

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

**Expert Group**

**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) 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 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 in three years' time for reporting to the Expert Group. Furthermore, AFCD was advised to carry

out a survey on the distribution profile of GM papayas in the territory, including the sampling of papaya seeds in seed or flower shops. 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. 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 on 8 May 2015. The second 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 GM 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, and it now contributes to

about 85% 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 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 China but only Huanong-1 has been 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.

10. In Taiwan, several virus-resistant GM papaya lines, including double resistance to both PRSV and the Papaya Leaf-distortion Mosaic Virus (PLDMV), have also been developed and conditionally approved for field testing since 2003, yet they failed to obtain the final approval from Council of Agriculture 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 (see paragraphs below).

11. Apart from developing virus-resistant papaya, field trial with terms and conditions was approved for delayed ripening transgenic Eksotika papaya in Malaysia since 2013 to reduce post-harvest loss and improve its quality and marketability. However, the GM papaya is still under experiments and yet to be commercialised.

### **GM Papayas in Hong Kong**

12. 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 158 imported papayas collected from the local markets in 2015-2018, up to 77% were found to be GM. 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 China Huanong-1 line. The TW-lines were the dominant GM lines found, which contributed up to 63% of the imported GM papayas in the study period. It was followed by the 55-1 and Huanong-1 which made up 35% and 2%, respectively, of the imported GM papayas (Table 1). 51% GM papayas imported from Mainland

China were derived mainly from the TW-lines (89%), whereas the Huanong-1 line was less common. On the other hand, 29% of GM papayas were imported from US or Hawaii, of which 91% belonged to 55-1 line (Table 2). All papaya samples from the Philippines in 2017-2018 were tested as GM-free. Almost all the GM papayas from Thailand and all the GM papayas from Malaysia belonged to the TW-lines and only one GM papaya from Thailand belonged to 55-1. On the other hand, all inspected papayas at Man Kam To in 2015-2018, imported for direct consumption as food, feed or for processing (FFP), were tested to be GMOs (Table 3).

Table 1. Percentage of GM papayas and the major GM lines sampled in the market from 2015 to 2018.

Year	Papaya sampled	Percentage of GM papaya	Percentage of the major GM lines among the sampled GM papaya		
			55-1	Huanong-1	TW-lines
2015-16	50	68%	32%	0%	68%
2016-17	65	89%	40%	2%	59%
2017-18	43	70%	30%	3%	67%
Total	158	77%	35%	2%	63%

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

Year	Percentage of GM papaya among all samples from China	Percentage of TW-lines among sampled GM papayas from China	Percentage of GM papaya among all samples from US/Hawaii	Percentage of 55-1 line among sampled GM papayas from US/Hawaii
2015-16	52%	100%	22%	100%
2016-17	52%	88%	40%	88%
2017-18	49%	76%	21%	89%
Total	51%	89%	29%	91%

Table 3. Percentage of imported papayas inspected at Man Kam To from 2015-2018.

Year	Papaya inspected	Percentage of GM papaya	Percentage of the major GM lines among the inspected papayas	
			TW-lines	Hybrids
2015-16	9	100%	100%	0%
2016-17	2	100%	100%	0%
2017-18	16	100%	94%	6%
Total	27	100%	96%	4%

13. Only one papaya seed sample was collected among the inspected seed and flower shops in the GMO Market Survey 2017-2018. It was found to be GM with the TW-lines. After receiving the written notification, the shop owner immediately stopped selling and destroyed the remaining and small stock of GM papaya seeds.

