

Application of Transgenic Technologies to Papaya: Developments and Biosafety Assessments in Thailand

Sunee Kertbundit^{1,2} • Miloslav Juříček^{2*}

¹ Department of Plant Science, Faculty of Science, Mahidol University, Rama VI Road, Payathai, Bangkok 10400, Thailand ² Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Na Pernikářce 15, CZ-16000 Prague, Czech Republic Concernenting author: * invisol:@uph.con.cr

Corresponding author: * juricek@ueb.cas.cz

ABSTRACT

Papaya (*Carica papaya*) is one of the staple foods in Thailand. Since 1975, papaya production in Thailand has been severely limited by *Papaya ringspot virus* (PRSV) which is now endemic. The great success of transgenic papaya resistant to PRSV from Hawaii in the 1990s signaled a new strategy to combat PRSV. However, transgenic papaya resistant to PRSV isolated from Hawaii is not resistant to PRSV isolated from Thailand and other countries. Consequently, three independent Thai research groups: the Department of Agriculture (DOA), Plant Genetic Engineering Unit (BIOTEC) and Mahidol University, set out to use transgene technology to develop papaya resistant to Thai isolates of PRSV. All obtained resistant papaya plants, but testing the levels of resistance to PRSV in the field has been thwarted because of the moratorium on field trials launched by Thai government in 2001. Only small experimental fields for research purposes are permitted. During 2004-2007, the entire experimental field test of transgenic papaya was banned on account of the argument of contamination of the environment by transgenic papaya materials from the DOA station. This ban was repealed in 2007 and currently a National Biosafety Law awaits ratification by the Thai parliament. If approved, this law will support the expansion of biotechnology research and commercialization of transgenic crops in Thailand.

Keywords: coat protein-mediated resistance, genetically modified organism, GMO, PRSV, Papaya ringspot virus, virus resistance

CONTENTS

INTRODUCTION	
DEVELOPMENT OF TRANSGENIC PAPAYA IN THAILAND	
Thai Department of Agriculture (DOA)	
Plant Genetic Engineering Unit (PGEU), BIOTEC	
Institute of Molecular Biology and Genetics (IMBG), Mahidol University	54
REGULATIONS OF TRANSGENIC PLANTS IN THAILAND	
CONTROVERSY OVER FIELD TESTING OF TRANSGENIC PAPAYA	
CONCERNS ON TRANSGENIC PAPAYA IN THAILAND	55
IS TRANSGENIC PAPAYA RESISTANT TO ALL THAI STRAINS OF PRSV?	
CURRENT SITUATION OF TRANSGENIC PAPAYA IN THAILAND	
Future Perspectives of Biotechnology in Thailand	56
FINAL REMARKS	
ACKNOWLEDGEMENTS	
REFERENCES	

INTRODUCTION

Papaya (*Carica papaya*), believed to be indigenous to tropical America, was introduced into South East Asia in the 16th Century (Storey 1969) and is now grown in all regions of Thailand. The favorite local papaya varieties in Thailand are 'Khak Dum' and 'Khak Nuan' that are consumed as a ripe fruit and as a green vegetable in spicy dishes such as "Som Tam". Although papaya is not a major commodity in Thailand, it is a staple food and a preferred backyard crop grown by subsistence farmers for daily consumption. Papaya production in Thailand in 2007 was 131,000 tons (FAOSTAT 2007), of which over 90% was used for domestic consumption and the rest exported, primarily to the EU and Japan as canned fruit, sauces and other products (Sriwatanapongse *et al.* 2007).

Papaya ringspot virus (PRSV), a member of the Potyviridae, is the causative agent of the most important disease of papaya. In Thailand, PRSV was reported for the first time in the North East province of Khon Kaen (Srisomchai 1975). Since then, PRSV has been found in several regions and now severely limits papaya production in Thailand. Several attempts to control the PRSV include eradication of infected papaya, mild strain cross-protection, conventional breeding and transgene technology.

The eradication approach was performed in Thailand between 1979-1981. However, the strategy was not successful in containing the disease because papaya growers did not want to destroy their infected papaya trees. Cross protection and conventional breeding against PRSV started in 1987 by collaboration between Thai Department of Agriculture, Ministry of Agriculture and Cooperatives (MOAC) and Cornell University, USA at the DOA research station in Tha Pra, Khon Kaen Province. Cross protection experiments using nitrous acid-induced mutation PRSV strain HA 5-1 and the naturally occurring mild strain did not result in sustainable control in the field trials (Sakuanrungsirikul *et al.* 2005). In a conventional breeding program, the local Thai variety 'Khak Dum' was crossed with the tolerant Florida variety 'Cariflora' (Conover *et al.* 1986). Three derivative lines, 'Tha Pra 1', 'Tha Pra 2' and 'Tha Pra 3', were thus tolerant but not resistant. Despite being still infected by PRSV, they produced fruit suitable for commercial sale. After testing for several generations, the 'Tha Pra 2' line was selected as the "recommended papaya variety" by DOA and was labeled as 'Khak Dum Tha Pra'. Since 1998, this variety has been available for seed propagation and sale to Thai farmers. However, 'Khak Dum Tha Pra' is yellow-fleshed and is much less desirable by the Thai market than the red-fleshed 'Khak Dum' variety.

