CP-Transgenic and non-Transgenic Approaches for the Control of Papaya Ringspot: Current Situation and Challenges

Gustavo A. Fermin1* · Luz T. Castro2 · Paula F. Tennant3

1 Centro Jardín Botánico, Faculty of Sciences, Universidad de Los Andes, Mérida 5101, Mérida, Venezuela
2 Department of Biology, Faculty of Sciences, Universidad de Los Andes, Mérida 5101, Mérida, Venezuela
3 Department of Life Sciences, University of the West Indies, Mona, Kingston 7, Jamaica

Corresponding author: *fermin@ula.ve

ABSTRACT

In the last decade, successful cases of managing plant virus diseases using the transgenic approach have been reported, with the best known example being the Papaya ringspot virus (PRSV)-resistant transgenic papayas in Hawaii. Use of the coat protein (cp) gene has proven effective with not only papaya, but with various plant-virus systems such as squash. Although other viral sequences are equally effective in conferring resistance, few transgenic plants engineered with these sequences have made their way into the market. In addition, opposition to genetic manipulation of crop plants has prevented wide application of the technology, despite the fact that many countries (including Jamaica, Brazil and Venezuela) have produced and characterized several generations of resistant transgenic papayas. Using the papaya-PRSV system as a case study, we examine the transgenic cropping systems available, constraints to the adoption of transgenic papayas in various countries, as well as the impact the technology has made on world production of this fruit crop. Alternative non-cp and non-transgenic approaches of managing PRSV are also presented.

Keywords: Carica papaya, biotechnology, GM, transgenic fruits
Abbreviations: cp, coat protein gene; DAS-ELISA, double antibody sandwich enzyme linked immunosorbent assay; HC-Pro, helper component- protease; npr1, neomycin phosphotransferase type II gene; PRSV, Papaya ringspot virus; PTGS, post transcriptional gene silencing; siRNA, small interfering RNA; uidA, β-glucuronidase gene

INTRODUCTION

Papaya (Carica papaya L.), the best known and most widely distributed species of the family Caricaceae, is cultivated mainly for its nutritious fruits in tropical and subtropical regions (Manshardt 1992). Among its pathogens, Papaya ringspot virus (PRSV) represents the most serious threat to production (Tripathi et al. 2008), with losses of up to 100% reported in some regions (Tennant et al. 2007). Although PRSV was first described in Hawaii in 1945, it has been recognized as a major threat to papaya production in many tropical and subtropical areas including South and Central America, Africa, Asia and the Caribbean (partially reviewed by Tripathi et al. 2008) before the 1940s. Moreover, PRSV still continues to be reported “for the first time” in many countries where, most likely, it has been overlooked for decades, as in the case of Mexico (Noa-Carrazana et al. 2006). Arguably the multiple names assigned to the etiological agent and the disease over the years has clouded the clear identity of isolates. Table 1 summarizes the “first report” of PRSV in different regions worldwide between 1910 and 2006. It is worth noting that in at least two cases the presence of PRSV in certain regions antedates the “first report”. In Australia, the virus was assumed to be reported for the first time in 1991 (Thomas and Dodman 1993), but recently Lima et al. (2001) note that a disease attacking papayas in the early 1920s could most likely be attributed to PRSV. Not surprisingly, in the decade of the 1930s many different reports in the Caribbean assumed that the virus was already present in Venezuela, Barbados and Jamaica (see Table 1). Interestingly, India, despite being reputed as a center of origin of the virus, has apparently served poorly as a source of the virus to neighboring countries since the disease only became evident in surrounding regions in the late 1970s (Thailand) or 2000s (Iran). Based on molecular analysis of PRSV isolates, Gibbs et al. (2008) argue that the most plausible explanation for the PRSV diaspora is the transmission of the virus by seeds (but not necessarily via papaya), an event that has apparently occurred at least three times, and probably 300 years ago to the Americas. Mutation, in addition to movement of PRSV, appears to be significant in the molecular evolution of the virus. Recombination, however, is emerging as an important factor affecting the genome architec-
Papaya’s world production is non-transgenic

Based on FAO’s statistics of area harvested, yield and gross production (2010), 59 countries worldwide produced some 9,095,875 MT of papaya in 2008 (Fig. 1). As shown in Fig. 2, world production of papaya has been increasing steadily, in an exponential fashion, over the last 48 years. However, scrutiny of the production levels in individual countries during this period reveals considerable variation.

In the period analyzed, papaya production increased by 687.58% or almost 7 times, presumably due to improved yields (that amounted to ca. 203.21% increase or 2 times), and to an augmentation in the area devoted to papaya cultivation (3.38 times increase). Of note, the strategy of growing more papaya differed between countries. Table 2 lists the major producers of papaya during the last nine years, from 2000 to 2008. The first 20 producers for 2008 account for 96% share of global production and none of these countries, apart from China perhaps, which is responsible for 1.32% of world production, cultivates transgenic papayas. Fig. 3 summarizes the production, yield and cultivated area of the major, mid- and minor producers of papaya. It is worth noting, for example, that in India (the first largest papaya producer) a surprisingly high increase in production can be entirely attributed to a huge increase of area harvested in response to a tremendous decrease in yield in the 1980s (that stills needs to recover the good figures in yield of the early 60s). In contrast, two other major producers, Brazil and Mexico, seemingly increased production by means of improving yields in combination with a moderate increase in the area harvested. Similarly, the mid-producers Venezuela and China employed much the same strategies to increase papaya production. On the contrary, the yield has remained more or less the same during the last 15 years (after a short period of increased yields) in the Philippines and this complements the trend of increased areas devoted to the cultivation of papaya. Finally, among selected small producers, the most notable case is the United States. The strategy adopted in the US involved the development and the introduction of transgenic papaya cultivars.

Table 1: Examples of “first reports” of Papaya ringspot virus in selected countries.

