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Genetically Modified Organisms (Control of Release) Expert Group Meeting

Review of the Exemption of Genetically Modified Papayas

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) discussed the risk assessments of GM papayas in Hong Kong in meetings held in 2011, 2015 and 2018. The risk assessments of GM papayas indicated that they were highly unlikely to pose any risk to the biodiversity of the local environment, and so the possible biosafety effect of GM papayas was deemed acceptable. Besides, in view of the widespread and scattered presence of home-grown papayas of which some were GM, it was considered impractical and highly undesirable for the authority to undertake enforcement against the maintenance of GM papayas in Hong Kong. In consideration of the above, 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 advised the Agriculture, Fisheries and Conservation Department (AFCD) to continue monitoring the latest progress and development of GM papayas and to carry out a review of the exemption of GM papayas regularly for reporting to the Expert Group. The Expert Group also recommended AFCD to continue to step up publicity on GM crops and organic farming to both the general public and the stakeholders. As such, AFCD has been distributing pamphlets on the Ordinance and GM crops to local farmers as well as seed and flower traders.

4. The Genetically Modified Organisms (Control of Release) (Exemption)

Notice took effect on 23 June 2012, and exempted 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 conducted a review of the exemption of GM papayas and consulted the Expert Group in 2015 and 2018. The third round of review has been completed by AFCD, and the results of the review are presented in the ensuing paragraphs.

Type of 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) which is naturally transmitted by aphids. PRSV would cause the appearance of circular spots on fruits and leaf distortion. 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 by inserting the PRSV coat protein genes into the papaya genome, together with an antibiotic resistance gene as a selection marker for screening and an expression reporter gene to confirm the proper expression of introduced gene cassettes. At present, several varieties of GM PRSV-resistant papayas were developed and some of them were commercialised.

8. The PRSV-resistant GM papaya CUH-CP551-8 (55-1 line) was developed and approved for commercial production in Hawaii in 1998. 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 CUH-CP631-7 (63-1 line) and UFL-X17CP-6 (X17-2 line) have also been deregulated by the United States Department of Agriculture (USDA), but they have not yet been commercialised.

9. Four lines of PRSV-resistant papayas (i.e. Huanong-1, ZS-1, ZS-2, 474) were developed in Mainland China but only Huanong-1 was approved for commercial

production in Guangdong Province in 2006. Huanong-1 was also grown in Hainan and Guangxi. It was reported that Huanong-1 showed less resistance to PRSV in recent years. In Taiwan, several virus-resistant GM papaya lines, including double resistance to both PRSV and the Papaya Leaf-distortion Mosaic Virus (PLDMV), have been developed and conditionally approved for field testing since 2003, yet they failed to obtain the final approval in 2015. In Hong Kong, two Taiwan lines of PRSV-resistant GM papaya, 16-0-1 line and 18-2-4 line, have been found among imported and locally grown papayas.

10. In Malaysia, field trial with terms and conditions was approved for delayed ripening transgenic Eksotika papaya since 2013 to reduce post-harvest loss and improve its quality and marketability. On the other hand, Eksotika papaya is also modified for resistance to Papaya Dieback Disease and its field trial with terms and conditions was approved in 2020. Both GM papayas are yet to be commercialised.

GM Papayas in Hong Kong

11. 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 214 imported papayas collected from the local markets in 2018-2022, up to 52% were found to be genetically modified. These imported GM papayas included the Taiwan 16-0-1 line and 18-2-4 line (hereafter TW-lines), Hawaii 55-1 line, and the Mainland China Huanong-1 line. The TW-lines were the dominant GM lines found, which contributed up to 66% of the imported GM papayas in the study period. It was followed by the 55-1 and Huanong-1 which made up 23% and 11%, respectively, of the imported GM papayas (Table 1). 67% of imported GM papayas were imported from Mainland China, of which were derived mainly from the TW-lines (73%), whereas the Huanong-1 line was less common. On the other hand, 25% of GM papayas were imported from US or Hawaii, of which 89% belonged to 55-1 line (Table 2).

