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# Transgene-mediated resistance to *Papaya ringspot virus*: challenges and solutions

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**Abstract** Global papaya production is severely affected by papaya ringspot disease caused by *Papaya ringspot virus* (PRSV). Management of this potyvirus is challenging, due to 1) its non-persistent transmission by numerous aphid species and 2) the diversity of PRSV strains that exists within a country or between different geographical regions. Papaya cultivars with transgenic resistance have reduced the impact of the disease. There are no effective alternatives to transgenic resistance available in areas where disease pressure is high. In Hawaii, transgenic papayas such as "SunUp" and "Rainbow" have remained resistant to PRSV more than two decades saving the commercial papaya industry. Following the success in Hawaii, researchers from other countries have focused on developing PRSV-resistant transgenic papaya. These transgenic cultivars often demonstrated an initial transitory resistance that was ultimately overcome by the virus. For other cases, resistance was inconsistent. That is, some transgenic lines were resistant while others were not. Transgenic cultivars are now losing PRSV-resistance for various reasons in China and Taiwan. In this review, we present an update on work with transgenic papaya with resistance to PRSV. The focus is on factors affecting transgenic resistance in papaya and our attempt to explain why the Hawaiian scenario of complete and durable resistance has not been replicated in other regions. The utilization of more recent technologies to the development of virus resistance in papaya is also discussed.

**Keywords** Transgenic resistance · Papaya ringspot disease

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## Introduction

Papaya (*Carica papaya* L.) is a unique edible fruit in the family Caricaceae. Although it originated in the Americas, it is now popular in tropical and subtropical areas worldwide (Badillo 1993; Gonsalves 2002; Hamim et al. 2014). Papaya is eaten ripe as a dessert fruit or unripe as a vegetable, and is a good source of nutrients. It is also used by some for its medicinal properties (Chan and Tang 1979; Sturrock 1940; USDA/ARS 2001; Ye and Li 2010), as papaya leaves are used to make tea to treat malaria (Titanji et al. 2008). In India, China, the Philippines, Brazil, Mexico, Australia, Thailand, South Africa and Indonesia, papaya is an established cash crop. The countries of India, Brazil, Indonesia, and Mexico together grow 10 million tons per year, valued at more than $200 million (USD) (FAOSTAT 2014). Papaya is one of the largest agricultural crops in Hawaii, USA, but only contributes 0.1% of the world's papaya production. However, the

development of transgenic cultivars resistant to *Papaya ringspot virus* (PRSV) has made Hawaii a pioneer in the papaya industry (Gonsalves 2002).

Many pests and diseases affect papaya production, but viruses are one of the greatest concerns potentially causing 100% loss in yield (Purcifull et al. 1984; Ye and Li 2010). Papaya ringspot disease, caused by PRSV, is the most important viral disease of papaya worldwide. Other viruses that infect papaya include: *Papaya leaf curl virus* (PLCV), *Papaya mosaic virus* (PapMV), *Papaya leaf distortion mosaic virus* (PLDMV), *Papaya meleira virus* (PMeV) and *Papaya lethal yellowing virus* (PLYV) (Paolla et al. 2015). These viruses are also of international importance, significantly reducing yield and fruit quality (Paolla et al. 2015).

PRSV was first reported in Hawaii in 1945 (Jensen 1949; Ferreira et al. 1992) and since then has become the primary concern of Hawaii's papaya industry. The incidence of PRSV in the field can reach 80 to 100% (Ye and Li 2010; Ventura et al. 2004; Hamim, *personal observation*). Papaya is often found growing as a "volunteer" along roadsides and in wild areas where it is a source of PRSV inoculum (Alabi et al. 2016).

PRSV is in the family *Potyviridae,* genus *Potyvirus* and has linear, flexuous rod-shaped virions (Purcifull et al. 1984; Yeh et al. 1992). The single-stranded, positive sense RNA genome is about 10,000 nucleotides in length and encodes a single large polyprotein. This protein is cleaved into eight smaller functionally active proteins: P1, HC-Pro, P3, CI, 6 K, NIa-Pro, NIb and CP. Moreover, there are few reports on the another protein 'P3N-PIPO' which is produced from a different small ORF, overlapped with P3 coding region (Chung et al. 2008; Fermin and Randle 2015). There are two types of PRSV: the P-type which infects papaya and the W-type which only infects the cucumber family (Yeh et al. 1992; Adams et al. 2005).

PRSV is spread from diseased to healthy plants in a non-persistent manner by several species of aphids (Jensen 1949; Tripathi et al. 2008; Purcifull et al. 1984) such as *Aphis nerii* (Boyer de Fonscolombe), *A. gossypii* (Glover), *A. spiraecola* (Patch), *Myzus persicae* (Sulzer), *Toxoptera aurantii* (Boyer de Fonscolombe), *A. craccivora (*Koch), and *Rhopalosiphum maidis* (Fitch) (Namba and Higa 1981; Prasad and Sarkar 1989; Wang 1981).

The virus systemically infects its host, causing severe mosaic symptoms on leaves and water-soaked streaks on leaf petioles and the trunk of the plant (Fig. 1). In severe cases, plants have distorted shoestring-like leaves, reduced photosynthetic capacity, and sometimes develop systemic necrosis and wilting (Purcifull et al. 1984). When PRSV infects papaya at an early stage, plants are stunted and do not develop fruit. Infected mature plants typically produce poor-quality fruit with bumps or ring-like spots and a low sugar concentration (Gonsalves 1998).

Many efforts have been made to manage papaya ringspot disease including: vector control by chemical or biological agents, disease-tolerant varieties, cross-protection, planting in virus-free areas, rouging, planting times at low populations of winged aphids, intercropping with barrier crops and growing papaya inside insect exclusion nets. None of these methods have proven effective in the field (Paolla et al. 2015; Yeh et al. 2010; Swain and Powell 2001; Yeh and Gonsalves 1994; Azad et al. 2014; Tripathi et al. 2008). Cross-protection was attempted on the island of Oahu and in Taiwan with a mild form of nitrous acid mutant from PRSV strain HA 5–1 from Hawaii (Yeh and Gonsalves 1994; Yeh et al. 2014**)**. This method did not provide long-lasting economic benefits, especially since it required continuous production of mild strains and inoculation of plants. Furthermore, the mild strain caused significant symptoms on some commercial cultivars including the popular 'Sunrise' **(**Ferreira et al. 1992**)**. Therefore, cross-protection was not used widely because of its shortcomings, initiating transgenic papaya research in 1985 (Gonsalves et al. 2004; Gonsalves 1998).

