Review Article

The Venturia Apple Pathosystem: Pathogenicity Mechanisms and Plant Defense Responses

Gopaljee Jha, Karnika Thakur, and Priyanka Thakur

Biotechnology Division, Institute of Himalayan Bioresource Technology, Council of Scientific and Industrial Research, Palampur 176061, Himachal Pradesh, India

Correspondence should be addressed to Gopaljee Jha, jmsgopal@ihbt.res.in

Received 27 March 2009; Revised 10 August 2009; Accepted 29 October 2009

Recommended by Sudhir Kumar Sopory

Venturia inaequalis is the causal agent of apple scab, a devastating disease of apple. We outline several unique features of this pathogen which are useful for molecular genetics studies intended to understand plant-pathogen interactions. The pathogenicity mechanisms of the pathogen and overview of apple defense responses, monogenic and polygenic resistance, and their utilization in scab resistance breeding programs are also reviewed.

Copyright © 2009 Gopaljee Jha et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

1. Introduction

Apple scab also known as black spot, caused by Venturia inaequalis (Cke.) Wint. is one of the most serious diseases of apple reported from almost all apple producing countries and causes huge economic losses (up to 70% reduction in apple production) [1, 2]. Scab infection leads to deformation in shape and size of the fruits, premature leaf and fruit fall, and enhances susceptibility of tree to chilling and freezing injuries. The oldest available report of scab is in the year 1819 by a Swedish scientist, Fries, however, apples showing scab symptoms are depicted in paintings of sixteenth century, suggesting existence of the disease at that time [3]. The pathogen has been placed into genus Venturia by Winter in 1880. These historical accounts and its taxonomy have been reviewed by MacHardy [2]. V. inaequalis is a heterothallic fungus and contains seven haploid chromosomes [4]. Fusicladium dendriticum and Spilocaea pomi are its anamorphs. Alike other obligate parasites, it generally infects and lives in association with living tissues. However, an ability to be cultured on laboratory medium, possibility of in vitro mating, existence of extensive population diversity, uninucleate conidia, genetically uniform progenies, stability of genotype and phenotype of the progeny even after multiple rounds of subculturing, and availability of standardized protocol for genetic manipulation, and so forth, make it a useful model to study the pathogenesis of obligate fungal pathogens which are generally not culturable. The ease of isolation of ascospores produced by a single meiotic event and their presence in a specific order in which they are produced [4] are the useful features for segregation analysis, centromere mapping, and understanding the processes of fungal meiosis. Furthermore, V. inaequalis and Apple provide an interesting system to study the molecular interactions of a fungal pathogen and a woody host. During the course of co-evolution, V. inaequalis has accumulated various pathogenic attributes (genes) that play a crucial role in invasion without causing much damage to apple [3]. Similarly, apple has evolved mechanisms to prevent severity of the disease. In this review, we summarize the recent literature about the life cycle, pathogenicity mechanisms, nutritional requirements, and hypervariability amongst races of V. inaequalis. The apple defense/resistance mechanisms and their prospects in scab resistance breeding are also discussed.

1.1. V. inaequalis Life Cycle and Pathogenicity. V. inaequalis primarily causes disease on apple cultivars, however, it also infects Malus (Crabapple), Cotoneaster integerrima, Crataegus oxycantha (Hawthorn), Loquat, Pyracantha (Firethorn), Sarcocephalus esculentus, Sorbus (Mountain Ash), and Viburnum. The characteristic disease symptoms on apple include
circular olive green velvety, necrotic or chlorotic lesions single or scattered on leaf surface, olivaceous spots on infected sepalas and pedicels, and dark colored sharply bordered brown and corky lesions on young fruits while small black spots termed as “pin-point scab” on the matured fruits. The disease symptoms on the fruit and leaf are depicted in Figure 1. Light brown blisters surrounded by whitish rings constitute the symptoms on infected twigs. However, sometimes during severe infections peeling of bark from the twigs occurs which is known as grind or scurf. 

V. inaequalis produces sexual and asexual spores, both capable of infecting apple. The pathogenic phase of disease starts with germination of ascospores (sexual spore) which serve as primary source of inoculum. Conidia, the asexual spores, are smooth, 0-1 septate, obpyriform to obclavate in structure, pale to mid-olivaceous brown in color, generally disseminated by wind or splashing rain, and serve as source of secondary infections [2]. Several cycles of conidia production and secondary infections take place within a single growing season of apple. At the beginning of winter, with the onset of leaf fall, the mycelium penetrates a bit deeper into leaf tissues and switches from vegetative to reproductive phase wherein the mycelia of two different mating types undergo sexual reproduction (mating). Following mating, a pseudothecium is produced which exhibits negative geotropism [5]. The asci of V. inaequalis are bitunicate, cylindrical, double walled, and loculcus. The ascospore consists of two unequal sized cells having thin light brown phenotype associated with the mutant, it can be speculated that the mutant might still be producing melanin sufficient enough for successful pathogenesis. Application of mechanical pressure is not apparent during cuticle penetration [7]. It is hypothesized that the pathogen uses enzymatic hydrolysis to breach cuticle (Table 1). Extracellular cutinases are produced by germinating conidia and mycelium [11, 12]. The observations that treatment of a specific cutinase inhibitor could prevent subcuticular growth and penetration of cuticle by the pathogen further corroborate the role of cutinase in host entry [11]. Esterase-like activity has been reported transiently during germination of conidia, which possibly leads to softening of cutin making it easier for the pathogen to penetrate the cuticle [13]. After penetration, the infection hyphae get differentiated into primary hyphae which further grow to form subcuticular stroma giving rise to conidiophores which bear conidia that bulge out from the host cells by rupturing epidermis. The conidia and conidiophores, together, give a characteristic velvety appearance to the young lesions of scab. The conidia of Venturia are capable of adhesion and germination on nonhost plants such as Pyrus communis, however further development to establish infections occurs only on the host plants [14]. Kucheryava et al. [15] have observed that conidia can germinate, form appressorium and subcuticular hyphae-like structures when grown on a cellophane membrane placed over the PDA (Potato Dextrose Agar) plates, thus mimicking growth during infection process. The growth of Venturia on cellophane could be used to identify genes involved in fungal pathogenesis. By using this system, the authors could identify two genes (namely, Cin1 and Cin3, being cellophane induced) and further demonstrated that they are highly upregulated during apple infections. Studies are underway to establish the role of these genes during pathogenesis on apple.

