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Role of Fungal Effector Proteins for Disease Expression in Plants

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ABSTRACT: The pathogenic fungi are highly diverse and utilize distinct strategies to interact with the host plant. Despite the diversity among these all fungi that colonize plants are recognized by the plant innate immune system, which elicits a host defense response. The innate immune perception triggers both local and systemic reactions, allowing a plant to respond to pathogen attack in a quick and localized manner over an extended period of time. It is accepted that most fungal avirulence genes encode virulence factors that are called effectors which are cysteine rich proteins. These proteins, first discovered because of their ability to trigger the hypersensitive response in resistant plants ("avirulence"activity), were later found to contribute to virulence in susceptible plants, typically host plants that lack effective resistance [*R*] genes. Over the past two decades, several novel *Avr* genes and cognate *R* gene have been discovered. The review provides a brief description of what are effector proteins, their types produced in the case of different host pathogen interaction, the mechanism involved and novel effectors. The focus has been given on effector molecules of biotrophic and hemibiotrophic fungi, responsible for triggering immunity or activating host defense response.

Key words: *Avr* genes, effectors, hypersensitive response, virulence

Pathogenic fungi have diverse growth lifestyles that support fungal colonization on plants. Successful colonization and infection for all lifestyles depends upon the ability to modify living host plants to sequester the necessary nutrients required for growth and reproduction. Secretion of virulence determinants referred to as "effectors" is assumed to be the key governing factor that determines host infection and colonization. Effector proteins are capable of suppressing plant defense responses and alter plant physiology to accommodate fungal invaders. Effectors can be defined as molecules that alter host cell structure and function, facilitating infection (virulence factors or toxins) and/or triggering defense responses (avirulence factors *Avr*).The gene-for-gene hypothesis proposed by Flor states that, for every dominant avirulence (*Avr*) gene in the pathogen, there is a cognate resistance (*R*) gene in the host, and the interaction between their gene products leads to the activation of host defense responses, such as the hypersensitive response (HR), that arrests the growth of biotrophic fungi(Flor, 1942). Since the pioneering genetic research of Flor,many plant pathologists have searched for molecular and biochemical evidence of the gene for gene concept. The molecular cloning of the first bacterial *Avr* gene was reported by Staskawicz *et al.*(1984), the first fungal *Avr* gene by Van Kan *et al.*(1991) and the first oomycete *Avr* gene was discovered by Tian *et al.*(2004). Over the past two decades, numerous novel *Avr* genes and cognate *R* genes have been discovered and our molecular understanding of the gene-for-gene relationship has increased considerably. It is now accepted that plants contain two lines of defense. The first line provides basal defenses against all potential pathogens and is based on recognition of conserved microbial features known as pathogen-associated molecular patterns (PAMPs) by so-called PAMP-recognition receptors (PRRs) that activate PAMP-triggered immunity (PTI) and prevent further colonization of the host (Jones and Dangi, 2006). To successfully facilitate infection, or to establish compatible interactions that lead to proliferation, fungi must be able to counteract PTI. To suppress the immune response and manipulate host cell physiology, plant pathogens secrete effector proteins (Stergiopoulos and de Wit, 2009; de Jonge *et al.*, 2011; Giraldo and Valent, 2013).

One of the best known microbial PAMPs is chitin, a major structural component of fungal cell walls, for

which two LysM-type of receptor-like kinases involved in its perception have been characterized in rice and *Arabidopsis,* respectively. Once the basal defense system of plants is overcome by pathogens, plants respond with the development of a more specialized recognition system based on effector perception by R proteins and subsequent activation of effector-triggered immunity (ETI) that leads to rapid and acute defense responses in plants, the hallmark of which is the hypersensitive response (HR). This triggers a second wave of co evolutionary arms race between pathogens and plants, during which pathogens respond by mutating or losing effectors, or by developing novel effectors that can avoid or suppress ETI, whereas plants develop novel R proteins mediating recognition of novel effectors.

