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# An Overview of the Safety Assessment of Transgenic Papaya for the Management of *Papaya ringspot virus* in Jamaica

**Paula F. Tennant[1,2*] • Simone E. Pinnock[2] • Melissa Powell[3] •
Andrew O. Wheatley[2,3] • Donna A. Minott[4]**

[1] Department of Life Sciences, The University of the West Indies, Mona, Jamaica
[2] The Biotechnology Centre, The University of the West Indies, Mona, Jamaica
[3] Department of Basic Medical Sciences (Biochemistry Section), The University of the West Indies, Mona, Jamaica
[4] Department of Chemistry, The University of the West Indies, Mona Campus, Jamaica

***Corresponding author*: * paula.tennant@uwimona.edu.jm**

## ABSTRACT

Papaya (*Carica papaya* L.) production in Jamaica suffered severe losses due to the type p *Papaya ringspot virus* (PRSV), causing devastation to over 40% of local papaya orchards in the past 15 years. Absence of genetic resistance in commercial cultivars has directed disease management efforts towards examination of transgenic resistance. Recently a number of transgenic lines were engineered with the coat protein gene ($cp_T$) of a Jamaican PRSV strain via microprojectile bombardment. Under field tests, $R_1$ transformants exhibited virus-resistant (70-75%) phenotypes. Trees were monoecious, producing red-flesh fruits similar in weight to the non-transgenic controls although some yellow-flesh fruits were obtained from two lines. Nutritional (e.g. vitamins, sugars) and anti-nutritional components (benzyl isothiocyanate, oxalate and cyanide) were assessed. With the exception of one $cp_T$ transgenic line, no significant differences to the control were observed in the levels of nutrients and anti-nutrients at various stages of maturity, although a few random variations were noted. A sub-chronic feeding study in rats revealed markers of general health, body weight, food intake, and activities of plasma, liver and kidney function enzymes, to be comparable for animals fed diets of $cp_T$ transgenic papaya and those of the control group. No effects were observed with the liver or kidney, in organ weights or histopathology. Although not statistically relevant, variations were recorded in some parameters. Thus, the $cp_T$ transgenic papaya lines possess nutritional attributes and resistance to PRSV that can be manipulated in subsequent generations for development of products with acceptable commercial performance. Factors affecting the deregulation and commercialization of the transgenic product are discussed.

**Keywords:** agronomic performance, *Carica papaya*, food safety, genetically engineered, nutritional and anti-nutritional components

**Abbreviations: BITC**, benzyl isothiocyanate; ***cp***, coat protein gene; **CP**, virus coat protein; $\boldsymbol{cp}_{\mathbf{NT}}$, untranslatable version of viral *cp*; $\boldsymbol{cp}_{\mathbf{T}}$, translatable version of viral *cp*; **GUS**, β-D-glucuronidase protein; **HCN**, hydrocyanic acid; ***nptII***, neomycin phosphotransferase II gene; **NPTII**, neomycin phosphotransferase II protein; **PRSV**, *Papaya ringspot virus*; ***uidA***, β-D-glucuronidase gene

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## INTRODUCTION

Two decades since the proposal of pathogen-derived resistance, virus resistant transgenic crops account for < 0.1% (~8,500 ha) of the total acreage of transgenic crops worldwide (James 2008). This strategy of using a portion of the virus' genetic material to confer resistance against the pathogen has proven effective and durable in the field (Fuchs and Gonsalves 2007) and has allowed for continued cost-effective production of some agricultural crops otherwise limited by destructive virus infections. Although a number of plant virus gene sequences have been evaluated for their efficacy of combating virus infections (e. g. RNA-dependent RNA polymerase, dysfunctional movement protein, inverted repeated sequences (Prins *et al.* 2008), most commercial cultivars carry a translatable coat protein gene ($cp_T$) of the virus. Recent studies have shown that the mechanism of resistance is dependent on > 90% sequence similarities between the virus transgene and the infecting virus and involves the degradation of transgene mRNA into small fragments (De Haan *et al.* 1992; Dietzen and Mitter 2006; Lin *et al.* 2007; Eamens *et al.* 2008).

Papaya (*Carica papaya* L.) is the first perennial, transgenic fruit crop released into commerce. The fruit is an essential part of the diet in many tropical and subtropical regions and a significant source of income for resource poor farmers. Increasingly papaya is being grown on a commercial scale and is as important as pineapple and mango in the international market, its success in the international market being due to its reputation as a healthy and dietetic fruit. In 1997, the Food and Agriculture Organization (FAO) estimated that papaya production accounted for 10% of the

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volume of the four major tropical fruits (including mangoes, pineapples, and avocados) produced worldwide. Since then papaya production has doubled. About 6.9 million metric tonnes were produced in 2007, with countries of Latin America and the Caribbean accounting for approximately 50% (FAO Statistics 2007).

