production is not regulated and it is known that

the two Taiwan lines of PRSV-resistant GM papaya, 16-0-1 and 18-2-4, (hereafter "TW-lines"), are known to be grown in China and available in the food market.

2.3 A summary of the characteristics of the eight GM papayas is in the table below.

	Transformation Event Code	Unique Identifier	Commercial Name	Common Name	References
a)	55-1	CUH-CP551-8	"SunUp"	Hawaii papaya	1, 2, 9, and 10
b)	63-1	CUH-CP631-7	Nil	Papaya	1, 2, 9, 10, and 11
c)	X17-2	UFL-X17CP-6	Nil	Papaya	6, 12, and 13
d)	ZS1	Not available	Nil	Papaya	7, 14 and 15
e)	ZS2	Not available	Nil	Papaya	7, 14 and 15
f)	Huanong-1	Not available	Huanong-1	Papaya	8, 16, 17, and 18
g)	16-0-1	Not available	Nil	Papaya	19, 20, 21 and 22
h)	18-2-4	Not available	Nil	Papaya	20, 21, 22 and 23

3. Recipient Organism

3.1 The recipient organism used for the genetic modification was *Carica papaya*, which is commonly called papaya and belongs to the Caricaceae family (24, 25). It is a soft-wooded perennial plant with a life span of about 5-10 years and can grow up to 10 metres.

3.2 It is speculated that papaya originates from the lowlands of eastern Central America where wild populations are found (26). It was dispersed to the Caribbean and South-east Asia by Spanish explorers in the 16th Century. Papaya cultivation is now practiced in all tropical countries and many sub-tropical regions of the world. Fruit production is optimal in areas with a minimum monthly rainfall of about 100 mm, minimum relative humidity of 66% and where temperatures range between 21℃ and 33℃ (24, 25).

3.3 Cultivated papaya plants have three forms with respect to sexual reproduction. Male plants bear only staminate flowers which produce pollen all year round but very rarely set fruit. Female plants only bear pistillate flowers, and they rely on pollen

from male or bisexual plants to set fruit. The third is the bisexual or gynodioecious plants which bear bisexual flowers that are self-compatible, i.e. they can set fruit by pollen from its own flowers (24, 25). The bisexual form is the preferred plant form for commercial production, although in certain places the male and female forms are preferred because of the year-round production of pollen and better quality of fruits.

3.4 Pollination is mediated by wind or insects, such as honeybees and hawkmoths. The viability of fresh pollens was determined to be around 90%. Pollen viability was found to be significantly affected by extremely high humidity or low temperature. On the other hand, papaya stigmas seem to be ready for reception throughout the year and are able to produce fruits even in winter (24).

3.5 The Caricaceae family comprises six genera (*Carica, Cylicomorpha, Jarilla, Horovitzia, Jacaratia* and *Vasconcellea*) and with about 33 species mainly distributed in Central and South America with the exception of the two African species of *Cylicomorpha*. *Carica* is a monotypic genus and papaya is the only extant species of the genus. The closest relatives of *Carica* are the genera *Jarilla* and *Horovitzia*. The two genera form a clade that split from the *Carica* about 25 million years ago (27). In the early efforts to improve papaya, the plant was inter-crossed with species from another Caricaceae genus, *Vasconcellea*, but the hybrids were mostly not viable in the field (25).

3.6 "Sunset", which is gynodioecious, is the parental variety used to develop the transgenic lines 55-1 and 63-1 (1, 9 and 11). The variety of papaya used to develop X17-2 is "F65" obtained from Taiwan (12). The parental plant of ZS1 is "Suizhonghong" (穗中紅), a yellow-fleshed papaya and the parental plant of ZS2 is "Sunrise", a red-fleshed papaya (15), whereas that of Huanong-1 is "Yuanyou-1(園優一號)", a cultivar developed in Guangdong (16). The variety of papaya used to develop the TW-lines (16-0-1 and 18-2-4) is the most popular commercial cultivar in Taiwan, "Tainung-2" (台農二號), which is a hybrid of cv. Sunrise and cv. Thailand (20).

