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Review Article

The *Venturia* Apple Pathosystem: Pathogenicity Mechanisms and Plant Defense Responses

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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.

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 coevolution, *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 esculantus*, *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 sepals 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 loculus. The ascospore consists of two unequal sized cells having thin brittle outer wall and a thick elastic inner wall which assists persistence of the pathogen during winter [2]. The fungi primarily perenate on fallen leaves, however perenation on twigs and fruits is also reported but in such cases ascospores are not produced and the conidia serve as primary inoculum. The ascospores mature at the time of apple bud break during which host tissues are most susceptible. Once matured, the ascospores are released from the asci and get disseminated by rain and wind. Interestingly, the presence of light is required for optimal discharge of the ascospores [3, 6]. This and several other favorable pathogenic attributes of *V. inaequalis* are reviewed by MacHardy et al. [3].

In order to colonize and establish disease, the pathogen needs to adhere, germinate, and form infection structures to penetrate the host. The conidia/ascospores of *V. inaequalis* attach to wet hydrophobic surface of apple and germinate by producing germ tubes, generally formed from the apical end of conidia or any of the two cells of the ascospore. *V. inaequalis* enters the host through cuticle but not through stomata [2]. Upon contact with cuticle, germ tube gets differentiated into an appressorium and produces adhesive mucilaginous substances predicted to facilitate attachment to the host surface [7]. The mucilaginous substances are composed of proteins and carbohydrates such as β-galactose and N-acetylglucosaminyl residues [8]. The presence of melanized ring at the base of growing appressorium is essential for successful pathogenesis [9]. However, Fitzgerald et al. [10] by silencing one of the melanin biosynthesis genes have observed that mutant exhibiting light brown phenotype is capable of infecting apple. Based upon the 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 pantothenic 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: 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 enzymes (CWDEs)	Promote pathogen entry into the host and facilitates nutrients uptake	[22, 23]
Cellulase	”	[22]
β-D-glucosidase	”	[22]
Polygalacturonase (endo-PG & Exo-PG)	”	[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 towards the site of infections	[24]
Cellophane induced		
Cin1	Induced during apple infections	[15]
Cin3	Induced during apple infections	[15]

(a) (b)

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
Race1	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 *Vm 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 *Vh*8 *R* gene

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

degrading plant cell walls also serves as a mark of infection and results in induction of plant defense response [25–27]. The controlled release of CWDEs might be a strategy of *Venturia* to prevent induction of host defense response which if successfully mounted would prevent growth of the pathogen. The role of CWDEs during pathogenicity on apple and their ability to induce defense responses are yet to be established. Melanin (generally present as melanoprotein) produced by *V. inaequalis* is speculated to tether CWDEs and facilitates their slow release [24]. The melanoprotein further assists in diverting the solute flow towards the site of infections, probably by altering the membrane permeability and solute transport system of apple to facilitate availability of nutrients for pathogen growth and development [2].

1.2. Hypervariability of V. inaequalis. The isolates of *V. inaequalis* are hypervariable and exhibit differential pathogenicity on apple cultivars (known as differential hosts). Based upon such differences, the pathogen has been categorized into eight physiological races [2, 16, 20]. The salient features of these races are summarized in Table 2. However, some of the isolates of *Venturia* are capable of growing on two different differential hosts and hence it is difficult to classify them to particular race. Recently, Bus et al. [28] have proposed a new nomenclature by reconsidering the differential host used in previous studies. They propose to replace the previously used differential host containing more than one *R* genes with new accession/selection which carries only one *R* gene. This system of nomenclature is robust, as per international standard, and has a feature to update the differential host each time a new *R* gene is discovered [28].

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 on 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 monogenic and polygenic 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 extent of resistance against isolates of the pathogen [40]. The matured leaves of apple demonstrate ontogenic resistance because of which the pathogen growth is suppressed immediately after cuticle penetration and appearance of disease symptom gets 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-glucosyloxydibenzofuran) and its aglycone and dibenzofuran 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 PR1 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 (*MpNPR*1-1, 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 *Rvik* (*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 *avrRvik*. 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		Source[a] /host	Linkage group	Resistance response[b]	Molecular marker (~Distance from the gene in cM)	Reference
	Old name	New name					
1	V_a	*Rvi10*	Antonovka Type PI 172623 Differential host: h10	LG-1	Class 1	B398480 (16)	[37]
2	V_b	*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	V_d	*Rvi13*	Durello di Forli Differential host: h13	LG-10 (Proximal end)	Class 2	OPAF07-880 (2.0) CH2b07 (9.0)	[37]
5	Vd_3		1980-015-025	LG1	—	CH-Vf1 (1) 67005F17 (7)	[63]
6	*Vdg*	*Rvi9*	J34; Differential host: h9	—	—		[64]
7	—	*Rvi14*	Dülmener Rosenapfel Differential host: h14	LG-6 (Proximal end)	Class 2	HB09TC (5)	[65]
8	V_f	*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	V_{fh}	*Rvi7*	*Malus floribunda* 821 Differential host: h7	LG-8	Class 1		[66]
10	V_g	*Rvi1*	Golden Delicious Differential host: h1	LG-12 (Distal end)	Class 2	MC105 (3.0) CH01D03 (0.5)	[37]
11	V_{h2}	*Rvi2*	*Malus pumila* R12740-7A (TSR34T15) Differential host: h2	LG-2 (Distal end)	Class 2	OPL 19 $_{433}$(1) Ch02b10 (8)	[37]
12	*Vh3.1*	*Rvi3*	Q71; Differential host: h3	—	—		[64]
13	V_{h4}/V_{r1}	*Rvi4*	*Malus pumila* R12740-7A (TSR33T239) Differential host: 4	LG-2 (Distal end)	Class 1	S22 (4) CH02c02 (5)	[37]
14	V_{h8}	*Rvi8*	*Malus sieversii* W193B Differential host: 8	LG-2 (Distal end)	Class 2	OPL19 (1.3)	[37]
15	V_m	*Rvi5*	*Malus micromalus* 245-38, *Malus atrosanguinea* 840 Differential host: h5	LG-17 (Distal end)	Class1	OPB12 (6)	[67]
16	V_{r2}	*Rvi15*	GMAL 2473 Differential host: h15	LG-2 (Proximal end)	Class 0 to 2	CH02c02a (0.0)	[37]

[a]The apple cultivar from where the gene has been isolated.
[b]The 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].

Beside *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

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 (Endo) & *nag70* (Exo)	*Trichoderma atroviride*	McIntosh	Exhibit Synergism in imparting scab resistance against mix of Races 1-5	[51]
		Galaxy, Araine	Transgenic plants are resistant to Race 1 and 6	[52]
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]

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), Vr2 (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.

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.

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