Papaya production worldwide has been primarily limited by the *Papaya ringspot virus* (PRSV), which induces plant stunting and causes a significant reduction in the yield and quality of fruits (Purcifull *et al.* 1984). Fruits that develop the diagnostic sunken ringspot blemishes do not accumulate sufficient sugars for an acceptable taste and are not organoleptically appealing to the consumer (Tipton *et al.* 1990; Gonsalves 1998). Conventional methods used in the management of PRSV such as, the removal and burning of infected plants and the use of insecticides against the insect vector, offer only temporary control (Gonsalves 1998).

Pathogen-derived resistance against PRSV has been extensively studied in Hawaii and has been shown to be an effective strategy for managing the virus. The first $cp_T$ transgenic line designated as line 55-1 was used to develop PRSV-resistant Hawaiian transgenic cultivars 'Rainbow' and 'SunUp' in the 1990s. By 1998, these two PRSV resistant papaya cultivars were released to growers (Fitch *et al.* 1992; Manshardt 1998). The $cp_T$ transgenic papayas have offered durable resistance to PRSV and have controlled the virus in Hawaii (Ferreira *et al.* 2002). Today they make up 80% of papaya acreage in the US state (Stokstad 2008). 'Rainbow' is a yellow-flesh $F_1$ hybrid of a cross between the $cp_T$ transgenic cultivar and non-transgenic cultivar, 'Kapoho solo' (Manshardt 1998; Gonsalves 2002), the preferred papaya cultivar in Hawaii.

Other countries, including Brazil, Jamaica, Venezuela, Thailand, Australia, Japan Malaysia and the Philippines (Tecson-Mendoza *et al.* 2008), have since used the technology and the $cp_T$ gene from the local virus strain to develop their own transgenic varieties. So far no $cp_T$ transgenic papaya cultivars have been commercialized in these regions. The reasons for this include but are not limited to, the cost of development and regulatory approval, international trade issues and issues relating to the perception of safety of genetically modified crops. Despite the benefits that genetic engineering offers, the technology has provoked public concern regarding the safety of the generated crops and plant products (Harlander 2002; Moseley 2002). Among the major concerns is whether or not genetically modified products pose a unique risk to human health and the environment (Paparini and Romano-Spica 2004; Lemaux 2008).

This review documents the substantive progress towards the introduction of $cp_T$ transgenic papaya in Jamaica. We recount the events that led to the development of transgenic papayas, summarize the field and safety testing conducted and address the challenges to be faced in commercial production of the $cp_T$ transgenic papaya in the country.

## DEVELOPMENT OF THE JAMAICAN TRANSGENIC PAPAYA

Papaya is one of the major nontraditional crops grown in Jamaica. Prior to the mid 1980s, there was no organized commercial activity for the fruit crop (Thomas 1993). Small backyard plantings, producing for the domestic market and home consumption, accounted for approximately 1200 metric tonnes. Following the promotion of papaya as a viable alternative to the traditional export crops (primarily sugar cane and banana), commercial production after the mid 1980s exceeded 20,000 metric tonnes (STATIN 2006). The 'Sunrise solo', the leading commercial variety, consists of small, red-orange flesh, pear-shaped fruit with a mass range of 300-700 g. Jamaican papaya, exported as fresh produce to the United States, Canada and Europe, is esteemed for its sweet, flavorful fruits. Large fruited varieties from Floridian and South American selections are cultivated mainly for use in the local processing industries (Thomas 1993).

The first epidemic of PRSV in Jamaica occurred in 1989 in two established papaya growing regions. Attempts at eradication failed to contain the virus, which subsequently spread to the major papaya producing regions in the West (Young 1994). By 2005, the virus was reported to be island-wide. Production decreased by 48% over a 10-year period (STATIN 2006).

Shortly after the major outbreak of PRSV in 1994, a papaya-breeding program with collaborators at the University of the West Indies (Mona), Cornell University, and the private sector organization, the Jamaica Agricultural Development Foundation (JADF), was initiated. The major goal of the program was to develop transgenic papaya varieties using virus *cp* genes. Somatic papaya embryos of the commercial variety were transformed via microprojectile bombardment with *cp* genes isolated from virus samples of one of the traditional papaya growing regions of the island (Tennant *et al.* 2002). Two versions of the viral *cp* were cloned; a translatable version ($cp_T$) and an untranslatable version ($cp_{NT}$). The literature, at that time, differentiated between CP-mediated and RNA-mediated protection elicited by $cp_T$ and $cp_{NT}$ viral genes, respectively. It was speculated that the mechanism of resistance with $cp_T$ was conferred by the CP, is broad spectrum against distantly related viruses (Namba *et al.* 1992; Pang *et al.* 1992; Maiti *et al.* 1993), and effective at both low and high levels of virus inoculum (Nelson *et al.* 1987; Providenti and Gonsalves 1995). Different lines of evidence also suggested that resistance exhibited by transgenic plants carrying $cp_{NT}$ versions of the viral *cp* is RNA-mediated and due to the induction of a specific cellular RNA-degradation mechanism which may also be induced by virus infection (Farinelli and Malnoë 1993; Kollar *et al.* 1993; Lindbo *et al.* 1993; Smith *et al.* 1994). Since the diversity of PRSV in the country was not known at that time, both *cp* versions were utilized in the transformation experiments to assure field resistance. The *cp* constructs were coupled to the 35S promoter and terminator of the *Cauliflower mosaic virus* and sandwiched between two marker genes, the selectable marker neomycin phosphotransferase II *(nptII*) and screenable β-glucuronidase (*uidA*) (Tennant *et al.* 2002).