***Avr* gene:** *Avr* genes were first identified by H. H. Flor in 1950.Mild genes of pathogen responsible for activation of certain defense response in host, lead to resistance including hypersensitive response. Effectors are key to the alterations of host structures and they act either in the intercellular space to handle the primary defense response or inside the host cell to execute functions such as reprogramming of the host to favour infection functions during infection (De wit *et al.*, 2009).

***R gene*:** Present in host plant that control a major step in the recognition of the pathogen and play a major role in expression of resistance. It also controls Gene-for-Gene interaction. R gene product can inactivate toxin (De wit *et al.*, 2009).

Effector Protein: Most fungal avirulence genes encode virulence factors that are called effectors. Most fungal effectors are secreted, cysteine-rich proteins, small molecules that alter host-cell structure and function. The Fungal Effector Proteins are of two types (a) Extracellular Fungal Effector Protein and (b) Cytoplasmic Fungal Effector Protein (Humira *et al.,* 2016)

(a) **Extracellular Fungal Effector Protein:** Extracellular effectors are secreted by the pathogen into the apoplast or xylem of their host plant.Some of the examples of extracellular fungal effector proteins are described here.

Cladosporium fulvum

Cladosporium fulvum(syn. *Passalora fulva*) is an asexual extracellular fungal pathogen of tomato. To date, four avirulence (*Avr*) genes have been cloned from *C. fulvum* that all encode small cysteine-rich proteins that are secreted during infection. These are Avr2, Avr4, Avr4E and Avr9 effector proteins whose recognition in tomato is mediated by the cognate Cf (*C. fulvum*) R proteins Cf-2, Cf-4, Cf- 4E, and Cf-9, respectively. Four additional extracellular proteins (Ecps), namely Ecp1, Ecp2, Ecp4 and Ecp5 have been characterized from *C. fulvum* that invoke an HR in tomato lines that carry a cognate *Cf-Ecp*gene, whereas Ecp6 and Ecp7 have also recently been identified (De Wit *et al.*, 1997 and Joosetan and De Wit, 1999). Karimi-Jashni *et al.* (2022) characterised a Ecp 20-2 as a novel effector protein responsible for complete virulence of *Cladosporium fulvum* on tomato.

Fusarium oxysporum* f. sp. *lycopersici

Six effectors *F. oxysporum*f. sp. *Lycopersici* (Fol) is an extracellular pathogen that colonizes the xylem vessels of tomato. Four small proteins, designated Six1 to Six4 (secreted in xylem), have been identified in Fol and are produced during infection. Six1 (currently renamed Avr3) is a small cysteine-rich protein of approximately 32 kDa that is N- and C terminally processed by either fungal and/or plant proteases into a 12 kDa mature protein or an alternative form of 22 kDa, and which is also proven to be an active form of the protein. Avr3 is required for full virulence on tomato, but it also behaves as an Avr factor that triggers HR in the presence of the cognate *I-3* resistance gene. The recent functional characterization of *Avr1* (previously known as *Six4*) might provide the answer to this question. Avr1 is a small cysteine rich secreted protein that confers avirulence to Fol strains on tomato lines carrying the *I* or *I-1*resistance gene. However, unlike Avr3, Avr1 is not required for full virulence of Fol strains on tomato plants that lack the cognate *R* gene (Rep *et al.*, 2004 and 2005).

Magnaportheoryzae

Species-specific *Avr* genes have been cloned and characterized from *M. oryzae*, including *Avr-Pita* (Kang *et al.,* 1995) that was originally called *Avr2-YAMO* (Valent *et al.*, 1991), *Avr1-CO39* (Farman and leong, 1998), *Pwl2* (Sweigard *et al.*, 1995, De Wit *et al.*, 1997), *Pwl1* (Kang *et al.*,1995) and *Ace1* (Bohnert, 2004). Avr-Pita effector encodes a presumably secreted preprotein of 223aa with homologyto fungal zinc-dependent metalloproteases.