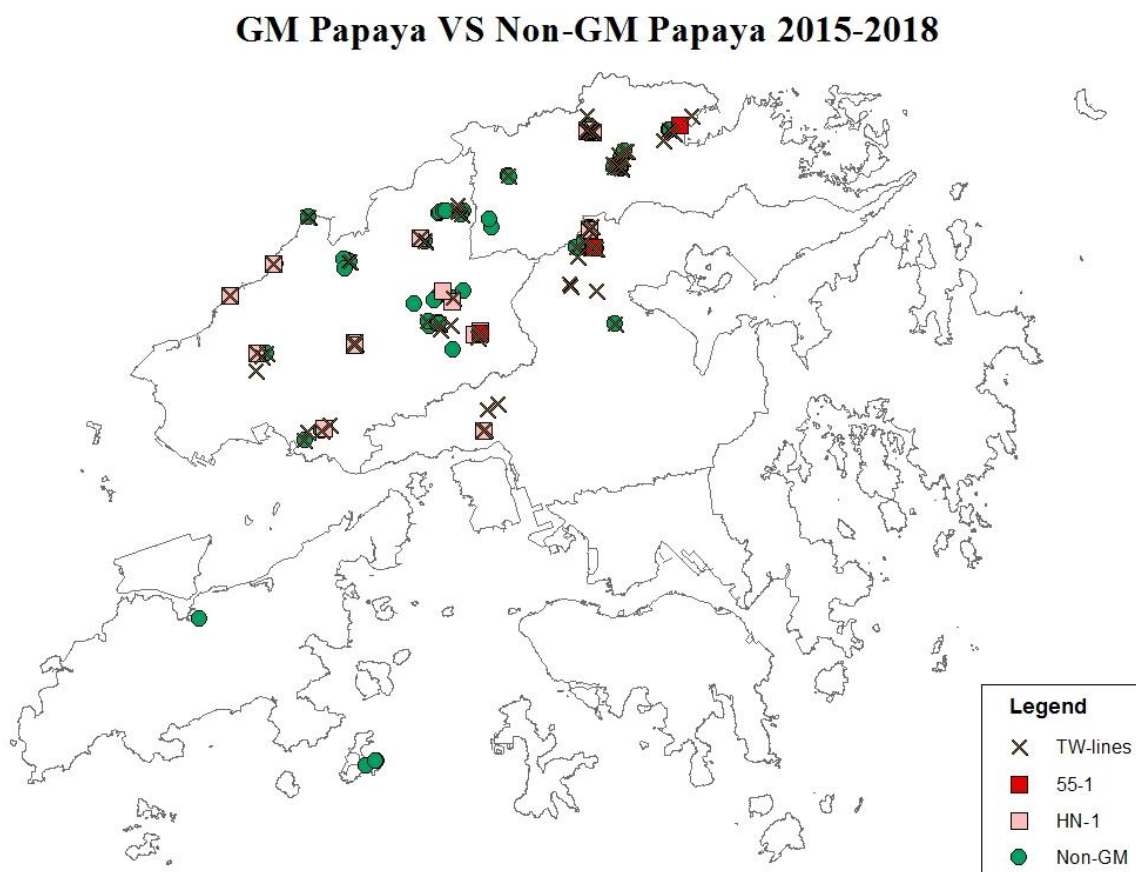
14. While it was not common to find seeds of GM papayas on sale in the markets, it is quite common for people living in the rural areas to grow papayas in their backyards or along the edges of farmlands from seeds obtained from consumed GM papaya fruits. Since GM papayas often show better growth performance due to their enhanced resistance to viral infection, papayas grown this way are more likely to be GM.

15. In 2015-2018, AFCD's survey for GMOs has collected over 748 samples of papayas from locally grown papaya plants, amongst which 55% were found to be GM (Table 4). 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. Data from 2015-2018 show that the TW-lines were most common, making up 89% of the locally grown GM papaya plants, followed by the Huanong-1 (8%), 55-1 (2%) and hybrids (1%) between them. Geographically, GM papaya plants have been found to be very widespread over the rural areas of Hong Kong especially for the TW-lines, and no specific geographical pattern of the GM strains was observed (Figure 1).

Table 4. Percentage of GM papaya and the major GM lines sampled among the locally grown papaya (from farm and rural area) from 2015 to 2018.

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
2015-16	43	56%	0%	17%	79%	4%
2016-17	135	64%	6%	15%	79%	0%
2017-18	570	53%	1%	6%	93%	0%
Total	748	55%	2%	8%	89%	1%

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



## **Risk Assessment of Genetically Modified Papayas**

16. 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 and 2015. An updated risk assessment was conducted as part of the present review (Annex), which additionally included three more GM lines of papaya – i) 474, ii) Double Resistance, and iii) Transgenic Eksotika papaya. 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.

17. 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.

18. As no native species of the Caricaceae family is known to occur in Hong Kong, 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. 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. 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**

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

**Agriculture, Fisheries and Conservation Department**  
**April 2018**



**Risk Assessment Report**  
**2018**  
**Genetically Modified Papayas**

## **1. Introduction**

1.1 The Genetically Modified Organism (GMO) surveys conducted by the Agriculture, Fisheries and Conservation Department (AFCD) during the period from 2015 to 2018 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 77% of imported papaya fruits in the local markets and about 55% 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 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).

2.3 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). Recently, a new transgenic papaya line 474 has been developed and undergoing field trials in Hainan where papaya is believed to be susceptible to PRSV infection (9, 10). 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. In addition, PLDMV is considered as a serious threat to papaya production even though papaya is genetically modified to be PRSV-resistant. As a result, the DR was developed to confer resistance against PRSV and PLDMV in Taiwan (11). Despite testing completion, it failed final approvals by Council of Agriculture (12).