Some 150 transgenic lines were generated, 39 of which were acclimatized under greenhouse conditions at Cornell University. Transgenic status and integrity of the *cp*, *nptII* and *uidA* transgenes were confirmed in regenerated plants via polymerase chain reaction (PCR) with oligonucleotides specific to the 5′ and 3′ ends of the respective genes. Products of the expected sizes (0.9, 1.0, and 1.7 Kb, respectively) were obtained, suggesting that full-length versions of the three transgenes were introduced. Probes of *cp*, *nptII*, *uidA* genes were used to determine the integration patterns of the transgenes; while a similar single insertion pattern of the *cp* and *uidA* genes was observed, there were multiple insertions of the *nptII* gene. Strong signals were recorded with the Jamaican transgenic papaya in total RNA membrane blots probed with PRSV *cp* gene (Tennant *et al.* unpublished).

Subsequent greenhouse inoculation experiments revealed high levels of resistance (78%) to manual inoculations with the homologous virus and plants carrying the $cp_T$ gene. Lower levels of resistance (10%) were obtained with transgenic plants carrying the $cp_{NT}$ and a recovery phenotype (15%). The latter phenotype was characterized by the development of chlorotic spots over the leaf lamina 20 to 58 days following inoculations with all subsequent new leaves free of symptom expression. $R_0$ clones that were not used in the infectivity assays were shipped to Jamaica under permit from the National Biosafety Committee.

## AGRONOMIC EVALUATION

Subsequent testing of the transgenic papayas in Jamaica was aimed at not only investigating field resistance, but also the agronomic performance of the trees. Guidelines on assessment of food safety of transgenic crops require comparative analysis of the transgenic crop with the conven-

**Fig. 1 Fruits of $cp_{\mathrm{T}}$ transgenic (lines 52.22, 52.24, 52.3) and non-transgenic (control) papaya trees**. Pear-shaped fruits of the transgenic papaya (**A**) and non-transgenic 'Sunrise solo' variety (**B**). Note PRSV-induced blemishes on the non-transgenic fruit.

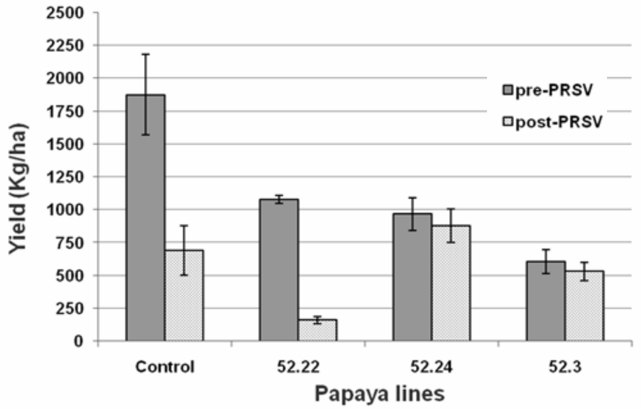

**Fig. 2 Yield parameters of $cp_{\mathrm{T}}$ transgenic (lines 52.22, 52.24, 52.3) and non-transgenic (control) papaya trees before and after introduction of PRSV into the experimental field plot.**

tionally bred parent crop that has a history of safe use (König *et al.* 2004). Thus transgenic lines carrying translatable $cp_{\mathrm{T}}$ constructs and non-transgenic papaya were established in a commercial papaya-growing region in Jamaica (Pinnock *et al.* unpublished data). Tree vigor, leaf nutrient content, root water content, fruit and yield parameters were examined over two years, before and after exposure to the virus. The transgenic papaya lines used in the study were derived from $R_0$ transformants carrying $cp_{\mathrm{T}}$ constructs. Three lines were selected based on good levels of resistance of the parent $R_0$ plants to the virus (Tennant *et al.* 2005).

During the field trial, all $cp_{\mathrm{T}}$ transgenic trees exhibited similar growth patterns to non-transgenic trees, but trees of one line displayed low vigor which was evidenced by smaller trunk diameters. Similar to previous reports on papaya (Klein *et al.* 1985), seasonal fluctuations in growth and fruit production associated with the crop were observed. Greatest increases in growth were obtained between 14 and 23 months. Apart from random variations, $cp_{\mathrm{T}}$ transgenic and non-transgenic papaya trees generally showed similar nutrient and water use patterns. Nitrogen and phosphorus levels higher than those reported for papaya (1.01-2.5% and 0.22-0.4%, respectively, Mills *et al.* 1991; Hardisson *et al.* 2001) were noted for both non-transgenic and $cp_{\mathrm{T}}$ transgenic samples. However, the micronutrient levels fell within literature ranges for both $cp_{\mathrm{T}}$ transgenic and non-transgenic papaya leaves, except for the iron content of the transgenic leaves which was higher than the expected range of 25-100 ppm (Pinnock *et al.* unpublished data).

