Seminars in Cell and Developmental Biology 148-149 (2023) 42–50

Contents lists available at ScienceDirect

Seminars in Cell and Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Recent developments in plant-downy mildew interactions

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ARTICLE INFO

Keywords:
Oomycetes
Obligate pathogens
Susceptibility genes
Effectors
SRNA
HIGS
SIGS

ABSTRACT

Downy mildews are obligate oomycete pathogens that attack a wide range of plants and can cause significant economic impacts on commercial crops and ornamental plants. Traditionally, downy mildew disease control relied on an integrated strategies, that incorporate cultural practices, deployment of resistant cultivars, crop rotation, application of contact and systemic pesticides, and biopesticides. Recent advances in genomics provided data that significantly advanced understanding of downy mildew evolution, taxonomy and classification. In addition, downy mildew genomics also revealed that these obligate oomycetes have reduced numbers of virulence factor genes in comparison to hemibiotrophic and necrotrophic oomycetes. However, downy mildews do deploy significant arrays of virulence proteins, including so-called RXLR proteins that promote virulence or are recognized as avirulence factors. Pathogenomics are being applied to downy mildew population studies to determine the genetic diversity within the downy mildew populations and manage disease by selection of appropriate varieties and management strategies. Genome editing technologies have been used to manipulate host disease susceptibility genes in different plants including grapevine and sweet basil and thereby provide new soucres of resistance genes against downy mildews. Previously, it has proved difficult to transform and manipulate downy mildews because of their obligate lifestyle. However, recent exploitation of RNA interference machinery through Host-Induced Gene Silencing (HIGS) and Spray-Induced Gene Silencing (SIGS) indicate that functional genomics in downy mildews is now possible. Altogether, these breakthrough technologies and attendant fundamental understanding will advance our ability to mitigate downy mildew diseases.

1. Introduction

Downy mildews are obligate oomycete pathogens in the order of Peronosporales within the formal family classification of Peronosporaceae [1]. Although only several hundred species have been classified in this family, they are responsible for serious losses in yield and reduction in quality in many important arable and horticultural crops including sunflower, onion, pea, faba bean, lettuce, spinach, hops, grapes, brassicas and cucurbits [2–8]. This family also contains the model downy mildew species *Hyaloperonospora arabidopsidis,* which infects the model plant *Arabidopsis thaliana* [9]. The most common downy mildew species are listed in Table 1 and representatives of downy mildews on different host plants are displayed in Fig. 1 and some of the infection structures are shown in Fig. 2.

Downy mildew pathogens undergo stereotypical primary and secondary infection cycles, albeit with some variation within species. Both primary and secondary infections contribute to downy mildew epidemics in crops. The primary infection cycle is initiated by sexually formed oospores, which reside within diseased tissues on the plant and overwinter or survive during undesired environmental conditions in the absence of a host [10,11] (Fig. 2A). Under cool and humid conditions, oospores germinate and initiate the primary infections through roots (e.g., *Hyaloperonospora brassicae* or *H. arabidopsidis* [12]), or in some cases through producing sporangia and zoospores that infect leaves (e.g.,

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https://doi.org/10.1016/j.semcdb.2023.01.010
Received 25 November 2022; Received in revised form 11 January 2023; Accepted 12 January 2023
Available online 18 January 2023
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Table 1
The most common downy mildews and their host plants.

Genus	Pathogen	Host	Reference
Bremia	*B. lactucae*	Lettuce	[100]
Hyaloperonospora	*H. arabidopsidis*	*Arabidopsis*	[101]
	H. brasicae	*Brassica campestris, B. juncea, B. napus, B. olaracea*	[102]
Peronospora	*P. belbahrii*	Sweet Basil	[103]
	P. destructor	Onion	[104]
	P. effusa	Spinach	[10]
	P. tabacina	Tobacco	[105]
	P. trifoliorum	Alfalfa, clover	[106]
	P. manshurica	Soybeans	[107]
	P. viciae f.sp. pisi	Pea	[5]
	P. viciae f.sp. fabae	Faba bean	[108]
Peronosclerospora	*P. maydis*	Maize	[109]
Plasmopara	*P. halstedii*	Sunflower	[22]
	P. viticola	Grapevine	[13]
Pseudoperonospora	*P. cubensis*	Cucurbits- Cucumber, Pumpkin, Zucchini, Squash, Melon, Watermelon	[110]
	P. humuli	Hops	[111]
Sclerophthora	*S. macrospora*	Rice, Sorghum, Wheat, Oat, Maize, Turf grass, maize	[112]
	S. rayssiae	Barley and Maize	[113]

Plasmopara viticola [13]). As with other oomycetes, intercellularly growing hyphae are coenocytic (contain several nuclei without the formation of cross-walls) [14] and their vegetative nuclei are diploid [15]. As hyphae branch and grow further, they develop haustoria into host cells (Fig. 2B) [13]. This colonisation of tissue is followed by the asexually formed sporangiophores, which carry sporangia or conidiophores (Fig. 2C) bearing conidiospores, commonly seen as a white, fluffy, downy growth on the abaxial (lower) surface of a leaf. This symptom gives the name "Downy" to the disease and the causative pathogens. The asexual spores initiate the secondary infection after being released from the sporophores by wind or rain drops. After landing on a leaf, conidiospores germinate (Fig. 2D) and form an appressorium and penetrates at the junction of two adjoining epidermal cells [16]. Some downy mildew species produce sporangiospores that produce motile zoospores, which encyst over stomata, produce a germ tube and an appressorium and penetrate through the stomata [13]. The full disease cycle is around 5–18 days [17].

As the nutrients deplete within the colonized tissues, formation of oospores is triggered through the development of antheridium and oogonium. Some downy mildews are homothallic (self-fertile, e.g., *Hyaloperonospora arabidopsidis*), while others are obligate outcrossers in which sexual reproduction happens only when complementary mating types grow together in intimate proximity (e.g., *Bremia lactucae* and *P. viticola,* [18]).

Throughout history, downy mildew outbreaks have caused significant economic impacts on diverse commercial crops and ornamental plants [13,19–22]. Traditionally, control of downy mildews relied on integrated strategies that utilize cultural practices, deployment of resistant cultivars, crop rotation, application of contact and systemic pesticides, and biopesticides [23–25]. Unfortunately, downy mildew populations can adapt quickly to selective pressure, leading to rapid breakdowns in host resistance [26] or insensitivity to chemical controls [18]. This adaptability necessitates new discoveries and exploitation of novel approaches such as CRISPR and RNA interference for mitigating the negative effects of disease.

These imperatives frame the remainder of this article. We focus on the recent research being carried out on molecular plant-downy mildew interactions, in particular the identification of new resistance sources, exploiting susceptibility genes, effectors, nutrient uptake, reverse genetics and population studies with translational aspects.

Fig. 1. Different downy mildews on their host plants. A) *Bremia lactucae* on lettuce, B) *Hyaloperonospora arabidopsidis* on *Arabidopsis thaliana*, C) *Peronospora viciae* f. sp. *pisi* on pea, D) *Peronospora viciae* f. sp. *fabae* on faba bean*,* E) *Plasmopara viticola on* grapevine, F) *Peronospora grisea* on hebe.

2. Analysis of sequenced genomes has illuminated diverse aspects of downy mildew biology

The inability to culture downy mildews on synthetic media has hampered genetic, molecular and physiological studies of these organisms. However, new light has been shed on downy mildews through exploitation of accessible, cost-effective technologies for assembling and analyzing genomes. The first downy mildew genome was published over a decade ago [27], and several additional species have been sequenced since then (e.g., [28–32]). Relevant links to available genome assemblies of different downy mildews are listed in Table 2. Long-read technologies helped resolve complex, repetitive regions and have been used to produce near-complete downy mildew genome assemblies [29,30]. Cost-effective short-read sequencing has supported genome resequencing and RNA-seq, and metagenomic filters can be utilized to remove the contaminating sequences that are inevitable in heterogenous samples obtained from the plant rather than from a pure culture [29,31]. Altogether, these and other advances have been exploited for increasingly high-quality assemblies, analyses of which have sparked research on several important aspects of downy mildew biology.

One such insight is provided by a report that convincingly demonstrates heterokaryosis for the lettuce downy mildew pathogen *B. lactucae* [29]. Heterokaryons are single cells, such as coenocytic oomycete hyphae, that contain multiple, genetically distinct nuclei. This manuscript presented a nearly complete genome assembly for *B. lactucae* and used resequencing, genetic segregation, and flow cytometry to demonstrate that a large proportion of field isolates are heterokaryotic. This represents yet another of many mechanisms through which genetic diversity can buffer oomycetes against selective pressures. Accordingly, the paper demonstrated enhanced fitness and virulence for heterokaryons compared to homokaryons. It will be of great interest to generalize these observations to other downy mildew species and to grapple with the implications for disease control strategies.