2.4 There is also one GM line of papayas developed and approved for field trials in Malaysia and the Philippines for delayed ripening but yet to be commercialized. It is named as “transgenic Eksotika papaya” and its transformation event code is unknown.

2.5 A summary of the eleven GM papayas is in the table below.

	Transformation Event Code/GMO	Unique Identifier	Commercial Name	Origin	References
a)	55-1	CUH-CP551-8	“SunUp”, “Rainbow”	Hawaii, US	1, 2, 13, and 14
b)	63-1	CUH-CP631-7	Nil	Hawaii, US	1, 2, 13, 14, and 15
c)	X17-2	UFL-X17CP-6	Nil	US	6, 16, and 18
d)	ZS1	Not available	Nil	Mainland China	7, 18 and 19
e)	ZS2	Not available	Nil	Mainland China	7, 18 and 19
f)	Huanong-1	Not available	Huanong-1	Mainland China	8, 20, 21, and 22
g)	474	Not available	Nil	Mainland China	9
h)	16-0-1	Not available	Nil	Taiwan	23, 24, 25 and 26
i)	18-2-4	Not available	Nil	Taiwan	23, 24, 25 and 27
j)	Double resistance	Not available	Nil	Taiwan	11, 12

k)	Transgenic Ekstotika papaya	Not available	Nil	Malaysia	28, 29, 30, 31
----	--------------------------------	---------------	-----	----------	----------------

### 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 (32, 33). 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 (34). It was dispersed to the Caribbean and Southeast Asia by Spanish explorers in the 16<sup>th</sup> 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 °C and 33 °C (32, 33).

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 (29, 32, 33). 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 (34). 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 (32).

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 (36, 37). In the early efforts to improve papaya, the plant was inter-crossed with woody highland species from another Caricaceae genus, *Vasconcellea*, but the hybrids were mostly not viable in the field (33).

3.6 “Sunset”, which is gynodioecious, is the parental variety used to develop the transgenic lines 55-1 and 63-1 (1, 15, 16). The variety of papaya used to develop X17-2 is “F65” obtained from Taiwan (16). 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 (18), whereas that of Huanong-1 is “Yuanyou-1(园优一号)”, a cultivar developed in Guangdong (20). The variety of papaya used to develop the TW-lines (16-0-1 and 18-2-4) and the DR is the most popular commercial cultivar in Taiwan, “Tainung-2” (台農二號), which is a hybrid of cv. Sunrise and cv. Thailand (11, 24). The variety of GM papaya developed in Malaysia is Eksotika, which is a backcross breeding of Hawaii “Sunrise” and locally adapted “Subang 6” (38).

#### **4. Donor Organisms: PRSV and PLDMV**

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 (39).

4.2 Since PRSV showed considerable geographical variation, and the effectiveness of the protection against the virus conferred by the genetic modification depends on the homology of the transgenes and the prevailing PRSV strain (40). 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 produced no conspicuous symptoms on papaya, a severe strain prevailing in Hawaii (14). The transgene of X17-2 was derived from a Florida isolate of PRSV, H1K (16). On the other hand, the transgene of Huanong-1 was also developed from a strain PRSV Ys, native to

Guangdong (20), while the donor strains of ZS1, ZS2 and 474 were not specifically mentioned. 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 (24, 25).

4.3 Papaya Leaf-Distortion Mosaic Virus (PLDMV) is another potyvirus that leads to severe symptoms of leaf-narrowing, distortion and stunting in papaya. It was first recorded in Okinawa, Japan and its scattered occurrence and close genetic relationship of the PLDMV isolates was later found in Taiwan (11, 42).

4.4 It has been reported that PRSV-resistant transgenic papaya lines were susceptible to the strain P-TW-WF of PLDMV in Taiwan, so DR was developed to protect the papaya against both PRSV and PLDMV (11, 42). It was found that back-crossed and hybrid progenies generated from the PRSV highly resistant lines 10-4 and 12-4 showed complete resistance to PLDMV P-TW-WF and PRSV YK. The hybrid progenies were claimed to be evaluated under field tests for commercial merits (11)

## 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  $\beta$ -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 (14, 41, 44).

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* (16, 17).

**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 (*Nlb*) 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 (18).

**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 (*Nlb*) (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 (20, 21, 22).

**5.5 474:** The vector pUCCRNAi first harbours the *PRSV CP* fragments to form hairpin structure with an intron, and the constructed CP hairpin structure was then inserted into the binary vector, pCAMBIA2300, to form the plant expression RNAi vector, regulated by the *CaMV 35S* promoter and the octopine synthase (*OCS*) terminator. The vector also contains the *nptII* gene controlled by the *CaMV 35S* promoter and the *NOS* terminator (9).

