# Chapter 15
# Genomics of Papaya Disease Resistance

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## Introduction

Papaya is a popular fruit in the tropics and subtropics that significantly contributes to the dietary intake of vitamins A and C (FAO 2009; Miller 1926). Although abundant year-round production of papaya is possible, its susceptibility to numerous diseases affects fruit quality and yield (Persley and Ploetz 2003) (Table 15.1). This may be partially attributable to its narrow genetic base. Presumably, papaya diverged from other species of the Caricaceae family as a result of being evolutionarily isolated in Central America (Aradhya et al. 1999). Archeological and paleoethnobotanical evidence indicates the presence of papaya in the region dating back to the Maya Classic Period (300–900 AD). The identification of maximal species richness of its close relative, the genus *Vasconcellea*, which occurs farther south in Colombia, Ecuador, and Peru, supports this hypothesis (Miksicek 1983; Lentz 1999; Scheldeman et al. 2007). As a consequence, *C. papaya* is the only member of the genus *Carica* (Aradhya et al. 1999; Kim et al. 2002) and is more vulnerable to disease than genera with greater genetic diversity.

Today, from a global production standpoint, papaya is no longer isolated anywhere in the world, and the severity and geographical distribution of some papaya diseases is highly variable. For example, papaya meleira virus (PMeV), which causes "sticky disease," is considered among the most severe diseases in Brazil (Ventura et al. 2004), but it is less prevalent elsewhere. Similarly, the acidic soils of Hawaii are thought to promote *Phytophthora* rot (Manshardt and Zee 1994), while in Malaysia where soil pH is also low, the disease is considered insignificant

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**Table 15.1** Major diseases of papaya

| Pathogen | Species | Prominent distribution |
|---|---|---|
| Bacteria | | |
| Bacterial canker and decline | *Erwinia* | Caribbean |
| Bacterial leaf spot | *Pseudomonas caricae-papayae* | Brazil |
| Internal yellowing | *Enterobacter cloacae* | Hawaii |
| Mushy canker | Erwinia | Northern Mariana Islands |
| Papaya bunchy top | *Rickettsia* | Puerto Rico, Caribbean, and Central and South America |
| Purple stain fruit rot | *Erwinia herbicola* | Brazil |
| Fungi and oomycetes | | |
| Alternaria fruit spot | *Alternaria alternate* | Israel and Hawaii |
| Anthracnose | *Colletotrichum gloeosporioides* | Most production areas |
| Asperisporium black spot | *Asperisporium caricae* | Australia, Africa, Central America, South America, India, and the USA |
| Black/dry rot | *Mycosphaerella caricae* | Most production areas |
| Brown spot/Corynespora leaf spot | *Corynespora cassiicola* | Most production areas |
| Cercospora black spot | *Cercospora papayae* | Most production areas |
| Collar rot | *Calonectria ilicicola* | Hawaii |
| Fusarium fruit rot | *Fusarium solani* | Hawaii, India, Israel, and the Philippines |
| Lasiodiplodia fruit and stem rot | *Diplodia theobromae* | Hawaii and India |
| Leaf spot, dry rot, end rot of fruits, wet fruit rot | *Phomopsis caricae-papayae*, *Phomopsis* sp. | Most production areas |
| Phytophthora fruit, root, and stem rot | *Phytophthora palmivora* | Most production areas |
| Powdery mildew | *Oidium caricae* | Most production areas |
| Soft rot | *Rhizopus stolonifer* | Most production areas |
| Stemphylium fruit rot | *Stemphylium lycopersici* | Most production areas |
| Nematodes | | |
| Reniform nematodes | *Rotylenchulus reniformis* | Most production areas |
| Root-knot nematodes | *Meloidogyne incognita, Meloidogyne javanica* | Most production areas |
| Phytoplasmas | | |
| Papaya dieback | *Candidatus* Phytoplasma australiense | Australia |
| Yellow crinkle and mosaic | *Candidatus* Phytoplasma australasia | Australia |
| Viruses | | |
| Leaf curl disease | *Papaya leaf curl virus* | India |
| Meleira or sticky disease | Virus | Brazil |
| Papaya droopy necrosis and papaya apical necrosis | Rhabdovirus | Florida and Venezuela |

(continued)

**Table 15.1** (continued)

| Pathogen | Species | Prominent distribution |
|---|---|---|
| Papaya leaf distortion mosaic | *Papaya leaf distortion mosaic virus* | Japan, Saipan, and Taiwan |
| Papaya lethal yellowing disease | *Papaya lethal yellowing virus* | Brazil |
| Papaya mild yellowing disease | *Papaya mild yellowing virus* | Venezuela |
| Papaya mosaic | *Papaya mosaic virus* | USA, Mexico, and South America |
| Papaya ringspot | *Papaya ringspot virus*-type P | Most production areas |
| Tomato spotted wilt | *Tomato spotted wilt virus* | Hawaii |

Data from Persley and Ploetz (2003) and Ventura et al. (2004)

(Personal communication: Dr. Chan Ying Kwok, Malaysian Agrifood Corporation). Such disparity in disease prevalence between environments might be explained by factors such as pathogen diversity, the concentration of disease vectors, the abundance of alternate hosts, or presence of natural barriers that affect pathogen movement and regional outbreaks, as was the case for papaya ringspot virus (PRSV-P) (Gonsalves 1998). In the case of pathogen diversity, the evolution of pathogenicity factors, including effector proteins, can intensify disease (Birch et al. 2006; Walton et al. 2009). Regardless of the mechanisms involved in a particular disease, local cultivars must be developed that can withstand pathogen pressure. To achieve this goal, sources of resistance can be obtained within *Carica* or related Caricaceae genera or by using bioengineering approaches.

Although *Carica* is monotypic, crosses of papaya cultivars have, in some cases, demonstrated that disease resistance is additive and selectable (Mosqueda-Vázquez et al. 1981; Mosqueda-Vázquez and Nakasone 1982). Markers linked to resistance loci are beginning to be developed (Noorda-Nguyen et al. 2010); with the papaya genome sequence now available (Ming et al. 2008), genetic resistance within the species may be more fully determined. Separately, 5 related genera, *Cylicomorpha*, *Horovitzia*, *Jacaratia*, *Jarilla*, and *Vasconcellea*, consist of 34 additional species (Scheldeman et al. 2007) that can be screened for resistance to papaya diseases (Tables 15.1 and 15.2). Transferring resistance genes from these species to papaya is difficult since hybrids often produce nonviable seed or parthenocarpic fruit caused by postzygotic barriers, such as abnormal endosperm development or ovule and embryo abortion (Mekako and Nakasone 1975; Manshardt and Wenslaff 1989a). Nevertheless, hybrids have been recovered (Manshardt and Wenslaff 1989b), and recently the introgression of PRSV resistance from a wild relative was successfully achieved (Siar et al. 2009). This advance provides encouragement that additional wild relative traits may be introgressed into papaya in the future.