Leptosphaeria maculans

At least nine distinct *Avr* genes, designated*AvrLm1* to *AvrLm9*, have been genetically identified in *L. maculans*, the causal agent of stem canker on oil seed rape, that cause avirulence on plants carrying the cognate *Rlm1* to*Rlm9 R* genes, respectively. These *Avr* genes have been mapped to at least four unlinked genomic regions, including the genetic clusters*AvrLm1-AvrLm2-AvrLm6* and *AvrLm3-AvrLm4-AvrLm7-AvrLm9* (Balesdent *et al.,* 2002). Therefore, itis currently unknown whether these effectors interact directly or indirectly with their cognate R proteins and whether recognition occurs outside or inside the host cells. In that respect, AvrLm6 might be secreted and reside in the apoplast as it contains six cysteine residues that could provide stability by forming disulfide bridges. In contrast, AvrLm1 contains only one cysteine residue, making it different fromAvrLm6 and the apoplastic cysteine-rich effector proteins of *C. fulvum* and *F. oxysporum* f. sp. *Lycopersici* discussed above, and therefore, uptake of AvrLm1 into the host cell seems more likely as has been observed for other fungi, *AvrLm1* is absent in races that are virulent on*Rlm1* cultivars (Gout *et al.,* 2006).

Rhynchosporium secalis

Rhynchosporium secalis, the causal agent of leaf scald on barley, secretes three low molecular weight peptides, designated Nip1 to Nip3, that function as nonspecific toxins on barley and several other plants. Nip1 and Nip3 are produced both *in vitro* and in plant, and their presence correlates with the formation of necrotic lesions that are most likely caused by an indirect stimulation of H+-ATPase activity in plant plasma membranes (Wevelsiep *et al.,* 1999). In addition, Nip1 triggers specific (non-HR) defense responses in barley cultivars carrying the *Rrs1* resistance gene (Hahn *et al.,* 1993). *Nip1* (also known as *Avr-Rrs1*) encodes a preprotein of 82 aa, which matures into a 60 aa protein after removal of the signal sequence. Ten cysteine residues are present in the mature Nip1 protein that is involved in five intramolecular disulfide bonds.

(b) Cytoplasmic Fungal Effector Protein

These are also called as Effectors from Haustorium-Forming Fungal Pathogens. Rusts and powdery mildews are obligate pathogenic fungi that produce haustoria in the epidermis (powdery mildews) or in mesophyll cells (rust fungi) of their hosts during infection. Haustoria are not only specialized feedings tructures required for acquisition of nutrients, but they also induce structural, cellular, and biochemical changes in the invaded host cells. Some examples are as follows.

Melampsoralini

The flax rust fungus *M. lini*is an obligate basidiomycete that infects flax (*Linum usitatissimum*) and other species of the genus Linum. To date, *Avr* genes have been cloned from four *M. lini*loci, namely *AvrL567*, *AvrM*, *AvrP123,* and *AvrP4* that code for haustorially expressed secreted proteins (HESPs) and elicit HR in flax plants that carry the cognate *R* genes (Catanzariti *et al.*, 2006).

Blumeria graminis* f. sp. *hordei

Blumeria graminis f. sp. *hordei*(Bgh) causes powdery mildew on barley and interacts with its host in a gene-for-gene manner. Two effector proteins, *Avrk1* and *Avra10* induce defense responses in barley varieties containing the cognate Mlk1 and Mla10R proteins, respectively (Bieri *et al.*, 2004).