The shape (**Fig. 1**) and mass of fruits from $cp_{\mathrm{T}}$ transgenic trees showed appreciable homogeneity with those from control trees except for those of one line which produced smaller fruits. Compared to the fruit yields of non-transgenic trees, lower yields were produced by $cp_{\mathrm{T}}$ transgenic trees. However, the yields of non-transgenic trees dramatically decreased following controlled introduction of virus in the plot, while $cp_{\mathrm{T}}$ transgenic trees of two lines continued to produce fruit under disease pressure (**Fig. 2**, Pinnock *et al.* unpublished data). As with transgenic papaya in other regions (Lines *et al.* 2002; Bau *et al.* 2003, 2004; Davis and Ying 2004), the Jamaican $cp_{\mathrm{T}}$ transgenic trees were up to 75% more resistant to PRSV than non-transgenic control trees. Trees of two lines were found more resistant to the virus, with about 25% of the trees exhibiting leaf symptoms as compared to 31% of the trees of the other lines (Chin and Tennant, unpublished data). Further work is required to determine the factors contributing to resistance in the $cp_{\mathrm{T}}$ transgenic plants. Generally *in planta* factors, such as transgene dosage, plant developmental stage and sequence identity (> 89.5%) between transgene and virus isolate populations, complicate transgenic virus resistance in papaya (Tennant *et al.* 2001). Other groups have suggested the involvement of the virus helper component – protease (HC-Pro), a general pathogenicity enhancer with the ability of suppressing PTGS, in cases where there is no correlation of resistance to higher degrees of sequence similarity with the transgene and the challenge isolate (Kasschau and Carrington 1998; Bau *et al.* 2003; Tripathi *et al.* 2004). It is also speculated that environmental conditions, notably low temperatures, high soil moisture, and infections with other pathogens (e.g. root rot fungi) can affect the physiological status of the transgenic plants and under these suboptimum conditions, compromise the mechanism of post-transcriptional gene silencing.

Given the precautions observed at the onset and throughout the field trial, the variations between the $cp_{\mathrm{T}}$ transgenic and non-transgenic papaya were not attributed to environmental influences but to intrinsic factors. One possibility is transgene insertion effects. Characterization of the insertion site of the three transgenes in the Jamaican $cp_{\mathrm{T}}$ transgenic papaya would reveal whether important endogenous plant genes have been disrupted. However, two observations made during the field trial point to other sources of the variation and suggest that the transgenic lines are not true breeding 'Sunrise solo' varieties. Firstly, a mixture of yellow and orange pulp in transgenic fruits was observed. Secondly, there were a number of staminate (male) trees in the field. True-to-type 'Sunrise solo' trees produce fruits with orange pulp and pistillate (female) and bisexual (hermaphrodite) progeny (Hamilton and Ito 1968). Staminate trees indicate out-crossing with other papaya varieties that produce pistillate and staminate trees. Although embryos of fruits from bisexual trees were used in the transformation and development of the transgenic lines, it seems likely that the fruits resulted from out-crossing which affects 5% of seed (Kim *et al.* 2002). Additionally, variation in morphological traits and agronomic performance can also be attributed to somaclonal variation. Somaclonal variation or phenotypic variation results from both pre-existing genetic variation within plant materials and variation induced during tissue culture (Larkin and Scowcroft 1981; Evans *et al.* 1984). Currently, the transformation technologies for papaya require the delivery of transgenes to cells grown *in vitro* and the induction of somatic embryogenesis from immature zygotic embryos using 2,4-dichlorophenoxyacetic acid (Fitch *et al.* 1990; Cai *et al.* 1999; Tennant *et al.* 2002). The *in vitro* environment (Karp 1994) and the plant growth regulator, 2,4-dichlorophenoxyacetic acid, are known to introduce variation.

Taken together, the general conclusion from the agronomic evaluation is that the $cp_T$ transgenic lines show some horticultural similarities to the nearest equivalent non-transformed 'Sunrise solo' papaya and exhibit varying levels of resistance against PRSV that can be manipulated in later generations to give a viable commercial product. If the observed variations in agronomic traits are a result of undesirable tissue culture induced genetic changes or out-crossing, backcrossing to the original parental variety and selection of progeny with appropriate horticultural (Dahleen *et al.* 2007) and PRSV resistant phenotypes can be adopted. Nonetheless, the transgenic fruits show characteristics suitable for distributors to the United States and Europe. The desired fruit characteristics for the export market as defined by the Jamaica Papaya Growers Association include pear-shaped fruits (shape index of 0.64-0.77) with red flesh, Brix of at least 12°, and a mass range of 385-535 g. Although similar fruit quality attributes are required by both the United States and European markets, buyers in the United States prefer fruits within the mass range of 274-744 g, while the market in the United Kingdom shows a preference for fruits in the range 224-535 g.