Several mutants of V. inaequalis, defective in biochemical requirements isolated by UV or nitrogen mustard-mediated mutagenesis, demonstrated differential pathogenicity on apple. The mutants demonstrating reduced/non-pathogenicity (auxotrophs of arginine, choline, histidine, methionine, proline, purines, pyrimidines, and riboflavin) might be unable to obtain the particular biochemical from host, whereas the mutants capable of pathogenicity (auxotrophs of nicotinic acid, biotin, reduced sulfur, inositol, and pantothentic acid) could derive the nutrients from the host. Both the pathogenic and non-pathogenic biochemical mutants were capable of host penetration and disease establishment. However, the non pathogenic mutants exhibited very limited growth and were unable to sporulate with the choline mutant as an exception. The growth and sporulation deficiency of non pathogenic mutants (adenine mutant being exception) were restored if the particular biochemicals were supplied on the host surfaces.

The most enigmatic feature of V. inaequalis is that it forms subcuticular stroma without significant damage to host tissues. It is hypothesized to use cell wall-degrading enzymes (CWDEs) to breach plant cell wall to derive nutrients without forming haustorium. However, that fact that no obvious damage to the host tissues is observed until pathogen starts conidiating suggests a minor role of

---

**Table 1:** Enzymes Produced by V. inaequalis

| Enzyme          | Function                                      | Occurrence |
|-----------------|-----------------------------------------------|------------|
| Cutinase        | Enables penetration of cuticle                | Transient  |
| Esterase        | Softens cutin for easier penetration          | Transient  |
| CWDEs           | Breaks plant cell wall                        | Continuous |
Table 1: Factors predicted to govern pathogenicity and virulence of *V. inaequalis*.

| Locus/Gene          | Associated functions                                                                 | References |
|---------------------|--------------------------------------------------------------------------------------|------------|
| Avirulence factors  |                                                                                      |            |
| avrVf (avrRvi6)     | Avirulent on apple cultivars containing Vf (Rvi6)                                    | [16]       |
| avrVg (avrRvi1)     | Avirulent on apple cultivars containing Vg (Rvi1)                                    | [16, 17]   |
| avrVm (avrRvi5)     | Avirulent on apple cultivars containing Vm (Rvi5)                                    | [18]       |
| avrVh2 (avrRvi2)    | Avirulent on apple cultivars containing Vh2 (Vr2; Rvi2)                              | [19]       |
| avrVfh (avrRvi7)    | Avirulent on apple cultivars containing Vfh (Rvi7)                                   | [16]       |
| avrVh8 (avrRvi8)    | Avirulent on apple cultivars containing Vh8 (Rvi8)                                   | [20]       |
| avrVd (avrRvi13)    | Avirulent on apple cultivars containing Vd (Rvi13)                                  | [21]       |
| Cell Wall Degrading | Promote pathogen entry into the host and facilitates nutrients uptake                | [22, 23]   |
| enzymes (CWDEs)     |                                                                                      |            |
| Cellulase           |                                                                                      | [22]       |
| β-D-glucosidase     |                                                                                      | [22]       |
| Polygalacturonase   |                                                                                      | [22, 23]   |
| Cutinase            | Assists pathogen in cuticle penetration and sub-cuticular growth                      | [11, 22]   |
| Esterase            | Assists pathogen in cuticle penetration by softening cutin                            | [13]       |
| Melanoprotein       | Assists in slow release of CWDEs and diverting the solute/nutrient flow to the site of infections | [24]       |
| Cellophane induced  |                                                                                      |            |
| Cin1                | Induced during apple infections                                                      | [15]       |
| Cin3                | Induced during apple infections                                                      | [15]       |

**Figure 1:** Symptoms of Apple Scab disease. (a) The dark colored sharply bordered, brown, and corky lesions are apparent on the infected apple fruit. (b) Scattered, olivaceous green and velvety, chlorotic sporulating lesions are observed on the infected leaf.

These enzymes in nutrient uptake. The cellulolytic, cutinolytic, pectinolytic, and β-D-glucosidase activities have been detected from the culture supernatant ([2], Table 1). The endo-polygalacturonase (PG) and exo-PG like activities are reported during in vitro growth of the pathogen [22, 23].

It is speculated that the CWDEs of *Venturia* might be tightly attached with its cell wall and are released in a controlled manner to degrade the host cell wall to facilitate nutrient uptake. In other plant-pathosystems, the CWDEs serve as important virulence factors. However, their action in
Table 2: Physiological races of *V. inaequalis*.

| Races       | Pathological characteristics on apple cultivars                                      |
|-------------|--------------------------------------------------------------------------------------|
| Race 1      | Non sporulating lesion on Dolgo, R 12740-7A (a Russian cultivar) and Geneva           |
| Race 2      | Sporulating lesions on Dolgo, Geneva and some progenies of R 12740-7A                 |
| Race 3      | Sporulating lesions on Geneva, and non sporulating lesion on Dolgo, R 12740-7A       |
| Race 4      | Non sporulating lesion on Dolgo, Geneva and sporulating lesion on those progenies of R12740-7A on which race 2 isolates cannot sporulate |
| Race 5      | Sporulating lesions on *Vrn R* gene containing cultivars                              |
| Race 6      | Sporulating lesions on Vf hybrids but cannot infect *Malus floribunda* 821 containing Vfh R gene |
| Race 7      | Can infect cultivars having Vf and Vfh R gene but cannot infect Golden delicious which contains Vg gene |
| Race 8      | Can infect Golden delicious, Royal gala, and cultivars containing Vfh R gene         |

References: Races 1 to 5: MacHardy, 1996 [2], races 6 and 7: Bénouf and Parisi, 2000 [16], race 8: Bus et al., 2005 [20].

The variations in sequences of internal transcribed spacer (ITS) region of the ribosomal DNA of *V. inaequalis*, presence of group one intron in this region [29–32] and various molecular markers such as RAPD, PCR-RFLP [32, 33], AFLP [34, 35], and Microsatellites [32, 33, 35, 36] have been used to reveal genetic diversity amongst the isolates collected from different regions of the world. Gladieux et al. [35] using microsatellite markers have revealed maximum diversity amongst the isolates from Asia followed by Europe. The authors speculated that the pathogen might have originated in Asia and from there spread to Europe and recently to other apple growing countries. Hypervariability and evolution of strains that have overcome host resistance are attributed to the ability of *Venturia* to recombine its genetic material every year.