Effector Delivery Mechanisms of Fungal Pathogens

Effectors can be defined as molecules that alter host cell structure and function, facilitating infection (virulence factors or toxins) and/or triggering defense responses (avirulence factors: Avr). Regardless of the effector type, efficient delivery of effectors to the plant is required for the infection process. Since pathogenic fungi have developed distinct lifestyles, they have also established diverse effector delivery systems upon infection. Biotrophic and hemibiotrophic fungal pathogens feed and live on living host cells, and secrete effectors that are targeted for the host apoplast or cytoplasm using specialized infection structures such as appressoria or haustoria (Kemen *et al.*, 2005; Catanzariti *et al.*, 2006; Rafiqi *et al.*, 2010; Koeck *et al.*, 2011; Petre and Kamoun, 2014). Obligate biotrophs such as rust fungi or powdery mildew fungi are only capable of propagating on living host tissue. After penetration of the epidermal cell wall, a lobed haustorium develops within the meshophyll (Manners and Gay, 1983; Mackie *et al.*, 1991; Harrison, 1998). The haustorium is completely surrounded by a membrane termed the extrahaustorial membrane, which appears to be an invagination of the plant plasma membrane (Garnica *et al.*, 2014). Interestingly, the biotroph *Ustilago maydis* does not establish bulbous feeding structures similar to the haustoria-forming rust fungi. During penetration, the host plasma membrane invaginates and completely encloses the intracellular hyphae, establishing an extensive area of interaction where the exchange of molecules between the fungus and host occurs (Djamei and Kahmann, 2012; Lo Presti *et al.*, 2015). Hemibiotrophic pathogens utilize abiotrophic phase early in the infection process followed by an ecrotrophic phase killing host cells to complete their lifecycle. For hemibiotrophic pathogen, *Magnaporthe oryzae*, two discrete secretion systems for delivery of apoplastic and cytoplasmic effectors are utilized (Giraldo *et al.*, 2013). For cytoplasmic effector delivery, these proteins appear to accumulate in the biotrophic interface complex (BIC) near the tip of the first bulbous cell formed after host cell penetration. Apoplastic effectors, on the other hand, are not associated with BIC, and once secreted they are dispersed in the extracellular space between the fungal cell wall and the extra-invasive-hyphal membrane (Giraldo *et al.*, 2013; Zhang and Xu, 2014). There have been no studies to elucidate effector delivery strategies employed by the pathogen *Leptosphaeria maculans,* which is a hemibiotroph that causes disease in *Brassica* plant species. However, one might speculate that it uses a mechanism similar to that of *M. oryzae*, where some of the cytoplasmic Avr effectors accumulate at the BIC. One particular review suggested that *L. maculans* was an apoplastic pathogen and unlike BIC-forming hemibiotrophs, it also triggers defense response where maximum expression of the effectors does not occur until after an initial endophytic growth phase (Stotz *et al.*, 2014).

Role of Effector Protein

Disease development: Effector protein plays an important role in disease development and act as a virulent factor in absence of *RGene*. Functional analysis indicates that in some cases effector proteins constitute genuine virulence factors that are required for full virulence of the pathogens on their host plants. Some effector genes are also reported to be clustered on pathogenicity islands, the presence of which iscorrelated with virulence (or avirulence) on specifichosts. Examples are the *Six1 (Avr3)*, *Six2,*and *Six3* genes of *F. oxysporum*f. sp. *lycopersici* that cluster in a genomic region that confers virulence on tomato plants (Van Der *et al.*, 2008).

Defense Activation: In resistance, plant where *R Gene* is present, the effector protein is recognised by them and causes the activation of defense responses HR. However, most plants have developed R proteins that monitor modifications in the plant targets of fungal effectors. In this so-called guard model,R proteins do not interact directly with an effector but guard its host target and respond to alterations in this target caused by the effector, which allows recognition of onlya limited set of structurally related effectors and activation of defense system. Classical example of the guard model is represented by the indirect interaction between Avr2 and Cf-2,

mediated by Rcr3 in the *C. fulvum* tomato interaction (Rooney *et al.,* 2005).

Molecular Basis for Gene for Gene Relationship
On the basis of molecular interactions involved in producing resistant/susceptible responses in the host, the gene-for-gene relationship may be classified into two general groups:

- Incompatible reaction
- Compatible reaction

Incompatible reaction

Found in biotrophic pathogens (obligate parasites) and is associated with hypersensitive response of the host. Since the products of *R gene* (R protein) and *Avr gene (*effector protein) would recognize and interact with each other, it would lead to the activation of the hypersensitive reaction due to programmed cell death (PCD).

Compatible reaction

In this case the product of *avr* gene goes undetected means receptor protein of host is unable to detect the effector protein of the pathogen and disease development takes place and as a result host become susceptible.