In the 1980s, Roger Beachy's research group reported that transgenic tobacco plants expressing the coat protein gene of *Tobacco mosaic virus* (TMV) postponed the expression of TMV (Powell-Abel et al. 1986). This discovery motivated research in the fields of agriculture and plant science to apply this approach to the development of virus resistant transgenic plants for commercial crops. The approach was initially called "parasite-derived resistance" (Sanford and Johnston 1985) and later changed to pathogen-derived resistance (PDR). In PDR, genes or sequences from the pathogen are inserted into the desired plants to develop resistance to pathogen and those closely related (Baulcombe et al. 1996; Beachy 1997). PDR in transgenic papaya governs gene expression through post-transcriptional gene silencing (PTGS) (Tennant et al. 2001; Ruanjan et al. 2007). Generally, gene silencing operates either at transcriptional or post-transcriptional stages employing genes that has the


**Fig. 1** Papaya plants severely affected by PRSV. **a** Leaf mosaic and chlorosis; **b** leaves may be "shoestring-like', narrow and distorted

regions of maximum similarity to the targeted genes and is evident by decreased amounts of steady state messenger RNA (mRNA) of the transgene and targeted gene (Fagard and Vaucheret 2000; Matzke and Matzke 1998). Transcriptional gene silencing results in reduced transcription due to termination of the promoter. However, with PTGS, transcription occurs but mRNA is degraded prior to translation resulting in reduced levels of gene product in the cytoplasm. Between the two types of gene silencing systems, PTGS (Carvalho et al. 1992) has been linked to RNA-mediated transgenic resistance (Baulcombe 1996). Therefore, the resistance is referred to either as homology dependent PTGS or homology dependent PDR (Baulcombe 1996) to define the specific resistance mechanism. Furthermore, scientists have confirmed post-transcriptional gene silencing in transgenic virus-resistant papaya by the presence small interfering RNA (siRNA) (Ruanjan et al. 2007), a hallmark of the PTGS mechanism.

Investigation by Beachy's group encouraged the research group of Dr. Gonsalves from Cornell University and Dr. Manshardt at the University of Hawaii at Manoa to collaborate on the development of PRSV *CP*-transgenic resistance in papaya in 1986 (Gonsalves 2002). The Hawaiian papaya industry was in a great crisis at that time. The mild Hawaiian PRSV strain HA 5–1 was used by Gonsalves and his collaborators as the source of the *CP* gene for the transgene construct since the aim was to generate transgenic resistant papaya to Hawaiian PRSV strains (Quemada et al. 1990). The transgene was designed to facilitate the *CP* gene translation, as it was thought that the *CP* protein was necessary for PDR (Table 1). When the *CP* of PRSV is generated through post-translational protease cleavage, there are no indigenous translation signals specifically available for the *CP* sequence. Thus, a chimeric gene was used to translate signals present in the leader sequence of the *Cucumber mosaic virus* (CMV) *CP* gene linked in frame to the sequence of the PRSV *CP* (Ling et al. 1991). In 1988, papaya researchers started using the biolistic approach to transform embryogenic cultures of papaya (Fitch et al. 1992). They focused on the transforming Hawaiian cultivars -'Sunrise', 'Sunset', and 'Kapoho'. These commercial cultivars bred true to type and are exclusively grown in main papaya production region of Hawaii. They worked excellently in transformation with the transgene. Fitch and associates (1992) were the pioneers in the transformation of papaya using the biolistic approach targeting embryogenic cultures, creating 17 independently transformed papayas (Fitch and Manshardt 1990). They immediately tested the resistance of the R0 lines against a severe Hawaiian strain called PRSV HA 5–1 (Fitch et al. 1992; Tennant et al. 1994, 2001). In 1991, they identified a single resistant line designated as '55–1' that was resistant to PRSV HA in greenhouse experiments (Fitch et al. 1992), '55–1' was the red-fleshed cultivar 'Sunset', a much less desirable fruit than the yellow-fleshed 'Kapoho'. In 1992, '55–1' was also found to be resistant to PRSV in a field tests in the Waimanalo Field Station of the University of Hawaii. In addition, two new transgenic cultivars-'SunUp' and 'Rainbow' were developed.

Homozygous 'SunUp' was produced by transforming red-fleshed 'Sunset' with the *CP* gene of mild Hawaiian PRSV strain HA 5–1 and hemizygous 'Rainbow' is a F1 hybrid developed by crossing 'SunUp' with the yellow-fleshed nontransgenic cultivar 'Kapoho' (Manshardt 1999). The trial in 1992 validated PRSV resistance of the transgenic plants in the field. In 1995, a crucial field trial of 'Rainbow' and 'SunUp' papaya was conducted in the Puna District of Hawaii Island where most commercially papaya is grown. The results conclusively displayed that the transgenic 'SunUp' and 'Rainbow' were resistant and were of acceptable commercial quality and gave superior yields compared to nontransgenic 'Kapoho' (Ferreira et al. 2002). Moreover, yellow-fleshed 'Rainbow' bore mature fruit earlier than the 'Kapoho', impressing the papaya growers of Puna. Within 4 years following the devffig

elopment of these varieties, papaya production in Hawaii had returned to pre-PRSV levels. In 1998, another popular papaya variety 'Kamiya' was transformed with the *CP* gene (untranslatable) derived from HA5–1 or its replicase gene. Transformants generated using both genes were found immune to PRSV in laboratory and green house test (Fitch 2010). Another complex hybrid is between Kamiya and Rainbow F2 plants, which was patented as 'Laie Gold' showed PRSV resistance in a field trial (Fitch 2010). Resistance to plant viruses through the development of transgenics is free from the problems of cross-protection, such as inoculation costs, possible mutation of the cross-protecting strain to a more virulent strain, movement of the mild strain of the virus to other crops, and adverse effects of attenuated strains on papaya plants (Yeh and Gonsalves 1984). Therefore, the Hawaiian scenario has confirmed that use of the PRSV-resistant transgenic papaya is a realistic solution for a control measure against PRSV. The transgenic papaya cultivars have been started growing commercially in Hawaii since 1998 and to date they maintained resistance to PRSV (Tripathi et al. 2008; Gonsalves 1998, 2002; Stokstad 2008; Yeh et al. 2010; Hu, *personal observation*).

PRSV isolates from different geographical regions of the world are genetically diverse (Tripathi et al. 2008). Zhao and associates (2016) inoculated transgenic papayas from Hawaii with PRSV strain from Hainan and observed infection with typical PRSV symptom development. Therefore, the Hawaiian *CP*-transgenic cultivars may not be useful in regions outside of Hawaii (Azad et al. 2014). The success of transgenic papaya in Hawaii has encouraged other papaya-cultivating states of the US and other countries to develop transgenic papayas resistant to their local PRSV isolates. Resistant transgenic varieties of papaya have been developed in Florida (US), Brazil, China, Jamaica, Indonesia, Malaysia, Thailand, Venezuela, the Philippines and Australia (Bau et al. 2003; Cheng et al. 1996; Davis and Ying 2004; Lines et al. 2002; Tripathi et al. 2008).