Bioengineering has played a major role in securing the production of papaya, most notably through coat protein-mediated resistance to ringspot virus (Fitch et al. 1992; reviewed by Gonsalves in Chap. 7 in this text). New transgenic strategies for controlling carmine spider mite and *Phytophthora palmivora* of papaya have

**Table 15.2** First reports and possible emerging diseases of papaya

| Disease | Location | Reference |
|---|---|---|
| Powdery mildew | Taiwan | Tsay et al. (2011) |
| Bacterial crown rot | Tonga | Fullerton et al. (2011) |
| Postharvest anthracnose | South Florida | Tarnowski and Ploetz (2010) |
| Fruit rot (*Colletotrichum magna*) | Brazil | Nascimento et al. (2010) |
| Scab | Taiwan | Chen et al. (2009) |
| *Erwinia* papayae/papaya dieback | Malaysia | Maktar et al. (2008) |
| Moroccan watermelon mosaic virus | Democratic Republic of Congo | Arocha et al. (2008) |
| Papaya leaf distortion mosaic virus infecting transgenic papaya resistant to papaya ringspot virus | Taiwan | Bau et al. (2008) |
| Atypical internal yellowing | Hawaii | Keith et al. (2008) |
| Ringspot virus | Côte d'Ivoire | Diallo et al. (2007) |
| 16SrII group phytoplasma | Ethiopia | Arocha et al. (2007) |
| 16SrII group phytoplasma | Cuba | Arocha et al. (2006) |
| Nivun Haamir dieback disease | Israel | Gera et al. (2005) |
| Ringspot virus | Bangladesh | Jain et al. (2004) |
| Leaf curl virus | Taiwan | Chang et al. (2003) |
| Phytoplasmas | Cuba | Arocha et al. (2003) |
| Ringspot virus | Iran | Pourrahim et al. (2003) |
| Black spot | Hawaii | Ogata and Heu (2001) |
| Papaya mosaic virus (Mexican isolate) | Mexico | Noa-Carrazana and Silva-Rosales (2001) |
| Collar rot | Baja California Sur, Mexico | Rodriguez-Alvarado et al. (2001) |
| Leaf blight, fruit rot, root rot | American Samoa | Roberts and Trujillo (1998) |
| Leaf curl disease | Pakistan | Nadeem et al. (1997) |

also been successful (Zhu et al. 2004; McCafferty et al. 2006; Zhu et al. 2007). Characterization of papaya's resistance genes, and those of its wild relatives, will likely provide additional sources of resistance (Porter et al. 2009a). At the same time, ecological control strategies, such as defensive mutualism, wherein a symbiont provides protection against pathogens, must be utilized to promote integrated disease management strategies to preserve resistance (Jaizme-Vega et al. 2006; Newcombe et al. 2010).

The genomics of papaya disease resistance will be discussed in this chapter in the context of the major diseases of papaya and the resources available to mitigate them. Genetic variation of resistance available within *C. papaya* and its wild relatives will be reviewed in addition to past and forthcoming transgenic approaches. Finally, pathogen diversity, emerging diseases, and strategies for promoting durable resistance will be addressed.

## Genetic Variation for Disease Resistance in *Carica papaya*

### *Papaya Ringspot Virus*

Papaya ringspot virus exists as multiple strains occurring worldwide and is among the most destructive diseases of papaya (see Chap. 7 in this text; Ventura et al. 2004). *C. papaya* lacks complete resistance to PRSV-P, but conventional breeding has developed partially resistant cultivars. In Florida, Conover et al. (1986) derived "Cariflora" from partially resistant dioecious lines (K2 and K3). Another partially resistant cultivar, Sinta, is an $F_1$ semidwarf hybrid developed by the Institute of Plant Breeding (College of Agriculture, University of the Philippines, Los Baños) (Siar et al. 2009). The level of resistance provided by "Sinta" is proposed to be sufficient for viable commercial production in areas where PRSV-P infection occurs (Siar et al. 2009). Although the resistance of lines developed from germplasm available within the *C. papaya* species is only partial, these genetic resources are valuable. The PRSV-P resistance of "Cariflora" (Conover and Litz 1978) and, likely, "Sinta" is multigenic and now more useful by using genomic tools (Ming et al. 2008). Markers for quantitative trait loci (QTLs) controlling PRSV resistance may be developed for breeding. New sources of resistance may be used to enhance protection against diverse virus isolates and contribute to the durability of deployed transgenic resistance (Fitch et al. 1990, 1992; Fitch 1993; Fitch and Manshardt 1990).

### *Phytophthora Fruit, Root, and Stem Rot*

*P. palmivora* is the causal organism of *Phytophthora* fruit, root, and stem rot of papaya and is thought to have originated in Asia (Persley and Ploetz 2003; Mchau and Coffey 1994). *P. palmivora* is classified as an oomycete which is distinct from fungi. Oomycetes are distinguished by being diploid and having nonseptate hyphae and cell walls that contain cellulose but little or no chitin (Latijnhouwers et al. 2003). Many *Phytophthora* species are devastating pathogens, and *P. palmivora*, with over 160 documented hosts (Erwin and Ribeiro 1996), is no exception. The pathogen produces infectious, biflagellate zoospores that are motile in water, making the disease particularly infective during wet conditions (Erwin and Ribeiro 1996). *P. palmivora* is particularly destructive in the southeast part of the island of Hawaii, which can receive >120 in. of rainfall per year (NOAA Climate Data 1971–2000).

Partial resistance to *P. palmivora* has been identified within the *C. papaya* species. After inoculating 1-month-old papaya seedlings with sporangia, Mosqueda-Vázquez et al. (1981) identified four partially resistant lines (Line 8, Waimanalo-23, Waimanalo-24, and Line 40) and two moderately resistant lines (Line 45-$T_{22}$ and Kapoho). Subsequently, "Waimanalo"-23, "Waimanalo"-24, "Line 40," "Line 45-$T_{22}$," and the susceptible cultivar Higgins were crossed in diallel (crosses in all

possible combinations) to determine the combining ability of *P. palmivora* resistance (Mosqueda-Vázquez and Nakasone 1982). $F_1$ progeny and parents were screened, and it was determined that there was significant general combining ability, suggesting that resistance is additive and selectable (Mosqueda-Vázquez and Nakasone 1982). In a separate study, field and greenhouse screenings identified the cultivars Tailandia Roxao and Cross Paris, which are larger "Formosa" types, as partially resistant and a separate group of "Solo" papaya as susceptible (Dianese et al. 2007, 2010). Interestingly, in Hawaii, the emergence of *P. palmivora* occurred when "Solo" (accession no. 2853) replaced the traditionally grown, more-resistant large-fruited cultivars (Parris 1941; Takeguchi et al. 1999). This suggests that marketing/educational strategies used to promote the production of both large- and small-fruited papaya might help overall crop resistance. Finally, molecular resources are being developed for marker-assisted selection. A segregating $F_2$ population derived from a cross of "Kamiya" (partially resistant) and "SunUp" (susceptible) was screened using amplified fragment length polymorphism (AFLP) analysis (Noorda-Nguyen et al. 2010). Several polymorphic DNA fragments linked to resistance were identified (Noorda-Nguyen et al. 2010) and may be converted to cleaved amplified polymorphic sequences (CAPS) to be used as markers to breed *Phytophthora* resistance.