Genome analysis has radically revised our understanding of downy mildew evolution. Downy mildews have been generally classified as sister taxon to the *Phytophthora* genus. However, recent analyses of genomic data support a more complicated history (e.g., [32–35]). Phylogenetic models place downy mildew pathogens in two clades, each nested within a different clade of *Phytophthora* species. Thus, it appears that downy mildews are comprised of at least two independent

Table 2
Links for available genome assemblies of downy mildew pathogens.

Pathogen	Links for Reference Genome Sequences
Bremia lactucae	https://www.ncbi.nlm.nih.gov/genome/?term=Bremia+lactacae
Hyaloperonospora arabidopsidis *Hyaloperonospora brasicae*	https://www.ncbi.nlm.nih.gov/genome/browse/#!/eukaryotes/2623/ http://protists.ensembl.org/Hyaloperonospora_arabidopsidis/Info/Index https://www.ncbi.nlm.nih.gov/genome/?term=Hyaloperonospora+brassicae
Peronospora belbahrii *Peronospora destructor* *Peronospora effusa* *Peronospora tabacina*	https://www.ncbi.nlm.nih.gov/genome/?term=Peronospora+ +belbahrii https://www.ncbi.nlm.nih.gov/genome/?term=Peronospora+destructor https://www.ncbi.nlm.nih.gov/genome/?term=Peronospora+effusa http://protists.ensembl.org/Peronospora_effusa_gca_003704535/Info/Index http://protists.ensembl.org/Peronospora_effusa_gca_003843895/Info/Index https://www.ncbi.nlm.nih.gov/genome/?term=Peronospora+tabacina
Plasmopara halstedii *Plasmopara viticola*	https://www.ncbi.nlm.nih.gov/genome/?term=Plasmopara+halstedii http://protists.ensembl.org/Plasmopara_halstedii_gca_900000015/Info/Index https://www.ncbi.nlm.nih.gov/genome/?term=Plasmopara+viticola
Pseudoperonospora cubensis *Pseudoperonospora humuli*	https://www.ncbi.nlm.nih.gov/genome/?term=Pseudoperonospora+cubensis https://www.ncbi.nlm.nih.gov/genome/?term=Pseudoperonospora+humuli

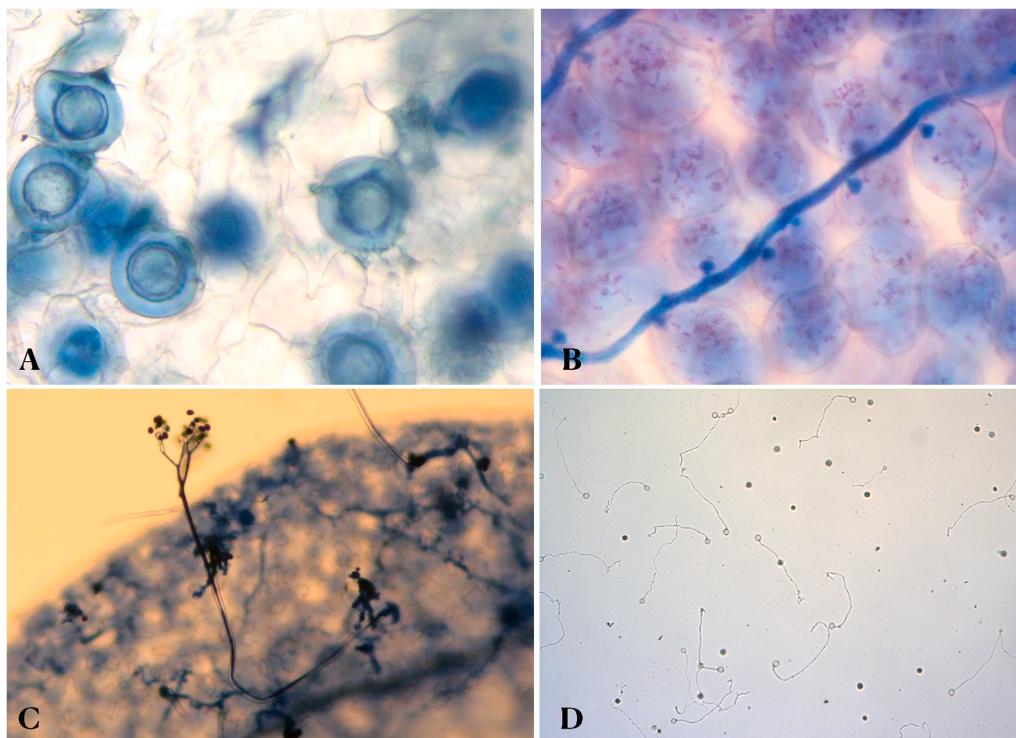

Fig. 2. Developmental stages of the *Hyaloperonospora arabidopsidis*. A) oospores on infected *Arabidopsis* leaves, B) Intracellular mycelia and haustorium in infected tissue, C) Conidiophore bearing conidiospores on infected tissue, D) Germinating conidiospores on glass slides.

evolutionary lineages that emerged from different *Phytophthora* lineages and convergently evolved very similar attributes. This raises questions about the taxonomy and classification of downy mildew species relative to *Phytophthora*, which are addressed thoughtfully in this recent commentary [36].

Another interesting insight from comparative genomics is that obligate oomycete lineages maintain reduced arsenals of virulence proteins compared to those of hemi-biotrophic and necrotrophic oomycetes [37]. For example, the number of predicted pectin lyase genes in genomes from three downy mildew species ranged from 6 to 22, compared with 82–122 for three *Phytophthora* species [34]. Such reductions are apparent in essentially every family of genes that encodes secreted virulence factors [28–32]. These reductions are generally interpreted as a signature of evolution to a mode of stealthy growth inside the host. In other words, selection has acted to reduce genes with activities that could alert the host to the presence of an invader (e.g., genes that contribute to the necrotrophic phase of hemi biotrophy.) Such signatures are ubiquitous in downy mildew pathogens as well as in fungal lineages that have evolved to obligate biotrophy, and probably represent general signatures of obligate biotrophy in phytopathogens. However, no virulence gene families are completely eliminated in any downy mildew genomes, and it will be of interest to investigate the functions of those virulence genes that remain and how they are deployed and perhaps camouflaged to maintain biotrophy.

Gene reductions have also been documented for a variety of metabolic pathways in downy mildews. The first such observation was for enzymatic pathways that assimilate nitrate and sulfate [27]. These pathways are broadly conserved in non-obligate oomycetes as well as in phytopathogenic fungi. Contrastingly, key enzymes are absent from many downy mildews as well as in another oomycete lineage (*Albugo* genus) in which obligacy has evolved independently. These losses have been interpreted as an evolutionary trajectory towards utilization of host-derived, organic sources of nitrogen (N) and sulfur (S), enabling the microbes to discard energetically expensive pathways for assimilation of inorganic N and S. Intriguingly however, these pathways appear to be intact in the genomes of *Plasmopara* species, although their functionality is unclear [28]. Additional metabolic deficiencies have been subsequently documented for downy mildews [28–32]. One recent, notable report describes a comprehensive comparison of metabolic pathways from a broad sample of oomycete species with diverse lifestyles [38]. Numerous gene losses were evident in obligate biotrophs, and network analysis suggested a general model of evolution towards “metabolic shortcuts” though which gene losses primarily impacted terminal branches in the metabolic network, perhaps to capitalize on host nutrients produced by redundant, parallel pathways in the host. Experimental interrogation of these predictions would provide important information about the metabolic interplay between downy mildews and their hosts and could identify metabolic “Achilles Heels” that could be exploited via chemical control or genetic resistance (e.g., manipulation of host disease susceptibility genes as described below).

Another notable deficiency in many downy mildew species is the loss of flagellar motility [39]. Corresponding genomic signatures are clearly apparent in many of these species, via the absence of genes encoding structural and regulatory components of flagellar motility (e.g., [34]). This is interpreted as an adaptation to foliar pathogenesis and atmospheric dispersal, eliminating the need for motility in aquatic habitats or soil. Interestingly, however, *Plasmopara* species retain flagellar genes and functional flagella [28]. Are these genes important for *Plasmopara* transmission, or are these genes on a trajectory towards obsolescence and loss?

3. Virulence factors: downy mildews reprogram host cells from the inside

As noted above, advances in genomics and bioinformatics have transformed our perspectives of virulence mechanisms for all oomycetes, including downy mildews. High quality genome assemblies have enabled identification of candidate virulence factors using bioinformatic tools. Candidates are then prioritized and their virulence functions are interrogated with molecular, cellular, and biochemical experiments, in some cases informed by structural biology [40].