Davis and Ying (2004) reported transformation of Papaya cultivar 'L. cv. F65' via *Agrobacterium*-mediated transformation with four different constructs containing the unmodified or modified *CP* gene i.e. sense orientation (S), antisense orientation (AS), sense orientation with a frame-shift mutation (FS), or sense orientation mutated with three-in-frame stop codons (SC) of Florida PRSV isolate H1K. They obtained 256 putative transgenic lines with the *CP* constructs and challenged these lines with PRSV H1K. Highly resistant lines were found in the different transgene groups (Table 1). These lines were crossed with six different genotypes of papaya. The transgenic lines derived from the FS/*CP* and SC/*CP* transgene groups showed high fertility, but the plants from the S/*CP* and AS/*CP* transgene groups were infertile. In the field test, 23.3% of fertile transgenic plants became naturally infected with PRSV; whereas, 96.7% of the nontransgenic control plants became infected (Davis and Ying 2004).

Zimmerman et al. (2005) transformed somatic embryos of papaya lines 'Washington' and 'Yuen Nong' via *A. tumefaciens* containing the *CP* gene of PRSV strain from the Virgin Islands (Table 1). Regenerated plants showed resistance to PRSV.

Lines and associates (2002) reported the development of two Australian transgenic papaya cultivars that were immune to infection with PRSV (Table 1). Somatic embryos from commercially grown cultivars ('GD3–1–19' and 'ER6–4') in Queensland were transformed via a particle inflow gun using a construct containing an untranslatable PRSV *CP* coding region. Two transgenic lines were demonstrated immune to PRSV following repeated inoculation in the greenhouse and field.

In the 1990s, transgenic papayas resistant to Brazilian PRSV were developed (Souza et al. 2005a, b) in the US by visiting Brazilian scientists *M. Souza* and associates (Table 1). Later, these were transferred to the Brazilian Agricultural Research Corporation (EMBRAPA) under a technology transfer program. Particle bombardment gun was used to insert translatable or nontranslatable *CP*

**Table 1** Transgenic lines developed in different countries to date and their resistant properties

| Country | Cultivar | Virus protien used in the construct | Transformation mechanism | Resistant properties | References |
|---|---|---|---|---|---|
| Australia | Local variety | Translatable CP | Biolistics | Immune to infection with PRSV | Lines et al. (2002) |
| Brazil | Sunrise or Sunset | Translatable or untranslatable CP | Biolistics | Mixed resistant reactions to isolates from Brazil, Hawaii and Thailand | Souza et al. (2005a, b) |
| China | Tai-nong-2 | 3′-truncated and 5′-extended RP gene fragments | *Agrobacterium tumefaciens* | Initially resistant to local PRSV isolates, but recently loss in resistance observed to newly emerged PRSV strains | Chen et al. (2001) |
| | Sunrise | A 544-bp conserved region of the PRSV CP gene | Biolistics | Resistance to PRSV local strains | Jia et al. (2017) |
| Florida (US) | CV.F65 | Translatable or untranslatable CP | *Agrobacterium tumefaciens* | Resistant to Florida isolate H1K of PRSV | Davis and Ying 2004 |
| Hawaii(US) | Sunset | Translatable CP | Biolistics | Resistant to a severe Hawaiian strain called PRSV HA 5-1 | Tennant et al. (2005); Ferreira et al. (2002) |
| Jamaica | Sunrise | Untranslatable CP or Translatable CP | Biolistics | Transgenic Lines with the translatable CP gene showed higher field resistance compared to lines with the non-translatable cp gene | Tennant et al. (2002); Tennant et al. (2005) |
| Taiwan | Tainung no. 2 | Translatable CP | *Agrobacterium tumefaciens* | Initially showed high resistance to PRSV YK strain, later PLDMV broke the resistance | Yeh et al. (1998, 2010) |
| | Tainung no. 2 | Untranslatable chimeric construct containing partial CP genes of PRSV and PLDMV | *Agrobacterium tumefaciens* | Initially resistant to PLDMV and PRSV-YK, later PRSV super strain 5-19 broke the resistance | Yeh et al. (2010); Kung et al. (2009) |

genes of the Brazilian PRSV strain on somatic embryos derived from the 'Sunrise' and 'Sunset' cultivars. A greenhouse study tested 26 regenerated lines containing translatable *CP* gene and 28 regenerated lines containing untranslatable *CP* gene. They were inoculated with three different virus isolates from Brazil, Hawaii and Thailand and revealed mixed resistant reactions (mono-, double- and triple-resistance) (Souza et al. 2005a, b).

Chen et al. (2001) introduced viral replicase (RP) gene conferring transgenic resistance to PRSV in papaya (Table 1). Embryogenic calli of cultivar 'Tai-nong-2' were transformed by *A. tumefaciens* harboring the pRPTW vector. This vector was constructed orientating the 3′-truncated and 5′-extended RP gene fragments under the control of the CaMV35 S promoter and nonpaline synthase gene (NOS) termination sequence in the mini Ti plasmid vector pRok. PRSV inoculation tests showed that the RP gene conferred resistance to PRSV in regenerated transgenic papayas. In 2006, the People's Republic of China deregulated this PRSV-resistant transgenic papaya as 'Huanong No. 1', for commercial production (Guo et al. 2009). This cultivar elicits PDR but uses the replicase gene from southern China instead of the CP gene (Azad et al. 2014; Tripathi et al. 2008). There was no loss of resistance observed in the transgenic papaya plants with replicase gene in the first 5 to 6 years (Mendoza et al. 2008). However, an example of resistance break down in 'Huanong No. 1' has been observed in recent years (Zhao et al. 2015).

In Jamaica, transgenic papaya lines containing either the insert of translatable or non-translatable *CP* genes were tested for PRSV resistance in the field (Tennant et al. 2005) (Table 1). Transgenic lines with the translatable *CP* gene showed much higher field resistance (80%) compared to lines with the non-translatable *CP* gene (44%).

Scientists in the University of the Philippines Los Banos initiated to the development of transgenic papaya with resistance to a virulent strain of PRSV isolated from Cavite, Philippines (Villegas et al. 2001) (Table 1). The transgenic lines were moderate to high susceptibility to PRSV in field evaluation.

