For Discussion on **Discussion Paper GMO 03/2011**
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Genetically Modified Organisms (Control of Release) Ordinance Cap. 607

Expert Group

Risk Assessment and Disposal of Genetically Modified Papayas

Purpose

This paper briefs members on the current status of genetically modified (GM) papayas in Hong Kong and their possible adverse effect on the local biodiversity, and invite members' view on the recommendation for the disposal of the GM papayas.

Background

2. To understand the prevalence of genetically modified organisms (GMOs) in the territory, AFCD has been conducting surveys for the presence of GMOs in various imported and locally grown crop produces since 2008. It was found that most of the crop produces examined were found to be non-transgenic. However, about 30% of imported papaya fruits from the local markets and about 50% of the home-grown/locally produced papayas were found to be genetically modified.

GM Papaya

3. Papaya (*Carica papaya*) is a soft-wooded perennial plant of the Caricaceae family. It is believed to be originated from eastern Central America and dispersed to the Caribbean and South-east Asia by Spanish explorers in the 16th Century. Papaya cultivation is now practiced in all tropical countries as well as many sub-tropical regions of the world.

4. Papaya Ringspot Virus (PRSV) is one of the major limiting factors for commercial production of papaya in many parts of the world. 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.

5. GM papaya was developed by inserting the PRSV genes into the papaya genome. The introduced gene expression cassette also included an antibiotic (Kanamycin) resistance gene and an expression reporter gene. At present, there are two GM varieties of papaya (i.e. "SunUp" and "Rainbow") available in the international food markets. The two GM varieties of papaya were commercialized in 1998 and now contribute to over 90% of papaya fruit production in Hawaii. Three other GM varieties (i.e. "CUH-CP631-7", "UFL-X17CP-6" and "Laie Gold") were also deregulated by the United States Department of Agriculture. However, they are either not commercialized or only available in the US markets. Besides, three varieties of PRSV-resistant papayas were developed in China but only one of the varieties (i.e. Huanong-1) was approved for commercial production.

6. Although the seeds of transgenic papayas are not readily available for sale in the international seed markets, they could be obtained from the imported fruits. As Hong Kong's climate is suitable for papaya cultivation, it is quite common for people living in the rural areas to grow papaya in their backyards or along the edges of farmlands. It is believed that the GM papayas found in the surveys were grown from the seeds obtained from the consumed GM papaya fruits, which are a popular choice for planting because of their better appearance and growth performance due to their enhanced resistance to viral infection, resulting in the prevalence of transgenic papaya plants in Hong Kong.

Risk Assessment

7. Due to the prevalence of GM papaya growing in the local environment, a risk assessment was undertaken to assess the possible adverse effect of GM papayas on the conservation and sustainable use of biological diversity in the local environment. The potential adverse biosafety effects of the cultivation of GM papayas in Hong Kong would include gene flow to wild relatives of papaya, potential to become a weed, production of harmful substances, horizontal gene transfer and impact on soil microbial diversity. The risk assessment indicated 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 is not expected. 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 vegetation. 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 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.

8. Based on the risk assessment, it is thus concluded that GM papayas are unlikely to pose any adverse biosafety effect on the biological diversity of the local environment. The detailed risk assessment report is attached at Annex.

Disposal

11. Under the Ordinance, during the transitional period (i.e. from 1 March 2011 to 31 August 2011), a person is not prohibited from knowingly maintaining the life of a released GMO that is in a state of being released into the environment. However, the person must, before that period expires, inform the Director of Agriculture, Fisheries and Conservation (the Director) of the maintenance by written notice or submit a GMO approval application. On the other hand, after the expiry of the transitional period, a person must not knowingly release an unapproved GMO or maintain the life of a released unapproved GMO. Anyone who has control of a GMO knows that the GMO is unapproved and has been released into the environment shall also inform the Director of the release. Besides, the Ordinance provides for the disposal of GMOs that the Director may either direct an authorized officer to dispose of the GMO or direct the responsible person to dispose of the GMO. Contravention of the above requirements may be liable to prosecution under the Ordinance.