## NUTRIENT AND ANTI-NUTRIENT COMPOSITION

Further assessment of the $cp_T$ transgenic fruits was conducted for assurance of food safety. The saporous papaya fruit being low in protein and fats but high in crude fiber and vitamins and minerals can be recommended as an interesting addition to a healthy diet. Although having a high amount of bound water (37 to 43% in comparison to the 10% found in most other fruits), it is combusted to produce large amounts of ash (De Calzada and Menchu 1980; Salunke and Desai 1984). A climacteric fruit like banana and mango, the final composition of the papaya fruit result from various metabolic processes that occur post harvest. It has been reported that while soluble solids, total carbohydrates, proteins and carotenoids, increase up to the full-ripe stage, a decrease in the moisture and fiber content occurs (Chan *et al.* 1979; Sankat and Maharaj 1997; Bari *et al.* 2006). In addition, papaya, as with many plants, produces several compounds such as oxalates, hydrocyanic acid (HCN) and benzyl isothiocyanate (BITC) (Umoh 1998), that are considered to be integrally involved in its chemical defense against pests and herbivores (Iason 2005). The effects of these anti-nutrients on human nutrition have been examined with different food crops and have been shown to vary from interference of the absorption of micronutrients and proteins to adverse impact on growth and development. Above a certain dose, toxicity may be exhibited (Welch and Graham 2000). Not all consequences are however deleterious, BITC in particular is a multifunctional antioxidant which has demonstrated the potential to prevent inflammation-related carcinogenesis (Nakamura 2008). Levels of the anti-nutrients, BITC, HCN and oxalates appear to be developmentally regulated and are reported to decrease during

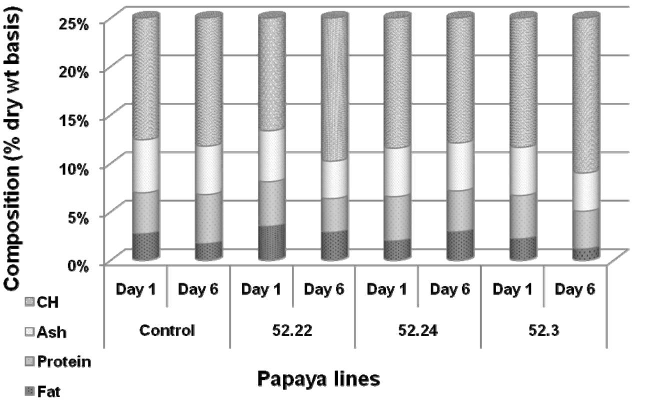

**Fig. 3 Changes in the relative composition of macro-nutrients in $cp_T$ transgenic (lines 52.22, 52.24, 52.3) and non-tansgenic papaya lines (control) with maturity.** Composition is reported on a dry weight basis. Scale of y-axis has been truncated at 25% in order to show the relative differences between the ash, fat and protein; the remaining 75% is due to carbohydrate which was calculated by difference: carbohydrate = 100 - (moisture + ash + protein + fat).

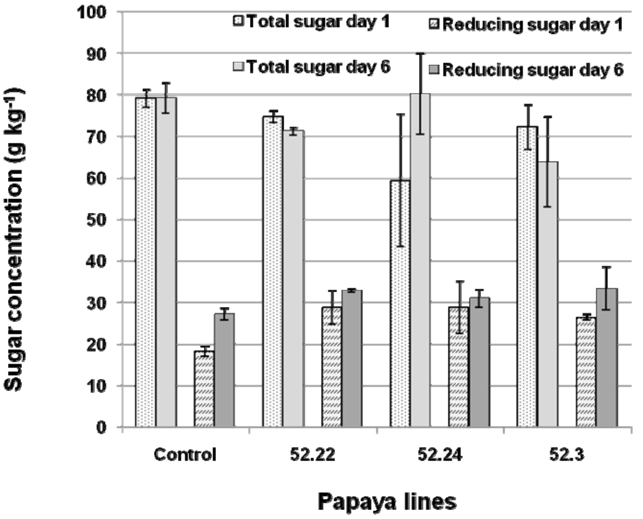

**Fig. 4 Total and reducing sugars variation in non-transgenic (control) and $cp_T$ transgenic (lines 52.22, 52.24, 52.3) papaya from commercial maturity (day 1) to eating maturity (day 6).**