In Taiwan, embryogenic tissues derived from immature zygotic embryos of the 'Tainung No. 2' cultivar were used for *Agrobacterium*-mediated transformation using the binary vector pBGCP (Cheng et al. 1996) containing the *CP* gene of the PRSV YK strain, a severe virus strain from Taiwan (Table 1). A total of 38 transgenic lines were tested for PRSV resistance and two lines demonstrated immunity, nine lines showed high resistance, and eight lines showed moderate resistance (Yeh et al. 1998). Three transgenic lines were selected for evaluation under field conditions (Yeh et al. 1998). Between 0 and 0.2% of the transgenic lines were infected with PRSV 12 months after planting, while the control plants were 100% infected 8 months after planting. To deal with the emerging problem of PLDMV infection, dual resistance was attempted in transgenic papaya carrying a chimeric construct containing partial *CP* genes of PRSV and PLDMV (Bau et al. 2008). Furthermore, a transgenic papaya line was generated using an untranslatable construct targeting the PTGS-suppressor protein (HC-Pro) of PRSV superstrain 5–19, which provided resistance to PRSV strains in Taiwan and other geographical locations (Yeh et al. 2010; Kung et al. 2015).

In Thailand, papaya cultivars 'Khak Dum' and 'Khak Nual' were transformed using microprojectile bombardment by Thai scientists at Cornell University (USA) in 1995 (Gonsalves 1998) (Table 1). Transformed lines were transferred to Thailand for virus resistance evaluations. The transformed 'Khak Nual' variety showed excellent field resistance to PRSV (97–100%) and had a higher yield than non-transgenic papaya (Sakuanrungsirikul et al. 2005). Another transgenic line 'Khakdam', showed 100% field resistance with higher yield compared to the non-transgenic variety (Phironrit et al. 2005).

In collaboration with Dennis Gonsalves, the first transgenic papaya in Venezuela was developed in 1993 by the University of Los Andes. They used the *CP* gene from two different PRSV strains isolated from local papaya cultivars and *Agrobacterium*-mediated transformation. Regenerated plants and their progenies were found to be resistant against the local PRSV strains in glasshouse testing (Fermin et al. 2004).

Despite these successes, there have been reports of failed transgenic resistance in papaya in China and Taiwan (Tripathi et al. 2008; Kung et al. 2015). Different factors may be involved in this loss of resistance including: loss of sequence identity between the transgene and virus populations, emergence of new viruses or strains, gene silencing suppressors, transgene dosage, plant developmental stage, and growing temperature during the cropping season. We will discuss these factors, including ways to establish a durable, nonspecific system to develop transgenic resistance in papaya. Moreover, it is work with the Hawaiian transgenic

papaya and transgenic papaya developed in other regions that has contributed to a better understanding of the factors affecting transgenic virus resistance in papaya. Therefore, knowledge from this review will give researchers further direction for effective management of PRSV.

## Factors affecting loss of resistance in transgenic papaya

### Sequence homology between transgene and infecting PRSV *CP* gene

To express successful resistance in transgenic papaya, high nucleotide homology is required between the transgene and the challenged virus *CP* gene. Transgenic virus resistance in papaya is sequence specific and provides resistance to only closely related strains of the virus (Baulcombe 1996; Tennant et al. 2001). The transgenic lines developed in Hawaii are highly resistant to local PRSV strains. These lines were obtained by transferring the *CP* gene of mild Hawaiian strain HA 5–1 into papaya and no loss of resistance has been observed in more than two decades (Yeh et al. 2010; Gonsalves 2002; Fitch et al. 1992; Lius et al. 1997; Fuchs and Gonsalves 2007; Hu, *personal observation*). However, these transgenic lines are susceptible to PRSV isolates from outside of Hawaii (Tennant et al. 1994). The continuing use of these transgenic cultivars in Hawaii is reliant on their resistance to Hawaii's PRSV strains and to foreign strains that may enter Hawaii. Thus, Dr. Tennant and her colleagues showed that the resistance of commercial transgenic papayas to PRSV is affected by the sequence homology of the invading strains to PRSV transgene (Tennant et al. 2001). They provided experimental evidence that PRSV HA 5–1, a strain from Hawaii could not overcome the resistance of 'Rainbow' or 'SunUp'. However, a recombinant virus of PRSV HA could infect 'Rainbow' and 'SunUp' (Chiang et al. 2001). In this recombinant virus, the *CP* gene was replaced with PRSV YK-CP gene. The *CP* of the resulting virus has less than 89% sequence homology to the original *CP* transgene.

Detailed comparative studies were made of *CP*-gene sequences from various PRSV isolates from different countries and the resistance responses of transgenic papaya cultivars (Tennant et al. 2001; Tripathi et al. 2008). Transgenic resistance was positively correlated with the degree of homology between the *CP* of the infecting PRSV strain and the transgene. PRSV isolates from Hawaii showed 97 to 100% sequence homology to the transgene CP, but isolates from other regions had only 89 to 93% *CP* sequence homology. Tennant et al. (2001) compared the nucleotide identities of the isolates from Hawaii (HA,OA, KA and KE), Jamaica (JA), Brazil (BR), and Thailand (TH) with the sequence of the severe Hawaiian strain PRSV HA 5–1 (Quemada et al. 1990) used in screening resistant lines in Hawaii. The identities ranged from 89.5% to 99.8%. Isolates from Hawaii had the highest sequence identities with PRSV HA 5–1 (96.7–99.8%). Interestingly, some of the isolates from Hawaii (OA, KA and KE) which had the lowest sequence homology to the transgene had the highest infection incidence on hemizygous plants. The most distantly related isolate to the transgene was PRSV isolate TH, which infected and caused severe symptoms on all transgenic papaya plants. As expected, the core region of the *CP* gene was most conserved between isolates with percent similarities from 97 to 99% between the Hawaiian isolates and PRSV HA 5–1, and 90 to 95% between PRSV HA 5–1 and isolates outside of Hawaii. Similarly, the *CP* C terminus was highly conserved among the isolates (91 to 100%). The most variable region was the N terminus region. Percent similarities in this N terminus among isolates outside of Hawaii and PRSV HA 5–1 were 83.7%, 84.4%, and 89.3% for isolates from Thailand, Brazil, and Jamaica, respectively. In contrast, similarities of 95.3 to 99.3% were observed among Hawaii isolates for the N terminal region (Tennant et al. 2001).