1.3. Apple Defense Responses. During the course of coevolution, apple has evolved mechanisms to prevent the severity of scab. The isolates of *V. inaequalis* provoke variable symptoms of these races are summarized in Table 2. However, some of the susceptible cultivars also demonstrate variable resistance against different regions of the world. Gladieux et al. [35] using different apple cultivars [2, 37, 38]. Based upon the extent of pathogen growth and nature of symptoms imparted by them on different apple cultivars, the responses are classified into class 0, 1, 2, 3a (syn. Class M), 3b (syn. Class3), and 4 (Table 3). The classes 0 to 3 are considered to be resistance responses while class 4 is a susceptible response. Several single monogenic loci capable of imparting scab resistance have been identified from wild cultivars of apple [2, 37, 39]. Interestingly, some of the susceptible cultivars also demonstrate variable resistance against isolates of similar pathogen [40]. The mature leaves of apple demonstrate ontogenic resistance because of which the pathogen growth is suppressed immediately after cuticle penetration and appearance of disease symptom is delayed [2]. The strengthened cell wall and cuticular membrane along with sub-cuticular pH of such leaves are speculated to play a role in governing such resistance. A breakdown of ontogenic resistance revealed by restored growth of the pathogen is observed in the old senescing leaves of apple. Detailed studies are needed to elucidate the functionality of such resistance and understand its breakdown mechanism.

Besides race specific and ontogenic resistance, apple employs diverse defense responses which provide resistance, albeit variable and suppress growth and spread of the pathogen. Ultrastructural studies have revealed that although conidia could germinate on apple leaves undergoing defense responses, formation of primary hyphae is delayed and growth of subcuticular stroma is suppressed resulting in reduced conidiation [2, 41]. Phenolics produced in response to *V. inaequalis* infections in apple are known to inhibit pathogen growth and are ascribed to be associated with defense mechanisms of scab resistant cultivars [2]. The inhibition of phenylalanine ammonia lyase (a key enzyme involved in the biosynthesis of phenolics) in scab resistant cultivar Sir Prize could render it susceptible to scab infections [42]. Phloridzin (a dihydrochalcone glycoside) produced by apple in response to *Venturia*, accumulated around the sites of infection, is postulated to get degraded into phloretin by pathogen and hinder its growth [2, 43]. Recently, efforts have been made to characterize genes involved in phloridzin biosynthesis in apple [44]. The treatment of yeast extract (a mimic of fungus infection) induces synthesis of six
new compounds in the cell suspensions of apple cultivar Liberty but not in McIntosh [43]. The chemical structures of several of these compounds have been identified and they are speculated to function as phytoalexins [43, 45]. One of these compounds, named Malusfuran (2,4-methoxy-3-hydroxy-9-O-β-D-glucosylxydibenzo furan) and its aglycone and dibenzo furan derivatives can suppress the germination and growth of conidia/mycelium of V. inaequalis.

Several pathogenicity related (PR) proteins including β-1,3-glucanase, chitinase, cysteine protease, osmotin-like protein, along with PRI1 and thaumatin-like protein are constitutively expressed in the apoplast of resistant apple cultivar Remo and are induced by V. inaequalis in the susceptible cultivar Elstar [46]. Degenhardt et al. [47] have identified several defense-related genes and genes involved in cellular detoxification upregulated in the uninfected young leaves of Remo as compared to Elstar. The constitutive expression of these genes in Remo is speculated to constitute a part of apple defense response. Interestingly a large number of metallothioneins (involved in metal ion detoxifications) were constitutively expressed in Remo, while they get induced following V. inaequalis infections in Elster. The molecular basis of constitutive expression of these genes in the resistant cultivar and their pathogen inducibility in susceptible cultivar should be explored to fish out the key regulatory molecules. The identified key genes if used to raise transgenic apple under pathogen inducible promoters can upregulate defense-related genes upon contact with the pathogen and suppress disease. Two subclasses of PR-10 gene (APa and APb) are induced by acibenzolar-S-methyl, a salicylic acid (SA, a plant defense hormone) analog in apple leaves [48]. Significantly higher expression of APa is induced following Venturia infection during resistant interactions than during compatible interactions and the kinetics of its expression coincide with appearance of visible necrosis [49]. The transgenic apple expressing either the endo (ech42) or the exo (nag70) chitinase gene of Trichoderma harzianum (syn. T. atroviride) have been found to impart scab resistance in a susceptible cultivar McIntosh ([50, 51], Table 4). The transgenic lines containing both endo and exo-chitinase genes exhibit synergism in promoting scab resistance ([51], Table 4). The overexpression of these genes could impart resistance in another susceptible cultivar Galaxy and strengthen resistance in the Vf gene containing cultivar Ariane against race 6 ([52], Table 4). This study has initiated a direction to combine defense related genes along with resistance genes to enhance the durability and efficacy of apple resistance.

Puroindoline B (PinB), a cysteine rich antifungal protein of wheat (Table 4), can suppress the growth of V. inaequalis [53]. The PinB gene when constitutively expressed in the cultivars Galaxy (susceptible to race 1) and Ariane could impart significant but variable resistance against race 6 of the pathogen in transgenic lines of both the cultivars, however, the transgenic Galaxy lines were still susceptible to race 1 [54]. This suggests that physiological differences between the strains can govern the PinB mediated resistance. Combining antifungal proteins along with resistance/defense-related genes can be a good strategy to enhance durability and efficiency of scab resistance in apple.

The NPR1 (Non-expressor of PR) protein plays a key role in systemic acquired resistance (SAR) in Arabidopsis thaliana [55]. Three AtNPR1 orthologs (MpNPR1-1, 2, and 3) have been identified from apple ([56], Table 4). The transgenic lines overexpressing MpNPR1-1 (considered as true apple orthog of AtNPR1) exhibit broad spectrum resistance against fungal (V. inaequalis and Gymnosporangium juniperi-virginianae, causative of cedar apple rust disease) and bacterial (Erwinia amylovora, causative of fire blight disease) pathogens, probably due to enhanced expression of PR proteins.