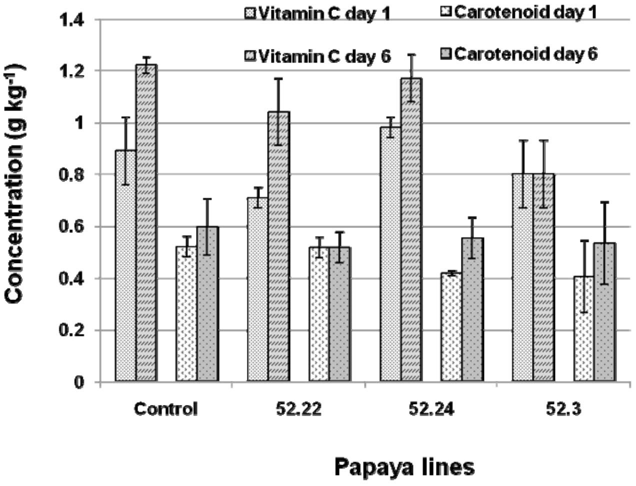

**Fig. 5 Vitamin C and carotenoid content of non-transgenic (control) and transgenic $cp_T$ transgenic (lines 52.22, 52.24, 52.3) papaya lines at commercial maturity (day 1) and eating maturity (day 6).**

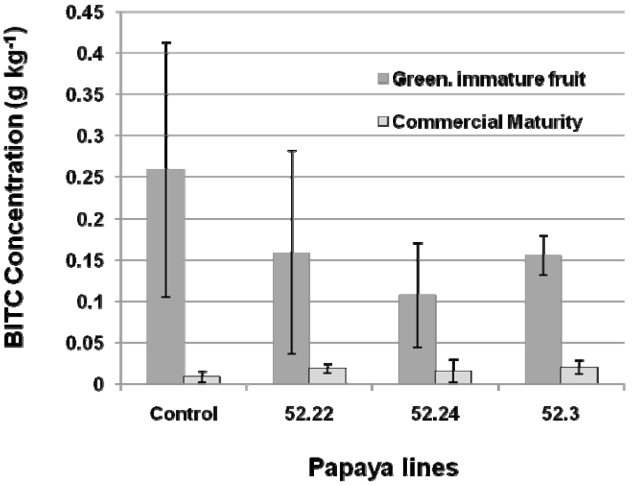

**Fig. 6 Comparative reduction in BITC levels in mature $cp_T$ transgenic (lines 52.22, 52.24, 52.3) and non-transgenic (control) papaya fruits.**

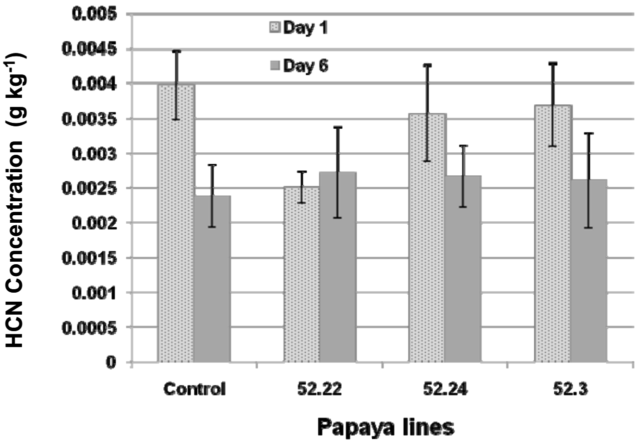

**Fig. 7 Variation in HCN content of non-transgenic (control) and $cp_T$ transgenic (lines 52.22, 52.24, 52.3) papaya lines from commercial maturity (day 1) through to eating maturity (day 6).**

ripening of papaya fruit (Tang 1971; Umoh 1998). Thus, the levels of the major nutritional and anti-nutritional components of $cp_T$ transgenic and non-transgenic papayas were compared over ripening using standard methods of analysis repeated for a minimum of triplicate samples unless otherwise indicated (AOAC 2000; Roberts *et al.* 2008).

Proteins in the papaya were determined using the Kjeldahl method whereas the fat content was analysed using a Soxhlet extractor in a semi-continuous extraction process. Reducing sugars were titrimetrically quantified using the Lane and Eynon general method while fiber residue was analysed gravimetrically after consecutive hydrolysis of the carbohydrates and proteins in the papaya samples with heat stable α-amylase, protease, and amyloglucosidase. Moisture analyses were carried out by drying the papaya samples in an air oven to constant weight and ash was determined by a gravimetric protocol. Individual mineral content (iron and calcium) in the papaya ash was assessed by atomic absorption spectroscopy but phosphorus was determined spectrophotometrically as its molybdovanadate complex. Vitamin C (ascorbic acid) was extracted and titrated against 2,6-dichloroindophenol in the presence of metaphosphoric acid solution. Carotenoid extracted from the papaya with ethanolic acetone was subject to colorimetric analysis (Gross 1991; Howard *et al.* 1996). The antinutrients hydrocyanic acid and oxalate were respectively titrated against silver nitrate and potassium permanganate, BITC content however was measured using Gas Chromatography-Mass Spectrometry (Tang 1971, 1973).