Several different families of virulence factors have been defined for phytopathogenic oomycetes [39,41]. Most of these families are evident in sequenced genomes of downy mildew species and include proteins that carry out functions in either the apoplast or the interior of plant cells. Apoplastic effectors are primarily comprised of enzymes that degrade macromolecules, for example proteases or carbohydrate-active enzymes [39]. As noted above, gene families encoding such factors are reduced in downy mildews. However, downy mildews encode robust complements of effectors that enter host cells. These are introduced and summarized in the following paragraphs.

The best understood virulence factors from downy mildew pathogens belong to the so-called “RXLR” superfamily [42]. These proteins are defined by a classical N-terminal secretory leader, followed by the consensus RXLR motif (Arg-any amino acid-Leu-Arg) and in many cases, an adjacent cluster of amino acids with acidic side chains (the “eeR” motif). RXLR proteins typically range in length from 100 to 300 residues, and do not contain domains suggestive of function other than, in some cases, subcellular targeting signals such as nuclear localization signals. However, many AVR proteins contain motifs called “W-Y” or “L-W-Y”, often arranged as tandem repeats that comprise evolvable interfaces for interaction with host proteins [43].

RXLR genes are prominent in the genomes of *Phytophthora* and downy mildew species. Annotations based on canonical RXLR motifs predict that *Phytophthora* genomes typically contain several hundred predicted RXLR genes, while downy mildew genomes contain ~40–150 genes [41,42]. However, recent studies have identified downy mildew effectors for which the RXLR sequence is degenerate or apparently absent [44]. In one example, a significant proportion of WY candidate effectors displayed virulence or avirulence activities when transiently expressed in lettuce, suggesting that they are *bona fide* effectors [45]. Based on these and other data, the authors proposed that previous bioinformatic screens based on the RXLR motif have likely underestimated the full complement of intercellular effectors for downy mildews, and to a lesser extent for *Phytophthora* species. Thus, an important challenge for the future is to improve the workflows for bioinformatic prediction of effectors for downy mildew pathogens.

Another challenge for research on downy mildew effectors is to experimentally validate the biological activity of predicted effector proteins and to undertake the requisite studies to understand the molecular mechanisms through which they promote virulence [42]. Because of the inability to transform downy mildew species, such studies have employed genetically tractable surrogates for effector delivery (e.g., phytopathogenic *Pseudomonas* bacteria) or by expressing the effector genes as transiently expressed or stably integrated transgenes in plant hosts. These approaches have been used to screen effector virulence and avirulence activity, map subcellular localization, and identify plant proteins that are targeted for manipulation [40,42]. Most of this work has focused on *Hyaloperonospora arabidopsidis* (*Hpa*) as a model, due to the genetic tractability of its host *A. thaliana* [9]. However, productive investigation of other downy mildew/host interactions are emerging (e.g., [45–47]).

Several excellent reviews have discussed progress on RXLR effector biology (e.g., [40,42]). Here, we summarize several generalities and highlight one interesting recent study. First, downy mildew RXLR effectors can localize to diverse subcellular addresses and can interact with a wide variety of plant host proteins. Many of these putative plant target proteins are known to be regulators of immunity, and several downy mildew effectors have been validated to suppress immunity by interacting with host immune regulators. In some cases, immune regulatory proteins or protein complexes are targeted by effectors from bacterial and/or fungal phytopathogens, further supporting the

importance of these targets for immune regulation [48]. Notably, host processes other than immunity have not been frequently discovered for downy mildew RXLR proteins. We consider this as an opportunity for productive future investigations.

A recent publication provides a good example of the methods through which downy mildew effectors can be studied, and how these studies can provide insights of broad significance to plant-oomycete interactions [49]. This study is based on large-scale functional comparisons of RXLR effectors from *Hpa* and from the late blight pathogen *Phythophthora infestans*. Effectors from each pathogen were studied in the native hosts (*Arabidopsis* and potato, respectively) as well as in the non-native hosts. In general, both sets of effectors displayed immune suppressive activity in the native host but not in the non-native host, suggesting that effectors evolve host-specific interactions with their target proteins. This was generalized by protein interaction screens, which demonstrated that effectors from *P. infestans* do not frequently interact with *Arabidopsis* homologs of the potato proteins with which the *P. infestans* effectors interact. Similarly, *Hpa* effectors did not frequently interact with potato homologs of their *Arabidopsis* targets. These findings suggest that the phenomenon of non-host resistance (e.g., *Arabidopsis* resistance to *P. infestans*) could be explained in part by lineage-specificity of interactions between effectors and host target proteins. This holds implications for genetic control of resistance: The authors demonstrated that substitution of a targeted protein in potato with a non-targeted homolog from *Arabidopsis* impaired *P. infestans* virulence in potato, due to its inability to manipulate the heterologous *Arabidopsis* protein [49]. In more general terms, effector-target specificity could provide guidance for replacement or editing of host susceptibility genes (see below) to hamper pathogen virulence [50].

Although most of the experimental attention on downy mildew effectors has focused on RXLR proteins, other interesting candidate virulence factors have been annotated [51]. These include so-called Crinkler effectors, along with elicitins and Necrosis- and ethylene-inducing peptide 1 (Nep1)-like proteins (NLP). Each of these families have been associated with virulence in *Phytophthora* but have only limited attention for downy mildews (e.g., [52]). Interestingly, each of these virulence protein families has been associated with cytotoxicity in *Phytophthora*, so it is somewhat surprising that they are maintained in downy mildews, even at low copy numbers. However, this conservation implies that they play important, biotroph-specific functions that are worthy of investigation. Perhaps the ability to suppress their expression via RNAi and thereby interrogate virulence activity will provide initial motivation to understand their modes of action.

4. Durable resistance and exploiting susceptibility genes for defence

Studies on plant-microbe interactions over the years revealed that molecules originating from the pathogen such as pathogen-associated molecular pattern molecules (PAMPs) or effectors are detected by cell surface and intracellular receptors, respectively [53]. Intracellular receptors, usually encoded by *R*-genes, encode nucleotide binding-leucine rich repeat (NB-LRR or NLR) proteins [54] and have been traditionally targeted by the breeders for the control of plant diseases.

R genes against downy mildew pathogens have been identifeid in diverse host plants such as lettuce [55], sunflower [56], spinach [57] and grapevine [58]. Many of these genes have been incorporated into breeding programmes using marker-assisted selection. However, a major drawback of these single major *R*-gene based disease control is the breakage of resistance by an evolved pathogen population often resulting from mutations in the corresponding avirulence (effector) gene [59]. To alleviate this problem, breeders can "stack" or pyramid" several *R*-genes in a single variety to generate a durable resistance, however, this system can also fail [60]. An alternative approach, which promises more durable resistance, is to use quantitative resistance where many genes are contributing from a genomic region, quantitative trait loci (QTL), from small to a moderate effect to resistance phenotype [61]. Several examples of QTLs against downy mildew diseases such as *P. cubensis* on cucumber [62,63], *P. humuli* on hops [64] and *P. belbahrii* on sweet basil [65] have been reported and genes contributing to resistance have been identified.

As disease occurs in a compatible interaction between a virulent and a susceptible host, plant host susceptibility genes (*S*-genes), are actively involved in the interaction and enable pathogen infection [66]. Manipulation of *S*-genes through mutation and gene editing technologies has been reported to provide resistance against pathogens, providing new opportunities for plant breeding [50]. For example, a forward genetic screen for reduced susceptibility to the downy mildew pathogen *H. arabidopsidis* in the highly susceptible *Arabidopsis* Ler-*eds1* accession revealed eight *dmr* (*downy mildew resistant*) loci that reduced the pathogen growth [67]. Subsequent map-based cloning studies of some of these *DMR* genes enabled a better understanding of the mechanistic basis of host susceptibility to downy mildews (Table 3). The best example is provided by *DMR6*, which encodes a 2-oxoglutarate (2OG)-Fe(II) oxygenase, associated with host defence [68,69]. *DMR1* encodes homoserine kinase (HSK), a key enzyme in the primary amino acid metabolism [70].