12. At present, GM papayas are not approved or exempted GMOs. Therefore, the above restrictions apply to GM papayas. However, the risk assessment undertaken for GM papayas indicates that they are unlikely to pose any risk to the biological diversity of the local environment. In other words, the possible biosafety effect of GM papayas is deemed acceptable. On the other hand, in view of the widespread and scattered presence of home-grown papayas in the territory, it is considered impractical and highly undesirable for the authority to undertake enforcement against the maintenance of the released GM papayas.

13. Section 46 of the Ordinance empowers the Secretary for the Environment to exempt any GMO from the application of section 5 (Restrictions on release into environment and maintenance of lives of GMOs), 7 (Restrictions on import of GMOs

intended for release into environment) or 23 (Restrictions on export of GMOs intended for release into environment) if the Secretary is satisfied that the possible adverse biosafety effect that may result from the exemption is acceptable or manageable.

14. Considering the above, it is recommended to grant exemption to GM papayas from the application of section 5 and 7 of the Ordinance. Once exempted, any person maintaining or growing GM papayas does not need to seek approval from or notify the Director.

Advice Sought

15. Members are invited to note the detail risk assessment report of GM papayas at the Annex and comment on the proposed exemption of GM papayas under the Ordinance.

Agriculture, Fisheries and Conservation Department
June 2011

Annex

Risk Assessment Report

<u>Genetically Modified Papayas</u>

Purpose

Based on the Genetically Modified Organism (GMO) surveys conducted by the Agriculture, Fisheries and Conservation Department during the period from 2008 to 2010, genetically modified (GM) papayas were found to be available for sale as food in the local markets and grown in the local environment. Of a total of 145 samples examined, about 30% of imported papaya fruits in the local markets and about 50% of the home-grown papaya were found to be transgenic. Due to the prevalence of GM papaya in the local environment, a risk assessment was undertaken to assess the possible adverse biosafety effect of GM papayas on the local environment.

This risk assessment report was prepared in accordance with Schedule 3 of 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.

Identities of the GMOs

At present, it is known that five GM varieties of papayas were developed in the United States and three GM varieties of papayas were developed in China. Three of the varieties developed in the United States (i.e. "SunUp", "Rainbow" and "Laie Gold") are commercially grown in Hawaii and a few other places in the United States. The remaining two non-commercialized varieties (i.e. "CUH-CP631-7" and "UFL-X17CP-6") 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. In China, the GM papaya varieties, ZS1 and ZS2, were approved for field trials while Huanong-1 was approved for commercial production in Guangdong. The identities of these GM papayas are as follows:

(i) Transformation Event Code: 55-1
Unique Identifier: CUH-CP551-8

Commercial Name: “SunUp”
Common Name: Hawaii papaya

(ii) Transformation Event: 55-1 x non-transgenic variety “Kapoho”
Unique Identifier: Not available
Commercial Name: “Rainbow”
Common Name: Hawaii papaya

(iii) Transformation Event: “Rainbow” x non-transgenic variety “Kamiya”
Unique Identifier: Not available
Commercial Name: “Laie Gold”
Common Name: Hawaii papaya
(Note: available in the US markets only)

(iv) Transformation Event Code: 63-1
Unique Identifier: CUH-CP631-7
Commercial Name: Nil
Common Name: Papaya

(v) Transformation Event Code: X17-2
Unique Identifier: UFL-X17CP-6
Commercial Name: Nil
Common Name: Papaya

(vi) Transformation Event Code: ZS1
Unique Identifier: Not available
Commercial Name: Nil
Common Name: Papaya

(vii) Transformation Event Code: ZS2
Unique Identifier: Not available
Commercial Name: Nil
Common Name: Papaya

(vi) Transformation Event Code: Not available
Unique Identifier: Not available
Commercial Name: Huanong-1
Common Name: Papaya

Recipient Organism

The recipient organism used for the genetic modification was *Carica papaya*, which is commonly called papaya and belongs to the Caricaceae family (1). It is a soft-wooded perennial plant with life span of about 5-10 years (2) and can grow up to 10 metres (3).