## *Other Diseases and Pests*

Genetic resistance has been reported for many other diseases and pests of papaya. Collar rot of papaya, caused by the fungus *Calonectria ilicicola*, is of notable concern in wet regions on the island of Hawaii (Persley and Ploetz 2003). Greenhouse inoculations identified the cultivar Kapoho Solo as partially resistant compared to the susceptible cultivars, Sunrise Solo and Waimanalo (Nishijima and Aragaki 1973). The fungal pathogen, *Colletotrichum gloeosporioides*, causes the postharvest disease of papaya known as anthracnose. While symptoms occur on the fruit after harvest, infection first occurs during fruit development (Alvarez and Nishijima 1987). "Sunrise Solo" displayed some resistance to *C. gloeosporioides* (Nakasone and Aragaki 1982). The fungus, *Asperisporium caricae*, causes black spot. Leaf infection decreases plant development, whereas the blemishes on infected fruit lessen marketability (Ventura et al. 2004). Dianese et al. (2007) found genotype Sekati to have the lowest severity of *A. caricae* foliage infection and "Sekati," "Tailandia Roxao," and "Tailandia Verde" to have the lowest levels of fruit infection.

In Hawaii, papaya to be exported to California must receive hot water or forced hot air disinfestation treatment to control fruit flies (*Toxotrypana curvicauda*) (Manshardt and Zee 1994), which are considered the most damaging insect pests of papaya (Pantoja et al. 2002). While fruit fly resistance has not been identified, Aluja et al. (1994) found more field infestation of a variety designated "Hawaiian" than two other cultivars, Cera Amarilla and Cera Roja. For all the diseases and pests mentioned previously, a genetic basis of resistance is worth exploring, especially as resources become available to associate molecular markers with these traits (Ming et al. 2008).

## Genetic Variation for Disease Resistance in Papaya's Wild Relatives

Wild relatives of papaya offer a source of genetic variation for traits such as fruit quality and disease resistance. The most diverse of the five genera related to *Carica* is *Vasconcellea*. It includes 21 species (Badillo 2000). Grown at higher elevations, they are commonly referred to as "highland papaya" (National Research Council 1989). *Vasconcellea* is thought to have originated in the region of Ecuador, Colombia, and Peru where maximum species diversity occurs (Scheldeman et al. 2007). Some *Vasconcellea* spp. are used in local cuisine for flavoring or cooked with sugar to make jams (National Research Council 1989). It may be possible to use these species for enhancing or altering papaya's flavor. Currently, the only highland papaya grown extensively outside of its region of origin is "Babaco," a sterile hybrid (*Vasconcellea* × *heilbornii*) (Kyndt and Gheysen 2007) that produces large parthenocarpic fruit that tastes like "strawberry with a hint of pineapple" (National Research Council 1989). "Babaco" has been evaluated for commercial production in a number of countries with some success, including New Zealand, Australia, Spain, France, the United Kingdom, Switzerland, Italy, the Netherlands, South Africa, and Canada (Scheldeman et al. 2007; Kempler and Kabaluk 1996). Before "Babaco" can be fully commercialized, a reduction in production cost and consumer education must be addressed (Kempler and Kabaluk 1996). In addition, greenhouse production of "Babaco" is limited by fusarium wilt (Ochoa et al. 2000). Possible sources of resistance for this pathogen and those affecting papaya are other members of *Vasconcellea*.

In the mid-1960s, a number of *Vasconcellea* species were screened for PRSV-P resistance. *V. cundinamarcensis* and *V. quercifolia* were found to be resistant (Conove 1964). A separate study found *V. cauliflora* and its $F_1$ hybrids from a cross with a susceptible species (*C. monoica*) resistant to PRSV-P (Horovitz and Jiménez 1967). Attempts to introgress this resistance into papaya through crosses with *V. cauliflora* have been mostly unsuccessful due to postzygotic barriers, including embryo abortion, abnormal endosperm development, and polyembryony (Manshardt and Wenslaff 1989a). In contrast, crosses of *C. papaya* to *V. quercifolia* results in fewer postzygotic disruptions and can be grown in the field (Manshardt and Wenslaff 1989b). In an attempt to improve the success rate of *C. papaya* × *C. cauliflora* hybridization, Magdalita et al. (1998) developed an efficient hybridization protocol, including the use of a more compatible *C. papaya* cultivar, higher quality pollen, and embryo isolation time at 90–120 days postfertilization. Combined with an improved embryo-rescue technique, this protocol resulted in a 94 % embryo germination rate, providing 485 hybrid plants with normal morphology (Magdalita et al. 1996). Unfortunately, although these hybrids were resistant to PRSV-P (Magdalita et al. 1997), none were fertile (Drew et al. 2005a). As a result, the focus for a source of resistance returned to *V. quercifolia*, which is more closely related to *C. papaya* (Jobin-Décor et al. 1997) so that there are fewer postzygotic barriers (Manshardt and Wenslaff 1989b).

Because the Philippine papaya industry was experiencing significant losses due to PRSV-P, the Institute for Plant Breeding (College of Agriculture, University of the Philippines at Los Baños) established a collaborative project with Griffith University (Nathan, Australia) in 2002 to prioritize the introgression of resistance from *V. quercifolia* into elite Philippine inbred lines (Siar et al. 2009). To initiate this process, a resistant male $BC_1$ plant (line 54) from a *C. papaya* × *V. quercifolia* cross was developed (Drew et al. 2005b). Unfortunately, although micropropagated clones of this line were resistant in Australia, the clones inoculated with a local PRSV-P strain in the Philippines succumbed to disease (Drew et al. 2005b). In a second attempt, a number of inbred lines and $F_1$ hybrids were crossed, and a $BC_1$ line found to be resistant after 12 months in a field in Los Baños was selected (Siar et al. 2009). Advanced backcrossing was conducted with this line, and the resulting plants were found to have only mild or delayed virus symptoms with little or no disease progression (Siar et al. 2009). This major accomplishment represents the first successful transfer of disease resistance from a wild relative to papaya and establishes a precedent for developing resistance to other diseases.

Significant levels of partial resistance to PRSV-P (Siar et al. 2009) and other pathogens may be improved by combining sources of resistance from the multiple *Vasconcellea* spp. One way to circumvent compatibility barriers and achieve this goal is by using a bridge species. *V. parviflora* is closely related to *C. papaya* and may be used for this purpose (Jobin-Décor et al. 1997). Resistance genes from more distantly related incompatible *Vasconcellea* spp. might be introgressed into *V. parviflora* and then into *C. papaya* (O'Brien and Drew 2010). In addition, to reducing the cost and variability associated with manual disease screening, molecular markers can be used to track the movement of *Vasconcellea* spp. resistance genes through breeding schemes; see Chap. 19 in this text for details of this process. Using an $F_2$ *V. cundinamarcensis* × *V. parviflora* mapping population, the PRSV-P resistance of *V. cundinamarcensis* was identified as being regulated by a single, dominant gene (*prsv-1*) (Dillon et al. 2005a). Using this population, a codominant marker (*PsiIk4*) linked to *prsv-1* was developed that can now be used to move resistance from *V. cundinamarcensis* and *V. pubescens* to papaya (Dillon et al. 2005b, 2006a; Drew et al. 2007; O'Brien and Drew 2010). Interestingly, the *PsiIk4* marker is not linked to *V. quercifolia* resistance, suggesting that separate gene(s) regulates this trait (Dillon et al. 2006b). This is encouraging because, if multiple sources of PRSV-P resistance exist, opportunities will exist for achieving more durable resistance by gene pyramiding. Although achieving resistance through interspecific and intergeneric hybridization requires years of work, it avoids the regulatory obstacles associated with transgenic approaches.