Thus far, it is evident that transgene technology is the most effective way to protect papaya from PRSV. Initial attempts with transgenic papaya plants to obtain resistance to PRSV were modeled on the coat protein-mediated resistance (CPMR) approach of (Powell-Abel et al. 1986). Papaya containing the coat protein (cp) gene of the mild PRSV strain HA 5-1 was reported to confer resistance to a severe Hawaiian strain of PRSV (Fitch et al. 1990; Fitch and Manshardt 1990; Fitch et al. 1992). However, this transgenic papaya is not resistant to PRSV isolated from Thailand and other countries (Tennant et al. 1994, 2001). These results led to the conclusion that the reported CPMR resistance is PRSV strain-specific and that this approach is best targeted to strains from the same geographical region. This review provides insight to the methods by which resistance to the virus has been attained and to some of the pitfalls that have been encountered.

DEVELOPMENT OF TRANSGENIC PAPAYA IN THAILAND

In Thailand, three independent research groups have applied CPMR technology for the development of papaya resistant to Thai isolates of PRSV. These are: the DOA at the Khon Kaen research station, the Plant Genetic Engineering Unit (PGEU) of the National Centre for Genetic Engineering and Biotechnology (BIOTEC) and faculty of Mahidol University. The research groups used different PRSV strains isolated from several provinces in Thailand. The evidence for the ability of transgenic papaya lines obtained by each of these groups to exhibit resistance to PRSV strains endemic in Thailand is discussed below.

Thai Department of Agriculture (DOA)

In 1994, the Thai DOA initiated collaboration with scientists at Cornell University to develop genetically engineered papaya resistant to PRSV. In September 1995, with financial support from the Thai Government, two scientists from DOA took seeds of the papaya varieties, 'Khak Dum', 'Khak Nuan', 'Tha Pra 1, 2, 3' and the PRSV isolated from Khon Kaen Province (Northeast of Thailand) to Cornell University to develop transgenic papaya. A plasmid containing the untranslatable cp gene was transformed into papaya somatic embryos by particle gun bombardment. Transgenic calli were selected on kanamycin containing media (Sarindu and Prasartsee 2000).

In 1997, 25 transgenic R_0 papaya plants and 487 plates of transgenic callus were sent back from Cornell University to the Thai DOA research station at Tha Pra, Khon Kaen, for screen house tests and selection of PRSV resistant plants. Only 5 of these 25 plants had shown PRSV resistance at Cornell laboratory (four 'Khak Nuan' and one 'Khak Dum'). The re-test of PRSV resistance under DOA's screen house conditions at Tha Pra, Khon Kaen, confirmed that only 3 of the 5 transgenic lines were PRSV resistant (all three were 'Khak Nuan'). From the 487 plates of transgenic callus, 50 plants were regenerated of which 4 plants were tolerant (infected for a period of time and then healthy), all were 'Tha Pra 2'. Three R_0 plants resistant to PRSV were both selfand cross-fertilized to generate progeny. One R_0 plant designated 319-1 KN was the most promising. The R_1 of this line (self) showed 12%-20% resistance to PRSV and two R_2 plants (from total of 89 plants) from R_1 self-pollinated (R_1 -179 and R_1 -181) showed 100% resistance to PRSV (Prasartsee, pers. comm). Four R_2 transgenic plants (R_2 319-1KN-179, R_2 319-1KN-180, R_2 319-1KN-181, R_2 319-1KN-182) derived from the same R_0 319-1KN line showed 90-100% resistance to PRSV isolates collected from five provinces in Thailand (Chantaburi, Ratchaburi, Chumporn, Chiang Mai and Khon Kaen) (Prasartsee *et al.* 2002a, 2002b).

The DOA report (Sakuanrungsirikul *et al.* 2005) indicated that there were 25 lines of 'Khak Nuan' and 2 lines of 'Khak Dum' in the R2 generation which showed various levels of resistance to PRSV and showed 97% PRSV resistance in R_3 319-1KN-180, R_3 319-1KN-181, R_3 319-1KN-182. It appears that all were progeny of 319-1 KN line.

Field trials at Tha Pra Research Station started in 1999. The transgenic lines showed excellent resistance under field conditions. The progeny from 'Khak Nuan' R_3 319-1KN-181 and 'Khak Dam' R_3 300KD-9 plants exhibited 90 to 100% resistance to PRSV (Sakuanrungsirikul *et al.* 2005). The two selected lines consisted of three gene inserts when analyzed by Southern blotting. Unfortunately, no molecular evidence has been presented on the assessment of the transgene copy number, organization or number of independent events. No coat protein was detected in dot blot analysis of leaf and tissues collected from ripe fruit that were probed with antibody specific to PRSV (Sakuanrungsirikul *et al.* 2005).