Table 1. Percentage of GM papayas among imported papayas sampled in the market from 2018 to 2022, and their major GM lines

Year	Imported Papaya sampled	Percentage GM	Percentage of the major GM lines among the imported GM papaya sampled		
			55-1	Huanong-1	TW-lines
2018-19	57	60%	29%	3%	68%
2019-20	60	67%	23%	25%	53%
2020-21	31	29%	56%	0%	44%
2021-22	66	42%	7%	4%	89%
Total	214	52%	23%	11%	66%

Table 2. Percentage and GM lines of sampled imported GM papayas from Mainland China and US/Hawaii

Year	Percentage of GM papaya among all samples from Mainland China	Percentage of TW-lines among sampled GM papayas from Mainland China	Percentage of GM papaya among all samples from US/Hawaii	Percentage of 55-1 line among sampled GM papayas from US/Hawaii
2018-19	50%	94%	32%	91%
2019-20	90%	47%	23%	89%
2020-21	44%	100%	56%	100%
2021-22	61%	100%	11%	67%
Total	67%	73%	25%	89%

12. While it was not common to find seeds of GM papayas available for sale in local 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 seeds obtained from consumed GM papaya fruits. Since GM papayas often show better growth performance due to their enhanced resistance to viral infection, papayas grown this way are more likely to be genetically modified.

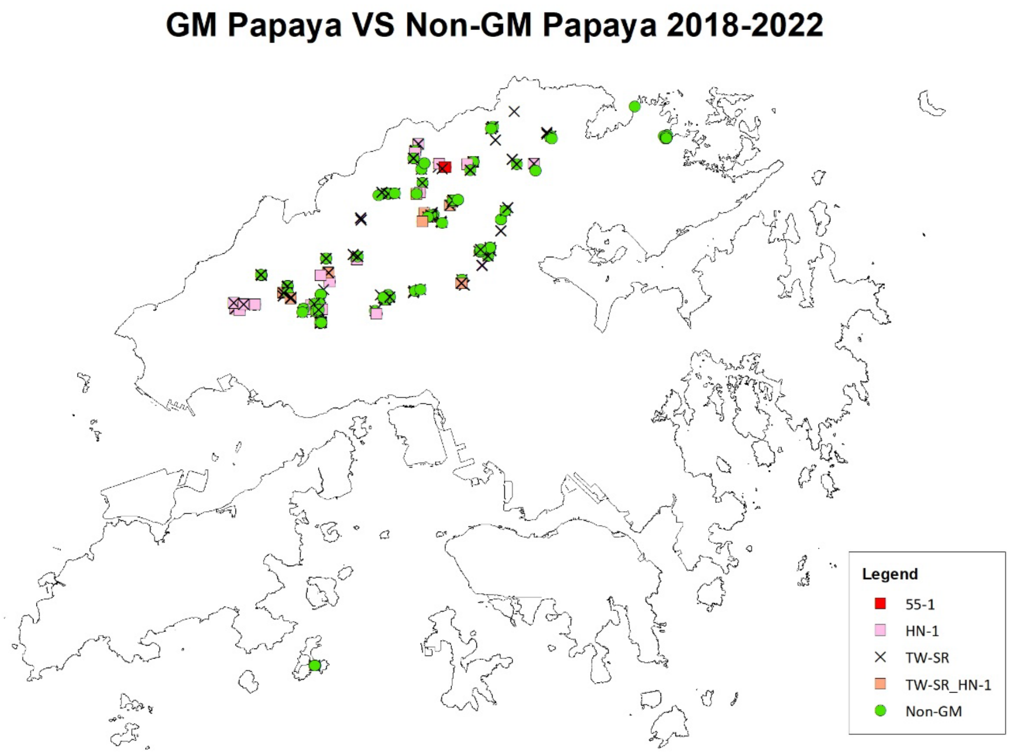
13. During the period 2018 to 2022, AFCD's survey for GMOs had collected 1024 samples of papayas from locally grown papaya plants, amongst which 69% were found to be genetically modified (Table 3). Data showed that among the GM papayas from locally grown papaya plants, 86% were found to be of the TW-lines, followed by

the Huanong-1 (10%) and the 55-1 (0.4%), and TW-lines and Huanong-1 hybrids accounts for about 2%. In general, GM papaya plants were found to be very widespread in the rural areas of Hong Kong, and no specific geographical pattern of the GM strains was observed (Figure 1).