4. Donor Organism: PRSV

4.1 Papaya Ringspot Virus (PRSV) is a potyvirus which infects papaya and causes severe damage to the plants. The disease starts with the yellowing and vein clearing in leaves, followed by severe blistering and leaf distortion. The fruits are marked

with dark concentric rings and spots or C-shape tattoos, which may turn tan-brown at the ripening of the fruits. The virus is spread from plant to plant by aphids (28).

4.2 Since PRSV showed considerable geographical variation, and the effectiveness of the protection against the virus conferred by the genetic modification depends very much on the homology of the transgenes and the prevailing PRSV strain (29). Local strains of PRSV had been used to develop the different transgenic lines of PRSV-resistant papaya. The transgenes of 55-1 and 63-1 transgenic lines were developed from PRV HA 5-1, a mild mutant strain derived from PRV HA, a severe strain prevailing in Hawaii (10). The transgene of the two TW- lines, 16-0-1 and 18-2-4, was developed from a severe PRSV strain from Taiwan, PRSV YK (20, 21). The transgene of X17-2 was derived from a Florida isolates of PRSV, H1K (12). The transgene of Huanong-1 was also developed from a strain PRSV Ys native to Guangdong (16). The donor strains of ZS1 and ZS2 were not specifically mentioned.

5. Vector

5.1 **55-1 and 63-1**: The vector used to produce 55-1 and 63-1 was pGA482GG/cpPRV-4, derived from pGA482GG. It contains three plant-expressible genes, namely PRSV coat protein (*PRSV CP*), neomycin phosphotransferase II (*NptII*), and β-glucuronidase (*UidA*) genes, and two tetracycline and gentamycin antibiotic resistance genes, which are expressed in bacteria only. The three plant-expressible genes are flanked by the right- and left- border regions derived from the *Agrobacterium tumefaciens* T-DNA. The expression of the PRSV *CP* gene is controlled by a promoter, a transcription terminator and polyadenylation signal sequences derived from the *35S* transcript of Cauliflower Mosaic Virus (CaMV). The *CP* gene sequences are fused to the 5' untranslated sequence and the first 39 nucleotides from the Cucumber Mosaic Virus coat protein *CMV CP* to enhance translation of the transgene mRNA. The promoter and terminator sequences of the *NptII* gene are derived from the nopaline synthase (*NOS*) gene of *A. tumefaciens*. The expression of *UidA* is controlled by 35S promoter region from CaMV and the NOS 3'-termination region (10, 30, 31).

5.2 **X17-2**: The vector used to produce X17-2 is pBI121fs which was constructed from the binary vector pBI121 (GenBank no. AF485783; Clontech, San Francisco, CA, USA), a derivative of pBIN19 (GenBank no. U09365). It contains two

plant-expressible genes located within the T-DNA region. The expression of the *PRSV CP* gene is controlled by the *35S* promoter from CaMV and the *NOS 3'* untranslated region from *A. tumefaciens*. The transcription of the *PRSV CP* gene is enhanced by the addition of the *UidA* sequence with an initiation codon placed at the 5' terminus of the gene. The expression of *NptII* is controlled by a promoter and a 3' untranslated region derived from the *NOS* gene of *A. tumefaciens* (12, 13).

5.3 **ZS1 and ZS2**: The vector used for the transformation was pRPTW, which was developed from pRoK. The pRPTW harbours a mutated replicase gene (*NIb*) from PRSV, flanked by the CaMV *35S* promoter and ended with the *A. tumefaciens NOS* terminator. The vector also contains the *NptII* gene controlled by the *NOS* promoter and the *NOS* terminator (14).

5.4 **Huanong-1**: The vector used for the transformation was developed from pUC18 (Sino-American Biotech) and pRoKII (Gene Company). The transgene contains the PRSV replicase gene (*NIb*) (GenBank Accession no. AF469604) regulated by the *CaMV 35S* promoter and the *A. tumefaciens NOS* terminator. The exogenous DNA insert also contains the *NptII* gene controlled by the *NOS* promoter and the *NOS* terminator (16, 17, 18).