1.4. Gene-for-Gene Interactions. Plants have evolved a set of genes to detect and mount resistance responses against pathogens. Such genes are known as resistance (R) genes, and the pathogenic factors which are detected by the products of these genes are called Avirulence (avr) factors because their presence renders the pathogen avirulent [57]. This type of interaction, popularly known as gene-for-gene interaction, is followed by Venturia-Apple pathosystem [2, 17]. Several R gene containing loci have been isolated from apple cultivars [2, 37] and efforts have been made to characterize the avr genes of V. inaequalis (Table 1). According to a new nomenclature proposed by Bus et al. [28], apple scab resistance genes are named as Rv (R refers to resistance gene, vi refers to Venturia inaequalis, and k refers to differential host) and the corresponding avr genes of the pathogen are named as avrRv. The new and the old names of the apple resistance genes along with their differential host are mentioned in Table 3. The avrVf (avrRvi6) and avrVg (avrRvi1) have been isolated as two independently segregating factors which could render the pathogen avirulent on the Vf (Rvi6) and Vg (Rvi1) R gene containing apple cultivars, respectively [16]. The position of the avrVg (avrRvi1) has been determined using molecular markers [58] and several cosegregating and flanking (both sides) molecular markers have been isolated for this gene [59]. A contig of 330 kb spanning avrVg (avrRvi1) has been identified following chromosome walking on a BAC library of V. inaequalis [59]. Experiments involving multiple approaches are in progress to identify, clone, and sequence the gene and analyze its functionality [59]. AvrVm (AvrRvi5) like activity (inducing resistance on Vm containing apple) has been identified from culture supernatant of race 1 isolate [18]. The activity is narrowed down to three proteins having low molecular weight and low isoelectric points, however further work is needed to identify and characterize the protein [18].

1.4.1. Vf(Rvi6) Gene. Vf (Rvi6) gene, isolated from wild crab apple Malus floribunda 821, is the most studied R gene of apple [2, 37]. The Vf (Rvi6) gene has been extensively used for breeding scab resistance [2, 37, 60, 61]. Races 6 and 7 of V. inaequalis have evolved to breach the Vf (Rvi6) resistance [33, 62], however, they exhibit lack of genetic diversity and are clonally propagated due to founder effect [34].
Table 3: List of apple R-genes imparting scab resistance.

| S.N. | R-Gene | Sourcea /host | Linkage group | Resistance responseb | Molecular marker (~ Distance from the gene in cM) | Reference |
|------|--------|---------------|---------------|----------------------|-----------------------------------------------|-----------|
| 1    | Va     | Rvi10         | Antonovka Type PI 172623 | LG-1 | Class 1 | B398480 (16) | [37] |
| 2    | Vb     | Rvi12         | Hansen’s baccata #2 Differential host: h12 | LG-12 (Distal end) | Class 2 to 3b | Hi02d05 (7.8) Hi07f01(13.7) | [37] |
| 3    | Vbj    | Rvi11         | Malus baccata jackii Differential host: h11 | LG-2 (Distal end) | Class 0 to 3b | CH05e03 (0.6) T6 (3.9) | [37] |
| 4    | Vd     | Rvi13         | Durello di Forli Differential host: h13 | LG-10 (Proximal end) | Class 2 | OPAF07-880 (2.0) CH2b07 (9.0) | [37] |
| 5    | Vd3    | 1980-015-025 LG1 — CH-Vf1 (1) | — | — | CH-Vf1 (1) 67005F17 (7) | [63] |
| 6    | Vdg    | Rvi9          | J34; Differential host: h9 | — | — | — | [64] |
| 7    | —      | Rvi14         | Dülmenner Rosenapfel Differential host: h14 | LG-6 (Proximal end) | Class 2 | HB09TC (5) | [65] |
| 8    | Vf     | Rvi6          | “Priscilla” Differential host: h6 | LG-1 (Distal end) | Class 0 to 3b | M18 (0.2) CH-Vf1 (0.0) AL07 (0.9) | [37] |
| 9    | Vfh    | Rvi7          | Malus floribunda 821 Differential host: h7 | LG-8 | Class 1 | — | [66] |
| 10   | Vg     | Rvi1          | Golden Delicious Differential host: h1 | LG-12 (Distal end) | Class 2 | MC105 (3.0) CH01D03 (0.5) | [37] |
| 11   | Vh2    | Rvi2          | Malus pumila R12740-7A (TSR34T15) Differential host: h2 | LG-2 (Distal end) | Class 2 | OPL 19.43s (1) Ch02b10 (8) | [37] |
| 12   | Vh3.1  | Rvi3          | Q71; Differential host: h3 | — | — | — | [64] |
| 13   | Vh4/Vr1 | Rvi4         | Malus pumila R12740-7A (TSR33T239) Differential host: 4 | LG-2 (Distal end) | Class 1 | S22 (4) CH02c02 (5) | [37] |
| 14   | Vh8    | Rvi8          | Malus sieversii W193B Differential host: 8 | LG-2 (Distal end) | Class 2 | OPL19 (1.3) | [37] |
| 15   | Vm     | Rvi5          | Malus micromalus 245-38, Malus atrosanguinea 840 Differential host: h5 | LG-17 (Distal end) | Class1 | OPB12 (6) | [67] |
| 16   | Vr2    | Rvi15         | GMAL 2473 Differential host: h15 | LG-2 (Proximal end) | Class 0 to 2 | CH02c02a (0.0) | [37] |

aThe apple cultivar from where the gene has been isolated.
bThe characteristics of resistance response imparted by these R genes on apple against scab infection. Class 0: no symptoms; Class 1: pit type hypersensitive response like reactions; Class 2: irregular edged chlorotic lesions with slight necrotic center with no sporulation; Class 3a: chlorotic and necrotic lesions with rare sporulation; Class 3b: prominent sporulations with chlorotic and necrotic lesions.