In addition to PRSV-P resistance, the diverse *Vasconcellea* species offer resistance for other pathogens as well. Black rot spore inoculation of green and ripened fruit in the field demonstrated that *V. goudotiana* has some resistance to *Mycosphaerella caricae* (Sanchez et al. 1991). *V. monoica*, *V. goudotiana*, and *V. cauliflora* are cited as being resistant to *Cercospora papayae* (black spot), while *V. quercifolia* is noted as being resistant to *Ascochyta caricae-papayae* (Ascochyta leaf spot). After conducting pathogenicity tests, Nishijima and Aragaki (1973) found a low incidence of collar rot

(*Calonectria ilicicola*) on *V. goudotiana*. As for "Babaco's" previously mentioned susceptibility to *Fusarium oxysporum*, *V. weberbaueri* and *V. monoica* offer a source of potential resistance (Scheldeman et al. 2003). *V. goudotiana* may be a possible source of *Phytophthora* resistance (Drew et al. 1998). In preliminary experiments, *V. goudotiana* exhibited rate-limiting resistance, characterized by mild symptoms associated with *P. palmivora* infection that was later outgrown (Zhu and Porter, unpublished data). This reaction is similar to that afforded by the nucleotide binding site-leucine-rich repeat (NBS-LRR) resistance gene, *RB*, isolated from wild potato (*Solanum bulbocastanum*) (Song et al. 2003). Additional studies must be conducted to further characterize this response. Finally, *V. cauliflora* may have another source of *Phytophthora* resistance (Erwin and Ribeiro 1996; Zentmyer and Mitchell 1985/1986).

High-throughput next-generation sequencing offers a means to survey transcriptomes for genes regulating this resistance, while microarray technology can monitor expression changes. We emphasize that because *Vasconcellea* spp. are an invaluable sources of diversity for papaya, it is of utmost concern that 5 of the 21 species are considered threatened (Scheldeman et al. 2007). Others suggest the number of threatened *Vasconcellea* spp. might be even higher due to the rate of deforestation, especially in the species-rich "hybrid zones" that exhibit high morphological variability (Kyndt and Gheysen 2007).

## Transgenic Resistance in *C. papaya*

### *Coat Protein-Mediated Resistance to Virus*

In papaya, coat protein-mediated resistance (CP-MR) has been remarkably effective; reviewed by Gonsalves 1998; see also Chap. 7 in this text. Preceding the development of this technology, one strategy for virus control involved exposing plants to a mild or "weaker" virus strain to achieve "cross protection" (Yeh et al. 1988). The exact mechanisms of cross protection are still being revealed (reviewed by Ziebell and Carr 2010), but the added labor costs, risk of mild symptom development, and risk of virus reversion to a more virulent strain led to low adoption rates (Gonsalves 1998). At the time, Sanford and Johnston (1985) proposed an alternative strategy, that if host cells themselves were engineered to produce key pathogen gene products, either in excess or in a dysfunctional form, pathogenicity could be disrupted. The laboratory of Dr. Roger Beachy validated this hypothesis in plants by expressing a tobacco mosaic virus coat protein gene in tobacco, resulting in delayed disease development and resistance (Abel et al. 1986). Like cross protection, the exact mechanism of CP-MR was unknown at the time, but the results were encouraging enough to justify evaluation of the strategy in papaya for controlling PRSV-P. Ultimately CP-MR was shown to be highly successful in papaya (discussed in Chap. 7 in this text), and, conceivably, such an analogous strategy could be applied

to control other diseases. However, before expanding the application of this approach, there are several lessons that can be learned from papaya CP-MR that must first be considered.

Of the two cultivars developed, SunUp, which is homozygous for the transgene, was found to be more resistant than Rainbow, an $F_1$ hybrid from SunUp which is hemizygous for the PRSV-P coat protein gene (Tennant et al. 2001; see Chap. 6 in this text for a historical discussion). The mechanism of resistance was discovered to be RNA-mediated homology-dependent posttranscriptional gene silencing (PTGS), which targets the virus in a dose-dependent manner (Tennant et al. 2001; Baulcombe 1996). While CP-MR has worked well for the virus strain in Hawaii, sequence divergence of the CP gene among p-type viruses was found to be as high as 12 % (Gonsalves 1998) so the same construct may not provide the same level of protection against other PRSV strains. When challenged with a virus isolate from Thailand having only 89.5 % homology to the Hawaii strain, "SunUp" resistance broke down (Tennant et al. 2001).

In addition to the problems associated with CP sequence divergence, potyvirus helper component-proteinase (HC-Pro) also contributes to the suppression of PTGS, providing another mechanism for resistance breakdown (Mangrauthia et al. 2010). Fortunately, PTGS is only one of many mechanisms of CP-MR. Expression of tobacco mosaic virus CP prevents the virus from uncoating and regulates viral movement protein production (Register and Beachy 1988; Ling et al. 1991; Bendahmane et al. 2002; Asurmendi et al. 2004). The CP-MR of potato virus X is not significantly dependent on PTGS (Bazzini et al. 2006). Therefore, a better understanding of the many control mechanisms will allow the design of multimodal virus protection constructs in the future. Meanwhile, constructs that target local strains and multiple virus types are providing resistance. Using the sequence of local PRSV isolates, CP-MR has been deployed in a number of countries including Jamaica, Venezuela, and Brazil (Tennant et al. 2005; Fermin et al. 2004; Júnior et al. 2005). In Taiwan, papaya lines have been developed with double resistance to PRSV and papaya leaf distortion mosaic virus (Kung et al. 2009; Kung et al. 2010).

In Hawaii, CP-MR currently targets a relatively homogeneous PRSV population (Tripathi et al. 2006, 2008). If a viral strain emerges that breaks down this resistance, additional transformation, perhaps combined with what has already been used, may be needed. As coevolution between transgenic systems and viruses occurs, resources such as selectable markers will need to be managed, especially when combining multiple constructs into the same plant line.

Transgene insertions occurred in three locations in the "SunUp" genome (Ming et al. 2008). If all three insertions contribute to resistance, this may allow for loss of function of some copies over time. In addition, gene divergence may occur, including alterations in promoter regions that could result in changes in gene regulation. These scenarios are interesting to consider from a plant–pathogen evolutionary standpoint and may be more plausible than expected considering the worldwide distribution of the technology.

### *Stilbene Synthase*

For the control of root rot, transgenic expression of the grapevine stilbene synthase (a resveratrol synthase) gene (*Vst1*) was evaluated in papaya (Zhu et al. 2004). Using the native grapevine pathogen-inducible promoter, transgenic lines produced the phytoalexin resveratrol and displayed increased resistance (Zhu et al. 2004). However, these plants failed to set fruit (unpublished data). Similar deleterious effects associated with excessive stilbene production have been previously reported, including abnormal pollen development, parthenocarpy, and male sterility (Ingrosso et al. 2011; Fischer et al. 1997). These effects are the result of resveratrol synthase competing for the same substrates as chalcone synthase, 4-coumaroyl CoA, and malonyl CoA (Fischer et al. 1997). Chalcone synthase requires these precursors to synthesize the scaffold required for the production of all flavonoids (Ferrer et al. 2008). In addition, these substrates are required in other pathways for the production of structural compounds, including lignin and sporopollenin (Ingrosso et al. 2011). Because of overproduction or mislocalization of resveratrol, synthase has the potential to impact other pathways. Genes encoding these enzymes are frequently pathogen/stress inducible and regulated in specific tissues. The stilbene synthase gene of sorghum, *SbSTS1,* is induced by host and nonhost pathogens (Yu et al. 2005). In grapevine, stilbene synthase is found in infected cells and in the exocarp of the berry where infection is likely to occur (Schnee et al. 2008; Fornara et al. 2008). Successful heterologous production of resveratrol synthase in papaya, therefore, will in part require tissue-specific, pathogen-inducible promoters. Expression characterizations of a number of papaya genes with promoters fitting these criteria have been identified (Porter et al. 2008, 2009b).