Compositional changes in the $cp_T$ transgenic papaya lines over three maturities (commercial, eating and an intermediate between the two) were, as expected, comparable to that previously reported (Yamamoto 1964; Chan *et al.* 1979, Bari *et al.* 2006). Reducing sugars, protein, vitamin C and carotenoids generally displayed an upward trend whereas a slight decline in moisture, ash, fiber, and fat content was observed at final stage of ripening (**Figs. 3-5**). Papaya samples from one line, however, consistently demonstrated different reducing sugar, carotenoid, calcium and phosphorus content compared to the non-transgenic 'Sunrise solo' variety. Nevertheless, in all cases, where lower values for the nutrients in question were obtained, the differences identified were small. Moreover, all of the other nutritional components showed no significant differences although there were random variations in the levels. Similarly, some variability in the concentrations of the three anti-nutrients, namely BITC, HCN and oxalate, was observed. In keeping with expectations, the levels of the anti-nutrients decreased with maturity and the values obtained were within the range of concentrations reported for the parental variety (**Figs. 6, 7**) (Tang 1971; Umoh 1998).

Thus, the nutritional analysis during ripening showed that $cp_T$ transgenic papayas are very similar to the non-transgenic 'Sunrise solo' papaya and it would appear that the transformation and expression of the viral *cp* and the two marker genes in transgenic papaya did not produce any major unintended alterations in composition, either with the nutrients or with the anti-nutrients. The random variations observed can be decreased by cross breeding with the conventional 'Sunrise solo' variety. It is known that natural biological variation of individual plants grown under the same conditions (as a result of differences in plant development, metabolism and biotic factors) influence nutritional composition (König 2004). But generally, variability in nutritional composition is attributed to differences in abiotic factors such as climate, soil type and fertility (Shewfelt 1990). Given the observations during the field trial previously described, it seems likely that the variations resulted from outcrossing and somaclonal variation (Shepherd *et al.* 2006).

## SUBCHRONIC TOXICITY ASSESSMENT USING RAT MODELS

Even though the compositional studies established the equivalence of the Jamaican $cp_T$ transgenic papaya to that of its nearest equivalent unmodified counterpart, a subchronic whole-food animal study using rat models was undertaken to substantiate nutritional equivalence and safety. Adult male and female Wistar rats were fed diets of marketed laboratory rodent diet supplemented with either $cp_T$ transgenic or non-transgenic papaya for 90 days. Papaya pulp and peel were incorporated into the diet at 100 g $kg^{-1}$, i.e. at the equivalent of twice the average daily human consumption of fresh papayas (CFNI 2000; WHO 2003). A control group of animals receiving a diet of only the marketed laboratory rodent diet for the same time period was included. Body weights and food consumption were monitored during the study in keeping with the findings of a review of the safety assessment of macronutrients. According to Borzelleca (1996), the single most effective way to evaluate the overall health of an animal is to observe the effects of treatment on body weight, food consumption and feed efficiency. Similarly, a review of the relationship between different chemical structures to toxicity noted that "no-observed effect levels" were more frequently based on body weight changes than other parameters measured in subchronic and chronic rodent toxicology studies (Munro *et al.* 1996). Nonetheless on termination, markers of general health and hepatotoxicity, including organ weights, activities of liver and kidney function enzymes and cholesterol and triglyceride levels, from $cp_T$ transgenic-fed and control animals were compared (Powell *et al.* 2008).

During the course of the study, animals of all groups appeared healthy and clinically normal. Food consumption and weight gain were comparable for treatments that received the control diet or diets supplemented with non-transgenic or $cp_T$ transgenic papaya. No differences in palatability of diet formulations containing non-transgenic or $cp_T$ transgenic papaya pulp or peel were observed. Significant differences in the absolute weights of liver, kidneys, spleen and intestines were not found at terminal examination. Similar levels of plasma protein were observed across all treatments of the test and reference groups relative to the control group. Likewise, liver and kidney function, as evaluated by acid phosphatase activity which has been shown to be sensitive to nutritional stress in rats (Adamson and Esionye 1987), was not significantly altered for the groups that consumed $cp_T$ transgenic diets relative to the control groups. Furthermore, the activities of alanine and aspartate aminotransferase, which have been widely employed as clinical diagnostic tools because they serve as sensitive indicators of tissue damage involving cellular necrosis or increased membrane permeability, were comparable among the different groups. Overall the plasma cholesterol levels, markers of cardiovascular risk, were similar to the control, as were triglycerides, the biomarkers for hepatotoxicity. Although not statistically relevant, variations were observed in the parameters monitored with the control and treatment groups, however, the values obtained were within the range of in house reference values and those reported for rats (Cousins and Miller 1985; Car *et al.* 2006). Histopathological analyses supported these observations. That is, the analyses revealed no gross or macroscopic differences between treatment groups and the control group.