5.5 **16-0-1 and 18-2-4**: Both TW-lines used the same vector, pBGCP for their transformation. The vector harbours the PRSV CP gene controlled by the CaMV *35S* promoter and the *A. tumefaciens NOS* terminator. The transcription of the *PRSV CP* gene is enhanced by the addition of the *UidA* sequence with an initiation codon placed at the 5' terminus of the gene. The vector also contains the *NptII* gene controlled by the *NOS* promoter and the *NOS* terminator (19, 23).

6. Insert and Modification

6.1 55-1 and 63-1 were produced by biolistic transformation of embryogenic cultures of the papaya cultivar 'Sunset' with DNA-coated tungsten particles (9, 10, 11). The modifications of X17-2, ZS1, ZS2, Huanong-1 and the TW-lines were introduced through *Agrobacterium*- mediated transformation (12, 13, 14, 16, 20).

6.2 55-1 and 63-1 were modified with the same vector but 63-1 lacks the β -glucuronidase (*UidA*) gene which functions as a reporter gene. Moreover, 63-1 has also taken up at least part of the genes for gentamycin and tetracycline resistance into its genome, although it is impossible for them to be expressed at 63-1 because of the

lack of their bacterial promoters (10, 31). Similarly, the two TW-lines share the same vector and they contain the same functional transgenes, but they have different insertion into the host genome as noted in their patent documents (19, 23).

6.3 PRSV Coat Protein - *PRSV CP: PRSV CP* encodes for the coat protein of the PRSV. For 55-1 and 63-1, the PRSV CP sequence was derived from PRSV HA 5-1, which is a mild mutant strain developed from the severe strain PRSV HA from Hawaii (10). For X17-2, the sequence was derived from the Florida isolate PRSV H1K and was inserted with a thymidine residue into its coding sequence to create a non-sense frame-shift to make it untranslatable. However, this insertion was lost subsequently and the sequence has unintentionally become translatable (12, 32). The two TW-lines (16-0-1 and 18-2-4) share the same transgene which contains coat protein encoding gene of the PRSV YK strain from Taiwan (20). These transgenic papaya lines transformed with *PRSV CP* all showed expression of the PRSC coat protein (10, 13, 20).

6.4 PRSV Replicase – *NIb:* In ZS1 and ZS2, the replicase gene is 3' truncated and 5'extended as compared to the original replicase gene (14). The replicase is thought to have polymerase activity. In Huanong-1, the replicase gene is not modified and it is expressed in the host plant (16).

6.5 Neomycin Phosphotransferase II – *NptII:* This is the antibiotic resistance gene from *Escherichia coli* encoding neomycin phosphotransferase II. It acts as the selection marker for screening in the laboratory, as it allows cells successfully introduced with the gene cassette to survive in the presence of the antibiotic kanamycin. It is present in the transgenes of all of the above-mentioned transgenic papaya lines, and the gene was expressed in all of the lines.

6.6 β-glucuronidase *UidA* (synonym: *gusA*): This gene encodes for β-glucuronidase of *E. coli*. It is a hydrolase which catalyses the cleavage of terminal glucuronic acid which is linked to mono-, oligo-, or poly- saccharides or phenols. *UidA* functions as a reporter gene to tell whether the introduced gene cassettes are expressed properly in the cells. It was used in the transformation vector for 55-1 and 63-1, but the gene was not integrated into the genome of 63-1 in the transformation (10, 31).

7. Differences between the Biological Characteristics of the GMO and those of the Recipient Organism

7.1 Because of the introduced *PRSV CP* or *NIb* gene, GM papaya plants have enhanced resistance to PRSV infection. The exact mechanism of the resistance is unknown. A hypothesis is that the expression of the RNA sequence in the cells of the GMO interferes with one of the first steps in viral replication by a process called post-transcriptional silencing (33). The expression of the *NptII* gene confers all GM papayas resistance to the antibiotic kanamycin. 55-1 and its hybridized progenies produce the enzyme *β*-glucuronidase, which allows the detection of the expression of the gene cassettes in the GM plants when the enzyme converts the added colourless compound X-Glu into a blue compound. On the other hand, 63-1 has no *β*-glucuronidase activity as the *UidA* gene was not integrated into its genome (10).