Table 3. Percentage of GM papaya and the major GM lines sampled among the locally grown papayas (from farms and rural areas) during 2018 to 2022.

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
2018-19	254	70%	0%	8%	90%	2%
2019-20	250	73%	0%	14%	80%	5%
2020-21	272	68%	0%	12%	84%	3%
2021-22	248	63%	2%	6%	90%	2%
Total	1024	69%	0.4%	10%	86%	3%

Figure 1. Geographical distribution of non-GM papayas and different lines of GM papayas.



Risk Assessment of Genetically Modified Papayas

14. Risk assessments were undertaken to assess the possible adverse effects of GM papayas on the conservation and sustainable use of biodiversity in the local environment in 2011, 2015 and 2018. There is one more GM line of papaya – papaya modified for resistance to papaya dieback disease. An updated risk assessment was conducted on this new GM line in the present review (**Annex**).

15. The risk assessment looked into potential adverse biosafety effects of the cultivation of GM papayas in Hong Kong, including:

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.

16. As no native species of the Caricaceae family is known to occur in Hong Kong, the potential of gene flow from GM papayas to other wild relatives of the Caricaceae family does not exist. In addition, 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. The potential for GM papayas to become a weed is deemed to be very low, as papaya is a domesticated plant and is easily outgrown by vines and other vegetation. Besides, no harmful substance is known to be produced as a result of genetic modifications of GM papaya plants. As shown in a number of studies, it was very unlikely that horizontal gene transfer from the GM papaya 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 papaya.

Advice Sought

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

Agriculture, Fisheries and Conservation Department
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Annex

Risk Assessment Report

2023

<u>Genetically Modified Papaya</u>

1. Introduction

1.1 The Genetically Modified Organism (GMO) surveys conducted by the Agriculture, Fisheries and Conservation Department (AFCD) during the period from 2018 to 2022 found that genetically modified (GM) papayas were available for sale as food in the local markets and grown in the local environment similar to previous years. About 52% of imported papaya fruits in the local markets and about 69% 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, most GM lines of papayas were developed for resistance against the Papaya Ringspot Virus (PRSV) and one was developed for double resistance (hereafter "DR") against PRSV and the Papaya Leaf-distortion Mosaic Virus (PLDMV), given that the natural self-defence system of the commercialized papaya varieties has been very weak.

2.2 There was a new GM line of Eksotika papaya reported to Biosafety Clearing-House in 2020, modified to have anti-pathogenic characteristics against a phytopathogenic bacteria, *Erwinia mallotivora*, which is also known as the causal agent of Papaya Dieback Disease (1). The risk assessment will focus on analysing the risks to the environment posed by this new GM papaya modified for resistance to Papaya Dieback Disease in the ensuing paragraphs.

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

	Transformation Event Code/GMO	Unique Identifier	Commercial Name	Origin
a)	55-1	CUH-CP551-8	"SunUp", "Rainbow"	Hawaii, US
b)	63-1	CUH-CP631-7	Nil	Hawaii, US

	Transformation Event Code/GMO	Unique Identifier	Commercial Name	Origin
c)	X17-2	UFL-X17CP-6	Nil	US
d)	ZS1	Not available	Nil	Mainland China
e)	ZS2	Not available	Nil	Mainland China
f)	Huanong-1	Not available	Huanong-1	Mainland China
g)	474	Not available	Nil	Mainland China
h)	16-0-1	Not available	Nil	Taiwan
i)	18-2-4	Not available	Nil	Taiwan
j)	Double resistance	Not available	Nil	Taiwan
k)	Transgenic Eksotika papaya	Not available	Nil	Malaysia
l)	Papaya modified for resistance to Papaya Dieback Disease ***(new)***	Not available	Nil	Malaysia

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 (2, 3). It is a soft-wooded perennial plant with a life span of about 5-10 years.

3.2 It is speculated that papaya originates from the lowlands of eastern Central America where wild populations are found (4). It was dispersed to the Caribbean and Southeast Asia by Spanish explorers in the 16^{th} Century. Papaya cultivation is now practised in all tropical countries and many sub-tropical regions of the world.