Functions and Host Targets of Effectors, Extracellular and Cytoplasmic Fungal Effectors

Fungal effector proteins can be roughly grouped into extracellular effectors that are secreted into the apoplast or xylem of their host plants and cytoplasmic effectors that are translocated into host cells (Humira *et al.,* 2016). Ace1 from *M. grisea* is the only known fungal effector protein that is not secreted by the fungus, but instead, it is suggested to be involved in the biosynthesis of a yet unknown secondary metabolite that is likely to be secreted (Bohnert *et al.*,2004). Despite the low degree of sequence conservation among fungal effectors, most of them code for small secreted proteins, of which some are translocatedinto host cells by a yet unknown mechanism.

The only two members of putatively cytoplasmiceffectors which lack a signal sequence are Avra10 and Avrk1 of *B. graminis* f. sp. *hordei* (Rohe *et al.*, 1995). Extracellular effectors are often N- and sometimes C-terminally processed by the plant and/or fungal proteases, but evidence for protein maturation is in some cases based only on *in-silico* predictions. In that respect, experimental evidence for secretion and processing of fungal effectors during infection is available only for the Avr and Ecp effectors of *C. fulvum*(De Wit *et al.*, 2009).

A second common feature of extracellular effectors, as well as some of the effectors that are active inside host cells, is the presence of multiple cysteine residues. The cysteine residues might be involved in disulfide bridge formation that provides protein stability in the harsh protease-rich environment of the host apoplast. Indeed, disulfide bonds between cysteine residues have been reported to be required for stability and activity of at least Avr4 and Avr9 of *C. fulvum*(Van Den *et al.*, 2003). However, mutational analysis of cysteine residues present in the Ecps from the same fungus suggested that not all cysteine residues are involved in disulfide bridges or are crucial for induction of HR on plants carrying the cognate Cf-Ecp proteins.

Direct and Indirect Interactions between Effectors and R Proteins

Direct interaction: Interaction between the cognate R protein and effector is referred to as the direct interaction eg. Avr-Pita effector interacts directly with cognate Pi-ta receptor protein in rice- *M. oryzae* pathosystem.

Indirect interaction:R proteins do not interact directly with effectors, but the effector binds with host target and this complex is identified by the R protein eg. Indirect interaction between Avr2 and Cf-2, mediated by Rcr3, in the *C. fulvum*–tomato pathosystem.

Molecular mimicry by effectors

A breakthrough discovery showed how the rice-blast fungus *Magnaportheoryzae* produces and secretes an analog of a phytohormone to modulate host immunity (Patkar and Naqvi.,2015).Phytopathogens such as *Fusarium oxysporum* and *Aspergillus flavus* are known to produce *oxilipins,* including the plant JA mimics (Fischer and Keller,2016).Many effectors produce analogs and mimics of plant hormones.

Necrotrophic fungi

Obligate and biotrophic fungi retrieve their nutrients

Table1:Examples of fungal Effector proteins (dE Wit, 2009)