7.2 It was reported that fruit development was faster for X17-2 in winter, probably because of larger leaf area and healthier plants (13, 32). No other difference such as mode of propagation, vegetative vigour, toxicity and allergenicity was reported (10, 16, 22). Field trials of 55-1, 63-1, and the two TW-lines all showed that they are susceptible to other papaya disease and pest like the non-transformed papaya (10, 13, 22).

8. Detection and Identification of the GMO

8.1 All the sequences of the introduced genes are available from the online genome databases. Specific PCR primers can be designed based on the published sequences and PCR can be employed for the detection and identification of the genetic modification with high sensitivity and reliability.

9. Intended Use of the GMO

9.1 The infection by PRSV is a disease of the greatest economic importance to papaya plants and is the limiting factor for commercial production around the world. Conventional methods, such as the use of insecticides against the aphid vectors, disposal of infected plants, quarantine of the infected area, selection for varieties with better disease tolerance and immunization with mild forms of the virus, have been employed to eliminate the disease or to reduce the production loss from the infection.

All of these methods have been proved to be ineffective in eradicating the disease from any region (28). The GM papayas were specially developed to confer resistance to PRSV.

10. Likely Potential Receiving Environment

10.1 The environmental requirements for the growth of GM papayas are the same as those for the parental organism (i.e. non-GM papaya). Hong Kong's environment is suitable for the growth of GM papayas, but it is located far away from the centre of origin of papayas, and there is no native plant species that are belonging to the same genus or family of papayas.

10.2 GM papayas are imported into Hong Kong as food. Some customers would retain the seeds and sow on their backyards or farmlands to grow the GM papayas. Based on AFCD's survey, GM papayas are known to be grown in local backyards and farmlands. About 51% of the home-grown papayas surveyed in 2011-2014 are GM.

11. Identification of any Novel Genotypic and Phenotypic Characteristics Associated with the GMO that may have an Adverse Effect on Biological Diversity in the Likely Potential Receiving Environment

11.1 The following novel genotypic and phenotypic characteristics were identified for
GMO papayas:

Novel Genotypic and Phenotypic Characteristics	**Associated Potential Adverse Effect**
Expression of *PRSV CP*, *NIb*, *NptII* and *UidA* gene	Potential for gene flow to wild relatives of papaya; Potential for horizontal gene transfer; Potential impact on soil microbial diversity
Resistance to PRSV	Potential to become a weed
Production of PRSV proteins, neomycin	Potential production of harmful

phosphotransferase II and β-glucuronidase	substances

12. Evaluation of the Likelihood of the Adverse Effect being Realized

12.1 Potential for gene flow to wild relatives: Gene transfer between GM varieties of papaya plants and wild relatives can take place through interspecific pollination. However, no native species of the Caricaceae family is known to occur in Hong Kong, and its closest related family, Moringaceae does not occur naturally in Hong Kong (34). Since crosses between papaya species with other genera in the same family failed to produce viable offspring, suggesting that inter-family cross is unlikely to be successful (25). As such, it is highly unlikely that other plant species in this region would be susceptible to potential gene contamination from GM papayas because of species barrier. The potential for gene flow of GM papayas to its wild relatives does not exist in Hong Kong.

12.2 Potential for horizontal gene transfer: Horizontal gene transfer is defined as a stable transfer of genetic material from one organism to another without reproduction or human intervention (35). This phenomenon could occur between bacteria and is considered a significant source of genome variation (36). Some concerns have been raised that engineered traits could be transferred to non-target organisms via horizontal gene transfer and thereby threaten environmental and animal safety.

12.3 This topic has received considerable attention from numerous expert panels held under the auspices of various national and regional regulatory systems as well as international bodies such as the Organization for Economic Cooperation and Development, the World Health Organization or the Food and Agriculture Organization. Based on available scientific evidence, horizontal gene transfer from GM plants to other organisms is deemed to be an extremely rare phenomenon and to date no environmental harm as a result has been reported (35, 37, 38, 39, 40, 41).