It is speculated that papaya originates from the lowlands of eastern Central America (3) and was dispersed to the Caribbean and South-east Asia by Spanish explorers in the 16th Century (4). 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 21ºC and 33ºC (1).

Papaya plants have two mating systems. They are either dioecious (having male and female flowers on different individuals) or gynodioecious (containing individuals that produce bisexual flowers and plants that produce only female flowers) (1). While pistillate plants of gynodioecious papaya are obligate out-crossers, hermaphrodites (bearing flowers that have both male and female reproductive organs) can out-cross occasionally. This is because staminate flowers of hermaphrodites continuously produce pollens during most of the year. Pollination is mediated by wind or insects, such as honeybees and hawkmoths (5).

The viability of fresh pollens was determined to be around 90% (5). But it was reduced to about 45% (even 4.5% in some lines) in winter. Pollen viability was found to be significantly affected by extremely high humidity or low temperature (5, 6). On the other hand, papaya stigmas seem to be ready for reception throughout the year and are able to produce fruits even in winter (5).

The Caricaceae family comprises five genera and 31 species. *Carica* is a monotypic genus and *Carica papaya* is the only extant species of the genus. In the early efforts to improve papaya, the plant was inter-crossed with relative species from other Caricaceae genera to acquire the traits of interest. However, the attempts gave rise to non-viable seeds (7, 8). Even with the closest relatives *Vasconcellea* spp., further development of the hybrid embryos was terminated by abortion of fertilized ovules or endosperm failure (9). The results of these attempts suggested the reproductive isolation of *Carica papaya* from the rest of the family (1).

"Sunset", which is gynodioecious, is the parental variety used to develop the transgenic lines 55-1 and 63-1. The variety of papaya used to develop X17-2 is "F65", which was a breeding selection obtained by a grower in Florida (10). The parental plant of ZS1 is "Suizhonghong", a yellow-fleshed papaya and the parental plant of ZS2 is "Sunrise", a red-fleshed papaya (18). The information about the parental variety of Huanong-1 is not available.

Donor Organism

The donor organism is Papaya Ringspot Virus (PRSV) which is a Potyvirus. PRSV infects papaya and causes severe damage to the plant. 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 (11). The virus is spread from plant to plant by aphids. Many species of aphids are capable of transmitting the virus and it takes only a few seconds of feeding time for an aphid to acquire the virus on its mouthpart. It spreads the virus to other plants during brief feeding probes. Other insects do not spread PRSV and the virus does not survive in soil or dead plant materials (12).

Vector

55-1 (and its hybridized progenies: Rainbow and Laie Gold) and 63-1

The vector used to produce 55-1 and 63-1 was pGA482GG/cpPRSV-4. It contains three plant-expressible genes (PRSV CP, *NptII*, and *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 (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 (13).

X17-2

The vector used to produce X17-2 is pBI121fs, which 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*.

ZS1 and ZS2

The vector used for the transformation is pRPTW, which harbours the mutated replicase gene (*NIb*) from PRSV, flanked by the 35S promoter and ended with the NOS terminator (18). The vector also contains the *NptII* gene controlled by the NOS promoter and the NOS terminator.

Huanong-1

The GM papaya was developed by the introduction of the PRSV replicase gene (*NIb*)

(19). The *NIb* gene is regulated by the 35S promoter and the NOS terminator. The exogenous DNA insert also contains an *NptII* gene cassette.

Insert and Modification

55-1 and 63-1 were produced by biolistic transformation of embryogenic cultures of the papaya cultivar 'Sunset' with DNA-coated tungsten particles. The modification of X17-2 was introduced through *Agrobacterium*-mediated embryo transformation (14).