Heterologous expression of grapevine stilbene synthase for the control of *Botrytis cinerea* infection of tobacco was first demonstrated more than 18 years ago (Hain et al. 1993). Since then, the strategy has been evaluated in a number of crops, including tomato, for the control of *Phytophthora infestans* (Thomzik et al. 1997), rice for the control of *Pyricularia oryzae* (Stark-Lorenzen et al. 1997), wheat and barley for a number of fungal pathogens (Leckband and Lörz 1998; Serazetdinova et al. 2005), alfalfa for the control of *Phoma medicaginis* (Hipskind and Paiva 2000), and other plant species (reviewed by Delaunois et al. 2009). However, to date, no crops transformed with stilbene synthase have received regulatory approval (CERA 2010). This may be due to the fact that the current state of the technology has only achieved partial resistance and failed to prove effective in the field. Transformation of tomato with grapevine stilbene synthase resulted in a range of disease reduction for *P. infestans* (between 38 and 68 %) but provided no significant control of *B. cinerea* and *Alternaria solani* (Thomzik et al. 1997). Control of *P. medicaginis* in alfalfa transformed with a cDNA encoding resveratrol synthase was demonstrated using leaf inoculations (Hipskind and Paiva 2000) but will require larger trials to determine production-scale disease control. Disease symptoms of wheat transformed with stilbene synthase following inoculation with *Puccinia recondita* f. sp. *tritici* were reduced by 19 ± 9 % to 27 ± 8 % (Serazetdinova et al. 2005). Finally, in papaya

expressing *Vst1*, 50 % of transgenic plants remained healthy following inoculation with *P. palmivora*, while 25 % of the untransformed controls remained healthy (Zhu et al. 2004).

Looking to the future, the use of stilbene synthases to control fungal and oomycete pathogens holds promise. Resveratrol synthase generates the backbone molecule, resveratrol, from which its derivatives, piceid, viniferins, and pterostilbene, are derived. Pterostilbene, a dimethylated derivative of resveratrol, was found to have threefold the activity of resveratrol and rapidly destroys the plasma membrane of *B. cinerea* (Adrian et al. 1997; Pezet and Pont 1990). Recently, a gene encoding a pathogen-regulated resveratrol O-methyltransferase (ROMT) for pterostilbene biosynthesis was isolated from grapevine (Schmidlin et al. 2008). Therefore, the use of *ROMT* in combination with resveratrol synthase is suggested to be a more effective strategy (as described next).

Genes encoding stilbene synthases are thought to have evolved independently from chalcone synthases in a diverse but relatively small number of plant species (Tropf et al. 1994; Austin and Noel 2003). Examples include peanut (Schöppner and Kindl 1984), pine (Schanz et al. 1992), grapevine (Sparvoli et al. 1994), whisk fern (Yamazaki et al. 2001), *Rheum tataricum* (Samappito et al. 2003), sorghum (Yu et al. 2005), *Polygonum cuspidatum* (Liu et al. 2011), and spruce (Hammerbacher et al. 2011). In the majority of cases, these genes are pathogen-inducible (Preisig-Müller et al. 1999; Yu et al. 2005; Hammerbacher et al. 2011). Regulation of this pathway, however, does not end at the production of resveratrol (or pinosylvin) backbone molecules. In *V. vinifera* and *Arachis hypogaea*, differential accumulation of resveratrol derivatives between genotypes demonstrates that regulation of enzymatic modifications, such as glycosylation, oxidation, and methylation (in the case of ROMT), is critical for effective defense responses (Pezet et al. 2004; Sobolev et al. 2007; Schmidlin et al. 2008). A transgenic approach involving multiple genes will likely be required to maximize disease resistance from stilbenes. This approach has begun to be evaluated in tobacco and *Arabidopsis* through the co-expression of genes for O-methyltransferase and stilbene synthase (Rimando et al. 2012). For the control of *P. palmivora* of papaya, an attractive model for evaluating early-stage multigene regulation of stilbenes might be *Arabidopsis* and *Hyaloperonospora arabidopsidis*, an oomycete pathogen (Chou et al. 2011).

## Dahlia merckii *Antimicrobial Peptide 1 (Dm-AMP1)*

First recognized in mammalian granulocytes, defensins are small, cysteine-rich, amphipathic peptides that permeabilize pathogen membranes, particularly those of fungi (Zeya and Spitznagel 1963; reviewed by Ganz 2003). Similar peptides have been identified in invertebrates, plants, and fungi, suggesting these ubiquitous components of innate immunity likely evolved from a common, ancient progenitor (reviewed by Wilmes et al. 2011; Zhu 2007). The defensin, *D. merckii* antimicrobial peptide 1 (Dm-AMP1), was first isolated from *D. merckii* (bedding dahlia) seed

(Osborn et al. 1995). Bioassays conducted using this defensin inhibited germ tube elongation rate, reduced hyphal thickness, and destroyed the cytoplasm of some fungi and inhibited the growth of *Bacillus subtilis* (Osborn et al. 1995). Interestingly, the binding of radioactively labeled Dm-AMP1 to *Neurospora crassa* and *Saccharomyces cerevisiae* cells can be blocked by preincubation with "cold" Dm-AMP1, but not by unrelated defensins (Thevissen et al. 2000a). This suggested that Dm-AMP1 binds a specific site on the target plasma membrane, a hypothesis supported by the identification of mutant *S. cerevisiae* that is resistant to Dm-AMP1 and demonstrates ten-fold less binding efficiency relative to wild type (Thevissen et al. 2000a). To determine the genetic basis of this loss of binding, a genomic library was constructed from susceptible, wild-type yeast and used to transform resistant mutants (Thevissen et al. 2000b). A clone encoding an enzyme that catalyzes the formation of sphingolipids (terminal sphingolipid mannosyldiinositolphosphophoceramide) was able to restore susceptibility in the mutants, suggesting this plasma membrane component is the Dm-AMP1 binding site (Thevissen et al. 2000b). This was confirmed using an enzyme-linked immunosorbent assay (ELISA), which demonstrated that Dm-AMP1 directly interacts with sphingolipids (Thevissen et al. 2003).

Constitutive expression of *Dm-AMP1* in papaya provided resistance to *P. palmivora* (Zhu et al. 2007). Leaf protein extract containing Dm-AMP1 inhibited hyphae growth by 35–50 %, and inoculated leaf discs from transformed plants had 40–50 % less infected area than controls (Zhu et al. 2007). The disease ratings of papaya plants expressing *Dm-AMP1* were significantly less than that of controls following root-drench inoculation (Zhu et al. 2007). Similarly, *Dm-AMP1* expressed in rice significantly suppressed the growth of *Magnaporthe oryzae* and *Rhizoctonia solani* (Jha et al. 2009). In *Solanum melongena*, Dm-AMP1 inhibited *Botrytis cinerea* in leaves, and root exudates containing the protein reduced the growth of *Verticillium albo-atrum* (Turrini et al. 2004a). Field trials will need to be conducted to evaluate the efficacy of *Dm-AMP1* in larger-scale production, with particular attention paid to gene durability.