### Emergence of recombinant strains

Plant viruses may undergo recombination and produce new strains expressing distinctly different disease symptoms. These recombination events can occur between similar viruses co-infecting a non-transgenic plant or between an infecting virus and a transgenic plant expressing mRNA with sequences from the target virus (Kung et al. 2009, 2015; Bau et al. 2003, 2008). These recombination events can initiate the emergence of new PRSV strains (Mangrauthia et al. 2008, 2010; Valli et al. 2007; Chaves-Bedoya and Ortiz-Rojas 2015) which are important events in PRSV evolution (Mangrauthia et al. 2008; Ohshima et al. 2007). The emergence of more virulent recombinant strains is a concern when growing transgenic papaya cultivars with PRSV resistance.

Chiang et al. (2001) reported that the infectivity of recombinant PRSV is influenced more by the position effect compared to the degree of sequence identity between the recombinant *CP* gene and the transgene. They demonstrated that recombinant strains of PRSV can overcome the resistance of transgenic papaya cultivars, producing mild to severe symptoms corresponding to the region of substitution within the *CP* gene. Furthermore, transgenic 'Rainbow' was resistant to PRSV HA but produced severe symptoms when challenged with PRSV YK from Taiwan or with a recombinant PRSV HA containing the *CP* gene of YK. PRSV HA recombinants with less than the entire *CP* gene segments of PRSV YK induce milder symptoms on 'Rainbow' than those of the entire gene. Interestingly, an HA recombinant containing a segment of the YK *CP* gene from the 5′ region, which has the lowest comparative nucleotide sequence homology to the transgene, induced very mild PRSV symptoms compared to recombinants containing a segment of the YK *CP* gene from the mid and 3′ end region (Chiang et al. 2001), suggesting lack of sequence homology is not the only factor in overcoming resistance.

The PRSV recombinant YK/AS contained a 263 nucleotide (nt) YK segment, which had 82–92% sequence identity to the corresponding region of the transgene. It induced very mild type symptoms on 'Rainbow', in contrast to the more prominent type symptoms induced by two other recombinants YK/SE and YK/EN, which have 89–95% and 82–100% nt similarity respectively to the transgene. Moreover, the length of the YK replacement segment did not account for the different symptoms induced, since the YK segments in the YK/EN and YK/AS recombinants were similar in length. The variable symptoms that the PRSV recombinants produced on 'Rainbow' were evidently not due to their inherent capability to replicate, as all the recombinants induced severe symptoms on non-transgenic papaya plants. Therefore, recombination is another factor apart from sequence homology in breaking down transgenic resistance. There is a possibility of emerging resistance-breaking PRSV strains through occurrence of recombination events between PRSV strains from Hawaii and transgenic papaya that express the *CP* or other genes of PRSV strains that could overcome the resistance of transgenic papaya in Hawaii (Chiang et al. 2001).

The transgenic lines targeting the YK *CP*-3′ untranslated region (UTR) developed in Taiwan showed resistance to PRSV strains from Hawaii, Mexico, and Thailand (Bau et al. 2003). However, Kung et al. (2015) constructed a virulent recombinant PRSV by replacing the PRSV HC-Pro region with that of PRSV 5–19 HC-Pro region to evaluate transgenic resistance. Results showed that the newly constructed PRSV recombinant strain broke down the transgenic resistance in a sequence homology independent manner. Interestingly, the sequences of the transgene transcript shared 97 ± 1% nucleotide identity with the genome of the infecting virus (Bau et al. 2003, 2004, 2008; Tripathi et al. 2008). This indicated that the resistance-evading phenomena did not depend on a difference in sequence divergence between the YK CP-3'UTR transgene sequence from the transgenic papaya lines and the respective PRSV strain (Tripathi et al. 2004; Kung et al. 2015). It has been suggested that *CP*-transgenic plants may also promote the development of mutants or variants within the natural PRSV population (Kung et al. 2015). The transgenic papaya 'Huanong No. 1' developed in China maintained PRSV resistance for several years in the field (Mendoza et al. 2008), but observations of resistance break down against field isolates of PRSV have been recently observed. Zhao et al. (2015) determined the complete genome sequence of PRSV isolate KF791028 from transgenic 'Huanong No. 1', which had the maximum sequence similarity (92%) to the other Hainan PRSV isolates- EF183499, HQ424465 and KF734962 and possessed the least similarity (81%) to the Hawaiian isolate- EU126128 (Lu et al. 2008; Zhang et al. 2014). It is assumed that this new PRSV variant evolved in transgenic papaya through recombination within Hainan's PRSV population (Kung et al. 2015; Zhao et al. 2015).

## Virus-encoded PTGS suppressors

Viruses with moderate to large genomes encode functionally distinct, highly diverse proteins. Some of these proteins help to suppress the PTGS pathway (Dıaz-Pendon and Ding 2008; Ding and Voinnet 2007). Many scientists accept that these suppressor proteins interfere with the biogenesis of siRNAs. The potyviral HC-Pro, for example, explicitly inhibits accumulation of secondary siRNAs, but not primary siRNAs (Dıaz-Pendon and Ding 2008). Several investigators agree that sequence homology of the virus to the transgene is not the only viral factor employed in defeating transgenic resistance produced by PTGS (Pruss et al. 1997; Anandalakshmi et al. 1998; Kasschau and Carrington 1998). They

consider viral suppressors as important factors in the failure of virus-resistant transgenic plants, along with increasing virus pathogenicity and enhanced synergism between viruses.

The HC-Pro of potyviruses changes the deposition of endogenous micro RNAs (miRNAs) indicating that symptoms caused by viruses might be the result of abnormal metabolism of miRNA (Mallory et al. 2002). Mangrauthia et al. (2009) reported that PRSV-resistant transgenic plants face a crucial barrier in obtaining resistance due to the involvement of PRSV-HC-Pro as a suppressor in RNA silencing mechanism. HC-Pro binds miRNA and interferes with the miRNA-directed regulatory pathways of plants that affect their developmental biology. The suppressor HC-Pro facilitates the establishment of the infecting PRSV and it also has strong positive synergism with other heterologous viruses. PRSV-HC-Pro does not bind dsRNA, but has a strong affinity toward miR171 duplexes. This distinct feature indicates that HC-Pro might use a sequestration model to operate suppressor function (Merai et al. 2006; Lakatos et al. 2006), in which viral suppressor proteins prevent siRNA from assembling with the RNA-induced silencing complex (RISC) responsible for PTGS. HC-Pro is a strong silencing suppressor which could break PRSV resistance in transgenic papaya (Tripathi et al. 2004; Ruanjan et al. 2007). The resistance of transgenic YK CP-3'UTR lines was overcome by the virulent strains (5–19, CS and TD2) due to the silencing suppressor gene HC-Pro (Kung et al. 2015). The mechanism of the homology-independent breakdown of *CP*-transgenic resistance by 5–19 strain was investigated in a separate study (Kung et al. 2015). An analysis of the reactions of single virus PRSV-resistant and double virus PRSV + PLDMV-resistant lines to the transgene-donating strain YK, the resistance-evading strain 5–19, and their recombinants has been completed. The results of the recombinant analysis indicated that 5–19 HC-Pro was a PTGS suppressor that may have suppressed RNA silencing mechanism in the YK CP-3'UTR transgenic lines resulting in the loss of PTGS-mediated resistance (Kung et al. 2015).