Effector Protein	Function	Role in virulence	R-gene (type)	Localization in plant
1. *Cladosporium fulvum*-Tomato				
Avr2	Protease inhibitor	Inhibits Rcr3 and other proteases	Cf-2 (eLRR TM)	Apoplast
Avr4	Chitin-binding	Protects against chitinases	Cf-4 (eLRR-TM	Not known
Avr4E	-	-	Hcr9-4E (eLRR-TM)	Apoplast
Avr9	Carboxypeptidase inhibitors	-	Cf-9 (eLRR-TM)	Apoplast
Ecp1	Turnor necrosis factor receptor	Disruption leads to Reduced virulence	Cf-Ecp1 Not cloned	Apoplast
Ecp2	-	Disruption leads to Reduced Virulence	Cf-Ecp2 Not cloned	Apoplast
2. Magnaporthe oryzae– rice				
Avr- pita	Homology to Metalloproteases	Not required for virulence on rice	Pi-ta (CCNBS- LRR)	Cytoplasm
Avr-Pita2	Avr-Pita	Not required for virulence on rice	Pi-ta (CCNBS- LRR)	Apoplast
Avr-Pita3	Homology to Metalloproteases	Probably not required for virulence on Rice	-	Cytoplasm
Pwl1	Glycine-rich hydrophilic protein	-	-	Apoplast
Pwl2	Glycine-richhydrophilicprotein	-	-	Apoplast
3. Melampsoralini– flax				
Avr L, 567 (A,B,C)	Unknown	Unknown	L5,L6 and L7	Cytoplasm
AvrM	Unknown	Unknown	M(NBS- LRR)	Cytoplasm
AvrP123	Kazal Ser protease inhibitor	Unknown	P, P1,P2	Cytoplasm
AvrP4	Cystine knotted peptide	Unknown	P4	Cytoplasm
Pwl2	Glycine-rich hydrophilic protein	-	-	Apoplast
4. Blumeria graminis f. sp. *hordei*– barley				
Avra 10	>30 paralogues in bgh and other formae species	Unknown	Mla10	Probably cytoplasm
Avrk 1	>30 paralogues in bgh and other formae species	Unknown	Mlk 1	Probably cytoplasm
5. Rhynchosporium secalis– barley				
Nip1	Non-specific toxin/induces necrosis and plasmamembrane H+ ATPase	Not required for virulence	*Rrs-1* Not cloned	Probably in apoplast
Nip2	Non-specific toxin/induces necrosis	Not required for full virulence	unknown	Probably in apoplast
Nip3	Non-specific toxin/induces necrosis	Not required for full virulence	unknown	Probably in Apoplast

from living host cells, whereas necrotrophic pathogens thrive on killed host cells. As necrotrophic pathogens produce proteinaceous effectors (some also known as host-selective toxins) that promote disease and the host produces receptors that are required for susceptibility. These systems are often seen as a mirror image of the classical gene-for-gene systems found in biotrophic pathosystems, where matching of dominant host and pathogen gene products triggers resistance (Friesen *et al.*, 2008). Karimi-Jashni *et al.* (2022) characterised a Ecp 20-2 as a novel effector protein responsible for complete virulence of *Cladosporium fulvum* on tomato. *Stagonospora nodorum* and *Pyrenophora tritici-repentis* are two necrotrophic fungal pathogens that produce several necrogenic host-specific peptide effectors which can be recognized by host susceptibility genes to cause disease. *Stagonospora nodorum* is a fungal pathogen of wheat, causing *Stagonospora nodorum* blotch (SNB) disease. SnTox1 was the first toxic peptide produced by *S. nodorum* that interacts with a corresponding host susceptibility gene *Snn1* (Friesen *et al.*, 2007; Liu *et al.*, 2006). SnToxA is encoded by a gene with a high degree of similarity to the PtrToxA gene from *P. tritici-repentis*, the causal agent of tan spot of wheat with the matching susceptibility gene *Tsn1*. Tsn1-disrupted mutants were insensitive to both PtrToxA and SnToxA, suggesting that both toxins are functionally similar as they are recognized by the samelocus in the host. Therefore, the Tsn1–ToxA interaction in thewheat–*S. nodorum* pathosystem parallels that of the wheat–tanspot system, and the wheat *Tsn1* gene serves as the major determinant for susceptibility to both SNB and tan spot (Liu *et al.*, 2006).