12.4 It was reported that the soil cultivated with transgenic papaya had increased populations of kanamycin resistant bacteria, actinomycetes and fungi compared with non-transgenic papaya (15). On the other hand, assessment of Huanong-1 has not shown any significant difference in count of bacteria, fungi and actinomycetes between the transgenic and non-transgenic plot (16). Since the soil already

contained kanamycin resistant microbes, there was no conclusive evidence to demonstrate the association of the genetic modification with the increase in the kanamycin resistant microbial populations, which could also be the result of increased total microbial populations in the soil.

12.5 Given the fact that antibiotic resistance genes, often located on mobile genetic elements, are already widespread in bacterial populations and that horizontal gene transfer events from transgenic plants to bacteria are supposed to occur at extremely low frequencies and have not yet been detected under field conditions, it is highly unlikely that antibiotic resistance genes used as markers in transgenic crops would contribute to any spread of antibiotic resistance in bacterial populations (42).

12.6 Potential impact on soil microbial biodiversity: There were reports suggesting that cultivation of transgenic papaya would result in the increase of total microbial populations (15, 43). However, there was no evidence to demonstrate the association of the increase of the microbial populations with the genetic modification. Other influences on the soil, such as the shift of chemical composition, pH, and the shading of the soil from UV sterilization from sunlight etc, could also contribute to the increase in total microbial populations.

12.7 Potential to become a weed: The wild varieties of papaya plants, which do not exist in Hong Kong, have weedy characteristics (prolific seed production, minimal edible flesh and seed dormancy (26, 44, 45). However, these plants cannot persist long in the natural successional cycle and are easily overgrown by vines and forest vegetation (44). Domesticated papaya plants are not considered as weeds because of their higher ratio of edible flesh to seed and a lack of seed dormancy. There is no evidence indicating that PRSV is a limiting factor in the development of escaped population of papaya, as papaya was not considered a weed before PRSV became a limitation to its production. The introduced genes and thus the conferred viral resistance are very unlikely to alter the parent plant's non-weedy characterization, as there is no scientific evidence to suggest that enhanced viral resistance would result in the emergence of a weed pest (10, 13, 16, 31, 32). The potential of GM papayas to become a weedy plant is considered very low.

12.8 Production of harmful substances: GM papayas will produce exogenous proteins due to the insertion of the transgene, but the level of such proteins is far lower than that in non-transgenic papaya infected with PRSV (10, 13). The parental variety of papaya plants is susceptible to PRSV infection and the concentration of the

viral protein in infected papaya fruits was reported to be higher than that accumulated in the GM papaya fruits. Those infected papaya fruits have a long history of safe consumption by both animals and humans. The products of the introduced *NptII* gene and *UidA* gene are not known to pose any risk to the environment (46). No harmful substance is known to be produced as the result of genetic modification by GM papayas.

13. Evaluation of the Consequences should the Adverse Effect be Realised

13.1 Potential for gene flow to wild relatives: As the inserted gene confers the recipient organism (i.e. papaya in this case) resistance to PRSV, it may provide the wild relatives competing advantages over their non-GM varieties. It may result in the reduction in the genetic diversity of the wild relatives. However, as mentioned above, the potential for gene flow of GM papayas to its wild relatives does not exist in Hong Kong.

13.2 Potential for horizontal gene transfer: The transgenes would be transferred to soil microorganisms. However, all of the transgenes come from microorganisms – the genes conferring the resistance (i.e. *CP* and *NIb*) were from the PRSV; *NptII* was isolated from transposon Tn 5 (*E. coli*) and was already widely present in soil microorganisms; *UidA* was also isolated from *E. coli*. Any of the above horizontal gene transfer would naturally take place even without the cultivation of the GM papaya.

13.3 Impact on soil microbial diversity: No adverse effect on the local soil microbial diversity has been identified.

13.4 Potential to become a weed: If GM papayas become weeds, they persist in natural habitats and compete for nutrients and space with other species, which may result in the reduction in the biological diversity of the environment that GM papayas occur. However, as mentioned above, the potential of GM papayas to become a weedy plant is considered very low.

13.5 Potential production of harmful substances: No harmful substance is known to be produced by GM papayas.

14. Estimation of the Overall Risk Posed by the GMO

14.1 Based on the above understanding that the identified potential adverse effects are unlikely to happen or do not exist, it is concluded that GM papayas are unlikely to pose any risk to the biodiversity of the local environment.

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