Extensive research has been made to characterise the Vf (Rvi6) gene, cumulation of which has led to the isolation and full length cloning of the gene [37]. The gene HcrVf2 (Homologs to C. fulvus R genes of the Vf region; syn. Vfa2) is considered to be the true Vf (Rvi6) gene which is constitutively expressed in apple [68–71]. Its expression under CaMV35S promoter into a susceptible cultivar Gala [68] or under its native promoter into the susceptible cultivars Gala [71, 72] and Elstar [71] has been found to impart scab resistance comparable to that of Vf cultivars. Variations in the length of HcrVf native promoter could influence the gene expression and level of imparted resistance [71]. Alike Vf (Rvi6) gene, the resistance imparted by HcrVf2 demonstrates race specificity being susceptible to race 6 and 7 of the pathogen [73]. Effort has been initiated to understand the molecular cascades triggered by pathogen attack in HcrVf2 transgenic lines. In order to identify the genetic network involved in downstream signaling imparted by HcrVf2 in the transgenic apple cultivar Gala upon pathogen attack, a cDNA-AFLP protocol has been optimized by Paris et al. [74].

Besides HcrVf2, several Vf paralogs have been identified from apple, and gene duplication events seem to have played a role in their evolution [37]. These paralogs are
thought to be reservoirs which apple might use following somatic recombination to develop resistance against rapidly evolving strains of the pathogen [76]. Recently, Malnoy et al. have raised transgenics of susceptible cultivars Galaxy and McIntosh expressing Vf (Rvi6) gene (Vfa2; syn. HcrVf2) and their paralogs Vfa1 (syn. HcrVf1) and Vfa4 under their respective promoters [77]. The Vfa1 and Vfa2 genes but not the Vfa4 gene imparted scab resistance in transgenic plants. The resistance imparted by Vfa2 expressed with its own promoter was less than that observed when the gene is expressed by CaMV35 or that exhibited by the Vf (Rvi6) cultivars obtained following classical breeding. The high level of expression by CaMV35 promoter and copresence of Vfa1 and Vfa2 genes in the Vf (Rvi6) introgressed cultivars are speculated to be a cause of observed variations. The cotransformation of Vfa1 and Vfa2 genes in apple should shed lights on synergism between them in imparting scab resistance.

1.5. Other Venturia Resistance Genes of Apple. Besides the Vf (Rvi6) gene, several R genes have been isolated which impart variable degrees of resistance ranging from class 0 to class 3b in apple. They are Va (Rvi10), Vb (Rvi12), Vbj (Rvi11), Vd (Rvi13), Vdg (Rvi9), Vfh (Rvi7), Vg (Rvi1), Vm (Rvi5), Vh2 (Rvi2), Vh3.1 (Rvi3), Vh4 (Rvi4), Vh8 (Rvi8), Vv2 (Rvi15), and Rvi14 (Table 3). Recently, Vd3 resistance gene has been identified on LG1, close to Vf (Rvi6) locus between CH-Vf1 and 67105F17 markers [63]. Almost all the characterized plant R genes contain Leucine-rich repeats [57]. An apple Leucine-rich repeat (LRR) receptor-like protein kinase, LRPKm1, having domain architecture atypical of LRR receptor is speculated to be associated with apple defense against Venturia infection ([75], Table 4). The gene exhibits rapid and early induction during resistance interactions whereas a slow but steady increase even after 72 hours of infection is observed during susceptible interactions.

Beside monogenic R genes, several polygenic sources of scab resistance are known. Several quantitative trait loci (QTLs) imparting scab resistance and a number of resistance gene analogs (RGA; containing LRR and Nuclotide Binding Site domains) have been identified from different apple cultivars [37, 65]. Interestingly, quite a few of these R genes map to the same locus. The colocalization of the RGAs, QTLs, and R genes is a useful feature which should be utilized in apple breeding programs to cotransfer them into susceptible varieties. Since the pathogen has rendered several of the monogenic R genes ineffective, it will be useful to pyramid several such genes or there combination with other source of resistance for imparting effective and durable scab resistance as evolving resistance against the cocktail of these genes might be a difficult task for the pathogen. The availability of closely linked molecular markers for most of the known monogenic resistance genes and several other resistance related genes will facilitate work in this direction.

In order to strategize breeding programmes, international effort “Monitoring of Venturia inaequalis virulences” has been initiated to analyse whether a particular apple R gene is breached by a particular race of pathogen and to what extent that race is spread ([64], http://www.vinquest.ch/). Furthermore, it has been proposed to standardize reporting of molecular markers associated in coupling with the new and previously reported R genes in relation with apple cultivars “Gala”, “Golden Delicious”, “Fiesta”, and “Prima” as standard.

2. Future Prospects

It might be evident from this review that V. inaequalis is an important plant pathogen because it causes huge economic losses and also has a very interesting lifestyle. It is an appropriate time to sequence whole genome of the pathogen. The availability of genome sequence will not only stimulate research in the field of Venturia-apple interactions and contribute to the basic understanding of this pathosystem but can also revolutionize the understanding of pathogenesis of other obligate pathogens. The genome sequence will help in identification of targets for development of new fungicides that are needed as the rapidly evolving pathogen has overcome most of the commonly used fungicides.

| Table 4: Genes shown to impart scab resistance in apple. |
|---------------------------------------------|
| Gene                        | Gene source               | Apple cultivar(s) used in study | Inference                                      | Reference |
|-----------------------------|---------------------------|---------------------------------|------------------------------------------------|-----------|
| Chitinase                   |                           |                                 |                                                |           |
| ech42 (Endo)                | Trichoderma atroviride    | McIntosh                        | Transgenic plants are resistant to mix of Races 1-5 | [50, 51]  |
| nag70 (Exo)                 | Trichoderma atroviride    | McIntosh                        | Transgenic plants are resistant to mix of Races 1-5 | [50, 51]  |
| ech42 & nag70 (Exo)         | Trichoderma atroviride    | McIntosh                        | Exhibit Synergism in imparting scab resistance against mix of Races 1-5 | [51]      |
| Puriondoline B (PinB)       | Wheat                     | Galaxy, Ariane                  | Transgenic plants are resistant to Race 6       | [53]      |
| MpNPR1-1                    | Apple                     | Galaxy                          | Transgenic plants are resistant to mix of Races 1-5 | [56]      |
| LRPKm1                      | Apple                     | Florina, Golden Delicious       | Upregulated by SA treatment and during Venturia infections | [75]      |
Understanding the mechanisms of *Venturia* pathogenesis and intricacies of its interaction with apple should provide important insights for developing new strategies to combat the disease. The whole genome mutagenesis screen should be initiated to identify key virulence factors. The availability of standardized transformation methodologies in *V. inaequalis* will facilitate such efforts. The mechanism involved in break down of *R* gene mediated resistance by the pathogen should be explored. Understanding defense response associated signal transduction pathway of apple and characterizing key genes involved in imparting resistance will be very useful in engineering scab resistant apple. Availability of microarray platform for apple could trigger research to characterize the defense response associated transcriptome. The proteomics approach can be an alternative for this purpose. Pyramiding different resistance and defense related genes into a single cultivar seems to be helpful in imparting effective and durable resistance. Conventional breeding might take years to achieve the goal; however, using transgenic approach the goal can be achieved in a lesser span of time. Apple promoters that are induced upon *Venturia* infection should be identified to use them in a transgenic approach to express the key defense/resistance related genes. Expressing genes under apple promoters and generating marker-free plants can enhance the acceptability of the transgenic/cisgenic apple amongst the consumers.