<table>
<thead>
<tr>
<th>Year</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circa 1930</td>
<td>Florida</td>
<td>The description of a viral disease of squash resembles that of Watermelon mosaic virus 1 (a previous synonym of PRSV), Anderson 1954</td>
</tr>
<tr>
<td>Circa 1930</td>
<td>Jamaica and Minor Antilles</td>
<td>In Jamaica in 1929 according to Jensen (1949), and the beginning of the 1930s as reported elsewhere (Marte and Thomas 1984)</td>
</tr>
<tr>
<td>1931</td>
<td>Tanganyika</td>
<td>Wallace 1936 (possibly PRSV)</td>
</tr>
<tr>
<td>Circa 1937</td>
<td>Venezuela</td>
<td>Muller 1941</td>
</tr>
<tr>
<td>Circa 1940</td>
<td>Puerto Rico</td>
<td>Aduar 1947; according to others (Marte and Thomas 1984), most probably during the 1930s.</td>
</tr>
<tr>
<td>1949</td>
<td>Hawaii</td>
<td>Jensen 1949</td>
</tr>
<tr>
<td>1952</td>
<td>Colombia</td>
<td>Torres and Giacometti 1966</td>
</tr>
<tr>
<td>1958</td>
<td>India</td>
<td>Capoor and Varma 1958, cited by Jain et al. 2004</td>
</tr>
<tr>
<td>1969</td>
<td>Brazil</td>
<td>Costa et al. 1969</td>
</tr>
<tr>
<td>1975</td>
<td>Mexico</td>
<td>Téllez-Ortiz et al. 1991</td>
</tr>
<tr>
<td>Circa 1975</td>
<td>Thailand</td>
<td>CharnoensHip et al. 2003</td>
</tr>
<tr>
<td>1979</td>
<td>Australia</td>
<td>Thomas and Dodman 1993</td>
</tr>
<tr>
<td>1992</td>
<td>According to symptomatology as reported by Shukla and Ward (1989) and re-interpreted by Lima 2001</td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>Indonesia</td>
<td>Pournahm et al. 2004</td>
</tr>
<tr>
<td>2002</td>
<td>St. Kitts</td>
<td>Chin et al. 2007</td>
</tr>
<tr>
<td>2004</td>
<td>Bangladesh</td>
<td>Jain et al. 2004</td>
</tr>
<tr>
<td>2004</td>
<td>Cook Islands</td>
<td>Davis 2004</td>
</tr>
<tr>
<td>2006</td>
<td>Ivory Coast</td>
<td>Diao et al. 2006</td>
</tr>
</tbody>
</table>
in Hawaii in the late 1990s. Although not all papayas grown in the US are transgenic, it is quite surprising that the yields have been fluctuating since the introduction of the transgenic cultivars. Apart from an initial recovery in 2000, papaya production has not reached its maximum. Decreases in both total area harvested and gross production are evident. A similar trend is observed with the yield data. It is pertinent to highlight that papaya consumption in the US has increased steadily after an explosive demand in the early 1990s. Moreover, the strategy selected to deal with the demand included increased importation of the fruit rather than increased internal production. Hawaiian papayas play a minor role in the economics of the crop and its consumption in the US (Suiyanata 2002); that is, in terms of total production.

Table 2 The 20 major papaya producer countries during the last 10 years, in tonnes, according data gathered from FAO.*

<table>
<thead>
<tr>
<th>Countries</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>1796960</td>
<td>2594000</td>
<td>2147200</td>
<td>1692100</td>
<td>2535100</td>
<td>2139300</td>
<td>2482100</td>
<td>2685900</td>
<td>2685900</td>
</tr>
<tr>
<td>Brazil</td>
<td>1439712</td>
<td>1489324</td>
<td>1597700</td>
<td>1714590</td>
<td>1612348</td>
<td>1573819</td>
<td>1897639</td>
<td>1811540</td>
<td>1900000</td>
</tr>
<tr>
<td>Nigeria</td>
<td>748000</td>
<td>748000</td>
<td>755000</td>
<td>755000</td>
<td>755000</td>
<td>755000</td>
<td>759000</td>
<td>765000</td>
<td>765000</td>
</tr>
<tr>
<td>Indonesia</td>
<td>42907</td>
<td>500571</td>
<td>605194</td>
<td>626745</td>
<td>732611</td>
<td>548657</td>
<td>643451</td>
<td>621524</td>
<td>653276</td>
</tr>
<tr>
<td>Mexico</td>
<td>672376</td>
<td>873457</td>
<td>876150</td>
<td>955694</td>
<td>787663</td>
<td>709477</td>
<td>798589</td>
<td>919425</td>
<td>638237</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>197300</td>
<td>223000</td>
<td>226000</td>
<td>230540</td>
<td>260000</td>
<td>260000</td>
<td>260000</td>
<td>260000</td>
<td>260000</td>
</tr>
<tr>
<td>Congo</td>
<td>213000</td>
<td>206222</td>
<td>210305</td>
<td>212180</td>
<td>214070</td>
<td>215980</td>
<td>217900</td>
<td>219840</td>
<td>225770</td>
</tr>
<tr>
<td>Colombia</td>
<td>112627</td>
<td>110764</td>
<td>89612</td>
<td>91608</td>
<td>103870</td>
<td>140346</td>
<td>164606</td>
<td>223945</td>
<td>207698</td>
</tr>
<tr>
<td>Guatemala</td>
<td>24040</td>
<td>39000</td>
<td>54000</td>
<td>69000</td>
<td>84000</td>
<td>99000</td>
<td>113277</td>
<td>184530</td>
<td>184530</td>
</tr>
<tr>
<td>Philippines</td>
<td>75896</td>
<td>77417</td>
<td>127680</td>
<td>130764</td>
<td>133876</td>
<td>146628</td>
<td>157120</td>
<td>162434</td>
<td>182907</td>
</tr>
<tr>
<td>Peru</td>
<td>159622</td>
<td>158910</td>
<td>172669</td>
<td>189793</td>
<td>193923</td>
<td>171055</td>
<td>175428</td>
<td>157771</td>
<td>157771</td>
</tr>
<tr>
<td>Venezuela</td>
<td>114234</td>
<td>130204</td>
<td>152738</td>
<td>148030</td>
<td>131753</td>
<td>118063</td>
<td>151335</td>
<td>132013</td>
<td>132013</td>
</tr>
<tr>
<td>Thailand</td>
<td>119000</td>
<td>120000</td>
<td>120000</td>
<td>125000</td>
<td>125000</td>
<td>131000</td>
<td>131000</td>
<td>131000</td>
<td>131000</td>
</tr>
<tr>
<td>China</td>
<td>154222</td>
<td>159207</td>
<td>162572</td>
<td>164559</td>
<td>157620</td>
<td>118475</td>
<td>115128</td>
<td>117914</td>
<td>120359</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>41000</td>
<td>44000</td>
<td>48000</td>
<td>47505</td>
<td>50615</td>
<td>240000</td>
<td>105245</td>
<td>95785</td>
<td>103609</td>
</tr>
<tr>
<td>Cuba</td>
<td>95503</td>
<td>135128</td>
<td>107240</td>
<td>120100</td>
<td>119000</td>
<td>91797</td>
<td>90309</td>
<td>89700</td>
<td>89400</td>
</tr>
<tr>
<td>Kenya</td>
<td>63410</td>
<td>77822</td>
<td>81811</td>
<td>86491</td>
<td>86000</td>
<td>87000</td>
<td>86000</td>
<td>86000</td>
<td>86000</td>
</tr>
<tr>
<td>Malaysia</td>
<td>60000</td>
<td>65000</td>
<td>70000</td>
<td>78000</td>
<td>75000</td>
<td>72000</td>
<td>72000</td>
<td>72000</td>
<td>72000</td>
</tr>
<tr>
<td>El Salvador</td>
<td>3000</td>
<td>3000</td>
<td>40000</td>
<td>53413</td>
<td>60470</td>
<td>63456</td>
<td>67264</td>
<td>65295</td>
<td>71117</td>
</tr>
<tr>
<td>Costa Rica</td>
<td>28786</td>
<td>27239</td>
<td>26458</td>
<td>31125</td>
<td>33815</td>
<td>35565</td>
<td>31090</td>
<td>41042</td>
<td>58408</td>
</tr>
<tr>
<td>World</td>
<td>6954812</td>
<td>8207372</td>
<td>8089108</td>
<td>7930846</td>
<td>8594281</td>
<td>806614</td>
<td>8913064</td>
<td>9210748</td>
<td>9095875</td>
</tr>
</tbody>
</table>