Tests of effects on the environment and food safety of transgenic papaya were performed between 2001 and 2004 (no indication was given as to which transgenic line was tested). The natural dispersal of the pollen was limited in the field and the probability of crossing between transgenic and non-transgenic papaya beyond a distance of 10 m was low. There were no differences in the number of Mycorrhiza spores, Rhizobium bacteria, some heterotrophic bacteria, Actinomyces bacteria, and filamentous fungi in the soil used for cultivating the transgenic papaya to the soil used for the non-transgenic papaya. Bee larvae (Apis mellifera) fed on the pollen of the transgenic papaya had normal growth rate and developed into normal adults as those fed on pollen from the non transgenic papaya. No abnormality was observed at any stage of development of the predatory mites, Amblyseius longispinosus (Evans), after feeding them with papaya pest mites Eutetranechus africanus (African red mite) that had consumed transgenic and non-transgenic papaya.

Food biosafety tests in rats (*Rattus norvegicus*) showed no differences in body weight, growth rate and reproduction of the rat fed on transgenic papaya fruit and non-transgenic papaya. The analysis of nutritional composition also showed no significant differences between transgenic and non-transgenic papaya (Sakuanrungsirikul *et al.* 2005).

Plant Genetic Engineering Unit (PGEU), BIOTEC

The PGEU is a research unit of the National Biotechnology and Genetic Engineering Center (BIOTEC), National Science and Technology Development Agency (NSTDA) under the Ministry of Science, Technology and Energy (MOST). To develop PRSV-resistant transgenic papaya, PGEU collaborated with Kasetsart University at Kamphaengsang and Queensland University of Technology, Australia. In 1997, with the support from ACIAR (Australian Center for International Agricultural Research), a PGEU scientist took seeds of 'Khak Dum' and 'Khak Nuan' and a PRSV isolate from Chiang Mai province (northern region of Thailand) to Queensland University of Technology to develop transgenic papaya through the purported CPMR approach. In 1999, the R₀ transgenic papaya plantlets were sent back to PGEU in order to test for PRSV resistance.

Transgenic papaya lines KN 1.2.3, KN 13.2.3 and KN 49 derived from 'Khak Nuan' variety were reported to show

potential resistance against PRSV isolated from different provinces of Thailand: Nakhon Pathom, Ratchaburi, Suratthani, Sakhonnakhon and Yasothon; KN 1.2.3 was found to be susceptible to the Yasothon isolate. There were no noticeable differences in the morphology of inflorescences, fruits and seeds between transgenic lines KN 1.2.3 and KN 13.2.3 and non-transgenic papaya under screen house conditions. Line KN 49, was a female plant which produced fruits consisting of slightly rough skin covered with scatter palegreen streaks (Warin et al. 2003). The other transgenic papaya resistant to PRSV obtained from PGEU was line KN116/5. The seedlings of R_2 generation of KN116/5 showed 35% of PRSV resistance in screen house. After two months in the field test, this R₂ line showed 97% PRSV resistance compared with non-transgenic plants that showed 100% PRSV-infection. Transgenic papayas displayed normal growth rate and gave 40 times higher fruit yield than that of the non-transgenic control (Phironrit et al. 2007). Southern blot analysis of the genomic DNA from R2 of KN116/5 showed 2-4 copies of the cp transgene insert and 2 copies of *npt*II transgenes. Coat protein expression was detected in all resistant plants by indirect sandwiched ELISA method suggesting a coat protein mediated mechanism of PRSV resistance (Phironrit et al. 2007).

The assessment of environmental biosafety of line KN116/5 R4 was evaluated under screen house conditions for papaya pollen vigor, the effects of abiotic factors, morphological and agronomical characters. Results showed that there were no significant differences between transgenic papaya and non-transgenic papaya (Phuangrat *et al.* 2008). Also, evidence of horizontal gene transfer and harmful effects of transgenic papaya plants to the rhizosphere soil bacteria under screen house conditions were not observed (Phironrit *et al.* 2008).

Institute of Molecular Biology and Genetics (IMBG), Mahidol University

The papaya research by this group started in 1994 with the collection and comparison of the cp gene and the 3' nontranslated regions of PRSV isolated from several regions in Thailand and other countries (Kertbundit, et al. 1998). The plasmid containing the expression cassette of the cp gene of PRSV isolated from Ratchaburi province (central region of Thailand) whose DNA sequence was the most similar to all other isolates, and was selected for transformation into somatic embryos of 'Khak Dum' papaya by particle gun bombardment. Eight transgenic lines were selected from bombarded calli under kanamycin selection. Transgenic lines G1 and T2 that expressed the coat protein were not resistant to PRSV, while the line G2, a transgenic line that did not express the coat protein, was found to be highly resistant to PRSV. This resistant line showed a high degree of rearrangement of the inserted coat protein cassette and a significantly low level of the mRNA of the coat protein was detected (Kertbundit et al. 2007). The suspected post transcriptional gene silencing as a cause of the resistance was confirmed by siRNA detection (Ruanjan et al. 2007). This line G2 resisted challenge inoculations with PRSV isolated from Ratchaburi, Nakhonratchasima and Surathani, but not to PRSV isolated from Ayuthaya, Khon Kaen and Nan (unpublished data).