Kung et al. (2015) also evaluated the gene silencing suppression mechanism of the HC-Pro gene of strain 5–19 in a transient expression system developed in the model plant *Nicotiana benthamiana*. They determined that 5–19 HC-Pro was a stronger suppressor of gene-silencing than YK HC-Pro. The 5–19 strain might have emerged in non-transgenic plants and then transmitted to transgenic papayas by aphids. The superior ability of the 5–19 strain to suppress PTGS allowed it to overcome *CP*-transgenic resistance in a sequence-homology-independent manner. Gene silencing suppressors can disrupt PTGS pathways at multiple points, inhibiting the plant's defense against their actions. This blocking inhibits host defense responses by acting against essential elements of the cellular silencing system resulting in over-simulating their normal cellular activities (Mangrauthia et al. 2008, 2009).

### Plant developmental stage

Different aspects of plant physiology can influence PTGS in the field. Sometimes, a temporary loss of resistance in transgenic plants appears during certain stages of growth and development (Davis and Ying 2004). Studies with papaya plants displaying RNA-silencing-mediated resistance suggest an influence of plant developmental stage on expressed resistance. Transgenic papaya plants were susceptible to inoculation with PRSV at a younger stage, but resistant at an older stage (Tennant et al. 2001). Some studies support the idea that younger transgenic plants accumulate fewer transgene-specific siRNAs than older plants, or correspondingly accumulate greater amounts of transgene-specific transcripts. These findings suggest that transgenic resistance against plant-infecting viruses is less efficient in younger plants (Kalantidis et al. 2002).

### Transgene copy number

The number of transgene copies plays a role in the level of virus resistance in transgenic papaya cultivars. Cultivars with a single transgene copy show less resistance to target viruses than cultivars with multiple copies (Tennant et al. 2001). This effect is also found with PTGS-mediated silencing and PRSV resistance in the original Hawaiian transgenic papayas (Lines et al. 2002). The R1 transgenic papaya of line 55–1 is resistant to PRSV isolates from Hawaii but susceptible to foreign strains. The 55–1 R1 plants like 'Rainbow', are hemizygous for the *CP* transgene and have a narrow, specific resistance to certain PRSV strains. When 'Rainbow' was inoculated with PRSV strains from Mexico in greenhouse trials it was resistant, but strains from Thailand, Australia, and Brazil produced symptoms of ringspot disease. Increasing the transgene copy number expanded resistance to foreign PRSV strains. 'SunUp',

is homozygous for the *CP* gene and resistant to some, but not all, PRSV strains outside of Hawaii (Tennant et al. 1994, 2001). Line 63–1 has a double insertion of the CP gene and is resistant not only to isolates of PRSV from Hawaii, but also to those from Jamaica, Thailand and Brazil (Tennant et al. 2005; Souza et al. 2005a, b).

### Environment

Environmental temperature influences virus resistance in transgenic plants. Ye and Li (2010) reported that transgenic papaya with high resistance to PRSV showed mosaic symptoms during the colder temperatures of early spring. Symptom expression and virus accumulation in papaya cultivar Pusa Nanha were greatest at temperatures between 26 and 31 °C (Mangrauthia et al. 2009). However, there was a marked reduction in virus accumulation and symptom expression at 10 °C above or below the 26 to 31 °C range. The HC-Pro protein of PRSV showed a temperature-related affinity for small RNAs (sRNAs). In one assay, recombinant PRSV HC-Pro bound to 21-nt double-stranded miRNAs more efficiently at ambient temperatures (25 °C) than at high (35 to 45 °C) or low (15 °C) temperatures (Mangrauthia et al. 2009). A mobility shift of sRNAs was not detected during incubation with PRSV HC-Pro at either high or low temperatures. This suggested a plausible role of HC-Pro in temperature-directed, host–virus interactions in papaya (Mangrauthia et al. 2009). Velázquez and associates (2010) observed that immediate shifting of temperature from 25 °C to 40 °C drastically reduced ssRNA synthesis, but the changes in temperature occasionally affected synthesis of dsRNA replication or intermediates forms until the synthesis of ssRNA had shut down. Higher amounts of dsRNA deposition after an increase in temperature could initiate PTGS.

### Emergence of new viruses

The introduction of a new virus into a region may create a synergistic interaction or an additive effect with an existing virus and may overcome single-copy transgene resistance in papaya (Kung et al. 2009, 2015; Bau et al. 2003, 2008). Multiple viral infections that may result in synergism or an additive effect frequently are found in nature, with unpredictable breakdown of trangenic virus resistance. Synergistic interaction is an effect resulting from mixed infections with two or more viruses in plants, resulting in an increase in the titers of one or both viruses which may result in enhanced symptoms (Karyeija et al. 2000; Pruss et al. 1997). Several scientists found that the introduction of a new virus results in an additive effect on the existing virus through a dramatic increase in virus titers and disease symptom expression (Calvert and Ghabrial 1983). In a complex situation, a pronounced synergistic effect could result in a drastic reduction in plant height, weight, and yield with severe disease symptoms leading to plant death (Murphy and Bowen 2006). Multiple infections of PRSV and one or more viruses, such as PLCV, PapMV, PLDMV, PMeV or PLYV can increase virus accumulation and symptom expression, reducing resistance in transgenic papaya (Bau et al. 2008; Daltro et al. 2012; Noa-Carrazana et al. 2006; Ventura et al. 2003; Kung et al. 2015). In Taiwan, a transgenic papaya with broad resistance was developed using *CP*-3'UTR from local PRSV strains. Infection by both PLDMV and PRSV interfered with plant resistance in the transgenic line. Both viruses cause similar symptoms in papaya, such as mosaic and leaf discoloration, distortion of leaves, water-soaked oily patches on petioles and ring like spots on fruits (Bau et al. 2008; Cruz et al. 2009), but in the mixed infection they can cause severe leaf distortion in both transgenic and non-transgenic papayas (Bau et al. 2008; Kung et al. 2009).