**cp TRANSGENIC PAPAYAS**

In 1985, Sanford and Johnston proposed the concept of pathogen-derived resistance (PDR) as a method of developing resistance against pathogens, such as viruses. It was theorised that the expression of a portion of the pathogen’s papaya be grown worldwide in the presence of the decimating PRSV, lacks support: transgenic papayas are grown commercially in very few countries (US, and maybe China), the area devoted to cultivating transgenic papayas is negligible, and most countries worldwide have learnt to cope with the economically devastating disease. Notwithstanding, as shown in the following sections, transgenic papayas have proven effective in controlling the virus and have furthered our knowledge of plant-virus interactions and the many ways scientific knowledge can be employed to solve practical issues related to agricultural production.
virus coat protein gene (cp) inhibit the pathogen. Subsequent studies initially examined genetic material, albeit in a dysfunctional form, could in- 
tobacco plants expressing the cp gene of Tobacco mosaic virus (TMV) exhibited phenotypes ranging from asympto-
matic to attenuated or delayed symptom expression follow-
ing inoculation with TMV. Later experiments by Lindbo and Dougherty (1992) with transgenic tobacco expressing 
mutant (untranslatable) cp of Tobacco etch virus (TEV) also demonstrated resistance against the challenge progenitor 
virus, therefore suggesting the involvement of the transgene RNA in conferring resistance. It was posited that sequence-
specific RNA degradation induced by viral transgenes target RNA species sharing sequence identity with the transgene, 
resulting in virus resistance (Lindbo et al. 1993). This model, coupled with the discovery that dsRNA induces a form of post transcriptional gene silencing (PTGS, Waterhouse et al. 1998), expanded and confirmed the original conception of PDR. Control of virus infections by the 
duction of a conserved, RNA-based plant antiviral defense response is achieved through small-interfering RNAs (siRNAs). Various reviews summarize the history of gene silencing research that contributed to our present understanding of plant virus resistance (Lindbo and Dougherty 2005; Prins et al. 2008; Eamens et al. 2008; Csorba et al. 2009).

Given the successful virus resistant development of transgenic plants, coupled with the complete characterization 
of PRSV and the difficulties associated with the intro-
gression and nature of PRSV resistance in relatives of papaya (Gonsalves et al. 2005), transgenesis of papaya was 
initiated by a research group at Cornell University and the University of Hawaii. The group, in applying the concept of 
PDR, used the virus cp gene. Other research groups soon followed suit, with some 12 countries documenting the de-
velopment and characterization of transgenic papaya varieties with indigenous PRSV isolates. Table 3 summarizes the characteristics of the transgenic varieties reported in the literature.

Similar cp constructs were used in the development of the transgenic lines in the respective countries. That is, 
translatable versions of the cp starting beyond the N1a-
pro tease cleavage site of the virus polyprotein. Translation 
signals of β-glucuronidase (uidA) or Cucumber mosaic virus (CMV), linked to the Cauliflower mosaic virus 35S promoter, were engineered to the cp in the region of the glutamine-serine (Q/S) cleavage site of the indigenous severe strain. The Hawaiian transgenic papaya, however, carry a slightly different construct consisting of 48 the nucleotides of the white leaf strain of CMV linked to the structural sequence of the mild cross protection strain, PRSV HA 5-1, a laboratory mutant derived from the severe PRSV HA strain.

The majority of the research groups have used microprojectile bombardment as the DNA delivery method into in vitro papaya materials and, consequently, the copy numbers and a range in insertion patterns, truncations and re-arrange-
ments of the transgenes are reported with transgenic lines from different regions (e.g. Lines et al. 2002; Kertbundit et al. 2007; Ruanjan et al. 2007). Complete single cp constructs have been obtained after bombardment, and in the case of the transgenics from Hawaii, nonfunctional, truncated 
clusters of the marker genes, neomycin phosphotrans-
ferase (nptII) and antibiotic (tet), resistance, were also found (Fermin 2002; Suzuki et al. 2008). Bau et al. (2003) 
reported on 1 to 2 copies following Agrobacterium transformation. Quite surprisingly, greater than 2 cp insertions were 
found in transgenics obtained by Agrobacterium transformation in Florida (Davis and Ying 2004). The insertion site 
in the papaya genome was characterized with the Hawaiian transgenic papaya. In keeping with reports of other trans-
genics (e.g. Arabidopsis and rice, Sawasaki et al. 1998; Matsuo et al. 2005), the sequences flanking the trans-
genies were identified as plastid DNA derived sequences in the nuclear genome (nuclear plastid sequences, nupts). Given the prokaryotic-like gene transcription and transla-
tion, it was deduced that these sequences do not represent functional or expressed genes (Fermin 2002; Suzuki et al. 2008), and thus, the transformation event did not result in a disruption of endogenous gene expression. This was further substantiated in field studies where the agronomic and nut-
ritional performance of transgenic trees and fruits were assessed (Ferreira et al. 2002). Superior agronomic perfor-
mance under virus disease pressure with yields three times higher than the industry average and fruits with percent soluble solids above the minimum (11%) required by com-
mercial fruit were noted.

Untranslatable constructs of PRSV cp containing cp se-
quences engineered with a stop codon or frame shift muta-
tions have also been used in the generation of transgenic 
papaya (Lines et al. 2002; Tennant et al. 2002; Davis and 
Ying 2004; Tennant et al. 2005). Untranslatable constructs derived from a mild strain of PRSV from Hawaii, PRSV
HA 5-1, were developed by engineering a frame shift mutation in the \textit{cp} gene. Somatic ‘Sunrise’ papaya embryos were transformed with non-linearized pGA482GG harboring the PRSV HA 5-1 untranslatable \textit{cp} gene (Cai et al. 1999). Embryogenic kanamycin-resistant clusters were treated as independent lines that allowed the subsequent establishment of 83 plants in the greenhouse. All 83 \textit{R}_0 transgenic lines were sequentially challenged twice with the nearly homolo-
gous, severe PRSV HA (99.8% similarity with the transgene). Each Rₐ line was represented by the fully developed plant plus its clonal cuttings. Different reactions were detected and subsequent generations were challenged with other isolates from the Bahamas, Brazil, Jamaica, Mexico and Thailand, and an additional heterologous Hawaiian isolate (C. Gonsalves, unpublished results, Fermin 2002). Untranslatable cp transgene-mediated resistance to PRSV was reported due to PTGS: no transgene-derived protein was detected in *in vitro*, nor in *in planta*, in a selected line which showed a high level and wide range of resistance against diverse geographical isolates of PRSV, and a high rate of *cp* transgene nuclear transcription was observed but its cytoplasmic mRNA was barely detectable. Finally, as observed in other transgenic papayas (Tennant et al. 1994; Chiang et al. 2001; Tennant et al. 2001) the level and range of resistance was largely homology-dependent. This work demonstrated that an untranslatable cp gene is able to confer resistance to the homologous strain of the virus, and also to some close geographical isolates of PRSV by PTGS. More