Transgenic approaches and genome editing have been used to assess whether *S*-genes could be manipulated for disease control in crop species (Table 3). The *Arabidopsis DMR6* orthologue from grapevine, *VviDMR6,* has been identified and demonstrated that *VviDMR6* can complement *Arabidopsis dmr6–1* resistant mutant [71], suggesting that mutation in this gene through gene editing technologies could be explored for downy mildew resistance in grapevine. Accordingly, editing of the *DMR6* orthologue from grapevine with CRISPR/Cas9 system improved resistance to downy mildew [72]. Similarly, editing of the orthologue of the *DMR6* in sweet basil led to a considerable reduction in the sporulation and biomass production of the downy mildew pathogen *P. belbahrii* [73]. The *ObDMR1* orthologue in sweet basil *ObDMR1*, was mutated using CRISPR/Cas9 system, but no pathology assay was reported [74]. However, using the same approach on *ObDMR1/ObHSK1*, another group showed that mutation in the gene significantly reduced susceptibility to the downy mildew disease [75]. Overexpression of the grapevine gene *VvNAC72*, a transcription factor, has been reported to show a strong resistance to the downy mildew pathogen *P. viticola* in grapevine [76]. These studies show that identifications of orthologues of *S*-genes in host plants could be explored in a wide range of plant-downy mildew pathosystems, providing a new generation of resistance breeding for crop improvements.

In addition to genome editing technologies, studies on susceptibility genes to provide resistance to downy mildews also included the RNA interference technology such as use of double stranded RNA (dsRNA). For example, [77] applied dsRNAs targeting the susceptibility gene *VviLBDIf7* in grapevine exogenously and demonstrated a significant decrease in downy mildew growth indicating an alternative strategy such as developing sRNAs as a pesticide targeting a susceptibility gene for disease control. Detailed use of small RNAs in downy mildew pathogens are given below.

5. Genetic manipulation

As downy mildew pathogens are obligate oomycetes, till now, it has proved difficult transform and manipulate these microorganisms, and thus genetic investigations such as Restriction enzyme-mediated integration (REMI) [78] with these microorganisms could not be explored for high throughput mutagenesis. Studies on other oomycete pathogens such as *P. infestans* and *P. sojae* have benefited from genetic transformation to investigate effectors and other genes within their native environment [79]. As noted above, alternative strategies had been developed for the obligate downy mildews to investigate the role and function of effectors. These included, a) co-bombardment assays into plant cells using the GUS gene to indicate avirulence activity [44], b)

Table 3
Putative susceptibility genes exploited for defence in plant-downy mildew interactions.

Downy mildew pathogen (DM)	Plant species/Host	Susceptibility gene	Function of gene	Phenotype	Method	References
H. arabidopsidis	*A. thaliana*	*DMR1* *DMR6* *DMR6/DLO1*	Homoserine kinase Encode a 2-oxoglutarate (2OG)-Fe (II)	Provide resistance Showed complete resistance Overexpression shows increased susceptibility	Map-based cloning Map-based cloning Transgenic approach	[70] [68] [69]
Peronospora belbahrii	*Ocimum basilicum* (Sweetbasil)	*ObDMR6* *ObDMR1/ObHSK*	Oxidoreductase Homoserine kinase	Enhanced resistance	Gene editing (CRISPR/Cas9)	[73] [75]
Plasmopara viticola	*Vitis vinifera* (Grapevine)	*VvDMR6.1* *DMR6* *VvNAC72* *VviLBDIf7*	Encode a 2-oxoglutarate (2OG)-Fe (II) Transcriptional repressor of *VvGLYI-4* Repressor of jasmonate-mediated defense mechanism	Complement *Atdmr6.1* Enhanced resistance Increased resistance Increased resistance	Transgenic approach Gene editing (CRISPR/Cas9) Transgenic approach RNAi approach	[71] [72] [76] [114]
Pseudoperonospora cubensis	*Cucumis sativus* (cucumber)	*CsSGR* (QTL locus)	Regulator of chloroplast degradation	Durable resistance	Map-based clonning	[63]

effector delivery from bacteria using Type III secretion [80], and c) creation of stably transformed plants expressing effector genes under control of inducible promoters [81]. However, these methods stripped the effector gene away from the pathogen where the expression level of a gene may not be comparable to that in the native background. Moreover, single-gene assays do not accurately capture gene function in the native milieu. For these reasons, RNAi-based tools for reverse genetics in downy mildews, described in the following section, are a welcome advance.

6. Exploiting RNA interference in plant-downy mildew interactions

RNA interference (RNAi) is a gene regulation mechanism conserved across different species [82], in which small interfering RNAs (siRNAs) generated from double stranded RNAs (dsRNAs) guide transcriptional and post-transcriptional gene silencing (TGS and PTGS, respectively) [83]. In recent years, studies have shown that plants communicate with interacting fungal and oomycete pathogens by using small RNAs (sRNAs). An elegant study by [84] on *Arabidopsis-Botrytis* interaction showed that host sRNAs are transported to pathogen cells inside extracellular vesicle (EV). Working on the model *Hpa-Arabidopsis* pathosystem, [85] used the host Argonaute I (AGO1) to pull down sRNAs originating from the downy mildew pathogen. They identified over a hundred *Hpa* sRNAs, 34 of which are targeting more than 49 *Arabidopsis* genes, indicating the possible involvement of sRNAs in virulence. This type of study indicates exchange of sRNAs between the pathogen and the host that lead to silencing of each other's genes through the process called Trans-Kingdom RNA silencing [86].

Exploitation of RNAi machinery by Host Induced Gene Silencing (HIGS) and Spray Induced Gene Silencing (SIGS) have shown promising results to investigate the role of pathogen genes in their native environment [87]. Although the number of sRNA-based studies on downy mildews are relatively small, the results are promising. [88] used HIGS on lettuce plants targeting the *Highly Abundant Message #34* (*HAM34*) or *Cellulose Synthase* (*CES1*) genes of the *B. lactucae* and reported significant reduction in growth and inhibited sporulation of the downy mildew pathogen. Using a SIGS approach and targeting the *DCL1 and DCL2* genes in grapevine downy mildew pathogen *P. viticola*, [89] observed reduction in transcript abundance from these genes as well as in the disease progress rate in an already-established infection. [90] provided the first report of successful SIGS against a downy mildew pathogen by targeting the *Cellulose synthase A3* (CesA3) gene in *Hpa*. They used sense and antisense sRNAs targeting the *Hpa-CesA3* gene in *Hpa* isolates and showed the antisense sRNAs inhibited spore germination and thus infection of *Arabidopsis*, while sense sRNAs had no obvious effect on *Hpa* pathogenicity. These small number of investigations using sRNAs on downy mildew pathogens open a long-closed door to much-needed reverse genetic studies on these obligate pathogens. This is a major area for exploitation in the future.

7. Population genetics

Population genetics aims to understand how polymorphisms in and between populations contribute to the dynamics of adaptation and evolution, due to various factors including genetic drift, recombination, mutation and selection [91]. Enhancing our understanding of intra-specific diversity at the genomic level, and the mechanisms regulating pathogenicity and virulence could help to manage the effects of disease more effectively and limit yield losses and wastage, through selection of appropriate varieties and management strategy. Whole genome sequencing provides a cost- effective technology to rapidly obtain genetic information for comparing diversity between individuals. As genome assemblies have been constructed for numerous commercially relevant downy mildew species, re-sequencing is now routinely employed to study oomycete pathogen populations [92]. For example, in *Peronospora effusa* (spinach downy mildew), sexual recombination is posited to drive changes in virulence at a regional scale, whereas asexual reproduction is the primary cause of polycyclic disease in crops at the field scale [93].

Genotyping by Sequencing (GbS) has been exploited similarly to WGS; recent studies in *P. destructor* (onion downy mildew) utilised GbS to investigate population structure and genetic variance, and identified greater genetic diversity observed within fields than between them (23.98%); a similar situation was observed within regional variance (82.07%) compared to differences between regions (17.93%) [94]. Locally, low levels of genetic diversity were observed, reflecting predominantly clonal reproduction. This is similar to *Pseudoperonospora humuli* (hop downy mildew), where minor, but significant genetic variation was observed between sites and plants at the same site [95]. Population genetic data was also used to investigate *P. destructor* epidemiology and indicated that asexual reproduction in over-wintering colonies appears to be the main source of new epidemics and that the disease is endemic, contrasting the initial theory that new infections were initiated primarily by spores spread on prevailing winds from crops in warmer regions in the USA [8].

Developing the capacity to accurately distinguish between downy mildew pathotypes is an important consideration for practical disease control to enable the most effective sources of resistance to be deployed. Some downy mildew species have a relatively wide host range, for example *P. halstedii* affects over 35 genera of Asteraceae [96] and *P. cubensis* infects cucurbits including squash, pumpkin, melon and

cucumber [97]. Contrastingly, many downy mildews demonstrate a high degree of/discrete host and race specificity(ies), reflecting co-adaptation in an evolutionary arms race. This is particularly evident in downy mildews affecting selectively bred crops including lettuce, spinach and pea; *Bremia lactucae, Peronospora effusa* and *P. viciae* f. sp. *pisi* have been found to exhibit complex race types (e.g. *B. lactucae: 37 races in EU; 16 races of P. viciae* [98,99]. Genetic diversity in pathogen populations was first characterised by describing the differential responses to host resistance through development of differential sets of host varieties to describe and monitor pathotypes prevalent in the populations. Differential screening is regularly used for determining prevalent races of downy mildew affecting lettuce (*Lactucae sativa*) and spinach (*Spinacia oleracea)* crops in Europe/UK and the USA to help guide plant breeders select resistant varieties, and enable growers deploy these effectively to reduce the negative effects from disease.