Taken together, differences between the animal groups were not regarded as reflecting a toxicologically meaningful effect. Thus, relative to the non-transgenic counterpart, it is reasonable to assume that the $cp_T$ transgenic papayas as whole foods had no evident effect on the parameters considered in this model. The findings add to the growing body of research into the potential risks posed by virus-resistant GM crops. There is a significant amount of evidence that GM crops have little to no detrimental impact on animal health beyond those of conventional agricultural crops (Craig *et al.* 2008). There is the documented safe release of transgenic squash over the past 12 years in the United States. Studies with this crop suggest that there is no significant risk on allergenicity and human health (Fuchs and Gonsalves 2007). Similarly, $cp_T$ transgenic papayas have been cultivated and consumed in Hawaii for over 10 years (Fuchs and Gonsalves 2007). Animal feeding studies with transgenic pepper and tomatoes give further support to the safety of virus-resistant transgenic crops expressing viral *cp* genes (Cai *et al.* 2003; Chen *et al.* 2003).

Of notable mention, structural and other characteristics of plant viral CP provide additional support that CP products are not regarded as toxicants or allergens: (i) sequence relatedness of 35% (or higher) or a continuous stretch of 8 amino acids is not shared with known allergens (Hileman *et al.* 2002), (ii) low levels or undetectable levels of CP are generally reported in *cp* transgenic plants because of the RNA silencing mechanism in virus resistant transgenic plants, resulting in low exposure levels and (iii) resistance to digestion under acidic conditions has not been demonstrated (Herman *et al.* 2006; Fuchs and Gonsalves 2007; Roberts *et al.* 2008). The marker proteins, NPTII and GUS, used extensively in the development of various transgenic plants, have been assessed based on sequence comparisons with allergens and toxins, *in vitro* digestion studies and acute oral toxicity studies (Fuchs *et al.* 1993; EPA 2000; CFIA 2006). These proteins are deemed safe when assessed individually and so far testing of whole foods with these constructs has demonstrated that their expression in a new host does not result in toxicology.

## CONCLUSIONS AND CHALLENGES

The studies with the Jamaican transgenic papaya were conducted as part of a risk assessment dossier required by the local National Biosafety Committee. The objectives of the studies were to characterize the resistance of the $cp_T$ transgenic papaya, to assess their agronomic performance, nutritional composition and safety of the transgenic fruit. Comparable agronomic performance, as well as variation in some nutritional traits, was observed with the $cp_T$ transgenic papaya and the non-transgenic counterpart. Presumably some of the variation can be attributed to biological variation, but further investigation of other parameters may be warranted as the biological significance is not known. Overall, the findings suggest that the Jamaican $cp_T$ transgenic papayas may not have adverse effects as regards the agronomic and safety parameters considered in this study.

The challenge then is to deregulate the Jamaican $cp_T$ transgenic varieties with acceptable commercial performance. Although the industry responded in a timely manner with the development of the $cp_T$ transgenic papaya shortly after the second major outbreak of the disease, a number of factors have slowed the process of deregulation and commercialization of the transgenic papaya. Firstly, there was the lack of support for a final field trial. Based on the observations of variations in agronomic and nutritional properties, crosses were made with non-transgenic 'Sunrise solo' papaya. The objective of the final trial was three-fold; to assess the agronomic traits and selected nutritional components of the progeny, examine their performance on farmers' orchards and to build seed supply. A further complication is acceptance of the transgenic product. Although a number of farmers involved in small papaya production are interested in receiving $cp_T$ transgenic papaya seeds and participating in setting up experimental plots on their farms, the major papaya growers are less supportive and are concerned about genetic contamination of their non-transgenic varieties and conceivably their European markets. And finally and more importantly, there are regulatory and policy constraints. Jamaica was ahead of its Caribbean counterparts having established a National Biosafety Committee in 1997, initiated field-testing of genetically modified papaya varieties in 1998 and having signed the Cartagena Protocol on Biosafety in 2001. The country, however, now appears to be lagging behind. In preparing to ratify the Cartagena Protocol there has been the development of the National Biosafety Framework, National Biosafety Policy and National Biosafety Act, however, public consultations have not been conducted nor documents presented to the Political Directorate. Thus, the deregulation of $cp_T$ transgenic papaya in Jamaica is on hold.

Despite the general recognition of the importance of biotechnology, Jamaica and most states in the Caribbean region have not yet fully embraced the opportunities. Undoubtedly the Agricultural sector can benefit from the application of biotechnology. The technology represents an arsenal of tools that can to be used to increase production or cut production costs, or develop product attributes desired by consumers. Co-ordination of agricultural research and development efforts nationally and regionally with links to international organizations, development of systems that can assist the region to access, generate and utilize new and appropriate technologies and the establishment of regulatory frameworks that would provide traceability and biosafety, are all areas in need of attention. Most importantly, state commitment and policy provisions for enabling public awareness would facilitate the adoption and utilization of the technology in the region. Notwithstanding, country import inhibitions and international product and process quality standards may yet pose a challenge to small island developing states.

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