**5.6 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 (23, 26).

**5.7 Taiwan Double-resistance:** The vector pPLDMVCP was used to amplify the strain R-TW-WF of *PLDMV CP* and it then cloned into PCRII-TOPO to generate pPLDMVstop of which the *NcoI/SmaI* fragment was ligated to the vector pBGCP

harbouring the transgene *PRSV CP*. The final construct contains the *nptII* gene controlled by the *CaMV 35S* promoter and the *NOS* terminator (11)

**5.8 Transgenic Eksotika papaya:** The vector, pASACO2E1, was derived from pGA643 and used to harbour the aminocyclopropanecarboxylate oxidase (ACO2) in an antisense orientation, at the 3' untranslated regions regulated by the *CaMV 35S* promoter, to suppress the endogenous ACO2 production. The vector also contains the *nptII* gene controlled by the *NOS* promoter and the *NOS* terminator (28).

## 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 (14, 15, 16) whereas the Hainan transgenic 474 line was produced via particle bombardment of embryogenic callus tissue from seeds of the papaya cultivar "Sunrise" (9). The modifications of X17-2, ZS1, ZS2, Huanong-1 and the TW-lines and transgenic Eksotika papaya were introduced through *Agrobacterium*-mediated transformation (16, 17, 18, 20, 24, 28, 30, 32).

6.2 55-1 and 63-1 were modified with the same vector but 63-1 lacks the *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 (11, 14). 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 (23, 26).

6.3 The transgenic Eksotika papaya was modified by inserting a transgene composed of an antisense portion of ACO2 coding sequence which suppresses the endogenous ACO2 enzyme to inhibit the production of ethylene gas, resulting in delayed fruit ripening (28, 30).

6.4 **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 (14). 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 (16, 42). In Haiwan, 474 line was produced to develop a broader-spectrum transgenic resistance by targeting the conserved domain of the *PRSV CP* gene (9). 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 (24). These transgenic papaya lines transformed with *PRSV CP* all showed expression of the PRSV coat protein (14, 17, 24).

**6.5 PRSV Replicase – *Nib*:** In ZS1 and ZS2, the replicase gene is 3' truncated and 5' extended as compared to the original replicase gene (18). 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 (20).

**6.6 PLDMV Coat Protein – *PLDMV CP*:** *PLDMV CP* encodes for the coat protein of the PLDMV. The *PLDMV CP* sequence, designated as P-TW-WF, which is serologically distinct from PRSV was derived from PRSV YK CP gene (11).

**6.7 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.

**6.8  $\beta$ -glucuronidase – *uidA* (synonym: *gusA*):** This gene encodes for  $\beta$ -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 (14, 43).

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

7.1 Because of the introduced *PRSV CP*, *PLDMV CP* or *Nib* gene, GM papaya plants have enhanced resistance to PRSV and PLDMV 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 gene silencing (44, 46). The expression of the *nptII* gene confers all GM papayas resistance to the antibiotic kanamycin. 55-1 and its hybridized progenies produce the enzyme  $\beta$ -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  $\beta$ -glucuronidase activity as the *uidA* gene was not integrated into its genome (14).

7.2 It was reported that fruit development was faster for X17-2 in winter, probably because of larger leaf area and healthier plants (17, 42). No other difference such as mode of propagation, vegetative vigour, toxicity and allergenicity was reported (14, 20, 26). 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 (14, 17, 26).

7.3 The transgenic Eksotika papaya would delay the ripening process for 8 – 20 days due to the suppression of ACO2 (28, 31). It was reported that the two major problems of the transgenic Eksotika papaya were the low rooting efficiency of regenerated shoots and low acclimatization rate of rooted transgenic papaya plants in the field (30).

## **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 or PLDMV 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 (32). The GM papayas were mostly developed to confer resistance to PRSV.

9.2 The delayed ripening characteristic of the transgenic Eksotika papaya is to extend the shelf life or storability of the fruit, allowing more time for import and export as well as for sale.

## **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 55% of the home-grown papayas surveyed in 2015-2018 are GM. The trend remains steady over the 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 papayas:

Novel Genotypic and Phenotypic Characteristics	Associated Potential Adverse Effect
Expression of <i>PRSV CP</i> , <i>PLDMV CP</i> , <i>Nlb</i> , <i>nptII</i> and <i>uidA</i> gene	Potential for gene flow to wild relatives of papaya; Potential for horizontal gene transfer; Potential impact on soil microbial diversity
Resistance to PRSV and PLDMV	Potential to become a weed
Production of PRSV proteins, neomycin phosphotransferase II and $\beta$ -glucuronidase	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 (47). 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 (29). 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 (48). This phenomenon could occur between bacteria in the environment or the digestive tract and is considered a significant source of genome variation (49). 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. But no finding has been reported on genetic contamination of GM papayas and its risks via bacteria.