## Potential ways of developing durable broad spectrum transgenic resistance against PRSV

### Use of transgenes from local or resistance-breaking PRSV strains

PRSV *CP*-mediated transgenic resistance relies on the homology between the transgene and the coat protein of the virus. Using the *CP* gene of a native, widespread strain of PRSV is a requirement for potent resistance in a specific geographic area (Gonsalves 2002). Successful use of the *CP* gene of a native PRSV strain to transform indigenous commercial papaya cultivars has been reported from several countries. An untranslatable PRSV *CP*-coding region used as a transgene to generate two transgenic papaya cultivars in Australia displayed immunity to the native PRSV isolate (Lines et al. 2002). Fermin et al. (2004) constructed PRSV-resistant transgenic papaya plants by transforming them with the *CP*

genes of PRSV isolates from two separate locations in Venezuela. All these transgenic lines and their progenies showed various levels of resistance to PRSV isolates from Hawaii and Thailand. Scientists in Florida developed PRSV resistant papaya lines using the *CP* gene of a local strain (Davis and Ying 2004). The resulting transgenic resistance was transferred to popular papaya cultivars through a traditional breeding program. Additionally, the researchers used truncated *RP* gene of PRSV as a transgene and developed a PRSV-resistant transgenic papaya via an *Agrobacterium*-mediated transformation system (Davis and Ying 2004). In 2015, scientists from Taiwan developed transgenic papaya plants using untranslatable constructs of HC-Pro from a highly virulent PRSV strain. The plants were resistant to the newly emerging, more virulent virus strains and recombinant strains known to break *CP*-mediated resistance in transgenic papaya plants (Kung et al. 2015).

### Use of multiple virus genes or virus segments

Several viruses, or strains of the same virus, can threaten papaya production in a specific area. Therefore, new transgenic papaya lines would include resistance to these viruses. Gene pyramiding inserts multiple virus transgenes into the same plant, producing a resistant plant to these viruses (Yeh et al. 2014). A research group from Taiwan used chimeric constructs to develop resistance to two different potyviruses, PRSV and PLDMV (Kung et al. 2009). This approach may protect papaya from new strains or non-target viruses if mRNA expressed by the transgenes can fold into hairpin structures inducing gene silencing (Schumann et al. 2013; Agrawal et al. 2003). Fermin and Gonsalves (2001) created tospovirus-resistant *Nicotiana benthamiana* by inserting a chimeric transgene consisting of a 200-bp N-gene segment from the tospovirus.

Another method of developing broad resistance in transgenic papaya is to use synthetic transgenes with a high nucleotide similarity to the targeted viruses (Fermin-Munoz 2002; Fermin and Gonsalves 2004). This novel approach does not use existing *CP* genes or chimeras and avoids multiple transformations with the desired sequences.

Fermin and Gonsalves (2001) developed transgenic plants using a synthetic transgene with 90% homology to *Tomato spotted wilt virus* and *Groundnut ringspot tospovirus* and the plant was resistant to both viruses. The reduction in sequence homology between the transgene and the invading virus or viruses resulted in a lower percentage of resistant lines. The Gonsalves group is refining this approach to provide papaya in Hawaii with durable resistance to PRSV (Fermin and Gonsalves 2004). Another group from Hawaii used an untranslatable, synthetic *CP* gene with more than 94% similarity to *Citrus tristeza virus* (CTV) strains from Hawaii and other countries to transform lime to generate transgenic resistant lines. The selected lines are currently being evaluated for resistance to CTV transmitted by brown citrus aphids (*Toxoptera citricida* Kiek) infecting citrus (Melzer and Hu, *unpublished*).

Bonfim et al. (2007) developed a transgenic line of common bean using transgenes containing virus sequences in an inverted orientation, which delayed and attenuated golden mosaic symptoms upon being challenged with viruliferous whiteflies. They worked on the idea of utilizing a construct with RNA interference, which silences the viral replication protein gene (AC1) and produce highly resistant transgenic common bean plants. Transgenic lines were obtained with an intron-hairpin construction and induced PTGS against the AC1 gene. Line −5.1 was highly resistance to repeated inoculation by viruliferous whiteflies at a very early stage of plant development. Transgene-specific siRNAs were detected in transgenic plants.

Jia et al. (2017) in China used an RNA interference (RNAi) approach to target a conserved *CP* region of the PRSV during virus replication to develop a broad spectrum resistance to PRSV isolates from Hainan (Table 1). By analyzing PRSV isolates from Hainan, they found a 544-bp region of the *CP* gene which shared 97 to 100% nucleotide identity among all isolates. Their results showed that the transgenic line produced siRNAs and showed resistance to PRSV local strains in greenhouse test. This research indicates that the newly developed transgenic papaya line using RNAi approach targeted a conserved *CP* gene region of the PRSV has a useful application against PRSV in the major papaya growing area.

### Increasing gene dosage

Increasing the transgene dosage strengthens and broadens virus resistance in papaya plants against both homologous and heterologous PRSV isolates (Souza et al. 2005a, b). It may be a factor in the homozygous ‘SunUp’ having a greater level of PRSV resistance than hemizygous ‘Rainbow’(Gonsalves et al. 2004).

Despite virus resistance of 'SunUp' to local and to some exotic PRSV strains, farmers prefer agronomic characteristics of 'Rainbow'. The 'Rainbow' cultivar is resistant only to a narrow range of PRSV strains, but has higher quality fruit, the preferred yellow flesh, earlier maturity and a greater market demand in Hawaii (Gonsalves 2004). Pyramiding transcriptionally active CP transgenes in the genome of transgenic papaya plant by super-transformation could be a potential option to increase the resistance in 'Rainbow'. This transgene management via recurrent selection is helpful to generate a highly resistant transgenic papaya plant to many PRSV isolates (Souza et al. 2005a, b).