The R_0 generation of 55-1, a red-fleshed female, was outcrossed with the non-transgenic "Sunset" and the R_1 progeny was self-pollinated. Homozygous R_4 were designated "SunUp" and crossed with non-transgenic "Kapoho", the yellow-fleshed Hawaiian industry standard. The resulting hybrid was designated as "Rainbow". "Laie Gold" is a cultivar obtained from the crossing between "Rainbow" and the large-fruited "Kamiya" (14).

The information about the methods of modification for ZS1, ZS2 and Huanong-1 is not available.

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 (15). For X17-2, the sequence was derived from the Florida isolate H1K of the PRSV and was inserted with a thymidine residue into its coding sequence to create a non-sense frame-shift. However, the frame-shift was corrected after the 5th generation (13).

PRSV *NIb*

In ZS1 and ZS2, the replicase gene is 3' truncated and 5'extended (20). The replicase is thought to have polymerase activity.

NptII

This is the antibiotic resistance gene from *Escherichia coli* encoding neomycin phosphotransferase II. It acts as the selective marker that allows cells introduced with the gene cassette to survive in the presence of the antibiotic kanamycin.

***UidA* (synonym: gusA) gene**

This gene encodes for β-glucuronidase of *E. coli*. It is a hydrolase which catalyzes the cleavage of terminal glucuronic acid which is linked to mono-, oligo-, or polysaccharides or phenols. *UidA* functions as a reporter gene to tell whether the introduced gene cassettes are expressed properly in the cells.

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

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 sequence in the cells of the GMO interferes with one of the first steps in viral replication (13). The expression of the *NptII* gene confers all GM papayas resistance to the antibiotic kanamycin. Both 55-1 (and its hybridized progenies) and X17-2 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, although the vector used to transform 63-1 was the same one used to transform 55-1, 63-1 has no β-glucuronidase enzyme activity as the *UidA* gene was not integrated into its genome.

It was reported that fruit development was faster for X17-2 in winter, probably because of larger leaf area and healthier plants (13). No other difference such as mode of propagation, vegetative vigour, toxicity and allergenicity was reported.

Detection and Identification of the GMO

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.

Intended Use of the GMO

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 (16). 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 (1). The GM papayas were specially developed to confer resistance to PRSV.

Likely Potential Receiving Environment

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.

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 50% of the home-grown papayas are genetically modified.

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

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* gene and *UidA* gene	Potential for gene flow to wild relatives of papaya; Potential for horizontal gene transfer; Impact on soil microbial diversity
Resistance to PRSV	Potential to become a weed
Production of PRSV proteins, neomycin phosphotransferase II and β-glucuronidase	Production of harmful substances

Evaluation of the Likelihood of the Adverse Effect being Realized

Potential for gene flow to wild relatives

Gene transfer can take place between non-GM varieties of papaya plants through intraspecific pollination. On the other hand, no native species of the Caricaceae family is known to occur in Hong Kong. It is highly unlikely that other plant species 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.

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 (21). This phenomenon could occur between bacteria and is considered a significant source of genome variation (22). 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.

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 (21, 23, 24, 25, 26, 27).

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 (17). However, 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.

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 (28).

Impact on soil microbial biodiversity

There were reports suggesting that cultivation of transgenic papaya would result in the increase of total microbial populations (17, 29). 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.

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).

However, these plants cannot persist long in the natural successional cycle and are easily overgrown by vines and forest vegetation (16). 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 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 (13).

The potential of GM papayas to become a weedy plant is considered very low.

Production of harmful substances

GM papayas will produce PRSV proteins due to the insertion of the PRSV genes. 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 (13). The products of the introduced *NptII* gene and *UidA* gene are not known to pose any risk to the environment.

No harmful substance is known to be produced as the result of genetic modification by GM papayas.

Evaluation of the Consequences should the Adverse Effect be Realized

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.

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.

Impact on soil microbial diversity

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

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.

Production of harmful substances

No harmful substance is known to be produced by GM papayas.

Estimation of the Overall Risk Posed by the GMO

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 biological diversity of the local environment.

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Agriculture, Fisheries and Conservation Department

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