### Table 3 Summary of the characteristics of transgenic papaya developed by various research groups.

<table>
<thead>
<tr>
<th>Country</th>
<th>Cultivar (ancestor of F65)</th>
<th>Construct</th>
<th>Transformation</th>
<th>Transgene copy number</th>
<th>Transgene expression</th>
<th>Resistance testing</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>Local variety</td>
<td><em>uidA</em> leader + CaMV 35S pro+ PRSVBridgeman cp from Q/S start</td>
<td>Biolistics</td>
<td>1- 4 reported on truncations &amp; rearrangements of the <em>cp</em> (no correlation copy number &amp; level of R but to level of degraded RNA in northern)</td>
<td>CP not detected in ELISA and low levels of <em>cp</em> detection in northerns</td>
<td>Truncated 1 copy 0% R to PRSV, Cruz das Amas</td>
<td>Lines et al. 2002</td>
</tr>
<tr>
<td>Brazil</td>
<td>Sunrise solo &amp; Sunset solo</td>
<td><em>uidA</em> leader + PRSV Bahia cp from Q/S start</td>
<td>Biolistics</td>
<td>nt</td>
<td>Low to high levels CP protein detected in ELISA</td>
<td>R, 46% R to PRSV Bahia, HA &amp; TH</td>
<td>na</td>
</tr>
<tr>
<td>Florida</td>
<td>cv. F65</td>
<td><em>uidA</em> leader + CaMV 35S pro+ PRSV H1K cp gene from Q/S start</td>
<td><em>Agrobacterium</em></td>
<td>1-&gt; 2</td>
<td>cp not detected in northern analysis</td>
<td>Rₐ, 63-1% R to PRSV Oahua</td>
<td>Davis and Ying 2004</td>
</tr>
<tr>
<td>Hawaii</td>
<td>Sunset solo</td>
<td><em>uidA</em> leader + PRSV HA 5-1 cp gene from Q/S start</td>
<td>Biolistics</td>
<td>1- 2 (correlation between R and sequence similarity with <em>cp</em> transgene, copy number, plant age)</td>
<td>Low to high levels CP protein &amp; transcript detected in ELISA &amp; Northern analysis</td>
<td>55-1: Rₐ NS; R₁ NS but showed symptoms against PRSV Oahu, 63-1: Rₐ, 40-50% resistance against Hawaiian isolates, but 26-39% resistance against BR, JA &amp; TH isolates</td>
<td>Fitch et al. 1992; Tennant et al. 1994; Ferreira et al. 2002; Tennant et al. 2005; Souza et al. 2005; Cai et al. 1999; Tennant et al. 2002, 2005 Bau et al. 2003; Tripathi et al. 2004</td>
</tr>
<tr>
<td>Jamaica</td>
<td>Sunrise Solo</td>
<td><em>uidA</em> leader + PRSV Caymanas cp from Q/S start</td>
<td>Biolistics</td>
<td>1-3</td>
<td><em>cp</em> RNA detected in northern analysis</td>
<td>Rₐ, 29-40% R to PRSV</td>
<td>Rₐ, 50-89% R to PRSV Cruz das Amas</td>
</tr>
<tr>
<td>Taiwan</td>
<td>Tainung No. 2</td>
<td><em>uidA</em> leader + CaMV 35S pro+ PRSV YK cp gene from Q/S start</td>
<td><em>Agrobacterium</em></td>
<td>1-2</td>
<td><em>cp</em> transcript detected in Northern (a relationship between R and the detection of CP &amp; <em>cp</em> transcript)</td>
<td>4 categories of reactions: delay &amp; then symptoms (40%), mild mottling (70-80%), immunity, susceptible</td>
<td>70-80% R to PRSV HA &amp; sequence similarity with <em>cp</em> transgene</td>
</tr>
<tr>
<td>Thailand</td>
<td>Khak Dum</td>
<td><em>uidA</em> leader + PRSV Ratchaburi province cp</td>
<td>Biolistics</td>
<td>Multiple insertions with rearrangements &amp; deletions of 8 lines of PRSV</td>
<td>CP not detected in ELISA and low levels of <em>cp</em> detection in northern analysis.</td>
<td>All lines susceptible except for one line (G2)</td>
<td>Kertbandit et al. 2007; Ruanjuan et al. 2007</td>
</tr>
<tr>
<td>Venezuela</td>
<td>Tailanda roja (Thailand red)</td>
<td><em>uidA</em> leader + PRSV EV &amp; VE from Q/S start</td>
<td><em>Agrobacterium</em></td>
<td>1</td>
<td>CP not detected in ELISA and low levels of <em>cp</em> detection in northern analysis.</td>
<td>All Rₐ plants with LA or EV cp R to PRSV LA and EV R, EV cp 7% R, 50-73% R &amp; 60-60% R to PRSV EV, LA and HA R, EV+LA cp 0% R, 31% R &amp; 38% R to PRSV EV, LA and HA R, EV cp 22-32% R</td>
<td>Kertbandit et al. 2007; Ruanjuan et al. 2007; Fermin et al. 2004</td>
</tr>
</tbody>
</table>
research is needed, however, to fully understand the biochemistry of viral resistance conferred by a transgene, that from its conception, is tagged for degradation just as nonsense mediated decay can also target untranslatable messengers for their destruction (Hilleren et al. 1999; Isshiki et al. 2001; Lykke-Andersen 2001).

One benefit of plants engineered with untranslatable transgenes is that a protein of viral origin is not present in the final product and thus the risks associated with potential allergenicity of transgenic products are avoided (Ruibal-Mendieta et al. 1997). Surprisingly, the NPTII protein was also not detected in some of the transgenic lines described, even though the plants showed high level of resistance to PRSV. A transcriptionally silenced nptII gene could account for the negative results by DAS-ELISA. It can also be that transgene mRNA degradation by PTGS is playing a role in limiting the level of the NPTII protein below the detection of DAS-ELISA. Recently, it has been demonstrated that plants harboring the same construct at the very same position display different patterns of transgene expression, and that the silencing of the introduced transgenes may be a stochastic event concomitant to plant transformation (Day et al. 2000). Alternatively, many epigenetic phenomena can be explained by transgene methylation (Matzke and Matzke 1998a, 1998b), but experiments were not performed to assess if the nptII gene was transcriptionally, instead of post-transcriptionally silenced (van Houdt et al. 2000). In the case under discussion some of the engineered transgenic papayas produce neither CP nor NPTII as translational products of their respective transgenes. The lack of a CP produced in planta precludes the possibility of heterocapsidation (transcapsidation) that has been claimed as a source of environmental concern when dealing with transgenic plants transformed with viral sequences (Robinson 1996; Hull 1998; Hammond et al. 1999).

With the development of transgenic papaya harboring similar CP constructs from different regions and seemingly different insertions, a number of conclusions on the phenomenon of CP transgenic virus resistance in papaya have been reported. It appears that no one set of characteristics contribute to the level of resistance, but rather an interplay of in planta, virus and external factors. Firstly, transformation with either translatable or untranslatable forms of the CP gene confers resistance against PRSV. However, higher levels of resistance (29-40% vs 15-29%) were obtained with transgenic plants harboring the translatable CP form in the Jamaican transgenic papaya (Tennant et al. 2005), whereas the converse was observed with transgenic papaya lines in Florida carrying translatable or untranslatable CP (5-12% vs 4.2-41.7%; Davis and Ying 2004).