However, the resulting pathotypic classes, and ability to discriminate between isolates depends greatly on the genetic composition of the host panel used to derive the differential phenotypes. Although limited in the degree of genetic information they yield, differential sets are still useful practical tools for investigating population diversity.

8. Conclusions and future perspectives

As additional genomic sequences of downy mildew became available, we expect further developments in effector research in the near future. Use of AlphaFold for accurate protein structure prediction has proved useful for effectors in other oomycetes such as *Phytophthora*, and we expect this machine learning approach to be extended to downy mildew effectors so the specialisation of these pathogens to their host plants could be elucidated in detail. Discovery of trans-kingdom spread of small RNAs has gained attention in the last few years and exploitation of this siRNA-mediated gene silencing in downy mildews opened the way for a high throughput gene silencing investigations in these obligate pathogens. Alternatives to *R* gene-mediated disease control, such as altering *S*-genes against downy mildews using genome editing technologies, are showing promising results and we would expect this to be applied to a wide range of plant-downy mildew pathosystems. Differential screening has been used routinely by breeders to determine prevalent races of downy mildew affecting crops. However, it is often laborious and provides only limited genetic information to the user. Pathogenomic approaches could be employed to investigate both inter- and intraspecific genetic variation in downy mildew species, helping to enhance our basic understanding and improve capability for practical diagnostics and monitoring.

Funding

Financial support from BBSRC grants BB/V014609/1 and BB/T016043/1 on Downy mildew research to M. Tör and BBSRC grant BB/T016019/1 to T. Wood is gratefully acknowledged. JMM was supported by project VA-160106 from the Virginia Agricultural Experiment Station and the Hatch Program of the National Institute of Food and Agriculture, U.S. Department of Agriculture.

Author contributions

MT, TW, AW, DG and JMM wrote the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interests.

Data Availability

Data will be made available on request.

References

[1] H. Voglmayr, Progress and challenges in systematics of downy mildews and white blister rusts: new insights from genes and morphology, Eur. J. Plant Pathol. 122 (1) (2008) 3–18.

[2] A.J. Biddle, J. Thomas, D. Kenyon, N.V. Hardwick, M.C. Taylor, The effect of downy mildew (Peronospora viciae) on the yield of spring sown field beans (Vicia fabae) and its control, The BCPC International Congress: Crop Science and Technology, Volumes 1 and 2 Proc. Int. Congr. held SECC, Glasg., Scotl., UK, 10-12 Novemb. 2003 2003 947 952.

[3] R. Michelmore, J. Wong, Classical and molecular genetics of Bremia lactucae, cause of lettuce downy mildew, Eur. J. Plant Pathol. 122 (1) (2008) 19–30.

[4] F. Runge, M. Thines, Reevaluation of host specificity of the closely related species Pseudoperonospora humuli and P. cubensis, Plant Dis. 96 (1) (2012) 55–61.

[5] J. Liu, T. Cao, K.-F. Chang, S.-F. Hwang, S.E. Strelkov, Virulence and diversity of Peronospora viciae f. sp. pisi in Alberta, Canada, Crop Prot. 43 (2013) 18–26.

[6] Q. Gascuel, Y. Martinez, M.-C. Boniface, F. Vear, M. Pichon, L. Godiard, The sunflower downy mildew pathogen Plasmopara halstedii, Mol. Plant Pathol. 16 (2) (2015) 109–122.

[7] C. Feng, K. Saito, B. Liu, A. Manley, K. Kammeijer, S.J. Mauzey, S. Koike, J. C. Correll, New races and novel strains of the spinach downy mildew pathogen peronospora effusa, Plant Dis. 102 (3) (2018) 613–618.

[8] H. Van der Heyden, P. Dutilleul, J.-B. Charron, G.J. Bilodeau, O. Carisse, Factors influencing the occurrence of onion downy mildew (peronospora destructor) epidemics: trends from 31 years of observational data, Agronomy 10 (5) (2020) 738.

[9] J. Herlihy, N.R. Ludwig, G. van den Ackerveken, J.M. McDowell, Oomycetes used in arabidopsis research, Arab. Book 17 (2019), e0188.

[10] B. Dhillon, C. Feng, M.I. Villarroel-Zeballos, V.L. Castroagudin, G. Bhattarai, S. J. Klosterman, J.C. Correll, Sporangiospore viability and oospore production in the spinach downy mildew pathogen, peronospora effusa, Plant Dis. 104 (10) (2020) 2634–2641.

[11] Y.-J. Zhang, Z.-J. Pu, Z.-W. Qin, X.-Y. Zhou, D. Liu, L.-T. Dai, W.-B. Wang, A study on the overwintering of cucumber downy mildew oospores in China, J. Phytopathol. 160 (9) (2012) 469–474.

[12] B. Mauch-Mani, A.J. Slusarenko, Arabidopsis as a model host for studying plant-pathogen interactions, Trends Microbiol. 1 (7) (1993) 265–270.

[13] K. Koledenkova, Q. Esmaeel, C. Jacquard, J. Nowak, C. Clement, E. Ait Barka, Plasmopara viticola the causal agent of downy mildew of grapevine: from its taxonomy to disease management, Front. Microbiol. 13 (2022), 889472.

[14] A.R. Hardham, Cell biology of plant-oomycete interactions, Cell Microbiol. 9 (1) (2007) 31–39.

[15] L.J. Grenville-Briggs, V.L. Anderson, J. Fugelstad, A.O. Avrova, J. Bouzenzana, A. Williams, S. Wawra, S.C. Whisson, P.R. Birch, V. Bulone, P. van West, Cellulose synthesis in Phytophthora infestans is required for normal appressorium formation and successful infection of potato, Plant Cell 20 (3) (2008) 720–738.

[16] M.E. Coates, J.L. Beynon, Hyaloperonospora Arabidopsidis as a pathogen model, Annu. Rev. Phytopathol. 48 (2010) 329–345.

[17] G.N. Agrios, Plant Pathology, Elsevier, London, 2005.

[18] Y. Cohen, A.E. Rubin, M. Galperin, Host preference of mating type in pseudoperonospora cubensis, the downy mildew causal agent of cucurbits, Plant Dis. 97 (2) (2013) 292.

[19] T. Caffi, G. Maddalena, B. Lecchi, F. Serina, S. Torcoli, S.L. Toffolatti, V. Rossi, G. Fedele, Oospore germination dynamics and disease forecasting model: an integrated approach for downy mildew management, BIO Web Conf. 50 (2022).

[20] R.A. Mouafo Tchinda, M.L. Fall, C. Beaulieu, O. Carisse, Competition between Plasmopara viticola clade riparia and clade aestivalis: a race to lead grape downy mildew epidemics, Plant Dis. (2022).

[21] C. Salgado-Salazar, N. LeBlanc, A. Ismaiel, Y. Rivera, C.Y. Warfield, J.A. Crouch, Genetic variation of the pathogen causing impatiens downy mildew predating and including twenty-first century epidemics on impatiens walleriana, Plant Dis. 102 (12) (2018) 2411–2420.

[22] L. Molinero-Ruiz, Sustainable and efficient control of sunflower downy mildew by means of genetic resistance: a review, Theor. Appl. Genet. (2022).

[23] C. Skrzypczak, Occurrence of downy mildews on ornamental plants and their control by chemical compounds, Commun. Agric. Appl. Biol. Sci. 72 (4) (2007) 801–804.

[24] S.L. Kandel, B. Mou, N. Shishkoff, A. Shi, K.V. Subbarao, S.J. Klosterman, Spinach downy mildew: advances in our understanding of the disease cycle and prospects for disease management, Plant Dis. 103 (5) (2019) 791–803.

[25] S.N. Raj, N.P. Shetty, H.S. Shetty, Seed bio-priming withPseudomonas fluorescensisolates enhances growth of pearl millet plants and induces resistance against downy mildew, Int. J. Pest Manag. 50 (1) (2004) 41–48.

[26] F. Delmotte, P. Mestre, C. Schneider, H.-H. Kassemeyer, P. Kozma, S. Richart-Cervera, M. Rouxel, L. Delière, Rapid and multiregional adaptation to host partial resistance in a plant pathogenic oomycete: evidence from European populations of Plasmopara viticola, the causal agent of grapevine downy mildew, Infect. Genet. Evol. 27 (2014) 500–508.