**Acknowledgments**

Karnika Thakur and Priyanka Thakur are supported by a research fellowship from the Council of Scientific and Industrial Research (CSIR) and University Grant Commission (UGC), Government of India, respectively. The authors acknowledge the inputs and suggestions given by Dr PS Ahuja and Shammi Bhatti during manuscript preparation. The work has been supported by research funding from CSIR. They acknowledge that, due to space constraints, they have not been able to cite the valuable work of several researchers in this review. IHBT publication number: 0876.

**References**

[1] A. R. Biggs, “Apple scab,” in *Compendium of Apple and Pear Diseases*, A. L. Jones and H. S. Aldwinckle, Eds., pp. 6–9, APS, St. Paul, Minn, USA, 1990.

[2] W. E. MacHardy, *Apple Scab, Biology, Epidemiology, and Management*, APS, St. Paul, Minn, USA, 1996.

[3] W. E. MacHardy, D. M. Gadoury, and C. Gessler, “Parasitic and biological fitness of *Venturia inaequalis*: relationship to disease management strategies,” *Plant Disease*, Vol. 85, no. 10, pp. 1036–1051, 2001.

[4] P. R. Day, D. M. Boone, and G. W. Keitt, “*Venturia inaequalis* (Cke.) Wint. XI. The chromosome number,” *American Journal of Botany*, Vol. 43, pp. 835–838, 1956.

[5] D. M. Gadoury and W. E. MacHardy, “Negative geotropism in *Venturia inaequalis*,” *Phytopathology*, Vol. 75, pp. 856–859, 1985.

[6] A. Stensvand, H. Eikemo, R. C. Seem, and D. M. Gadoury, “Ascospore release by *Venturia inaequalis* during periods of extended daylight and low temperature at Nordic latitudes,” *European Journal of Plant Pathology*, Vol. 125, no. 1, pp. 173–178, 2009.

[7] K. J. Smereka, W. E. MacHardy, and A. P. Kausch, “Cellular differentiation in *Venturia inaequalis* ascospores during germination and penetration of apple leaves,” *Canadian Journal of Botany*, Vol. 65, pp. 2549–2561, 1987.

[8] C. F. A. Schumacher, U. Steiner, H.-W. Dehne, and E.-C. Oerke, “Localized adhesion of nongerminated *Venturia inaequalis* conidia to leaves and artificial surfaces,” *Phytopathology*, Vol. 98, no. 7, pp. 760–768, 2008.

[9] U. Steiner and E.-C. Oerke, “Localized melanization of appressoria is required for pathogenicity of *Venturia inaequalis*,” *Phytopathology*, Vol. 97, no. 10, pp. 1222–1230, 2007.

[10] A. Fitzgerald, J. A. L. van Kan, and K. M. Plummer, “Simultaneous silencing of multiple genes in the apple scab fungus, *Venturia inaequalis*, by expression of RNA with chimeric inverted repeats,” *Fungal Genetics and Biology*, Vol. 41, no. 10, pp. 963–971, 2004.

[11] W. Költer, D. M. Parker, and C. M. Becker, “Role of cutinase in the penetration of apple leaves by *Venturia inaequalis*,” *Phytopathology*, Vol. 81, pp. 1375–1379, 1991.

[12] W. Költer and D. M. Parker, “Purification and characterization of cutinase from *Venturia inaequalis*,” *Phytopathology*, Vol. 79, pp. 278–283, 1989.

[13] R. L. Nicholson, J. Kuc, and E. B. Williams, “Histochemical demonstration of transitory esterase activity in *Venturia inaequalis*,” *Phytopathology*, Vol. 62, pp. 1242–1247, 1972.

[14] M. Chevalier, C. Bernard, M. Tellier, et al., “Host and non-host interaction of *Venturia inaequalis* and *Venturia pirina* on *Pyrus communis* and *Malus* s. domestica,” *Acta Horticulturae*, Vol. 663, pp. 205–208, 2004.

[15] N. Kucheryava, J. K. Bowen, P. W. Sutherland, et al., “Two novel *Venturia inaequalis* genes induced upon morphogenetic differentiation during infection and in vitro growth on cellophane,” *Fungal Genetics and Biology*, Vol. 45, no. 10, pp. 1329–1339, 2008.

[16] G. Bénouf and L. Parisi, “Genetics of host-pathogen relationships between *Venturia inaequalis* races 6 and 7 and *Malus* species,” *Phytopathology*, Vol. 90, no. 3, pp. 236–242, 2000.

[17] D. M. Boone, “Genetics of *Venturia inaequalis*,” *Annual Review of Phytopathology*, Vol. 9, pp. 297–318, 1971.

[18] J. Win, D. R. Greenwood, and K. M. Plummer, “Characterisation of a protein from *Venturia inaequalis* that induces necrosis in *Malus* carrying the *Vrn* resistance gene,” *Physiological and Molecular Plant Pathology*, Vol. 62, no. 4, pp. 193–202, 2003.

[19] V. G. M. Bus, E. H. A. Rikkerink, W. E. van de Weg, et al., “The *Vh2* and *Vh4* scab resistance genes in two differential hosts derived from Russian apple R12740-7A map to the same linkage group of apple,” *Molecular Breeding*, Vol. 15, no. 1, pp. 103–116, 2005.