Tennant et al. (2001) summarized virus and in planta factors that complicate transgenic virus resistance in papaya. It was reported that transgene dosage, plant developmental stage and sequence identity (> 89.5%) between transgene and virus isolate populations can affect the level of transgenic resistance. Young and older Hawaiian hemizygous PRSV HA 5-12% CP plants were resistant to the homologous PRSV HA (99.8% homology to CP transgene), while only

---

### Table 3 (Cont.)

<table>
<thead>
<tr>
<th>Country</th>
<th>Cultivar</th>
<th>Construct</th>
<th>Transformation</th>
<th>Transgene copy number</th>
<th>Transgene expression</th>
<th>Resistance testing</th>
<th>Field</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UNTRANSLATABLE cp</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>Sunrise</td>
<td>CaMV 35S + CMV leader+ PRSV Bahia</td>
<td>Biolistics</td>
<td>nt</td>
<td>nt</td>
<td>R: 100% R to PRSV Bahia &amp; PRSV HA, 72% R PRSV TH</td>
<td>na</td>
</tr>
<tr>
<td></td>
<td>solo &amp;</td>
<td>cp from Q/S start in antisense</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sunset</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>solo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>cv. F65</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(ancestor of F65)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>uidA leader + CaMV 35S pro+ PRSV H1K cp gene from Q/S start in antisense</td>
<td>Agrobacterium</td>
<td>1-2</td>
<td>cp not detected in northern analysis</td>
<td>12-15%</td>
<td>Nt</td>
</tr>
<tr>
<td>Florida</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hawaii</td>
<td>CaMV 35S + CMV winner + PRSV H1K cp gene from Q/S start with frame shift mutation</td>
<td>Biolistics</td>
<td>nt</td>
<td>nt</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Jamaica</td>
<td>CaMV 35S + CMV leader+ PRSV Caymanas untranslatable cp</td>
<td>Biolistics</td>
<td>nt</td>
<td>cp not detected in northern analysis</td>
<td>4-42%</td>
<td>71-90%</td>
</tr>
</tbody>
</table>

R resistant; S susceptible; nt not tested; na not available
older plants were resistant to the other Hawaii isolates (96.7% homology). However, all inoculated hemizygous cp plants were susceptible to PRSV isolates collected from Jamaica, Brazil, and Thailand. In contrast, homozygous cp plants were resistant to all PRSV isolates, except the isolate from Thailand, regardless of the plant developmental stage. Resistance to the Thailand isolate, which shares 89.5% homology to the transgene, was observed only with plants inoculated at the five-leaf stage (Chiang et al. 2001). Initial experiments involving manipulating the sequence similarity between the transgene and its target virus. Gene constructs derived from the third fourth of the nucleocapsid N gene (3/4N) nucleotide sequence of Tomato spotted wilt virus (TSWV), differing in sequence similarity and location of nucleotide changes, were used to transform Nicotiana benthamiana explants. Leaf explants were transformed with constructs designed and synthesized with 5, 10, 15 and 20% changes evenly scattered along the transgenes, or with constructs (5 and 15% changes) with nucleotide changes clustered at the 5’ or 3’ end, or the middle of the transgene. R0 plants were tested for resistance. The construct with 5% scattered changes gave 2-5 fold more resistance than those with 10, 15, or 20% changes. Only the 5% scattered construct had 20 nt long stretches that were identical to the gene of PTGS (Chiang et al. 2007). Constructs with clustered changes conferred similar levels of resistance as the transgene that had no changes. Subsequently, a custom-designed construct with 10% of changes, not evenly scattered but strategically located in the construct to have 2-3 stretches at least 23 nt long identical to different tospoviruses 3/4N genes was designed. This synthetic, short transgene was 90% similar to the TSWV and Groundnut ringspot virus (GRSV) 3/4N genes, that otherwise share only a 79% similarity for this sequence. This construct was able to confer resistance to TSWV and GRSV demonstrating the feasibility of engineering multiple virus resistance with short, synthetic constructs (Femin 2002). Further applications will tell whether the strategy can be successfully used to engineer multiple resistant transgenic papaya plants transformed with a short, synthetic transgene.

**FACTORS CONTRIBUTING TO THE ADOPTION OF TRANSGENIC PAPAYA**

Despite the potential of transgenic varieties for the control of plant virus diseases, less than 0.1% (~8,500 ha) of some 117 million ha under cultivation with transgenic crops is planted with virus resistant transgenic crops (James 2008). Transgenic papaya varieties as well as tomato and sweet pepper transgenic papaya resistant to CMV and papaya resistant to PRSV have been released and adopted in the US and China, respectively (Stone 2008). Reports out of Hawaii describe a high adoption rate by farmers (Gonsalves et al. 2007). Farmers who have acquired seeds of the transgenic papaya varieties tout excellent performance of trees under virus pressure. Some produce transgenic papaya for sale or use the transgenic papaya as a buffer zone to assist in the economical production of non-transgenic papaya (Gonsalves et al. 2004; Tripathi et al. 2007). Despite the benefits, the rate of adoption of virus resistant transgenic crops is extremely low. Moreover, 14 countries have developed their own transgenic papaya varieties utilizing the cp gene from their region (Tecson Mendoza et al. 2008). Challenges facing the adoption of the technology as it relates to papaya appear to be associated with the application of the biotechnological protocols for the development of the transgenic product as well as the subsequent stages involving the development of a commercially viable product, notably regulatory issues and trade regulations.

A number of steps are critical to the development of a virus resistant transgenic crop; identifying the gene of interest, transformation of the crop plant, characterization of the new phenotype and multiplying seed. Unlike the development of the other transgenic varieties that involves the search for a suitable herbicide tolerant gene or a combina-
tion of genes capable of conferring adequate levels of insect resistance, the development of virus resistant transgenic papaya involves the isolation of the virus gene and engineering a transgenic functional in planta. Most challenging is the following step, notably the delivery of the transgene to in vitro plant materials and obtaining a viable number of transformation events. Whereas reasonable transformation efficiency rates of 5-30% are reported with herbaceous crops (Borzani, Ishida et al. 2002; Cortina and Culiáñez-Maciá 2004), lower rates of about 1% are generally obtained with papaya (Cabrera-Ponce et al. 1995; Cai et al. 1999). Thus, most of the research groups involved in transgenic papaya technology have formed collaborations with groups in the US (either the International Service for the Acquisition of Agri-biotech Applications (ISAAA), the Gonsalves laboratory or Australia (Australian Centre for International Agricultural Research ACIAR), in order to easily and successfully pursue the development of a transgenic papaya product. Collaborations, typically involving the initial steps of the tissue culture of the progenitor plant species and transformation with the engineered virus gene in the host laboratory, have been successful in transferring the technology; that is, transgenic plants were regenerated and characterized. Manipulation, lastly, depends on the transformation methods that are used in these situations (Tecson Mendoza et al. 2008), but rather the final steps leading to field testing and the commercial release of the transgenic product; namely the biosafety regulatory issues and social acceptance of the technology.