[27] L. Baxter, S. Tripathy, N. Ishaque, N. Boot, A. Cabral, E. Kemen, M. Thines, A. Ah-Fong, R. Anderson, W. Badejoko, P. Bittner-Eddy, J.L. Boore, M.C. Chibucos, M. Coates, P. Dehal, K. Delehaunty, S. Dong, P. Downton, B. Dumas, G. Fabro, C. Fronick, S.I. Fuerstenberg, L. Fulton, E. Gaulin, F. Govers, L. Hughes, S. Humphray, R.H.Y. Jiang, H. Judelson, S. Kamoun, K. Kyung, H. Meijer, P. Minx, P. Morris, J. Nelson, V. Phuntumart, D. Qutob, A. Rehmany, A. Rougon-Cardoso, P. Ryden, T. Torto-Alalibo, D. Studholme, Y. Wang, J. Win, J. Wood, S. W. Clifton, J. Rogers, G. Van den Ackerveken, J.D.G. Jones, J.M. McDowell,

J. Beynon, B.M. Tyler, Signatures of adaptation to obligate biotrophy in the *Hyaloperonospora arabidopsidis* genome, Science 330 (6010) (2010) 1549–1551.
[28] Y. Dussert, I.D. Mazet, C. Couture, J. Gouzy, M.C. Piron, C. Kuchly, O. Bouchez, C. Rispe, P. Mestre, F. Delmotte, A high-quality grapevine downy mildew genome assembly reveals rapidly evolving and lineage-specific putative host adaptation genes, Genome Biol. Evol. 11 (3) (2019) 954–969.
[29] K. Fletcher, J. Gil, L.D. Bertier, A. Kenefick, K.J. Wood, L. Zhang, S. Reyes-Chin-Wo, K. Cavanaugh, C. Tsuchida, J. Wong, R. Michelmore, Genomic signatures of heterokaryosis in the oomycete pathogen Bremia lactucae, Nat. Commun. 10 (1) (2019) 2645.
[30] K. Fletcher, O.H. Shin, K.J. Clark, C. Feng, A.I. Putman, J.C. Correll, S. J. Klosterman, A. Van Deynze, R.W. Michelmore, Ancestral chromosomes for family peronosporaceae inferred from a telomere-to-telomere genome assembly of Peronospora effusa, Mol. Plant Microbe Interact. 35 (6) (2022) 450–463.
[31] J. Klein, M. Neilen, M. van Verk, B.E. Dutilh, G. Van den Ackerveken, Genome reconstruction of the non-culturable spinach downy mildew Peronospora effusa by metagenome filtering, PLoS One 15 (5) (2020), e0225808.
[32] M. Thines, R. Sharma, S.Y.A. Rodenburg, A. Gogleva, H.S. Judelson, X. Xia, J. van den Hoogen, M. Kitner, J. Klein, M. Neilen, D. de Ridder, M.F. Seidl, G. van den Ackerveken, F. Govers, S. Schornack, D.J. Studholme, The genome of peronospora belbahrii reveals high heterozygosity, a low number of canonical effectors, and TC-rich promoters, Mol. Plant Microbe Interact. 33 (5) (2020) 742–753.
[33] T.B. Bourret, R.A. Choudhury, H.K. Mehl, C.L. Blomquist, N. McRoberts, D. M. Rizzo, Multiple origins of downy mildews and mito-nuclear discordance within the paraphyletic genus Phytophthora, PLoS One 13 (3) (2018), e0192502.
[34] K. Fletcher, S.J. Klosterman, L. Derevnina, F. Martin, L.D. Bertier, S. Koike, S. Reyes-Chin-Wo, B. Mou, R. Michelmore, Comparative genomics of downy mildews reveals potential adaptations to biotrophy, BMC Genom. 19 (1) (2018) 851.
[35] K. Fletcher, O.-H. Shin, K.J. Clark, C. Feng, A.I. Putman, J.C. Correll, S. J. Klosterman, A. Van Deynze, R. Michelmore, Ancestral chromosomes for the Peronosporaceae inferred from a telomere-to-telomere genome assembly ofPeronospora effusa, bioRxiv (2021).
[36] C. Brasier, B. Scanu, D. Cooke, T. Jung, Phytophthora: an ancient, historic, biologically and structurally cohesive and evolutionarily successful generic concept in need of preservation, IMA Fungus 13 (1) (2022) 12.
[37] J.M. McDowell, Genomes of obligate plant pathogens reveal adaptations for obligate parasitism, Proc. Natl. Acad. Sci. U.S.A. 108 (22) (2011) 8921–8922.
[38] S.Y.A. Rodenburg, D. de Ridder, F. Govers, M.F. Seidl, Oomycete metabolism is highly dynamic and reflects lifestyle adaptations, bioRxiv (2020).
[39] H.S. Judelson, A.M.V. Ah-Fong, Exchanges at the plant-oomycete interface that influence disease, Plant Physiol. 179 (4) (2019) 1198–1211.
[40] R.A. Wilson, J.M. McDowell, Recent advances in understanding of fungal and oomycete effectors, Curr. Opin. Plant Biol. 68 (2022), 102228.
[41] J. McGowan, D.A. Fitzpatrick, Genomic, network, and phylogenetic analysis of the oomycete effector arsenal, mSphere 2 (6) (2017).
[42] R.G. Anderson, D. Deb, K. Fedkenheuer, J.M. McDowell, Recent progress in RXLR effector research, Mol. Plant-Microbe Interact. 28 (10) (2015) 1063–1072.
[43] S. Dong, W. Ma, How to win a tug-of-war: the adaptive evolution of Phytophthora effectors, Curr. Opin. Plant Biol. 62 (2021), 102027.
[44] K. Bailey, V. Cevik, N. Holton, J. Byrne-Richardson, K.H. Sohn, M. Coates, A. Woods-Tor, H.M. Aksoy, L. Hughes, L. Baxter, J.D. Jones, J. Beynon, E. B. Holub, M. Tor, Molecular cloning of ATR5(Emoy2) from Hyaloperonospora arabidopsidis, an avirulence determinant that triggers RPP5-mediated defense in Arabidopsis, Mol. Plant Microbe Interact. 24 (7) (2011) 827–838.
[45] K.J. Wood, M. Nur, J. Gil, K. Fletcher, K. Lakeman, D. Gann, A. Gothberg, T. Khuu, J. Kopetzky, S. Naqvi, A. Pandya, C. Zhang, B. Maisonneuve, M. Pel, R. Michelmore, Effector prediction and characterization in the oomycete pathogen Bremia lactucae reveal host-recognized WY domain proteins that lack the canonical RXLR motif, PLoS Pathog. 16 (10) (2020), e1009012.
[46] A.J.E. Pelgrom, C.N. Meisrimler, J. Elberse, T. Koorman, M. Boxem, G. Van den Ackerveken, Host interactors of effector proteins of the lettuce downy mildew Bremia lactucae obtained by yeast two-hybrid screening, PLoS One 15 (5) (2020), e0226540.
[47] T. Ma, S. Chen, J. Liu, P. Fu, W. Wu, S. Song, Y. Gao, W. Ye, J. Lu, Plasmopara viticola effector PvRXLR111 stabilizes VvWRKY40 to promote virulence, Mol. Plant Pathol. 22 (2) (2021) 231–242.
[48] M.S. Mukhtar, A.-R. Carvunis, M. Dreze, P. Epple, J. Steinbrenner, J. Moore, M. Tasan, M. Galli, T. Hao, M.T. Nishimura, Independently evolved virulence effectors converge onto hubs in a plant immune system network, Science 333 (6042) (2011) 596–601.
[49] H. McLellan, S.E. Harvey, J. Steinbrenner, M.R. Armstrong, Q. He, R. Clewes, L. Pritchard, W. Wang, S. Wang, T. Nussbaumer, B. Dohai, Q. Luo, P. Kumari, H. Duan, A. Roberts, P.C. Boevink, C. Neumann, N. Champouret, I. Hein, P. Falter-Braun, J. Beynon, K. Denby, P.R.J. Birch, Exploiting breakdown in nonhost effector-target interactions to boost host disease resistance, Proc. Natl. Acad. Sci. U.S.A. 119 (35) (2022), e2114064119.
[50] H. Garcia-Ruiz, B. Szurek, G. Van den Ackerveken, Stop helping pathogens: engineering plant susceptibility genes for durable resistance, Curr. Opin. Biotechnol. 70 (2021) 187–195.
[51] J. McGowan, D.A. Fitzpatrick, Recent advances in oomycete genomics, Adv. Genet. 105 (2020) 175–228.