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 (48, 50, 51, 52, 53, 54).

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 (19). On the other hand, assessment of Huanong-1 did not show any significant difference in count of bacteria, fungi and actinomycetes between the transgenic and non-transgenic plots (20). 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 (55).

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 (19, 56). 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) (35, 57, 58). However, these plants cannot persist long in the natural successional cycle and are easily overgrown by vines and forest vegetation (57). Domesticated papaya plants are not considered as weeds because of their higher ratio of edible flesh to seed and a lack of seed dormancy. 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 (11, 14, 17, 20, 42). 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 (14, 17). 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 PRSV-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 (59). 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 or PLDMV, 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. *PRSV CP*, *PLDMV 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:** 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.

**Agriculture, Fisheries and Conservation Department**  
**April 2018**

## References

1. Gonsalves, D. 1998. Control of papaya ringspot virus in papaya: a case study. *Annu. Rev. Phytopathol.* 36:415–37.
2. USDA/APHIS. 1996. *USDA/APHIS petition 96-051-01P for the determination of nonregulated status for transgenic sunset' papaya lines 55-1 and 63-1: environmental assessment and finding of no significant impact.* [http://www.aphis.usda.gov/brs/aphisdocs2/96\\_05101p\\_com.pdf](http://www.aphis.usda.gov/brs/aphisdocs2/96_05101p_com.pdf) .
3. Manshardt, R.M. 1998. 'UH Rainbow' papaya. University of Hawaii College of Tropical Agriculture and Human Resources *New Plants for Hawaii-1*, p2.
4. Fitch, M.M.M. 2002. *U.S. Patent No. US PP12481 P2*. Washington, DC: U.S. Patent and Trademark Office. Available online: <https://www.google.com/patents/USPP12481?dq=US+PP12481+P2&hl=en&sa=X&ei=tVlaVaXYE-LPmwXYsILABA&ved=0CB4Q6AEwAA> .
5. Hawaii Papaya Industry Association. 2009. *Hawaiian Papaya Varieties Webpage.* <http://www.hawaiipapaya.com/choices.html> . Accessed on 30 January, 2015.
6. USDA/APHIS. 2009. *Finding of no significant impact: petition for nonregulated status for University of Florida X17-2 papaya.* Available online: [http://www.aphis.usda.gov/brs/aphisdocs2/04\\_33701p\\_com.pdf](http://www.aphis.usda.gov/brs/aphisdocs2/04_33701p_com.pdf)
7. Ye, C.M., Wei, X.D., Chen, D.H., Lan, C.Y. and Chu, L.M. 2003. Analyses of virus resistance and transgenes for transgenic papaya. *Hereditas (Beijing)* 25(2): 181-184. (In Chinese with English abstract)
8. Ministry Of Agriculture of China. 2010. *2010年第二批农业转基因生物安全证书批准清单.* (in Chinese) Available online: <http://www.stee.agri.gov.cn/biosafety/spxx/P020101018682476807732.pdf> .
9. Jia, R., Zhao, H., Hung, J. Kong, H., Zhang, Y., Guo, J., Huang, Q., Guo, Y., Wei, Q., Zuo, J., Peng, M. and Guo, A. 2017. Use of RNAi technology to develop a PRSV-resistant transgenic papaya. *Scientific Reports* 7: 12636. DOI:10.1038/s41598-017-13049-0.
10. Zhao, H., Jia, R., Zhang, Y., Zhu, Y., Zeng, H., Kong, H. McCafferty, H., Guo, A. and Peng, M. 2016. Geographical and genetic divergence among Papaya ringspot virus populations within Hainan Province, China. *Phytopathology* 106: 937-994.
11. Kung, Y., Bau, H., Wu, Y., Huang, C., Chen, T., and Yeh, S. 2009. Generation of transgenic papaya with double resistance to Papaya ringspot virus and Papaya leaf-distortion mosaic virus. *Phytopathology* 99: 1312-1320.