Line G2 was female plant. It was crossed with nontransgenic 'Khak Dum' papaya for the R1 generation. The PRSV resistance and *cp* transgene insertion in transgenic line G2 were inherited in the R₁ generation. Thirty of 60 plants (50%) in R₁ generation of line G2 contained the *cp* gene, and all showed PRSV resistance. The R₂ and R₃ progenies from self pollinated R₁ and R₂ plants showed 48-97% PRSV resistance.

A small field trial of transgenic papaya was performed between 2000-2004 at the Institute's screenhouse and experimental plot. While the R_0 generation of G2 papaya was found to be fully resistant to PRSV infection, PRSV was able to break the resistance in R_1 and subsequent generations by suppressing post-transcriptional gene silencing (PTGS) (Ruanjan *et al.* 2007). Risk assessment of transgenic papaya line G2 aimed for commercialization has not yet been performed.

REGULATIONS OF TRANSGENIC PLANTS IN THAILAND

There are several steps to move from development of transgenic plants to deregulation and commercialization. The term "Biosafety" has been used to describe the policies that ensure the safe development and application of biotechnologies and their products, including genetically modified living organisms (GLMOs) and genetically modified organisms (GMOs), and minimum risk to plant genetic resources, plant, animal or human health, or the environment. Biosafety regulations vary in different countries.

In Thailand, the National Center for Genetic Engineering and Biotechnology (BIOTEC), established in 1983, has played an important role in biosafety regulation. The first Biosafety guidelines, (1) Genetic Engineering and Biotechnology for Laboratory Work and (2) Biosafety Guidelines in Genetic Engineering for Field Work and Planned Release, were issued by BIOTEC in June 1992 and revised in November 2004.

The National Biosafety Committee (NBC) was established in 1993 to monitor implementation of the guidelines with BIOTEC as secretariat. Universities, public and private research institutes were encouraged to set up their own Institutional Biosafety Committees (IBC) to be responsible for research work at their own institute, in consultation with the NBC.

The importation of transgenic crops into Thailand requires the approval of the Director-General of the Department of Agriculture, who relies on the NBC for its recommendations.

The importation of transgenic plants into Thailand is regulated by Plant Quarantine Act, B.E. 2507 (1964) 2^{nd} Amendment on B.E. 2542 (1999) and 3rd Amendment on B.E. 2551 (2008), and Plant Varieties Protection Act B.E. 2542 (1999). Originally these acts regulated the importation and export of plant varieties and were amended for the regulation of transgenic plants. Under the Plant Quarantine Act, transgenic plants can only be imported into Thailand for research with permission from the Director-General of DOA, who relies on the NBC for its recommendations. Approval for field testing transgenic plants is granted based on a collaborative arrangement between the DOA and NBC. There is no regulation on the importation of Genetically Modified Organisms (GMOs) or Living Modified Organisms (LMOs) for food and feed or for processing especially GM corn and soybeans, but their products are subjected to labeling requirements under the Ministry of Public Health announcement No. 251 B.E. 2545 (2002).

The first transgenic plant that was approved for field testing in Thailand was the Flavr Savr tomato, a delayed ripening tomato, in 1994. Since then, several transgenic crops have been approved for study in Thailand, including cotton and corn containing the toxin gene from *Bacillus thuringiensis*, (B.T. cotton and B.T. corn), herbicide resistant corn, virus resistant papaya (Sriwatanapongse *et al.* 2007). However on July 2001, Thai cabinet imposed a ban on field testing transgenic plants based on the controversy on biosafety regulation of the field test of B.T. cotton.

CONTROVERSY OVER FIELD TESTING OF TRANSGENIC PAPAYA

B.T. cotton was approved for importation into Thailand in 1995 and a field trial was started in March of 1996. It was expected to be the first transgenic plant to be deregulated and commercialized in Thailand soon after the field trial.

However in October 1999, non-governmental organizations (NGOs) reported that B.T. cotton was spreading from Monsanto's approved site to the fields of local farmers'. In March 2000, an alliance of 35 farmer groups, environmental lawyers and NGOs requested the government to call off tests on transgenic cotton for fear of possible cross-pollination between transgenic and non-transgenic plants. The pressure from NGOs led the cabinet to declare a moratorium and banned all field trials of transgenic plants on 3 April 2001 until the biosafety law will be enacted.

According to this ban, only the small contained field trials of transgenic papaya at DOA research station, PGEU and Mahidol University were allowed to continue under the IBC regulations. In July 2004, Greenpeace reported that the 'Khak Dam Tha Phra' seeds distributed by the DOA's research station at Tha Pra, Khon Kaen were mixed with transgenic papaya seeds. Greenpeace then accused the DOA of illegally distributing transgenic papaya seeds and demanded Thai government to immediately destroy all papaya trees, fruit, seedlings and seeds at the research station to prevent further spread of contamination. The claim was ignored by DOA. Greenpeace activists illegally entered the confined field of DOA and they removed transgenic papaya fruit from the trees and put them in hazardous material containers. DOA officials sued the Greenpeace activists with trespassing, theft, and destruction of property. However, the activists were acquitted in 2006 (Bangkokbiznews 2006).