## Development of virus-resistant papaya using gene editing technology

The novel CRISPR/Cas9 technology can expedite the development of virus-resistant papaya either by targeting the virus directly, or editing one or more host genes essential for virus replication. There is no complete resistance to PRSV in any known papaya cultivars. CRISPR/Cas9-mediated mutations of host plants can confer resistance against many invading viruses (Green and Hu 2017). CRISPR/Cas9 system causes sequence-specific double-stranded break (DSB) to modify the targeted DNA sequences using a single guide RNA (sgRNA), which directs a Cas9 nuclease (Hsu et al. 2014). Chandrasekaran and others (2016) used the CRISPR/Cas system to introduce targeted mutations in the eukaryotic translation initiation factor (*eIF4E gene*) of cucumber (*Cucumis sativus)*. The homozygotic T3 generation plants were immune to *Cucumber vein yellowing virus* and resistant to two other potyviruses *Zucchini yellow mosaic virus* and PRSV-W (Chandrasekaran et al. 2016). A CRISPR/Cas9 system has been inserted successfully into *A. thaliana* to create mutations in *eIF(iso)4E*, an isoform of *eIF4E*. The insertion provided high levels of resistance to *Turnip mosaic virus* (TuMV) (Pyott et al. 2016). RNA viruses that infect plants, like PRSV, require host factors (*eIF4E* or *eIF(iso)4E)* (Lellis et al. 2002; Nicaise et al. 2003; Ruffel et al. 2006) to continue their life cycle as it interacts with viral protein genome –linked (VPg) in the hosts. The *eIF4E* complex binds to the potyviral 5′ m7G cap structure and 3′ polyA tail of mRNA for translation. Disruption of this interaction by either mutagenesis or silencing stops further virus infection (Léonard et al. 2000; Sanfaçon 2015; Jiang and Laliberté 2011; Duprat et al. 2002; Lellis et al. 2002; Rodríguez-Hernández et al. 2012; Sato et al. 2005). The CRISPR/Cas9 system was used to knockout the target gene from host factors in different plants. Broad spectrum resistance to RNA viruses resistance has been demonstrated by silencing or mutations of the *eIF4E* and *eIF(iso)4E* genes in various crops i.e. tomato and melon (Mazier et al. 2011; Rodríguez-Hernández et al. 2012; Gómez et al. 2009).

CRISPR/Cas9 can also be used to specifically target the dsDNA of a geminivirus (Ji et al. 2015). Circular single-stranded DNA (ssDNA) of geminiviruses replicate within the nuclei of plant cells and can cause serious production losses for many dicotyledonous crop plants (Hsu et al. 2014; Manssor et al. 1999; Moffat 1999). The ssDNA is converted to a double-stranded DNA (dsDNA) intermediate during geminivirus replication, from which new ssDNA is generated by rolling-circle replication. These dsDNA can then be specifically target by CRISPR/Cas9 to inhibit virus replication and confer virus resistance to host plants (Ji et al. 2015). The CRISPR/Cas9 systems have also been used in *A. thaliana* and *N. benthamiana* to confer resistance to geminiviruses (Hanley-Bowdoin et al. 2013). In transgenic *N. benthamiana*, reduced virus accumulation and symptom expression were produced in plants challenged with *Bean yellow dwarf virus* (BeYDV) and *Beet severe curly top virus* (BCTV) (Baltes et al. 2015; Ji et al. 2015). Ali et al. (2015) reported that transgenic *N. benthamiana* engineered with a CRISPR/Cas system targeting *Tomato yellow leaf curl virus* (TYLCV) slowed virus production or decreased its titer and eliminated or substantially reduced symptoms.

This technology has benefits over the commonly used non-specific integration methods of *Agrobacterium*-mediated transformation or microprojectile bombardment. CRISPR/Cas technology can be used, for example, to integrate transgenes into selected loci, avoiding undesired positional effects and averting the interruption of innate gene activities. The widespread application of CRISPR/Cas9 to plant disease problems is an indicator of its future potential in this field (Green and Hu 2017).

## Strengthening quarantine systems and field monitoring

Strengthening quarantine procedures can help to reduce the introduction of foreign viruses or new virus strains into an area. Detection techniques with greater sensitivity like single-tube nested PCR can improve quarantine

efforts and disease monitoring. They also can provide an early warning of emerging viruses or virulent strains in transgenic papayas (Hamim et al. 2017; Dey et al. 2012; Kung et al. 2015; Tripathi et al. 2008; Yeh et al. 2014; Hu *personal observation*). It is important to regularly survey papaya-producing regions to identify existing viruses and virus strains and monitor their movement to other papaya-producing areas (Paolla et al. 2015). Frequent monitoring is also needed to detect changes in virus populations in papaya production areas (Paolla et al. 2015). "Rainbow" and "SunUp" have proven, durable transgenic resistance to PRSV in Hawaii. The emergence of new viral strains locally or by the introduction of foreign divergent strains could break down resistance. Therefore, it is essential to monitor the diversity and the arrival or the emergence of new and more virulent strains in the PRSV population. The recent advances in research on PRSV gene sequences in different geographical regions will be helpful for devising effective PRSV management strategies, including designing the most effective transgene for achieving broad resistance to PRSV isolates in specific regions (Tripathi et al. 2008; Wang et al. 2017a, b). Monitoring and quarantine should be implemented on a local, national and international level.

### Integrating transgenic technology with integrated pest management (IPM)

To date, transgenic papaya cultivars are used in Hawaii and other countries commercially, but their use in an IPM program has been limited. This is the consequence of the gap between the reductionist approaches of biotechnology and the holistic approaches of IPM (Kos et al. 2009). PRSV-resistant transgenic papayas have been claimed as being the sole solution to PRSV problem, whereas proponents of IPM strategies have found that a single solution is not sufficient to provide durable PRSV management. Indeed, current transgenic papaya based on PTGS can solve PRSV problems in a region for a certain period, but introduction of new or foreign strain of PRSV can break the transgenic resistance.

Some scientists already demonstrated that IPM components worked successfully to reduce PRSV infection in papaya. The use of insecticides is unlikely to effectively control PRSV because of the erratic and unpredictable non-persistent transmission of the virus by aphids. Fermin et al. (2010) reported that application of silver reflective plastic mulches in the papaya orchard has been working effectively in repelling aphids from young papaya plants; as a result, reducing or delaying virus infection. Another successfully adopted cultural practice in Colombia and Brazil is eradication or rouging of curcurbits and weeds from inside and around the borders of papaya orchard. These plants served as aphid hosts allowing populations to build (Fermin et al. 2010). Considering transgenic papayas as an important component of IPM of PRSV, we believe that existing and future transgenic papaya cultivars will have extensive potential, when incorporated into IPM systems.

## Conclusions

Papaya ringspot disease, caused by PRSV, is the most significant constraint to papaya production worldwide. Newly emerged virulent PRSV strains, or the increased virulence of existing strains that arise from synergistic interactions with other viruses, have caused severe disease outbreaks among transgenic cultivars in many papaya-growing areas. PRSV-resistant transgenic plants are still the most effective means of controlling papaya ringspot disease. Several biotic and abiotic factors may cause partial or total loss of virus resistance in transgenic papaya lines. CRISPR/Cas9 and other new technologies may lead to the development of durable, broad-spectrum, PRSV-resistant transgenic cultivars. Transgenic resistance should be combined with appropriate elements of an IPM program. This combination will maximize the effect of transgenic virus resistance and extend its viability. Local, national and international quarantine efforts can help reduce the risk of resistance breakdown by avoiding the introduction of new viruses or strains from other regions.

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**Compliance with ethical standards**

**Conflict of interest** There is no conflict of interest regarding the publication of this article in Phytoparasitica.

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