3.3 Cultivated papaya plants have three forms with respect to sexual reproduction - i) male plants bear only staminate flowers which produce pollen all year round but very rarely set fruit, ii) female plants only bear pistillate flowers, and they rely on pollen from male or bisexual plants to set fruit, and iii) 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 (2, 3). 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 skipper butterflies, thrips, honeybees and hawkmoths (4). The viability of fresh pollens was determined to be around 90%. Papaya stigmas seem to be ready for reception throughout the year and are able to produce fruits even in winter (2).

4. Donor Organism

4.1 *Erwinia mallotivora* is a phytopathogenic bacteria which infects papaya and causes the Papaya Dieback Disease, of which the typical symptoms are greasy, water-soaked lesions and spots on leaves. The lesions can lead to secondary infection, possibly killing the papaya plant eventually (1).

5. Vector

5.1 The vector used to produce papaya modified for resistance to Papaya Dieback Disease is pCAMBIA2301. It contains genes for bacterial and plant selection with kanamycin and the β-glucuronidase (*gusA*) reporter construct. The construct uses *Escherichia coli gusA* with an intron inside the coding sequence to ensure that expression of glucuronidase activity is derived from eukaryotic cells, not from expression by residual *A.tumefaciens* cells. This vector is suitable for insertion of other genes of interest containing their own promoter and terminator (6).

6. Insert and Modification

6.1 The transgenic Eksotika papaya was modified by inserting two acyl-homoserine lactonase genes from *Bacillus cereus* CHB37 and *Bacillus thuringiensis* SP24, which disrupt quorum sensing (bacterial cell-to-cell communication) through the hydrolysis of acyl-homoserine lactone and thus reducing expression of virulence genes and disease symptoms (1).

6.2 **Neomycin Phosphotransferase II – *nptII*** was also inserted into the papaya genome acting 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.

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

7.1 The Eksotika papaya (*Carica papaya* L. var. Eksotika) has been modified to have anti-pathogenic characteristics against *Erwinia mallotivora*. The expression of the *nptII* gene confers all GM papayas resistance to the antibiotic kanamycin.

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 anti-pathogenic characteristic of the transgenic Eksotika papaya prevents infection of Papaya Dieback Disease and thus increases papaya production in Malaysia.

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 is suitable for the growth of GM papayas although it is located far away from the origin of papayas, i.e. Central America. There is no native plant species that are belonging to the same genus (*Carica*) or family (Caricaceae) of papayas, so native plants are not likely to be genetically polluted.

10.2 GM papayas are imported into Hong Kong for direct consumption 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 69% of the home-grown papayas surveyed in the period 2018 to 2022 are GM, nevertheless, a general downward trend in the percentage of GM papayas out of all home-grown papayas surveyed (from 70% to 63%) was observed in recent years.

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 assessed:

Novel Genotypic and Phenotypic Characteristics	Associated Potential Adverse Effect
Expression of *nptII* gene and acyl-homoserine lactonase gene	Potential for gene flow to wild relatives of papaya; Potential for horizontal gene transfer; Potential impact on soil microbial diversity
Production of phosphotransferase II	Potential production of harmful substances

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

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 (7). 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 (8). 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 (9). 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 (10).

12.3 **Potential impact on soil microbial biodiversity:** There were reports suggesting that cultivation of transgenic papaya would result in the increase of total microbial populations (11). However, there was no evidence to demonstrate the association of the increase of the microbial populations with the genetic modification.

12.4 **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) (12, 13, 14). However, these plants cannot persist long in the natural successional cycle and are easily overgrown by vines and forest vegetation (13). Domesticated papaya plants are not considered as weeds because of their higher ratio of edible flesh to seed and a lack of seed dormancy. 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. The products of the introduced *nptII* gene is not known to pose any risk to the environment (15). 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 on the recipient organism (i.e. papaya in this case) reduction in expression of virulence genes and resistance to kanamycin, 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 from 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, *nptII* was isolated from transposon Tn 5 (*E. coli*) which was already widely present in soil microorganisms. Any of the above horizontal gene transfer would naturally take place even without the cultivation of the GM papaya. The risk of horizontal gene transfer of marker genes to soil micro-organisms was examined and rated as negligible (16).

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:** 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 the GM papaya is unlikely to pose any risk to the biodiversity of the local environment.

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