In August 2004, the Thai prime minister reversed the ban on field trial of transgenic plants. This led to public opposition from the NGOs, Buddhist communities and the Thai organic business groups. Ten days later, the prime minister retracted his decision and called for the creation of a national panel of academics to look into the matter of the transgenic papaya contamination. The DOA was forced by the National Human Rights Commission, Greenpeace and farmer groups to publicly reveal the list of the 2,669 farmers in 37 provinces who bought papaya seeds from DOA research station at Tha Pra, Khon Kaen.

On 14 September 2004, the DOA found one positive sample of 239 from farmers who had purchased papaya seeds from the DOA research station. The minister ordered the eradication of all trees on the test-positive farm, and the testing of plants from all registered recipients of papaya seeds from the station (Davidson 2008). The next day, the minister ordered the DOA to remove all transgenic papaya trees in the Tha Pra research station and the surrounding plots. An investigating committee was set up to determine whether the transgenic papaya seeds were smuggled out of the station or had been the result of cross pollination. The prime minister subsequently ordered the destruction of all field trials in the country, following a cabinet decision to place a moratorium on all confined field trials in Thailand, in addition to the 2001 ban on open field trials.

In 2005, Greenpeace and the National Human Rights Committee reported contaminated papaya fields in several provinces including Rayong, Kamphaeng Phet, Kalasin, Chaiyaphum, Mahasarakham and Ubonratchathani. In October 2006, Greenpeace Southeast Asia petitioned the Administrative Court against the DOA and its director for negligence in preventing the spread of transgenic papaya seeds from its research station in Khon Kaen in 2004. This was Thailand's first liability lawsuit on GMOs. The charge was acquitted on July 2008. On August 27, 2007, Greenpeace activists tried to stop the MOAC plan to submit a proposal to the Thai cabinet to waive the ban on open-field trials on the day after (Greenpeace 2007).

CONCERNS ON TRANSGENIC PAPAYA IN THAILAND

In the case of transgenic papaya, trade loss and intellectual properties were significant issues raised by Greenpeace and other NGOs. They claimed that migration of transgenic papaya into farmers' plantations in Thailand would cause problems for export of Thai papaya and other farm products to countries that ban the importation of GM products (Organic Consumers Association 2004). This situation was reported in Hawaii. A decrease in papaya exports to the EU, Japan and China resulted following the introduction of GM papaya to Hawaii in 1998. Offsetting this is the expense of showing that the non-transgenic papaya trees have not been contaminated by spreading of pollen from transgenic papaya (Samabuddhi 2004). Further, organic papaya presently sells for three times the price of transgenic papaya.

The second concern raised by Greenpeace is the issue of intellectual property (Greenpeace Southeast Asia 2004). Naturally, several patent claims over papaya on wide range of aspects have been applied by Monsanto, Seminis and Cornell University in the US Patent and Trademark Office (USPTO). Greenpeace disclosed that Cornell Research Foundation has submitted patent applications covering the transgenic papaya to the USPTO and the World Intellectual Property Organization (WIPO). One application (US 6750382) was approved by the USPTO on June 15, 2004. In 2006, USPTO granted a patent (US 7078586) on Papaya ringspot virus genes assigned to Cornell Research Foundation covering a broad range of DNA constructs and methods for creation of PRSV resistance (Patent Strom 2006). This makes transgenic 'Khaek Dam' and 'Khaek Nuan' papaya varieties technically the property of Cornell even if the original material including the virus strains used in the research were brought from Thailand and its development was done by the Thai DOA.

Intellectual issues are also exemplified by interactions between Thai DOA and Cornell University and scientists engaged in the project. To enable Thai farmers to use the papaya without violating intellectual property rights, it was agreed that a memorandum of understanding (MOU) covering these aspects would be worked out.

In a meeting organized by the Biotechnology Alliance Association, Khon Kaen University and the United States embassy on November 18, 2005, Dennis Gonsalves, the PRSV project leader, told the Thai farmers that the GM papaya would 'make ringspot virus a thing of the past' and that they should ask the government to reconsider the ban on GM crops (Wongruang 2005), also reported online by the Thai newspaper "Manager" (Manager 2005).

IS TRANSGENIC PAPAYA RESISTANT TO ALL THAI STRAINS OF PRSV?

It has been shown that PRSV resistance in transgenic papaya is mediated by post transcriptional gene silencing (Tennant *et al.* 2001; Ruanjan *et al.* 2007). This mechanism is very effective but is highly strain-specific (Tennant *et al.* 2001). Comparison of the 3' non-translated and coat protein coding regions of PRSV isolated from several Thai provinces and from other countries showed 95-99% sequence similarity among PRSV Thai isolates and up to 13% variation between PRSV isolates from Thailand and other countries (Kertbundit *et al.* 1998). Unlike Hawaii that is an isolated island, Thailand's neighboring countries also grow papaya infected that is infected by various strains of PRSV. These findings raises the question if transgenic papaya developed by above mentioned research groups can resist to all PRSV strains in Thailand.