Previously, plant defensins were evaluated in other crop–pathogen systems (Terras et al. 1995). Expression of a pea defensin (*DRR230*) in Canola targeted *Leptosphaeria maculans* (Wang et al. 1999). Monsanto Company successfully demonstrated the use of an alfalfa defensin (*alfAFP*) in potato for the control of *Verticillium dahliae* (Gao et al. 2000). Although *alfAFP* proved particularly effective in controlling *V. dahliae*, Monsanto's potato biotechnology program was halted in 2001 due to lack of market support (Gao et al. 2000; Kilman 2001). Nevertheless, as Dm-AMP1 and other defensins progress toward production-scale applications, strategies to promote durability should be prioritized. These peptides play a key role in innate immunity. *S. cerevisiae* mutants were resistant to Dm-AMP1 (Thevissen et al. 2000a), so defensin vulnerability to pathogen mutation under high selection pressure could undermine endogenous resistance in papaya and other species. For long-term durability, simply expressing defensins constitutively at high levels may be found to be too simplistic an approach.

Natural expression is more complex. The radish defensin genes *Rs-AFP3* and *Rs-AFP4*, for example, are pathogen-inducible in leaves, while Rs-AFP1 and

Rs-AFP2 accumulate in specific cell layers of the seed and are released during germination (Terras et al. 1995). Similarly, *PDF1.2*, an *Arabidopsis* pathogen-inducible defensin gene, is regulated by a jasmonate-dependent/salicyclic acid-independent pathway (Penninckx et al. 1996; Thomma et al. 1998). This suggests that, in nature, defensins are highly regulated and that avoiding resistance breakdown may require regulated expression and/or more complex multigene strategies.

## Manduca sexta *Chitinase*

Chitin is an abundant biological polymer found in many organisms including fungi, arthropods, and crustaceans. Modification and destruction of this structural polysaccharide occurs in part by hydrolysis of its glycosidic bonds, catalyzed by chitinases. Chitinases are near ubiquitous in nature, occurring in organisms with and without endogenous chitin, including mammals, amphibians, arthropods, nematodes, fungi, bacteria, and baculoviruses. In organisms with chitin, chitinases are generally used for developmental purposes, whereas other organisms have evolved chitinases for defense or pathogenicity. Chitotriosidase, for example, is a human chitinase secreted from phagocytes as part of the immune system for the degradation of chitin-containing pathogens (Boot et al. 2001). Examples of chitinases contributing to pathogenicity come from the malaria parasite and a baculovirus. *PfCHT1*, a gene from the human malaria parasite (*Plasmodium falciparum*), encodes a chitinase that contributes to disease transmission by allowing the pathogen to escape the midgut of mosquitoes (Vinet et al. 1999). Cathepsin (a cysteine protease) and chitinase A from the baculovirus AcMNPV act together in the liquefaction of insect hosts (Hawtin et al. 1997). Finally, in insects, chitinase activity is highly regulated in precise fashion for elaborate developmental processes such as molting. Recently, in the red flour beetle (*Tribolium castaneum*), it was shown that Knickkopf protein protects new cuticle formation from chitinase found in molting fluid (Chaudhari et al. 2011). Disruption of such processes can be deleterious. Downregulation of the gene encoding Knickkopf protein is lethal, making it a potential target for biocontrol (Chaudhari et al. 2011). Similarly, ectopic expression of chitinase in plants can be used as a control strategy as demonstrated by overexpression of *M. sexta* (tobacco hornworm) chitinase in tobacco for the control of tobacco budworm and hornworm (Ding et al. 1998).

*C. papaya* was transformed with *M. sexta* chitinase (MSCH) under the control of the constitutive (CaMV 35S) promoter (McCafferty et al. 2006). Ten weeks post-inoculation in the laboratory with carmine spider mites (*Tetranychus cinnabarinus* Boisd.), all transgenic lines had a significantly higher number of leaves relative to the susceptible donor cultivar "Kapoho" (McCafferty et al. 2006). However, only one transgenic line (T-24) had significantly fewer mites per leaf than the control. This most likely occurred as a result of the control having fewer leaves, forcing the mites to migrate to the transgenic plants (McCafferty et al. 2006). Conversely, in the field, all transgenic lines expressing *MSCH* had fewer mites than the control, which

suggests that when the mites have a choice, they prefer to avoid chitinase-expressing lines (McCafferty et al. 2006). These results are particularly encouraging. Rather than functioning as an insecticide, MSCH appears to deter feeding and encourage migration. Because *T. cinnabarinus* has a large host range, including many weed species (Goff 1986), movement of mites from transgenic plants to alternate hosts could, in theory, occur with minimal selection pressure, effectively promoting *MSCH* durability.

Recently, corn plants engineered to express the insecticidal *Bacillus thuringiensis* (Bt) toxin Cry3Bb1 for the control of western corn rootworm (*Diabrotica virgifera virgifera*) were found to be susceptible in some fields in Iowa, illustrating the consequences of high selection pressure (Gassmann et al. 2011). Plants expressing Cry34/35Ab1 were found to be resistant to the problem rootworm, but pathogen resistance could emerge for this line as well (Gassmann et al. 2011). A combination of resistance sources combining Cry3Bb1 and Cry34/35Ab1 (SmartStax) may delay the evolution of pathogen resistance (Gassmann et al. 2011; EPA 2009). Strategies such as combining genes for chitinase and scorpion toxin, which have been determined to cause high larvae mortality, should be evaluated to determine if this selective combination is durable (Wang et al. 2005). Finally, the environmental impact of transformations using chitinase genes should be considered. In papaya, confirmation is needed to ensure that pollinating insects are unaffected by *MSCH*. In addition, papaya expressing *MSCH* should be evaluated for resistance to fruit flies and mites other than *T. cinnabarinus*. Although aphids do not colonize papaya, they transmit PRSV-P to papaya in a nonpersistent manner by conducting exploratory probes (Pantoja et al. 2002; Kalleshwaraswamy and Kumar 2008). The possible influence that MSCH may have on this behavior should be explored as well.

## Papaya Mutualistic and Protective Endophytes

To ensure that beneficial microbes are not affected by transgenic modifications for disease resistance, it is sometimes necessary to survey and select for lines that maintain compatibility with mutualistic endophytes. Up to 90 % of terrestrial plants form mycorrhizal-root associations (Fitter and Moyerson 1996), but some, including papaya, are considered highly dependent upon arbuscular mycorrhizal fungi (AMF) for inorganic phosphorus ($P_i$) uptake (Miyasaka and Habte 2001). In addition, some endophytes also provide protection against insects, nematodes, and other pathogens (Vega et al. 2008; Jaizme-Vega et al. 2006; Stein et al. 2008).