[52] S. Oome, T.M. Raaymakers, A. Cabral, S. Samwel, H. Bohm, I. Albert, T. Nurnberger, G. Van den Ackerveken, Nep1-like proteins from three kingdoms of life act as a microbe-associated molecular pattern in Arabidopsis, Proc. Natl. Acad. Sci. U.S.A. 111 (47) (2014) 16955–16960.
[53] M. Tor, M.T. Lotze, N. Holton, Receptor-mediated signalling in plants: molecular patterns and programmes, J. Exp. Bot. 60 (13) (2009) 3645–3654.
[54] J. Kourelis, T. Sakai, H. Adachi, S. Kamoun, RefPlantNLR is a comprehensive collection of experimentally validated plant disease resistance proteins from the NLR family, PLoS Biol. 19 (10) (2021), e3001124.
[55] L. Parra, K. Nortman, A. Sah, M.J. Truco, O. Ochoa, R. Michelmore, Identification and mapping of new genes for resistance to downy mildew in lettuce, Theor. Appl. Genet. 134 (2) (2021) 519–528.
[56] Y. Pecrix, L. Buendia, C. Penouilh-Suzette, M. Marechaux, L. Legrand, O. Bouchez, D. Rengel, J. Gouzy, L. Cottret, F. Vear, L. Godiard, Sunflower resistance to multiple downy mildew pathotypes revealed by recognition of conserved effectors of the oomycete Plasmopara halstedii, Plant J. 97 (4) (2019) 730–748.
[57] G. Bhattarai, W. Yang, A. Shi, C. Feng, B. Dhillon, J.C. Correll, B. Mou, High resolution mapping and candidate gene identification of downy mildew race 16 resistance in spinach, BMC Genom. 22 (1) (2021) 478.
[58] P. Fu, W. Wu, G. Lai, R. Li, Y. Peng, B. Yang, B. Wang, L. Yin, J. Qu, S. Song, J. Lu, Identifying Plasmopara viticola resistance Loci in grapevine (Vitis amurensis) via genotyping-by-sequencing-based QTL mapping, Plant Physiol. Biochem. 154 (2020) 75–84.
[59] E. Peressotti, S. Wiedemann-Merdinoglu, F. Delmotte, D. Bellin, G. Di Gaspero, R. Testolin, D. Merdinoglu, P. Mestre, Breakdown of resistance to grapevine downy mildew upon limited deployment of a resistant variety, BMC Plant Biol. 10 (1) (2010) 147.
[60] R. Stam, B.A. McDonald, When resistance gene pyramids are not durable-the role of pathogen diversity, Mol. Plant Pathol. 19 (3) (2018) 521–524.
[61] J.A. Corwin, D.J. Kliebenstein, Quantitative resistance: more than just perception of a pathogen, Plant Cell 29 (4) (2017) 655–665.
[62] J.A. Berg, F.W.K. Hermans, F. Beenders, L.N. Lou, W.H. Vriezen, R.G.F. Visser, Y. L. Bai, H.J. Schouten, Analysis of QTL DM4.1 for downy mildew resistance in cucumber reveals multiple subQTL: a novel RLK as candidate gene for the most important subQTL, Front. Plant Sci. 11 (2020).
[63] Y. Wang, J. Tan, Z. Wu, K. VandenLangenberg, T.C. Wehner, C. Wen, X. Zheng, K. Owens, A. Thornton, H.H. Bang, E. Hoeft, P.A.G. Kraan, J. Suelmann, J. Pan, Y. Weng, Staygreen, stay healthy: a loss-of-susceptibility mutation in the staygreen gene provides durable, broad-spectrum disease resistances for over 50 years of US cucumber production, New Phytol. 221 (1) (2019) 415–430.
[64] J.A. Henning, D.H. Gent, M.C. Twomey, M.S. Townsend, N.J. Pitra, P. D. Matthews, Precision QTL mapping of downy mildew resistance in hop (Humulus lupulus L.), Euphytica 202 (3) (2015) 487–498.
[65] R. Pyne, J. Honig, J. Vaiciunas, A. Koroch, C. Wyenandt, S. Bonos, J. Simon, A first linkage map and downy mildew resistance QTL discovery for sweet basil (Ocimum basilicum) facilitated by double digestion restriction site associated DNA sequencing (ddRADseq), PLoS One 12 (9) (2017), e0184319.
[66] M.A. Mahmood, R.Z. Naqvi, S. Mansoor, Engineering crop resistance by manipulating disease susceptibility genes, Mol. Plant 15 (10) (2022) 1511–1513.
[67] M. Van Damme, A. Andel, R.P. Huibers, R. Panstruga, P.J. Weisbeek, G. Van den Ackerveken, Identification of arabidopsis loci required for susceptibility to the downy mildew pathogen Hyaloperonospora parasitica, Mol. Plant Microbe Interact. 18 (6) (2005) 583–592.
[68] M. van Damme, R.P. Huibers, J. Elberse, G. Van den Ackerveken, Arabidopsis DMR6 encodes a putative 2OG-Fe(II) oxygenase that is defense-associated but required for susceptibility to downy mildew, Plant J. 54 (5) (2008) 785–793.
[69] T. Zeilmaker, N.R. Ludwig, J. Elberse, M.F. Seidl, L. Berke, A. Van Doorn, R. C. Schuurink, B. Snel, G. Van den Ackerveken, Downy mildew resistant 6 and DMR6-LIKE OXYGENASE 1 are partially redundant but distinct suppressors of immunity in Arabidopsis, Plant J. 81 (2) (2015) 210–222.
[70] M. van Damme, T. Zeilmaker, J. Elberse, A. Andel, M. de Sain-van der Velden, G. van den Ackerveken, Downy mildew resistance in Arabidopsis by mutation of homoserine kinase, Plant Cell 21 (7) (2009) 2179–2189.
[71] C. Pirrello, G. Malacarne, M. Moretto, L. Lenzi, M. Perazzolli, T. Zeilmaker, G. Van den Ackerveken, S. Pilati, C. Moser, L. Giacomelli, Grapevine DMR6-1 is a candidate gene for susceptibility to downy mildew, Biomolecules 12 (2) (2022).
[72] L. Giacomelli, T. Zeilmaker, S. Scintilla, U. Salvagnin, J.R. Van Der Voort, C. Moser, Vitis Vinifera Plants Edited in DMR6 Genes Show Improved Resistance to Downy Mildew, Cold Spring Harbor Laboratory, 2022.
[73] J.A.R. Hasley, N. Navet, M. Tian, CRISPR/Cas9-mediated mutagenesis of sweet basil candidate susceptibility gene ObDMR6 enhances downy mildew resistance, PLoS One 16 (6) (2021), e0253245.
[74] N. Navet, M. Tian, Efficient targeted mutagenesis in allotetraploid sweet basil by CRISPR/Cas9, Plant Direct 4 (6) (2020), e00233.
[75] X. Zhang, Y.C. Low, M.A. Lawton, J.E. Simon, R. Di, CRISPR-editing of sweet basil (Ocimum basilicum L.) homoserine kinase gene for improved downy mildew disease resistance, Front. Genome Ed. 3 (2021), 629769.
[76] T. Li, X. Cheng, X. Wang, G. Li, B. Wang, W. Wang, N. Zhang, Y. Han, B. Jiao, Y. Wang, G. Liu, T. Xu, Y. Xu, Glyoxalase I-4 functions downstream of NAC72 to modulate downy mildew resistance in grapevine, Plant J. 108 (2) (2021) 394–410.
[77] D. Marciano, V. Ricciardi, E. Marone Fassolo, A. Passera, P.A. Bianco, O. Failla, P. Casati, G. Maddalena, G. De Lorenzis, S.L. Toffolatti, RNAi of a putative grapevine susceptibility gene as a possible downy mildew control strategy, Front. Plant Sci. 12 (2021), 667319.
[78] S. Liu, R. Wei, T. Arie, I. Yamaguchi, REMI mutagenesis and identification of pathogenic mutants in blast fungus (Magnaporthe grisea), Chin. J. Biotechnol. 14 (3) (1998) 133–139.
[79] J. Cao, M. Qiu, W. Ye, Y. Wang, Phytophthora sojae transformation based on the CRISPR/Cas9 system, Bio Protoc. 12 (6) (2022), e4352.