[20] V. G. M. Bus, F. N. D. Laurens, W. E. van de Weg, et al., “The *Vh8* locus of a new gene-for-gene interaction between *Venturia inaequalis* and the wild apple *Malus sieversii* is closely linked to the *Vh2* locus in *Malus pumila* R12740-7A,” *New Phytologist*, Vol. 166, no. 3, pp. 1035–1049, 2005.

[21] L. Parisi, V. Fouillet, H. J. Schouten, et al., “Variability of the pathogenicity of *Venturia inaequalis* in Europe,” *Acta Horticulturae*, Vol. 663, pp. 107–113, 2004.

[22] A. Kollar, “Characterization of an endopolygalacturonase produced by the apple scab fungus, *Venturia inaequalis*,” *Mycological Research*, Vol. 102, no. 3, pp. 313–319, 1998.

[23] C. Valsangiacomo and C. Gessler, “Purification and characterization of an exo-polygalacturonase produced by *Venturia*
inaequalis, the causal agent of apple scab,” Physiological and Molecular Plant Pathology, vol. 40, no. 1, pp. 63–77, 1992.

[24] E. S. Jacobson, “Pathogenic roles for fungal melanins,” Clinical Microbiology Reviews, vol. 13, no. 4, pp. 708–717, 2000.

[25] G. Jha, R. Rajeshwari, and R. V. Sonti, “Bacterial type two secretion system secreted proteins: double-edged swords for plant pathogens,” Molecular Plant-Microbe Interactions, vol. 18, no. 9, pp. 891–898, 2005.

[26] C. A. Ryan and E. E. Farmer, “Oligosaccharide signals in plants: a current assessment,” Annual Review of Plant Physiology and Plant Molecular Biology, vol. 42, no. 1, pp. 651–674, 1991.

[27] B. Le Cam, L. Parisi, and L. Arene, “Evidence of two formae speciales in Venturia inaequalis, responsible for apple and Pyracantha scab,” Phytopathology, vol. 92, no. 3, pp. 314–320, 2002.

[28] G. Schnabel, E. L. Schnabel, and A. L. Jones, “Characterization of ribosomal DNA from Venturia inaequalis and its phylogenetic relationship to rDNA from other tree-fruit Venturia species,” Phytopathology, vol. 89, no. 1, pp. 100–108, 1999.

[29] I. Tenzer and C. Gessler, “Subdivision and genetic structure of four populations of Venturia inaequalis in Switzerland,” European Journal of Plant Pathology, vol. 103, no. 6, pp. 565–571, 1997.

[30] I. Tenzer and C. Gessler, “Genetic diversity of Venturia inaequalis across Europe,” European Journal of Plant Pathology, vol. 105, no. 6, pp. 545–552, 1999.

[31] F. Güérin and B. Le Cam, “Breakdown of the scab resistance gene Vf in apple leads to a founder effect in populations of the fungal pathogen Venturia inaequalis,” Phytopathology, vol. 94, no. 4, pp. 364–369, 2004.

[32] X. Xu, J. Yang, V. Thakur, A. Roberts, and D. J. Barbarea, “Population variation of apple scab (Venturia inaequalis) isolates from Asia and Europe,” Plant Disease, vol. 92, no. 2, pp. 247–252, 2008.

[33] P. Glaudeux, X.-G. Zhang, D. Afoufa-Bastien, R.-M. V. Sanchez, M. Baghli, and B. Le Cam, “On the origin and spread of the scab disease of apple: out of central Asia,” PLoS ONE, vol. 3, no. 1, article e1455, 2008.

[34] F. Güérin, P. Franck, A. Loiseau, M. Devaux, and B. Le Cam, “Isolation of 21 new polymorphic microsatellite loci in the phytopathogenic fungus Venturia inaequalis,” Molecular Ecology Notes, vol. 4, no. 2, pp. 268–270, 2004.

[35] C. Gessler, A. Patocchi, S. Sansavini, S. Tartarini, and L. Gianfranceschi, “Venturia inaequalis resistance in apple,” Critical Reviews in Plant Sciences, vol. 25, no. 6, pp. 473–503, 2006.

[36] M. Chevalier, Y. Lespinasse, and S. Renaudin, “A microscopic study of different classes of symptoms coded by the Vf gene in apple for resistance to scab (Venturia inaequalis),” Plant Pathology, vol. 40, pp. 249–256, 1991.

[37] E. B. Williams and J. Kuc, “Resistance in Malus to Venturia inaequalis,” Annual Review of Phytopathology, vol. 7, pp. 223–246, 1969.

[38] H. Sierotzki, M. Eggenschwiler, O. Boillat, J. M. McDermott, and C. Gessler, “Detection of variation in virulence toward susceptible apple cultivars in natural populations of Venturia inaequalis,” Phytopathology, vol. 84, no. 10, pp. 1005–1009, 1994.

[39] F. Ortega, U. Steiner, and H.-W. Dehne, “Induced resistance to apple scab: microscopic studies on the infection cycle of Venturia inaequalis (Cke.) Wint,” Journal of Phytopathology, vol. 146, no. 8–9, pp. 399–405, 1998.

[40] U. Mayr, S. Michalek, D. Treutter, and W. Feucht, “Phenolic compounds of apple and their relationship to scab resistance,” Journal of Phytopathology, vol. 145, no. 2–3, pp. 69–75, 1997.

[41] G. Hazdina, W. Bojarska-Wysoki, and C. Lister, “Phytotoalexin production in an apple cultivar resistant to Venturia inaequalis,” Phytopathology, vol. 87, no. 8, pp. 868–876, 1997.

[42] G. Heczko, G. Heczko, and C. Stich, “Biosynthesis of phloridzin in apple (Malus domestica cv. Elstar) after infection by Venturia inaequalis and constitutive expression of PR genes in the resistant cultivar Remo,” European Journal of Plant Pathology, vol. 110, no. 7, pp. 703–711, 2004.

[43] J. Degenhardt, A. N. Al-Masri, S. Kürkçüoğlu, I. Szankowski, and A. E. Gau, “Characterization by suppression subtractive hybridization of transcripts that are differentially expressed in leaves of apple scab-resistant and susceptible cultivars of Malus domestica,” Molecular Genetics and Genomics, vol. 273, no. 4, pp. 326–335, 2005.