Fermin et al. (2004) compared the adoption of transgenic papaya in three countries, Hawaii, Jamaica and Venezuela. The product was similarly developed, that is, papaya embryos were transformed with a translatable version of the cp gene from the respective countries. However, the rates of adoption in the respective countries vary. Fermin et al. (2004) attribute the differences in adoption to demand-side factors, the presence of an established biosafety regulatory framework as well as the acceptance of the technology by the consumer.

The Hawaiian story is well known. The papaya industry was in crisis in wake of the movement of PRSV in 1992 from the island of Oahu to the Puna region of the Hawaii island, the largest producing region of the country that accounted for 95% of total production. Within two years after the detection of PRSV in this region, the disease was rampant and efforts at eradication were abandoned by the Hawaiian Department of Agriculture. Concurrently, transgenic varieties, carrying the cp of a mild strain of PRSV were generated from ‘Sunset’ papaya embryos and the first field infections with transgenic lines established in 1992. The transgenic papayas proved highly resistant to virus field infections and subsequent efforts were focused on stabilizing the line and the development of a commercially acceptable product. R3 generations were obtained, thus creating the ‘SunUp’ variety which is homozygous for the cp gene and crosses were made with the R1 ‘Sunset’ and ‘Kalapohi’, the preferred yellow flesh variety in Hawaii (Gonsalves 1998; M. Paton 1998). Given their performance, tolerance against field isolates, deregulation of the transgenic papaya was initiated by the research team to government agencies, USDA’s Animal and Plant Health Inspection Service, under the Plant Protection Act (APHIS), Environment Protection Agency (EPA) and the Food and Drug Administration Agency (FDA). The legal considerations, that is, licenses for the use of the intellectual property rights for the process and components (patents, pntII, uidA, cp) used in the development of the transgenic product, and financial considerations were addressed by the industry’s papaya administrative committee and its legal counsel (Gonsalves 1998). Within three years, transgenic seeds were distributed to farmers in Hawaii. The rapid development and adoption of the transgenic papaya in less than ten years in Hawaii is attributed to the dedication of the research team as well as the dedication and acceptance by the farmers. Moreover, and most significant, the industry was in crisis; all available commercial varieties were highly susceptible to the virus which was now widely distributed, mild strain cross protection strategies did not translate to long lasting economic benefits, neither did tolerant varieties and no significant progress had been achieved in the introduction of resistance genes from other members of the Caricaceae family (Gonsalves 2005). The transgenic varieties appeared to be the only available solution for continued production of papaya in Hawaii. Jamaica ranks first in the production of papaya in the Caribbean (FAO Statistics 2010) and is one of the few countries that supplies its domestic and international markets. Papaya is regarded as one of the cash crops in the agricultural industry that is competitive globally and can provide food security. Yields higher than those reported in other Caribbean countries are obtained in Jamaica (e.g. > 220,184 t/ ha, FAO Statistics 2010) and the fruit is esteemed for its high quality. First described in Jamaica in 1929 (Jensen 1949), PRSV remained a disease of minor importance until outbreaks in the traditional papaya producing regions in the late 1980s. By the mid 1990s, the disease spread to all papaya-growing regions in Jamaica. Based on the reported success in Hawaii, the industry moved forward by initiating collaborations with the Gonsalves laboratory and the NBC was given a statutory mandate under the Plants Importation Control Regulations (1997) and oversight responsibilities for the importation and research on transgenic plants. Within one year, permission was obtained and field testing of genetically modified papaya varieties initiated. The results were not as dramatic as in Hawaii; however, resistance against PRSV was identified in some transgenic varieties as well as a tolerant cultural change that could be manipulated in later generations for the development of a commercial product (Tennant et al. 2005). Further, the findings of the safety assessments suggested that the Jamaican transgenic papayas may not have adverse effects as regards the nutritional and toxicological parameters considered (Powell et al. 2008; Roberts et al. 2008). Thus, the next stage of the project was to field test subsequent generations, conduct the necessary field trials of ‘SunUp’ and to develop seed supply. However, the legislative and regulatory mechanisms needed to facilitate this stage, and the later stages of commercialization, were still not in place. In preparing to ratify the Cartagena Protocol, which was signed in 2001, there was the development of the National Biosafety Framework, National Biosafety Policy and National Biosafety Act. Nonetheless, public consultations were not conducted, nor were permits presented to the Political Director. Thus, the deregulation of transgenic papaya cannot proceed until these mechanisms are in place. Moreover, unlike the situation in Hawaii in the mid 1990s, production of papaya is still possible in Jamaica without transgenic papaya. Production has decreased by 48% because of PRSV (STATIN 2006), but the industry has maintained viability and a few farmers continue to satisfy domestic markets and exports to the US, Canada, and other Caribbean countries, ensuring markets and prices without much competition. Management
practices involving vigilant scouting coupled with the prompt removal of infected trees in their immediate vicinity have contributed to this sustained production in regions with low disease pressure. Given the hurdles, deregulation costs and possible trade restrictions with Europe, it may be a while before transgenic papayas become common place in Jamaica.

Quite another situation exists in Venezuela where papaya is mainly a cash crop grown for local consumption. Over 7,000 ha are under cultivation and 130,000 tonnes produced on average (FAO Statistics 2010). Following the engineering of the cp gene constructs in collaboration with the Gonsalves lab, Agrobacterium transformations were initiated in Venezuela and transgenic ‘Thailand Red’ were developed in house (Fermín et al. 2004). A special permit was obtained from the Ministry of Health in 2001 for a small field trial with transgenic plants. Special permission was required because there are no biosafety regulations and guidelines for research, development and transboundary movement of genetically modified crops and their products in the country. In the absence of a National Biosafety Committee (still under creation), the Ministry of Health was the principal agency involved in biosafety related matters (it is presently the Ministry of Environment). Once in the field, resistance data against the virus were collected for up to 4 months but 4 months following, opponents of the technology gained access to the secure plot and destroyed all the plants. Data on the durability of resistance and horticultural characteristics were not collected after eight years of work and expenditure in excess of $40,000. The production of papaya continues in Venezuela. Similar to the situation in Hawaii in the early outbreaks of PRSV, the farmers continually move out of heavily infected areas to virus-free areas and establish new papaya orchards. Additionally, as in many other countries (for instance Ecuador; Convenio MAG/IICA 2001, and Mexico, Rivas-Valencia et al. 2008), papaya is treated as an annual or biannual crop, after reaching its reproductive stage, and all general cultural practices associated with these crops are employed. Given the land mass, this may prove a viable method of growing papaya for a number of years to come.

Of note, some transgenic crops have made their way to the fields in Latin America. For example, cotton in Argentina and Mexico, corn in Argentina and Uruguay, and soybean in Argentina, Brazil, Mexico, Paraguay and Uruguay. The great imponderable question is why have some crops been accepted while others, such as papaya, remain as regulated articles, have not received clear acceptance from the government, and granted permission for limited greenhouse or field testing of the article (Bárcena et al. 2004; FORAGRO/IICA 2006). In the case of Venezuela it might be that the acceptance of the transgenic papayas is considered by some GMO opponents as the Trojan horse to include and accept others developed outside the country or in the country itself (for example, rice, plantain, coffee, casava, mango and papaya). In the case of Brazil, it appears that the non-delivery of transgenic papaya is because of the slow and inadequate development of transgenic technology, delays in the production of transgenic papaya plants in the Gonsalves lab, and the bureaucratic process required because there are no biosafety regulations in place.