[80] G. Fabro, J. Steinbrenner, M. Coates, N. Ishaque, L. Baxter, D.J. Studholme, E. Körner, R.L. Allen, S.J.M. Piquerez, A. Rougon-Cardoso, D. Greenshields, R. Lei, J.L. Badel, M.-C. Caillaud, K.-H. Sohn, G. Van Den Ackerveken, J.E. Parker, J. Beynon, J.D.G. Jones, Multiple candidate effectors from the oomycete pathogen hyaloperonospora arabidopsidis suppress host plant immunity, PLoS Pathog. 7 (11) (2011), e1002348.
[81] A. Woods-Tor, D.J. Studholme, V. Cevik, O. Telli, E.B. Holub, M. Tor, A Suppressor/Avirulence gene combination in hyaloperonospora arabidopsidis determines race specificity in Arabidopsis thaliana, Front. Plant Sci. 9 (2018) 265.
[82] J. Ricaño-Rodríguez, J. Adame-García, S. Portilla-Vázquez, J.M. Ramos-Prado, E. Hipólito-Romero, The extraordinary nature of RNA interference in understanding gene downregulation mechanism in plants InTech 2016.
[83] X. Zhang, T. Lai, P. Zhang, X. Zhang, C. Yuan, Z. Jin, H. Li, Z. Yu, C. Qin, M. Tor, P. Ma, Q. Cheng, Y. Hong, Mini review: Revisiting mobile RNA silencing in plants, Plant Sci. 278 (2019) 113–117.
[84] Q. Cai, L. Qiao, M. Wang, B. He, F.-M. Lin, J. Palmquist, S.-D. Huang, H. Jin, Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes, Science 360 (6393) (2018) 1126–1129.
[85] F. Dunker, A. Trutzenberg, J.S. Rothenpieler, S. Kuhn, R. Pröls, T. Schreiber, A. Tissier, A. Kemen, E. Kemen, R. Hückelhoven, A. Weiberg, Oomycete small RNAs bind to the plant RNA-induced silencing complex for virulence, eLife 9 (2020).
[86] M. Knip, M.E. Constantin, H. Thordal-Christensen, Trans-kingdom cross-talk: small RNAs on the move, PLoS Genet. 10 (9) (2014), e1004602.
[87] O. Bilir, D. Gol, Y. Hong, J.M. McDowell, M. Tor, Small RNA-based plant protection against diseases, Front. Plant Sci. 13 (2022), 951097.
[88] M. Govindarajulu, L. Epstein, T. Wroblewski, R.W. Michelmore, Host-induced gene silencing inhibits the biotrophic pathogen causing downy mildew of lettuce, Plant Biotechnol. J. 13 (7) (2015) 875–883.
[89] Z.M. Haile, D.E. Gebremichael, L. Capriotti, B. Molesini, F. Negrini, M. Collina, S. Sabbadini, B. Mezzetti, E. Baraldi, Double-stranded RNA targeting dicer-like genes compromises the pathogenicity of plasmopara viticola on grapevine, Front. Plant Sci. 12 (2021), 667539.
[90] O. Bilir, O. Telli, C. Norman, H. Budak, Y. Hong, M. Tor, Small RNA inhibits infection by downy mildew pathogen Hyaloperonospora arabidopsidis, Mol. Plant Pathol. 20 (11) (2019) 1523–1534.
[91] G.M. Michael, CHAPTER 12: contributions of population genetics in plant disease epidemiology | population biology of plant pathogens: genetics, ecology, and evolution, Gen. Plant Pathol. (2017).
[92] R. Lyon, J. Correll, C. Feng, B. Bluhm, S. Shrestha, A. Shi, K. Lamour, Population structure of Peronospora effusa in the Southwestern United States, PLoS One 11 (2) (2016), e0148385.
[93] P. Skiadas, J. Klein, T.Q. Monnens, J. Elberse, dR. Jonge, dG.V. Ackerveken, M.F. Seidl, Sexual reproduction contributes to the evolution of resistance breaking isolates of the spinach pathogen Peronospora effusa, 2021, p. 2021.11.05.467349.
[94] H. Van der Heyden, P. Dutilleul, M.-O. Duceppe, G.J. Bilodeau, J.-B. Charron, O. Carisse, Genotyping by sequencing suggests overwintering of Peronospora destructor in southwestern Québec, Canada, Mol. Plant Pathol. 23 (3) (2022) 339–354.
[95] D.H. Gent, N. Adair, B.J. Knaus, N.J. Grünwald, Genotyping-by-sequencing reveals fine-scale differentiation in populations of pseudoperonospora humuli, Phytopathology 109 (10) (2019) 1801–1810.
[96] Y. Rivera, C. Salgado-Salazar, T.J. Gulya, J.A. Crouch, Newly emerged populations of plasmopara halstedii infecting rudbeckia exhibit unique genotypic profiles and are distinct from sunflower-infecting strains, Phytopathology 106 (7) (2016) 752–761.
[97] J.C. Bello, M.K. Hausbeck, M.L. Sakalidis, Application of target enrichment sequencing for population genetic analyses of the obligate plant pathogens Pseudoperonospora cubensis and P. humuli in Michigan, Mol. Plant-Microbe Interact. 34 (10) (2021) 1103–1118.
[98] A. Lebeda, V. Zinkernagel, Evolution and distribution of virulence in the German population of Bremia lactucae, Plant Pathol. 52 (1) (2003) 41–51.
[99] J.E. Thomas, D. Kenyon, Evaluating resistance to downy mildew (Peronospora viciae) in field peas (Pisum sativum L.) and field beans (Viciae fabae L.) Proc. 5th Eur. Conf. Grain Legumes, Dijon., Fr. 1 2004 81 82.
[100] H. Su, A.H. van Bruggen, K.V. Subbarao, H. Scherm, Sporulation of bremia lactucae affected by temperature, relative humidity, and wind in controlled conditions, Phytopathology 94 (4) (2004) 396–401.
[101] M. Göker, A. Riethmüller, H. Voglmayr, M. Weiss, F. Oberwinkler, Phylogeny of Hyaloperonospora based on nuclear ribosomal internal transcribed spacer sequences, Mycol. Prog. 3 (2) (2004) 83–94.
[102] J.S. Lee, H.B. Lee, H.-D. Shin, Y.-J. Choi, Diversity, phylogeny, and host-specialization of hyaloperonospora species in Korea, Mycobiology 45 (3) (2017) 139–149.
[103] Y. Ben-Naim, L. Falach, Y. Cohen, Transfer of downy mildew resistance from wild basil (Ocimum americanum) to sweet basil (O. basilicum), Phytopathology 108 (1) (2018) 114–123.
[104] K. Natesan, J.Y. Park, C.-W. Kim, D.S. Park, Y.-S. Kwon, C.-G. Back, H. Cho, High-quality genome assembly of peronospora destructor, the causal agent of onion downy mildew, Mol. Plant-Microbe Interact. 33 (5) (2020) 718–720.
[105] M. Blanco-Meneses, I. Carbone, J.B. Ristaino, Population structure and migration of the tobacco blue mold pathogen, peronospora tabacina, into North America and Europe, Mol. Ecol. 27 (3) (2018) 737–751.
[106] J.R. Yaege, D.L. Stuteville, Reactions in the annual medicago core germ plasm collection to two isolates of peronospora trifoliorum from Alfalfa, Plant Dis. 84 (5) (2000) 521–524.
[107] H. Dong, J. Tan, M. Li, Y. Yu, S. Jia, C. Zhang, Y. Wu, Y. Liu, Transcriptome analysis of soybean WRKY TFs in response to Peronospora manshurica infection, Genomics 111 (6) (2019) 1412–1422.
[108] D.J. Gaag, H.D. Frinking, Survival, germinability and infectivity of oospores of Peronospora viciae f.sp. fabae, J. Phytopathol. 145 (4) (1997) 153–157.
[109] R. Lukman, A. Afifuddin, T. Lübberstedt, Tracing the signature of Peronosclerospora maydis in maize seeds, Austral Plant Path 45 (1) (2016) 73–82.
[110] S. Sun, S. Lian, S. Feng, X. Dong, C. Wang, B. Li, W. Liang, Effects of temperature and moisture on sporulation and infection by pseudoperonospora cubensis, Plant Dis. 101 (4) (2017) 562–567.
[111] S. Purayannur, D.H. Gent, T.D. Miles, S. Radisek, L.M. Quesada-Ocampo, The hop downy mildew pathogen Pseudoperonospora humuli, Mol. Plant Pathol. 22 (7) (2021) 755–768.
[112] H.C. Kim, K.H. Kim, K. Song, J.Y. Kim, B.M. Lee, Identification and validation of candidate genes conferring resistance to downy mildew in maize (Zea mays L.), Genes 11 (2) (2020).
[113] M.L. Putnam, Brown stripe downy mildew (Sclerophthora rayssiae var. zeae) of maize, Plant Health Prog. 8 (1) (2007).
[114] D. Marciano, V. Ricciardi, E.M. Fassolo, A. Passera, P.A. Bianco, O. Failla, P. Casati, G. Maddalena, G. De Lorenzis, S.L. Toffolatti, RNAi of a putative grapevine susceptibility gene as a possible downy mildew control strategy, Front. Plant Sci. 12 (2021).