There are instances of transgenes affecting AMF. Tobacco constitutively expressing a pathogenesis-related protein (PR-2) delayed *Glomus mosseae* colonization, whereas *G. mosseae* was resistant to constitutive chitinase expression in tobacco and *Nicotiana sylvestris* (Vierheilig et al. 1993, 1995). Because defensins can inhibit a range of fungi (Osborn et al. 1995), transformations using genes such as *Dm-AMP1* might inhibit endophytes. Fortunately, Dm-AMP1's inhibition of pathogen growth has been shown to spare some beneficial mycorrhizae. *Solanum melongena*

transformed with *Dm-AMP1* inhibited the pathogenic fungi, *Botrytis cinerea* and *Verticillium albo-atrum,* while the arbuscular mycorrhizal fungus *G. mosseae* was able to established host recognition, initiate symbiosis, and promote host plant growth (Turrini et al. 2004a, b). Examination of the possible effects of Dm-AMP1 on other beneficial, nontarget microorganisms in other host systems, such as papaya and rice (Zhu et al. 2007; Jha et al. 2009), will determine if the observed AMF resistance is an exception or trend. In papaya, *G. mosseae* not only contributes significantly to plant phosphorus uptake but, along with *G. manihotis*, significantly reduces the reproduction of the parasitic nematode *Meloidogyne incognita* (Jaizme-Vega et al. 2006; Rodriguez-Romero et al. 2011). A comprehensive study of these AMF in papaya expressing *Dm-AMP1* will require phosphorus evaluations and nematode bioassays. In maize, one line (*Bt* 176) with high expression of *CryIAb* toxin negatively affected *G. mosseae* pre-symbiotic hyphal growth and appressoria development, but another line, *Bt* 11, was indistinguishable from the non-transgenic control (Turrini et al. 2004b).

This suggests that selection for AMF-compatible lines is possible. The next step is the development of more rapid high-throughput monitoring. Arnold et al. (2000) used plating techniques to isolate endophytes representing 347 genetically distinct taxa from the leaves of two tropical tree species, *Heisteria concinna* (Olacaceae) and *Ouratea lucens* (Ochnaceae). Screening techniques have been developed for evaluating the impact of transgenes on AMF (Turrini et al. 2004b), but a comprehensive DNA-based screen that captures difficult-to-culture microorganisms may be needed (Mlot 2004). While the elimination of endophytes is one concern, another possible consequence of transgene selection pressure is conversion of endophytes from mutualists to pathogens. Mutation of a single NADPH oxidase gene was shown to disrupt reactive oxygen species (ROS) production in the endophyte *Epichloë festucae*, causing the death of its host *Lolium perenne* (perennial ryegrass) (Tanaka et al. 2006). Conversely, Freeman and Rodriguez (1993) used UV mutagenesis to demonstrate conversion of the pathogen *Colletotrichum magna* into a protective endophyte (Freeman and Rodriguez 1992; Freeman and Rodriguez 1993; Redman et al. 1999). In the future, transgenic strategies designed to promote mutualistic and protective endophytes in papaya may enhance yield and pathogen resistance. One particularly attractive candidate for this application is *Piriformospora indica*. An AMF isolated from woody shrubs from Rajasthan's Thar Desert, *P. indica*, has been associated with disease resistance and higher yield (Verma et al. 1998; Verma and Sharma 1999; Waller et al. 2005; Shahollari et al. 2007; Stein et al. 2008).

## The Nucleotide Binding Site-Leucine-Rich Repeat (NBS-LRR) Gene Family and *P. palmivora* Resistance

*Solanum* spp. and *P. infestans* provide an analogous host–pathogen system for guiding the development of *P. palmivora* resistance in papaya. Wild potatoes and *Vasconcellea* spp. both occur in the tropical highlands at average altitudes of

~1,500 m and ~2,800 m, respectively, with overlapping geographical regions of species richness (Hijmans and Spooner 2001; Scheldeman et al. 2007). The diversity of *Solanum* species is highest in Mexico, Peru, Bolivia, and Argentina, while the maximum diversity of *Vasconcellea* spp. is found in Ecuador, Colombia, and Peru (Hijmans and Spooner 2001; Scheldeman et al. 2007). To date, 21 *P. infestans* resistance genes have been cloned from *Solanum* spp., reflecting extensive coevolution with a pathogen that shares a center of origin in the central highlands of Mexico (Vleeshouwers et al. 2011; Grünwald and Flier 2005). Eighteen of these genes originate from species found in Mexico, and four originate from species from Argentina (Vleeshouwers et al. 2011).

The story of *P. palmivora* is somewhat more complex in that the duration of its coevolution with papaya's wild relatives (i.e., *Vasconcellea* spp.) is uncertain. It has been suggested that *P. palmivora* originated from Central or South America (Zentmyer 1988), but the diversity of isolates identified from coconut (*Cocos nucifera*), durian (*Durio zibethinus*), and other Southeast Asia hosts points instead to a Southeast Asia center of origin (Mchau and Coffey 1994). If this is true, *P. palmivora* may have only recently spread from Asia, and the evolution of *Vasconcellea* spp. resistance gene(s) specificity may be the result of more modern selection pressure. Nevertheless, what appears to be a rate-reducing form of resistance similar to that of *Rpi-blb1* (*RB*) (Song et al. 2003; van der Vossen et al. 2003) has been identified in *V. goudotiana* (Zhu and Porter, unpublished data). *Rpi-blb1* is generally considered a broad-spectrum, durable source of resistance, and it would be encouraging to find similar resistance for papaya. The only exceptions are two *P. infestans* isolates from Mexico (PIC99189 and PIC99177) lacking an effector variant (class I *ipiO*) that were recently determined to be virulent in the presence of *Rpi-blb1* (Champouret et al. 2009). Although resistance can break down and *P. infestans* has the reputation of being an "R gene destroyer" (Fry 2008), the *P. palmivora* resistance observed in *V. goudotiana* is worth exploring and if isolated, perhaps combined with known sources of partial resistance (Noorda-Nguyen et al. 2010; Dianese et al. 2007, 2010).

All *P. infestans* resistance genes cloned to date belong to the nucleotide binding site-leucine-rich repeat (NBS-LRR) gene family (Vleeshouwers et al. 2011). Possibly, the *P. palmivora* resistance genes observed in *V. goudotiana* and some papaya genotypes (Zhu and Porter, unpublished data; Noorda-Nguyen et al. 2010; Dianese et al. 2007, 2010) are also members of this family. From the draft genome of *C. papaya*, 54 NBS class resistance genes have been identified (Ming et al. 2008; Porter et al. 2009a). This is substantially fewer than the number found in other plant genomes (Table 15.3), including *Arabidopsis*, which has 174 NBS genes (*Arabidopsis* Genome Initiative 2000).

While few in number, papaya's NBS genes represent both Toll/interleukin-1 receptor (TIR) and non-TIR subclasses found as clusters and single genes throughout the genome (Fig. 15.1) (Porter et al. 2009a). Unlike *Arabidopsis*, whole genome duplication has not occurred in the papaya lineage since its divergence from *Arabidopsis* (Ming et al. 2008; Sémon and Wolfe 2007; see also discussion in Chap. 11 of this text). The lack of genome duplication may partially explain the

**Table 15.3** The total number of predicted NBS-encoding genes identified in five sequenced angiosperm genomes

| Species | Total number of predicted protein-encoding genes | Total number of predicted NBS-encoding genes | Genome size (Mb) | Source |
|---|---|---|---|---|
| *Carica papaya* | 24,746 | 54 | 372 | Ming et al. (2008) |
| *Arabidopsis thaliana* | 25,498 | 174 | 125 | Arabidopsis Genome Initiative (2000) |
| *Vitis vinifera* | 30,434 | 535 | 487 | Jaillon et al. (2007) |
| *Oryza sativa* | 37,544 | 519 | 389 | International Rice Genome Sequencing Project (2005) |
| *Populus trichocarpa* | 45,555 | 416 | 485 | Tuskan et al. (2006) |