In the above mentioned meeting in 2005, Dennis Gonsalves conceded that GM papaya might not be resistant to all strains of PRSV present in Thailand. At the same seminar, Vilai Prasartsee, director of the Agricultural Research and Development Office Region 3 in Khon Kaen, said GM papaya developed from the 'Khak Dam' and 'Khak Nuan' varieties could resist virus strains taken from 18 Thai provinces. However, she admitted that resistance to PRSV might break down with time as transgenic papaya in the confined field at Khon Kaen became infected after 2 years and 3 months. Research from the Mahidol University group revealed that while transgenic papaya line G2 was highly resistant to virus infection during 3 years of intensive testing, the PRSV virus was able to break this resistance in subsequent generations by suppressing post-transcriptional gene silencing (PTGS) (Ruanjan *et al.* 2007).

The above situation raises important questions: Should

the commercial papaya growers pay the royalty to Cornell University if the transgenic papaya does not exhibit long term resistance to PRSV? Moreover, how will the country manage a situation where the released transgenic variety is not resistant to PRSV? Although the latter concern could be addressed by implementing the wining Hawaiian scenario, that is the distribution of PRSV resistant seedlings to farmers and strictly following practices to minimize the possibility of a breakdown in resistance. However, this approach is applicable to or practical in Thailand.

CURRENT SITUATION OF TRANSGENIC PAPAYA IN THAILAND

Though Greenpeace tried to stop the field trials of transgenic plants in Thailand, on December 25, 2007, the Thai Cabinet revoked the field trial ban. Future field trials will be conducted under new restrictive controls and surveillance, including confining trials to government owned properties only and conducting public hearings with individuals in the neighborhood of the field area prior to initiating new field trials. Furthermore, field trial proposals must be submitted to the Cabinet for case-by-case approval. Each proposal has to state the exact type of GM plant that will be conducted in field testing and the exact plantation area (Technical Biosafety Committee 2008).

The Technical Biosafety Committee under BIOTEC prepared a specific guideline called, "Models for field trial of genetically modified papaya, tomato, pineapple and corn". The guideline was developed from the Biosafety Guidelines for Work Related to Modern Biotechnology or Genetic Engineering in order to provide public assurance on the released field trials of 4 targeted genetically modified crops in Thailand (Technical Biosafety Committee 2008). The guidelines should be submitted to DOA for a final review and then will be submitted to the Cabinet for approval.

Future Perspectives of Biotechnology in Thailand

The delay in adopting transgenic technology in Thailand reflects the lack of comprehensive biosafety laws, public skepticism concerning transgenic plants and the lack of trust in the capacity of public agencies to regulate biosafety. This restriction dissuades Thai researchers from using modern biotechnology to improve the quality and quantity of food production in Thailand. The delay or loss of opportunities to capture potential added values through improved productivity, increased income, and higher competitiveness come at a high cost.

The present biosafety guidelines are not law, meaning that there are no provisions to impose penalties to any party not following the guidelines. It would seem that a comprehensive biosafety law is needed to resolve the situation. Steps taken towards developing a National Biosafety Law include:

(1) The establishment in 2003 of The Sub-Committee on Drafting under the supervision of the Ministry of Natural Resources and Environment (MONRE).

(2) On 29 January 2004, Thailand became party effective to the Convention on Biological Diversity (CBD).

(3) On 8 February 2006, Thailand became party effective to the Cartagena Protocol on Biosafety (CPB). Upon becoming Party to the CBD Thailand embarked on the development of a National Biosafety Frame work (NBF) to ensure the safety of agricultural biotech products in Thailand. Development of the NBF is supervised by the Steering and Advisory Working Group appointed by Office of Natural Resources and Environmental Policy and Planning (ONREP), Ministry of Natural Resources and Environment (MONRE).
(4) On November 7, 2007, the draft of National Biosafety Policy was approved by the Compliance Committee under the Cartagena Protocol on Biosafety. The policy covers eight concepts:

(a) Public Awareness, education and participation: Requiring the involvement of affected parties in policylevel decision-making on the sustainability, advantages and risks of the technology in question.

(b) Sustainability: Sustainable bioresource management must be taken into account the sustainability of the ecology, preservation of species and genetic pool.

(c) Risk Assessment and Management: Risk acceptability will be assessed and managed on a case-by-case basis according to the Guidelines on Biosafety which will be based on scientific grounds first and foremost.

(d) Risk Characterization: Characterizing risks for the management and control of biotech materials will depend on the outcome of risk assessment.

(e) Risk Communication: Risk communication will be based on basic scientific concepts simplified for the public in order to lessen the concerns of affected parties, increase public trust in research results, as well as curb possible panic from sensitive or contradictory information.

(f) Precautionary Principle: Avoid unnecessary damage from the lack of reliable scientific data on possible effects of biotech materials on the conservation and utilization of biodiversity, environment, and health care.