[44] F. Ziai, P. Poupard, M.-N. Brisset, J.-P. Paulin, and P. Simoneau, “Characterization of apple scab in two subclasses of PR-10 transcripts inducible by acibenzolar-S-methyl, a functional analogue of salicylic acid,” Physiological and Molecular Plant Pathology, vol. 59, no. 1, pp. 33–43, 2001.

[45] P. Poupard, L. Parisi, C. Campion, S. Ziai, and P. Simoneau, “A wound- and ethephon-inducible PR10 gene subclass from apple is differentially expressed during infection with a compatible and an incompatible race of Venturia inaequalis,” Physiological and Molecular Plant Pathology, vol. 62, no. 1, pp. 3–12, 2003.

[46] J. P. Bolár, J. L. Norelli, K.-W. Wong, C. K. Hayes, G. E. Harman, and H. S. Aldwinckle, “Expression of endochitinase from Trichoderma harzianum in transgenic apple increases resistance to apple scab and reduces vigor,” Phytopathology, vol. 90, no. 1, pp. 72–77, 2000.

[47] J. P. Bolár, J. L. Norelli, G. E. Harman, S. K. Brown, and H. S. Aldwinckle, “Synergistic activity of endochitinase and exochitinase from Trichoderma atroviride (T. harzianum) against the pathogenic fungus (Venturia inaequalis) in transgenic apple plants,” Transgenic Research, vol. 10, no. 6, pp. 533–543, 2001.

[48] M. Faize, M. Malnoy, F. Dupuis, M. Chevalier, L. Parisi, and E. Chevreau, “Chiitinases of Trichoderma atroviride induce scab resistance and some metabolic changes in two cultivars of apple,” Phytopathology, vol. 93, no. 12, pp. 1496–1504, 2003.

[49] E. Chevreau, F. Dupuis, C. Ortolan, et al., “Transformation of apple for durable scab resistance, expression of a puroindoline gene in a susceptible and resistant (VF) genotype,” Acta Horticulturae, vol. 560, pp. 323–326, 2001.
[54] M. Faize, S. Sourice, F. Dupuis, L. Parisi, M. F. Gautier, and E. Chevreau, “Expression of wheat puroindoline-b reduces scab susceptibility in transgenic apple (Malus x domestica Borkh.),” *Plant Science*, vol. 167, no. 2, pp. 347–354, 2004.

[55] H. Cao, J. Glazebrook, J. D. Clarke, S. Volko, and X. Dong, “The Arabidopsis NPR1 gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats,” *Cell*, vol. 88, no. 1, pp. 57–63, 1997.

[56] M. Malnoy, Q. Jin, E. E. Borejsza-Wysocka, S. Y. He, and H. S. Aldwinckle, “Overexpression of the apple MpNPR1 gene confers increased disease resistance in Malus x domestica,” *Molecular Plant-Microbe Interactions*, vol. 20, no. 12, pp. 1568–1580, 2007.

[57] J. L. Dangl and J. D. G. Jones, “Plant pathogens and integrated defense responses,” *Nature*, vol. 411, pp. 826–833, 2001.

[58] B. Le Cam, L. Parisi, M. Devaux, et al., “Identification and characterization of molecular markers linked to the avirulence avrVg of Venturia inaequalis,” in *Proceedings of the 9th International Congress of Molecular Plant-Microbe Interactions*, Amsterdam, The Netherlands, 1999.

[59] G. A. L. Broggini, B. Le Cam, L. Parisi, et al., “Construction of a contig of BAC clones spanning the region of the apple scab avirulence gene AvrVg,” *Fungal Genetics and Biology*, vol. 44, no. 1, pp. 44–51, 2007.

[60] J. A. Crosby, J. Janick, P. C. Pecknold, et al., “Breeding apples for scab resistance: 1945–1990,” *Fruit Variety Journal*, vol. 46, pp. 145–166, 1992.

[61] G. J. King, S. Tartarini, L. Brown, F. Gennari, and S. Sansavini, “Introgression of the Vf source of scab resistance and distribution of linked marker alleles within the Malus gene pool,” *Theoretical and Applied Genetics*, vol. 99, no. 6, pp. 1039–1046, 1999.

[62] L. Parisi, Y. Lepinusae, J. Guillaume, et al., “A new race of Venturia inaequalis virulent to apples with resistance due to the Vf gene,” *Phytopathology*, vol. 83, pp. 533–537, 1993.

[63] J. M. Soriano, S. G. Joshi, M. van Kaauwen, et al., “Identification and mapping of the novel apple scab resistance gene Vd3,” *Tree Genetics and Genomes*, vol. 5, no. 3, pp. 475–482, 2009.

[64] A. Patocchi, A. Frei, J. E. Frey, and M. Kellerhals, “Towards improvement of marker assisted selection of apple scab resistant cultivars: Venturia inaequalis virulence surveys and standardization of molecular marker alleles associated with resistance genes,” *Molecular Breeding*, vol. 24, no. 4, pp. 337–347, 2009.

[65] V. Soufflet-Freslon, L. Gianfranceschi, A. Patocchi, and C.-E. Durel, “Inheritance studies of apple scab resistance and identification of Rvi14, a new major gene that acts together with other broad-spectrum QTL,” *Genome*, vol. 51, no. 8, pp. 657–667, 2008.

[66] C.-E. Durel, “Genetic localisation of new major and minor pest and disease factors in the apple genome,” in *Proceedings of the 3rd Rosaceae Genomics Conference*, Napier, New Zealand, March 2006, abstract OP5.

[67] F. S. Cheng, N. F. Weeden, S. K. Brown, et al., “Development of a DNA marker for Vm, a gene conferring resistance to apple scab,” *Genome*, vol. 41, pp. 208–214, 1998.

[68] E. Belfanti, E. Silfverberg-Dilworth, S. Tartarini, et al., “The HcrVf2 gene from a wild apple confers scab resistance to a transgenic cultivated variety,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 3, pp. 886–890, 2004.

[69] M. Xu and S. S. Korban, “A cluster of four receptor-like genes resides in the Vf locus that confers resistance to apple scab disease,” *Genetics*, vol. 162, no. 4, pp. 1995–2006, 2002.