Values for *Arabidopsis thaliana*, *Vitis vinifera*, *Oryza sativa*, and *Populus trichocarpa* were previously summarized by Yang et al. (2008). The total number of predicted protein-encoding genes and the genome size of each species are also provided (see source column for references)

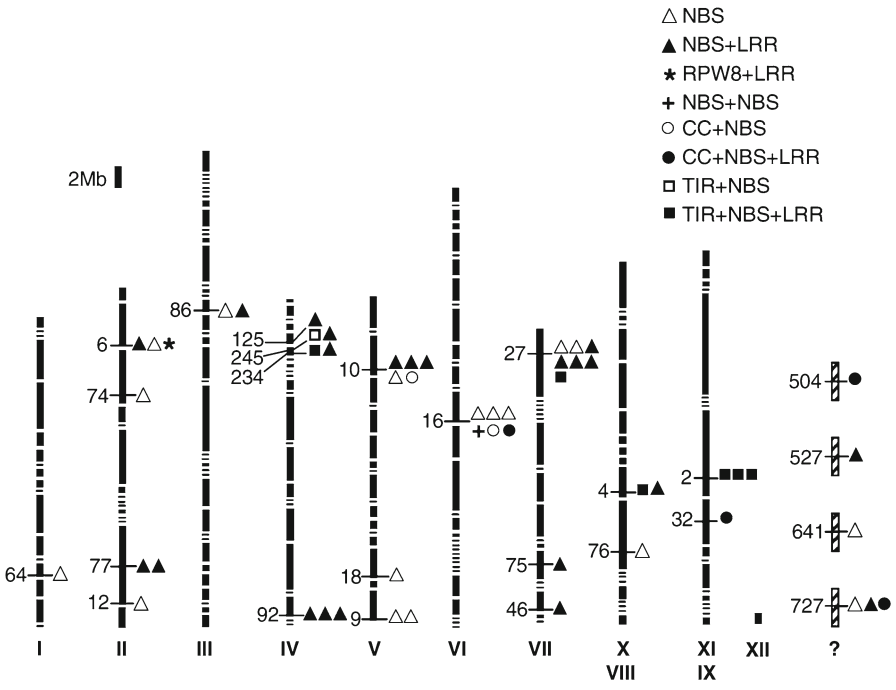


**Fig. 15.1** Distribution of predicted *Carica papaya* NBS-encoding genes across linkage groups. The papaya genome sequence was anchored to the 12 papaya linkage groups as described by Ming et al. (2008) [reproduced with kind permission of Springer Science+Business Media from Porter et al. (2009a)]

scarcity of NBS-LRR genes, but it is also possible that papaya preferentially relies upon surveillance, or "guarding," of common effector targets to detect large numbers of pathogens with relatively few NBS genes (van der Biezen and Jones 1998; Dangl and Jones 2001; DeYoung and Innes 2006; McDowell and Simon 2006). Papaya also has a lower total number of genes than other sequenced angiosperms (Ming et al. 2008; *Arabidopsis* Genome Initiative 2000; Jaillon et al. 2007; International Rice Genome Sequencing Project 2005; Tuskan et al. 2006), so it is possible that fewer NBS genes are required for surveillance (Porter et al. 2009a).

Finally, some NBS genes may reside in the limited portion of the genome lacking sequence coverage, but because 90 % of the euchromatic chromosomal regions have been sequenced, finding a significant number of additional NBS genes is considered unlikely (Ming et al. 2008; Porter et al. 2009a). Now that papaya's NBS genes have been mapped (Fig. 15.1), susceptible and partially resistant cultivars (Noorda-Nguyen et al. 2010; Dianese et al. 2007, 2010) may be compared using targeted sequencing techniques (reviewed by Mamanova et al. 2010).

In the future, native R-genes may be ineffective for the control of *P. palmivora*, but a better understanding of the molecular basis of *Phytophthora* pathogenicity may provide opportunities to modify NBS genes or effector targets to achieve resistance. A first step in this process is determining pathogen host recognition and translocation of effectors from *Phytophthora* haustoria into the plant cell. Two N-terminal-conserved motifs identified in *P. infestans* effectors are RXLR and EER, which serve as a host cell uptake (penetration) signal. In *P. infestans*, 425 genes of this protein class have been identified (Birch et al. 2006; Whisson et al. 2007). The exact mechanism of effector entry is uncertain, and recent studies have reached contradictory conclusions (Ellis and Dodds 2011). Kale et al. (2010) suggest that phospholipid, phosphatidylinositol-3-phosphate (PI3P), found on the surface of plant cell plasma membranes mediates effector entry. Yaeno et al. (2011) suggest that PIP binding contributes intracellularly, promoting effector stabilization, accumulation, and virulence function. Resolving the exact mechanism of this process is important as it may lead to upstream resistance strategies to block effector entry. The virulence functions of *P. infestans* effectors are beginning to be revealed. AVR3a, for example, has been shown to act upstream at the plasma membrane by inhibiting the host ubiquitin E3-ligase, CMPG1, required for plant immunity (Bos et al. 2010; Gilroy et al. 2011). Interestingly, in papaya, *P. palmivora* infection is associated with reduction of a transcript encoding a putative aquaporin (Porter et al. 2009a, b). Similar aquaporin repression has been reported in other plant systems, including cotton following *Fusarium oxysporum* f. sp. *vasinfectum* inoculation (Dowd et al. 2004). Aquaporins play a role in hydraulic permeability and have been shown to be targets of bacterial effector regulation in animal disease (Guttman et al. 2007). Further investigation will be required to determine if *P. palmivora* effectors regulate papaya aquaporins, either directly or indirectly. Regardless, once effector targets are determined, they may be modified for resistance.

## Emerging Diseases of Papaya

Adaptation and evolution increase pathogen diversity, a process that often begins with the spread of disease into new environments. The first reports of disease in papaya (Table 15.2) suggest that this phenomenon is active. In 2001, for example, black spot disease of papaya [*A. caricae* (Speg.) Maulbl.] was discovered on the island of Maui and subsequently on other Hawaiian Islands (Ogata and Heu 2001). Outbreaks of black spot now require the application of costly fungicides. Early detection of emerging diseases can provide an opportunity to implement cultural practices to help delay the spread of disease until tolerant cultivars are obtained for production. Maintaining genetic diversity in the field will hedge against losses and slow disease spread. Expanding niche markets, such as those that utilize larger-fruited papaya, is one example of how diversification may be achieved. The management of alternate hosts, including weeds, provides another means to mitigate and monitor pathogen movement (Chin et al. 2007). Genetic characterization of pathogen diversity can also be used to predict the likelihood of disease outbreaks (Gibb et al. 1998; Maoka and Hataya 2005). Ultimately, however, an understanding of the molecular basis of host–pathogen interaction will be needed to allow for resistance to be engineered or selected for.

Recently, a proteinase (NIaPro) of the virus nuclear inclusion body was shown to regulate PRSV host specificity (Chen et al. 2008), which offers insight for the possible disruption of host recognition. Separately, PRSV helper component-proteinase (HC-Pro) was found to interact with papaya calreticulin, suggesting the involvement of calcium signaling in infection or defense (Shen et al. 2010). This and other host–pathogen interactions may be regulated for creating resistance.

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