12. Chiou Mey Perng 2015. Taiwan Agricultural Biotechnology Annual – Gain Report. Available online:  
[https://gain.fas.usda.gov/Recent%20GAIN%20Publications/Agricultural%20Biotechnology%20Annual\\_Taipei\\_Taiwan\\_7-15-2015.pdf](https://gain.fas.usda.gov/Recent%20GAIN%20Publications/Agricultural%20Biotechnology%20Annual_Taipei_Taiwan_7-15-2015.pdf) .
13. Fitch, M.M., Manshardt, R.M., Gonsalves, D., Slightom, S.L. and Sanford, J.C. 1992. Virus resistant papaya plant derived from tissues bombarded with the coat protein of papaya ringspot virus. *Nature Biotechnology* 10:1466-1472.
14. Gonsalves, D. and Manshardt, R. 1996. *Petition for determination of non-regulated status for transgenic papaya lines 55-1 and 63-1 and their derivatives*. USDA-APHIS Biotechnology Regulatory Service.  
[http://www.aphis.usda.gov/brs/aphisdocs/96\\_05101p.pdf](http://www.aphis.usda.gov/brs/aphisdocs/96_05101p.pdf) .
15. Tennant, P., Souza, M.T., Gonsalves, D., Fitch, M.M., Manshardt, R.M. and Slightom S.L. 2005. Line 63-1: a new virus-resistant transgenic papaya. *HortScience*. 40(5)1196-1199. Available online:  
<http://hortsci.ashspublications.org/content/40/5/1196.full.pdf> .
16. Davis, M. and Ying, Z. 2004. Development of papaya breeding lines with transgenic resistance to papaya ringspot virus. *Plant Disease* 88:352-358. Available online:  
<http://apsjournals.apsnet.org/doi/abs/10.1094/PDIS.2004.88.4.352> .
17. Davis, M. 2007. *Petition for determination of nonregulated status for the X17-2 line of papaya: a Papaya ringspot virus – resistant papaya*. USDA-APHIS Biotechnology Regulatory Service. Available online:  
<http://cera-gmc.org/docs/decdocs/09-055-005.pdf> .
18. Chen, G., Ye, C., Huang, J., Yu, M. and Li, B. 2001. Cloning of the papaya ringspot virus (PRSV) replicase gene and generation of PRSV-resistant papayas through the introduction of the PRSV replicase gene. *Plant Cell Reports*. 20: 272-277.
19. Wei, X.D., Zou, H.L., Chu, L.M., Liao, B., Ye, C.M., Lan, C.Y. 2006. Field released transgenic papaya effect on soil microbial communities and enzyme activities. *J Environ Sci (China)*.18(4):734-40.
20. South China Agricultural University. 2009. *Safety Certificate for the National Application of “Huanong No. 1”: a transgenic papaya with Papaya ringspot virus replicase gene (转番木瓜环斑病毒复制酶基因的番木瓜华农1号在全国应用的安全证书)*. (in Chinese) Available online:  
<http://www.moa.gov.cn/ztl/zjyqwgz/spxx/201307/P020141202585761605739.pdf> .
21. Jiang, D.-G., Zhou, F., Yao, J., Mu, H. and Mei, M.-t. 2009. Establishment of event-specific qualitative PCR detection method for transgenic papaya

- “Huanong No. 1”. *Journal of South China Agricultural University* 30 (1): 37-41. (In Chinese with English abstract)
22. Guo, J., Yang, L., Liu, X., Guan, X., Jiang, L. and Zhang, D. 2009. Characterization of the Exogenous Insert and Development of Event-specific PCR Detection Methods for Genetically Modified Huanong No. 1 Papaya. *J Agric Food Chem.* 57:7205-7212.
  23. Bau, H.-J., Yeh, S.-D., Su, T.-T., Fan, C.-C., Cheng, Y.-H., Chen, S., Kung, Y.-J. 2011. *C.N. Patent No. CN102071208A*. Beijing, China, PRC State Intellectual Property Office. Available online: <https://www.google.com/patents/CN102071208A?cl=en> .
  24. Cheng, Y.-H., Yang, J.-S. and Yeh, S.-D. 1996. Efficient transformation of papaya by coat protein gene of Papaya ringspot virus mediated by *Agrobacterium* following liquid-phase wounding of embryogenic tissues with caborundum. *Plant Cell Reports* 16:127-132.
  25. Bau, H.-J., Cheng, Y.-H., Yu, T.-A., Yang, J.-S. and Yeh, S.-D. 2003. Broad-spectrum resistance to different geographic strains of papaya ringspot virus in coat protein gene transgenic papaya. *Phytopathology* 93 (1): 112-120.
  26. Bau, H.-J., Cheng, Y.-H., Yu, T.-A., Yang, J.-S., Hsiao, C.-H., Lin, C.-Y. and Yeh, S.-D. 2004. Field evaluation of transgenic papaya lines carrying the coat protein gene of *Papaya ringspot virus* in Taiwan. *Plant Disease* 88(6): 594-599.
  27. Yeh, S.-D., Bau, H.-J., Cheng, Y.-H., Fan, C.-C., Kung, Y.-J., Chen, S. and Su T.-T. 2012. *U.S. Patent No. US 8,232,381 B2*. Washington, DC: U.S. Patent and Trademark Office. Available online: <https://www.google.com/patents/US8232381> .
  28. Papaya modified for delayed ripening (Record ID 104889) Available online: <https://bch.cbd.int/database/record.shtml?documentid=104889> . Data retrieved on 31 August 2017.
  29. The Biology of *Carica papaya* L. (papaya, papaw, paw paw). 2008. *Department of Health and Ageing Office of the Gene Technology Regulator*, Australian Government.
  30. Sekeli, R., Abdullah, J.O., Namasivayam, P., Muda, P., Bakar, U.K.A. 2013. Better rooting procedure to enhance survival rate of field grown Malaysian Eksotika Papaya transformed with 1-Aminocyclopropane-1-Carboxylic Acid Oxidase Gene. *ISRN Biotechnology* Article ID 958945.
  31. Sekeli, R., Abdullah, J.O., Namasivayam, P., Muda, P., Bakar, U.K.A., Yeong, W.C. and Pillai, V. 2014. RNA Interference of 1-Aminocyclopropane-1-carboxylic Acid Oxidase (ACO1 and ACO2) Genes