(g) Freedom of Choice: In utilizing biotech materials for everyone, including consumers, entrepreneurs, academics, farmers, as well as the general public with interested concerns. The state must encourage transparency, accuracy and up-to-date public data for an informed freedom of choice.

(h) Capacity Building: Capacity-building on the national level for the consistent development of biosafety and modern biotechnology on the same ground, to increase national strengths in understanding, utilization and management capacity for the public, business and general sector via studies and development.

(5) On 23 January 2008, the draft "Biosafety of Modern Biotechnology Act B.E. 2551 (2008)" was approved by the Cabinet.

(6) In April 2008, the draft act was forwarded to the Office of the Council of State for legal review. After legal review, final legislation will be submitted to the House of Representatives for enactment and enforcement (Office of Natural Resources and Environment 2009). If approved, this law will support the expansion of biotechnology research and commercialization of transgenic crops in Thailand. The research community is hopeful for the passing of this law so they can continue their research, which stands to benefit Thailand.

To date, the Office of the Council of State maintains dialogue with the Ministry of Natural Resource and Environment (MONRE) towards making adjustments to the draft law. It is anticipated that the review process may not be finalized until early 2010. After legal review, final legislation will be submitted to the House of Representatives for review and approval. This process normally can be terminated anytime if the Prime Minister decides to dissolve the House of Representatives, a political tool used in the past to circumvent political difficulties.

FINAL REMARKS

The importance of modern biotechnology and its wide acceptance is well illustrated by the fact that, worldwide, over 70% soybean, 24% corn and 46% cotton are genetically modified (ISAAA 2008). The technology is an indispensable tool for increasing the efficacy and competitiveness of Thailand's future agricultural and food industries. As has been reviewed, strong opposition from non-government organizations (NGOs) and fear that Thailand might lose food export markets if GM products are commercialized have slowed the adoption of agricultural biotechnology for research and development of the country. The National Biosafety Framework and control is one key to the success use of the technology. However, precautionary measures must be enforced to assure the public that the risks associated with the use of gene technology are minimal to human health and the environment.

ACKNOWLEDGEMENTS

We thank Timothy Hall for critical reading and suggestions and Sathin Kunawasen for updated information.

REFERENCES

- Bangkokbiznews (2006) Available online:
- http://www.bangkokbiznews.com/2006/09/16/a001_137921.php?news_id=13 7921
- Conover R, Litz R, Malo S (1986) 'Cariflora': a *Papaya ringspot virus*-tolerant papaya for south Florida and the Caribbean. *HortScience* **21**, 1072
- Davidson SN (2008) Forbidden fruit: transgenic papaya in Thailand. Plant Physiology 147, 487-493
- FAOSTAT (2007) Available online: http://faostat.fao.org/site/339/default.aspx
- Fitch M, Manshardt R, Gonsalves D, Slightom J, Sanford J (1990) Stable transformation of papaya via microprojectile bombardment. *Plant Cell Reports* 9, 189-194
- Fitch MMM, Manshardt RM (1990) Somatic embryogenesis and plant regeneration from immature zygotic embryos of papaya (*Carica papaya* L.). *Plant Cell Reports* 9, 320-324
- Fitch MMM, Manshardt RM, Gonsalves D, Slightom JL, Sanford JC (1992) Virus resistant papaya plants derived from tissues bombarded with the coat protein gene of *Papaya ringspot virus*. *Bio/Technology* **10**, 1466-1472
- Greenpeace Southeast Asia (2004) Unfinished Business: New US Patents on GE Papaya in Thailand. Greenpeace Southeast Asia, Bangkok, Thailand. Available online:
- http://www.greenpeacesoutheastasia.org/en/seanews125.html Greenpeace (2007) Available online:
- http://www.greenpeace.org/seasia/th/news/papaya-dumping-moac ISAAA (2008) Available online:
- http://www.isaaa.org/resources/publications/briefs/39/pptslides/Global-Status-Map-2008.pdf
- Kertbundit S, Attasart P, Panyim S, Juříček M (1998) Sequence comparison of coat protein and 3' non-translated region of *Papaya ringspot virus* isolates from Thailand. *Asia-Pacific Journal of Molecular Biology and Biotechnology* 6, 191-197
- Kertbundit S, Pongtanom N, Ruanjan P, Chantasingh D, Tanwanchai A, Panyim S, Juříček M (2007) Resistance of transgenic papaya plants to Papaya ringspot virus, Thai isolate. Biologia Plantarum 51, 333-339
- Manager (2005) Available online: http://www.manager.co.th/science/viewnews.aspx?NewsID=9480000160303. Office of Natural Resources and Environment (2009) Available online:
- http://www.unep.org/Biosafety/Documents/NBFs/NBF_%20Letter_of_undert aking_and_Executive_summary.pdf
- Organic Consumers Association (2004) Available online:
- http://www.organicconsumers.org/ge/papaya091604.cfm

Patent Strom (2006) Available online: http://www.patentstorm.us/patents/7078586.html