Non-transgenic Approaches

Even though transgenic papayas have proven feasible for controlling PRSV, their limited use on a global scale implies that there must be other ways of fighting against the disease. It would appear that most of the resources have been directed towards the development of transgenic cultivars in some regions precluding active study into understanding other ways that can be used for controlling PRSV. But what many may have seemingly overlooked has been addressed in various Latin American countries that account for more than 50% of global papaya production. These regions have directed much effort to increasing and improving papaya production. Many scientific publications are available, albeit to a limited audience as they are written in Spanish or Portuguese.

For more than 20 years one of the authors has visited papaya orchards south of Lake Maracaibo and only recently located small, healthy papaya orchards free of PRSV (Fig. 4) despite the presence of infected fields less than 20 km away. To rule out the presence of illegal transgenic plants, samples were collected and tested for the cp gene. In all polymerase chain reactions conducted, using appropriate controls and primers directed to different parts of the cp gene, amplification of PRSV-derived sequences were negative (Castro and Fermín, unpublished results). Apparently the culture of intercropping plantain in alternate rows with papaya is a potential management strategy against non-persistent PRSV disease and warrants further investigation. In other regions of Venezuela, papaya is grown as an annual or bi-annual crop since capital return is guaranteed.

In Brazil, non-transgenic control of the disease relies mainly on avoidance, curcitur eradication, rouging, and cross protection (Lima et al. 2001). Similar strategies are adopted in Colombia, particularly the rouging of curcubits. Many farmers blackberry that these strategies are the foundation of commercial technology and have contributed to the viability of papaya in these areas, and weeds, like Momordica charantia, in close proximity to commercial papaya orchards were positive for PRSV. Apparently the curcitur plants tested served as feeding and virus acquisition hosts for A. gossypii (Arango et al. 2000). Also in Colombia, this time in the Caribbean region, early rouging, along with the use of grass barriers and the elimination of yellow leaves have been applied with some success (Pazo et al. 2003). El Salvador, which according to FAO (2010) touts the highest yield for papaya production, achieves control primarily through careful selection of land, use of barriers plants, crop rotation, careful selection and germination of seeds (not necessarily certified seeds), rouging, avoidance of aphids by planting seedlings when the vector population is at its lowest, weed eradication, balanced fertilization and the use of insect traps (Rodríguez 2004). Epidemiological studies in Mexico clearly show that transplanting schedules may serve to delay the onset of
epidemics, and hence, reduce the incidence of the disease (Mora-Aguilera et al. 1996), as do rouging and crop low density (Hernández-Castro et al. 2003).

In Puerto Rico, management practices are directed towards the aphid vector. Work with plastic mulch has proven effective in decreasing aphid populations in papaya orchards, and hence disease incidence. Trees in plots with protective mulching showed delayed appearance of symptoms, but more importantly increased yields (measured as marketable fruits) of more than a 100% (Robles et al. 2006). The study of the biology of the aphids that vector PRSV can contribute to the development of strategies for controlling the PRSV disease in papaya. It is well known that aphids do not colonize papaya plants; however, it is during the initial feeding probe that the virus is acquired on the insects’ stylet. In this regard it is necessary to evaluate the different aphids that vector PRSV and subsequently devise a strategy to target these populations. In Venezuela, Vegas et al. (1985) reported that efficacy in transmitting PRSV varies largely between Myzus persicae and Aphis gossypii (80% transmission), Toxoptera aurantii (40%), and a group comprised by A. craccivora, A. nerii, Pentalonia nigronervosa and Rhopalosiphum maidis (20% transmission). More recently Kalleshwaraswamy and Krishna Kumar (2007) reevaluated the efficiency of three aphid species in transmitting PRSV and demonstrated that this varied among the species analyzed (A. gossypii, A. craccivora and M. persicae). Based on their work in India, efforts will have to be directed to the main vector, A. gossypii, in the subcontinent.

Barrier plants have also been tested as deterrents of plant virus vectors in many crop management systems (Hooks and Fereres 2006), including papaya. In Mexico, for example, Rivas-Valencia et al. (2008) demonstrated that barrier plants interfered with the efficiency of PRSV transmission by delaying the beginning and progress of disease epidemics. The authors proposed the use of a number of different species (e.g. corn [Zea mays] and roselle [Hibiscus sabdariffa]) rather than the use of a single species. It was recommended that a combination of different varieties be used at different times during the papaya crop cycle, in addition to applications of non-damaging-to-the-plant “insecticides” (e.g., mineral oils like Safe-T-Side®). The cultivation of organic papayas, a growing trend in countries like Mexico (20,551 tonnes per year), is setting an example for other countries in Central America and the Caribbean (Pohlman et al. 2007).

Since symptom development in PRSV-infected papayas is dependent on temperature, it is very difficult to envision a strategy aimed at manipulating this abiotic factor to cope with the virus. Mangrauthia et al. (2009) reported that the expression of symptoms is maximal between 26 and 31°C, which is also the ideal temperature range for growing papaya. The authors also analyzed the fate of virus induced siRNAs and their interaction with HC-Pro and discovered a temperature-regulated host–virus relationship. A study on manipulating the activity of HC-Pro through crop nutrition and other abiotic factors may generate novel ways of knocking out counter-silencing strategies in favor of plant defense against virus attack. Along the same vein, since HC-Pro is an important factor in facilitating infections of alternate hosts by PRSV-W (Yap et al. 2009), investigations into controlling the activity of its ortholog in PRSV-P might be helpful for limiting PRSV infections of papaya (and cucurbits).

In some regions, cross protection is used to produce papaya commercially. This strategy is based on the assumption that infection with an attenuated strain of the virus protects the challenged plant from the detrimental effects of a second infection by a more virulent variant of the same virus. Cross protection in papaya has been tried in the past in Hawaii, Taiwan (Yeh and Gonsalves 1984, 1994; Sheen et al. 1998), Brazil (Lima et al. 2001), and Venezuela (Vegas et al. 2000; González et al. 2002) with mixed results. In almost all cases, cross protection must be accompanied with other control measures to be effective. The main reasons for limited adoption of the strategy have been summarized elsewhere (Yeh et al. 2007): the need of a large-scale inoculation program, break down of resistance by more virulent isolates of the virus, financial constrains of small producers, and, we must add, national legislation oversieving controlled infections in the field. Cross protection must be reevaluated, however, since a comprehensive analysis of the epidemiology of the disease under this kind of management has not been conducted as thoroughly as in the successful case of cross protection against Citrus tristeza virus and Barley yellow dwarf virus (Zhang and Holt 2001).

Finally, in a recent report Srivastava et al. (2009) showed that PRSV infections can be suppressed through the use of a systemic antiviral protein isolated from the prickly myrtle plant, Clerodendrum aculeatum (Srivastava et al. 2009). Although preliminary, these results are encouraging since the lack of symptom development in mechanically challenged papaya plants seems to be due to an inhibition of virus replication in planta.