- Expression Prolongs the Shelf Life of Eksotika (*Carica papaya* L.) Papaya Fruit. *Molecules* 19(6): 8350-8362.
32. Garrett, A. 1995. *The Pollination Biology of Papaw (Carica papaya L.) in Central Queensland*. PhD Thesis. Central Queensland University, Rockhampton.
  33. OECD. 2005. *Consensus document on the biology of papaya (Carica papaya)*. *Series on Harmonisation of Regulation Oversight in Biotechnology no. 33*. OECD Environment, Health and Safety Publication. Available online at: <http://www.oecd.org/science/biotrack/46815818.pdf> .
  34. “GFruit” - The Transfer of Global Information System on Tropical Fruits with Special Focus on Africa. *International Tropical Fruits Network (TFNet)*. Available at: <http://www.itfnet.org/gfruit/Templates%20English/papaya.biology.htm> . Access on 30 Aug 2017.
  35. Brown, J.E., Bauman, J.M., Lawrie, J.F., Rocha, O.J. and Moore, R.C. 2012. The structure of morphological and genetic diversity in natural populations of *Carica papaya* (Caricaceae) in Costa Rica. *Biotropica* 44, 179 – 188.
  36. Carvalho, F.A. & Renner, S.S. 2015. IV. A dated phylogeny of the papaya family (Caricaceae) reveals the crop’s closest relatives and the family’s biogeographic history. *Molecular phylogeny, biogeography and an e-monograph of the papaya family (Caricaceae) as an Example of Taxonomy in the Electronic Age*. pp 49-81.
  37. Teixeira da Silva, J.A., Rashid, Z., Nhut, D.T., Sivakumar, D., Gera, A., Souza Jr., M.T., Tennant, P.F. 2007. Papaya Biology and Biotechnology. *Tree and Forestry Science and Biotechnology* 1(1): 47-73.
  38. Anem, M. 2015. Papaya exotica – from Malaysia. *Anim Agriculture Technology*. Available online: <http://animagro.blogspot.hk/2015/06/papaya-exotica-from-malaysia.html> .
  39. Gonsalves, D., S. Tripathi, J. B. Carr, and J. Y. Suzuki. 2010. Papaya Ringspot virus. *The Plant Health Instructor*. DOI: 10.1094/PHI-I-2010-1004-01 <http://www.apsnet.org/edcenter/intropp/lessons/viruses/Pages/PapayaRingspotvirus.aspx> .
  40. Tennant, P. F., Gonsalves, C., Ling, K. S., Fitch, M., Manshardt, R., Slightom, J. L., and Gonslaves, D. 1994. Different protection against papaya ringspot virus isolates in coat protein gene transgenic papaya and classically cross-protected papaya. *Phytopathology* 84:1359-1366.
  41. Suzuki, J.Y., Tripathi, S., Fermin, G.A., Jan, F.-J., Hou, S., Saw, J.H., Ackerman, C.M., Yu, Q., Schatz, M.C., Pitz, K.Y., Yepes, M., Fitch, M.M.M.,