- Powell-Abel PP, Nelson RS, De B, Hoffmann N, Rogers SG, Fraley RT, Beachy RN (1986) Delay of disease development in transgenic plants that express the Tobacco Mosaic Virus coat protein gene. *Science* 232, 738-743
- Phironrit N, Chowpongpang S, Attathom S, Warin N, Bhunchoth A (2007) Small-scale field testing of PRSV resistance in transgenic papaya line KN116/5. Acta Horticulturae 740, 169-176
- Phironrit N, Phuangrat B, Burns P, Kositratana W (2008) Monitoring transgenic papaya line KN116/5 R4 planted under screen house condition for assessing horizontal gene transfer to soil bacteria. In: 10th International Sym-

posium on the Biosafety of Genetically Modified Organisms. Symposium Handbook Biosafety Research of GMOs: Past Achievements and Future Challenges, 16–21 November 2008, Wellington, New Zealand, Poster presentation (P8.2310)

- Phuangrat B, Phironrit N, Burns P, Kositratana W (2008) Risk assessment of ringspot virus (PRSV) resistant under screen house conditions using pollen biology and morphological and agronomical characters. In: 10th International Symposium on the Biosafety of Genetically Modified Organisms. Symposium Handbook Biosafety Research of GMOs: Past Achievements and Future Challenges, 16–21 November 2008, Wellington, New Zealand, Poster presentation (P3.23)
- Prasartsee V, Sarindu N, Sakuanrungsirikul S, Chaikiatiyos S, Siriyan R, Gonsalves D (2002a) Green house and field tests in Thailand identify transgenic papaya resistant to papaya ringspot virus. In: *Proceedings of the Khon Kaen University Annual Agricultural Seminar for the Year 2002*, 28-29 Jan 2002, Khon Kaen, Thailand, 2002, pp 82-89
- Prasartsee V, Sarindu N, Sakuanrungsirikul S, Chaikiatiyos S, Siriyan R (2002b) The development of PRSV-resistance transgenic papaya program of the Department of Agriculture. In: *Horticultural Research Institute Annual Conference*, March 26–30, 2002, Petchaburi, Thailand, 10 pp
- Ruanjan P, Kertbundit S, Juříček M (2007) Post-transcriptional gene silencing is involved in resistance of transgenic papayas to *Papaya ringspot virus*. *Biologia Plantarum* 51, 517-520
- Sakuanrungsirikul S, Sarindu N, Prasartsee V, Chaikiatiyos S, Siriyan R, Sriwatanakul M, Lekananon P, Kitprasert C, Boonsong P, Kosiyachinda P, Fermin G, Gonsalves D (2005) Update on the development of virus-resistant papaya: virus-resistant transgenic papaya for people in rural communities of Thailand. Food and Nutrition Bulletin 26, 422-426
- Sarindu N, Prasartsee V (2000) Papaya resistance to Papaya ringspot virus: The success of Thai agriculture genetic engineering. Thai Agricultural Development versus Biotechnology 2000, 214-222 (in Thai)
- Samabuddhi K (2004) Hawaiians warn against GM papaya: Species' introduction may hit native strain. In: *Bangkok Post*, July 4, 2004, Thailand. Available online: http://www.gmwatch.org/archive2.asp?arcid=4337
- Srisomchai T (1975) Studies on Papaya ringspot virus. NEROA (Northeast Regional Office of Agricalture) Report, pp 228-232 (in Thai)
- Sriwatanapongse S, Iamsupasit N, Attathom S, Napasintuwong O, Traxler G (2007) The Study of Agricultural Benefits in Thailand. *Biotechnology Alliance Association*, Bangkok, Thailand, pp 1-65
- Storey WB (1969) Papaya (Carica papaya L.). In: Ferwerda FP, Wit F (Eds) Outline of Perennial Crop Breeding in the Tropics, H. Veenman en Zonen BV, Wageningen, pp 389-407

Technical Biosafety Committee (2008) Available online:

- http://home.biotec.or.th/NewsCenter/my_documents/my_files/22C14_Thaila nd_White_Paper.pdf
- Tennant P, Gonsalves C, Ling K, Fitch M, Manshardt R, Slightom J, Gonsalves D (1994) Differential protection against *Papaya ringspot virus* isolates in coat protein gene transgenic papaya and classically cross-protected papaya. *Phytopathology* 84, 1359-1365
- Tennant PG, Fermin MM, Fitch RM, Manshardt JL, Slightom JL, Gonsalves D (2001) Papaya ringspot virus resistance of transgenic Rainbow and SunUp is affected by gene dosage, plant development, and coat protein homology. European Journal of Plant Pathology 107, 645-653
- Warin N, Chowpongpang S, Bhunchoth A, Romyanon K, Rodaree R, Kositratana W, Attathom S (2003) New papaya cultivars for *Papaya ring-spot virus* resistance. In: *Proceedings of the 41st Kasetsart University Annual Conference*, 3-7 February, 2003, Bangkok, pp 539-546
- **Wongruang P** (2005) GM PAPAYA/RESEARCH BENEFITS Patents rested with Cornell 'from outset'. In: *Bangkok Post*, November 20, 2005, Bangkok, Thailand. Available online:

http://www.munlochygmvigil.org.uk/new_2005.htm