Other regions, namely the US and Australia, are focused on developing genetic resistance against papaya ringspot. Genetic resistance continues to be the first choice for disease control. When resistance genes are not present in the commercial cultivars, breeders opt for introgression from related species by means of controlled crosses. With papaya this approach has always been difficult because of reproductive barriers. Recent advances offer the promise of overcoming reproductive isolation of the species. Although some PRSV tolerant papaya lines have been developed by introgression to selected cultivars, for example “Cariflora” (Conover et al. 1986), the pursuit of fully resistant cultivars has proven to be more difficult. The most consistent efforts at breeding resistant papayas have been in Australia and some artificial interspecific hybrids between C. papaya and
Vasconcellea cauliflora, V. querciflora and V. cindamar-ncensis (Magdalita et al. 1997a, 1997b; Drew et al. 2005) have been recovered after embryo rescue and micropropo- dation (Manshardt and Weslaff 1989). However, the F1 plants regenerated were infertile except for the progeny of crosses with V. quercifolia (Drew et al. 1998). These plants showed a level of fertility that facilitated backcrossing with C. papaya, giving rise to male plants resistant to PRSV. In a different study published in 2000, PRSV resistance was reported on the results of several interspecific crosses between C. papaya and diverse Vasconcellea (C. papaya x V. pub- escens; C. papaya x V. parviflora (F1); V. pubescens x V. parviflora (F1); V. pubescens x V. parviflora (F2); V. pub- escens x V. parviflora (F1); RR); C. papaya x V. pubescens x V. parviflora (F1); (V. pubescens x V. parviflora (F2 RR) x V. parviflora (BC1); (V. pubescens x V. parviflora (F2 RR) x V. parviflora) BC1 x V. parviflora (BC2). Many of the hybrids were morphologically normal and a few showed resistance to PRSV upon mechanical inoculation. In others, the presence of molecular markers (cleaved amplified polymorphic sequences [CAPS]) linked to resistance was demon- strated (Dillon et al. 2006). Although V. parviflora is not resistant to PRSV, the species is being used as a bridge to introgress resistance genes from Vasconcellea species to C. papaya (O’Brien and Drew 2009). The generation of hybrids through intergeneric crosses is ongoing in Venezue- ela by Vegas et al. (2003) with C. papaya and V. cauliflora using the methods of Magdalita et al. (1997a, 1997b) in- volving embryo or ovule rescue. Intergeneric hybrids have proven useful in obtaining not only resistance against PRSV, but also in the introgression of other useful genes into C. papaya.

Another way of dealing with the papaya ringspot dis- ease involves the analysis and use of tolerant varieties, which do exist for papaya. In pioneering efforts to analyze PRSV tolerance in papaya, Conover and Litz (1978) evaluated 95 papaya accessions from around the world. It was demonstrated that tolerance was inherited quantitatively, not qualitatively, and dependent on the phenotype of the parents used in the cross. However, crosses required to generate true breeding tolerant strains are unpredictably cumbersome and has precluded or impeded consistent efforts to develop new varieties. Nonetheless, success was attained in Mexico with the development of the PRSV- tolerant hybrid ‘Azteca’ using genotypes derived from accessions collected in Tab- asco in 2003. These plants appear to be performing well under ongoing field assessments (Mirañantes and Azpélúa 2008).

CONCLUDING REMARKS

Plant disease management is a human activity that is as complex as it is unavoidable in agriculture (Gilligan 2008), particularly with virus diseases (Jones 2009). Almost every available management strategy has been employed in at- tempts to control PRSV; some measures are more effective than others and various countries have deployed different strategies against the disease, including transgenic cultivars. The latter strategy has been touted as a biotechnological success although few regions have embraced the technology in a country already driven by the easy accep- tance of new technologies. While also noting the advances in knowledge of the popular tropical fruit culminating in the sequencing of its genome (Ming et al. 2008). These later accomplishments, and future developments in papaya engi- neering, as well as a guaranteed increase of the knowledge of its basic biology, are due in part to the timely application of transgenic technology, e.g. ripening, as reported by López-Gómez et al. (2009) and many more to come (AK- hond and Machray 2009). The research has furthered our knowledge of the popular tropical fruit culminating in the sequencing of its genome (Ming et al. 2008). These latter accomplishments, and future developments in papaya engi- neering, as well as a guaranteed increase of the knowledge of its basic biology, are due in part to the timely application of transgenic technology, e.g. ripening, as reported by López-Gómez et al. (2009) and many more to come (AK- hond and Machray 2009). The research has furthered our knowledge of the popular tropical fruit culminating in the sequencing of its genome (Ming et al. 2008).

Notwithstanding the poor adoption of transgenic papa- yas resistant to PRSV, the technology behind the creation of the transgenic papaya is sound and effective, and has opened the door to other applications that include traits not related to virus biology, e.g. ripening, as reported by López-Gómez et al. (2009) and many more to come (AK- hond and Machray 2009). The research has furthered our knowledge of the popular tropical fruit culminating in the sequencing of its genome (Ming et al. 2008). These later accomplishments, and future developments in papaya engi- neering, as well as a guaranteed increase of the knowledge of its basic biology, are due in part to the timely application of transgenic technology, e.g. ripening, as reported by López-Gómez et al. (2009) and many more to come (AK- hond and Machray 2009). The research has furthered our knowledge of the popular tropical fruit culminating in the sequencing of its genome (Ming et al. 2008).

REFERENCES


Davies LO, Kuma-Holopola L, Holopola L, Ow D (2000) Transgene integration into the same很简单 systematic location can produce alleles that express at a predictable level, or alleles that are differentially silenced. Genes and Development 14, 2689-80


Jensen D (1949) Papaya diseases with special reference to Papaya ringspot virus, Phytopathology Supplement; 39, 141-130


Lindbo JA, Dougherty WG (1992) Translaminar transfer of the Tobacco etch virus coat protein gene sequence can interfere with tobacco etch virus replication in transgenic plants and protoplasts. Virology 189, 725-733


Magdalita PM, Persley DM, Godwin ID, Drew RA, Adkins SW (1997b) Screening Carica papaya x C. cauliflower hybrids for resistance to Papaya ringspot virus-type P. Plant Pathology 46, 837-841


Evolución de barreras vegetales en el manejo integrado de la mancha anular del papayo en Michoacán, México. Summa Phytopathologica 34, 307-312
Tennant P, Ahmad MH, Gonsalves D (2002) Transformation of Carica papaya L. with virus coat protein genes for studies on resistance to Papaya ringspot virus from Jamaica. Tropical Agricultural (Trinidad) 79, 105-113
Torres R, Giacomelli D (1966) Virosis de la papaya (Carica papaya L.) en el valle del Cauca. Agricultura Tropical (Colombia) 22, 27-33
Tripathi S, Bau HH, Chen LF, Yeh SD (2004) The ability of Papaya ringspot virus strains overcoming the transgenic resistance of papaya conferred by the coat protein gene is not correlated with higher degrees of sequence divergence from the transgene. European Journal of Plant Pathology 110, 871-882


Waterhouse PM, Graham MW, Wang M-B (1998) Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. Proceedings of the National Academy of Sciences USA 95, 13959-13964

Yap YK, Duangjit J, Panyim S (2009) N-terminal of Papaya ringspot virus type-W (PRSV-W) helper component proteinase (HC-Pro) is essential for PRSV systemic infection in zucchini. Virus Genes 38, 461-467