- Manshardt, R.M., Slightom, J.L., Ferreira, S.A., Salzberg, S.L., Alam, M., Ming, R., Moore, P.H., Gonsalves, D. 2008. Characterization of insertion sites in Rainbow Papaya, the first commercialized transgenic fruit crop. *Tropical Plant Biology* 1: 293-309
42. Bau, H., Kung, Y., Raja, J. A. J., Chan, S., Chen, K., Chen, Y., Wu, H., and Yeh, S. 2008. Potential threat of a new pathotype of Papaya leaf distortion mosaic virus infecting transgenic papaya resistant to Papaya ringspot virus. *Phytopathology* 98:848-856.
  43. Center for Environmental Risk Assessment. 2014. *GM Crop Database: 55-1/63-1*. Published online: <http://cera-gmc.org/GmCropDatabaseEvent/55-1%2F63-1> . Data retrieved on 19 December 2014.
  44. Center for Environmental Risk Assessment. 2014. *GM Crop Database: X17-2*. Published online: <http://cera-gmc.org/GmCropDatabaseEvent/X17-2> . Data retrieved on 19 December 2014.
  45. Meins, F. Jr. 2000. RNA degradation and models for post-transcriptional gene silencing. *Plant. Mol. Biol.* 43: 261-273.
  46. Azad, M.A.K., Amin, L. and Sidik, N.M. 2014. Gene technology for papaya ringspot virus disease management, *The Scientific World Journal* 2014. Article ID 768038.
  47. Missouri Botanic Garden. 2015. *Angiosperm Phylogeny Website*. Version 13. <http://www.mobot.org/MOBOT/research/APweb/> . Assessed on 4 February 2015.
  48. Kesse, P., 2008. Risks from GMOs due to Horizontal Gene Transfer. *Environ. Biosafety Res.* 7: 123-149.
  49. Conner, J. A., Glare, R. T, and Nap, J-P, 2003. The release of genetically modified crops into the environment, part: Overview of ecological risk assessment. *The Plant Journal* 33: 19-46.
  50. Nielsen, K. M., Gebhard, F., Smalla, K., Bones, A.M., Van Elsas, J.D., 1997. Evaluation of possible horizontal gene transfer from transgenic plants to the soil bacterium *Acinetobacter calcoaceticus* BD413. *Biotechnology* 13, 1094 – 1098.
  51. Nielsen, K.M., Bones, A.M., Smalla, K., Van Elsas, J.D., 1998. Horizontal gene transfer from transgenic plants to terrestrial bacteria – a rare event? *FEMS Microbiol. Rev.* 22: 79-103.
  52. OECD, 2007. *Consensus document on safety information on transgenic plants expressing Bacillus thuringiensis-derived insect control protein*. Env/JM/MONO(2007)14. <http://www.epa.gov/opp00001/biopesticides/pips/reg-biotech.pdf>

53. Schlüter, K., Fütterer, J., Potrykus, I. 1995. "Horizontal" Gene Transfer from a Transgenic Potato Line to a Bacterial Pathogen (*Erwinia chrysanthemi*) Occurs-if at All- at an Extremely Low Frequency. *Bio/Technology* 13: 1094 – 1098.
54. Savadogo, M. *Environmental Issues Related to Genetically Modified Crops*. <http://www.nepadbiosafety.net/for-regulators/resources/subjects/environmental-biosafety/environmental-issues-gm-crops> .
55. Smalla, K, Borin, S, Heuer, H, Gebbard, F, Elsas, van JD and Nielsen, KM. 2000. Horizontal transfer of antibiotic resistance genes from transgenic plants to bacteria: are there new data to fuel the debate? In: C Fairburn, G Scoles and A McHughen, Editors, 6th *International Symposium on the Biosafety of GMOs*. University Extension Press University of Saskatchewan, Saskatoon.
56. Hsieh, Y.T., Pan, T.M. 2006. Influence of planting papaya ringspot virus resistant transgenic papaya on soil microbial biodiversity. *J Agric Food Chem*. 11:130-137.
57. Paz, L., Vazquez-Yanes, C., 1998. Comparative seed ecophysiology of wild and cultivated *Carica papaya* trees from a tropical rain forest region in Mexico. *Tree Physiol*. 18, 277 – 280.
58. d'Eeckenbrugge, C.G., Restrepo, M.T., Jimenez, D. 2007. Morphological and isozyme characterization of common papaya in Costa Rica. *Acta Hort*. 740: 109-120.
59. OGTR (Office of the Gene Technology Regulator, Australia). 2014. *Risk assessment reference: marker genes in GM plants*. Available online: <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/marker-genes-ref-1-htm> . Accessed on 5 February, 2015.