The SnTox2–*Snn2* interaction was the third gene pair identifiedin this system. SnTox2 is a small secreted peptide of about7 kDa. Sensitivity to SnTox2 is conferred by the single dominant gene *Snn2*. In contrast with the classical gene-for-gene model,the Tsn1–SnToxA and Snn2–SnTox2 interactions are additive in their contribution to susceptibility. SnTox3 is the fourth necrosis-inducing peptide that gives *Snn3*-dependent necrosis. However, unlike the SnToxA–Tsn1 andSnTox2–Snn2 interactions, which both have largely additive effects relative to each other; both the SnToxA–Tsn1 andSnTox2–Snn2 interactions are epistatic to the SnTox3–Snn3interaction (Friesen *et al.*, 2008; Zhang *et al.*, 2009). ToxA was the first peptide toxin produced by the most common races of *P. tritici-repentis*. PtrToxA is a 13.2-kDa proteinthat causes necrosis in particular genotypes of wheat (Ciuffetti *et al.*, 1997). ToxA interacts with a high-affinity binding sitepresent on wheat mesophyll cells through the Arg-Gly-Asp(RGD)-containing, solvent-exposed loop, resulting in toxin internalization causing cell death (Manning *et al.*, 2007). ToxAinteracts with a chloroplast ToxA binding protein 1 (ToxABP1). ToxABP1 contains a lysine-rich region within a coiled-coil domain that is similar to the phosphatidylinositol binding sitespresent in animal proteins involved in endocytosis. ToxABP1protein is present in both chloroplast membranes and chloroplaststroma. Surprisingly, *ToxABP1* is expressed at similar levels and encodes an identical protein in both ToxA-sensitive and ToxA-insensitive cultivars, indicating that ToxA should have othertargets apart from ToxABP1 (Manning *et al.*, 2007).

Just like the effectors of biotrophic fungal pathogens, toxicpeptides from necrotrophic pathogens represent effectors that interact with host targets. However, the effectors of necrotrophic pathogens cause necrosis, facilitating disease, and might thereforebe considered as basic effectors for which no true gene-forgene-based resistance has been developed to date as a resultof a low level of co-evolution. It could be that host targets of effectors of necrotrophic and biotrophic pathogens are intrinsically similar, but that those of biotrophic pathogens have evolved further as a result of co-evolution and no longer inducenecrosis, but manipulate host targets in a gentler manner.

Effectors of Ectomycorrhizal Fungi

Little is known about effectors from ectomycorrhizal fungi that shows both saprophytic and biotrophic lifestyle. Recently, the genome of *Laccariabicolor* was sequenced, which revealed thatthis

basidiomycete shows highest sequence similarity with the sequenced basidiomycete plant pathogens *U. maydis* and *M. lini* (Martin and Selosse, 2008; Martin *et al.*, 2008). The 65-Mbgenome is expected to code for approximately 20,000 proteins, approximately 3000 of which are predicted to be secreted,including approximately 300 effector-type SSPs. A number of these SSPs show homology to HESPs of rust fungi. Some of thegenes encoding SSPs are strongly up-regulated during infection,whereas others are down-regulated, suggesting a complex regulation of SSPs during colonization of the host. Many SSPs arealso cysteine-rich and probably belong to the cystine-knottedpeptides, such as Avr9 of *C. fulvum* (van den Hooven *et al.*,1997). One of those mycorrhiza-induced cysteine-rich SSPs of 7 kDa (MISSP7) was highly up-regulated in the mycorrhizal tipsof mycelium present in the Hartig net. However, additional studies are required to learn more about its function and thatof other MISSPs. Further functional analysis of the *L. bicolor* genome will provide more insight into the differences betweensymbiotic, saprophytic and pathogenic fungi.

Discovery of Novel Effector

Recently, whole-genome sequencing of fungal pathogens has provided an enormous amount of data that can be analysed for the presence of putative (secreted) effectors. Enrichmentfor effector candidates can be achieved by the integration of genome, transcriptome, proteome and metabolome data, when available. Comparative secretome analysis and BLAST sequence similarity searches can also be used to identify putative effectors in sequenced genomes, but will only prove to be successful when sufficient homology exists among effector genes, such as seen for Avr4, Ecp6 and the functional homologue of ToxA peptide from *S. nodorum* in *P. tritici-repentis* (Friesen *et al.*, 2008). In addition, the genome of *L. bicolor* has identified some MISSPs homologous to effectors in rust and other fungi (Martin *et al.*, 2008). However, despite the great potential of genomewide searches for the identification of candidate effector genes, their function still needs to be confirmed experimentally by overexpression, gene disruption or silencing in fungal isolates and subsequent virulence assays on host plants.

Factors that affect Evolutionary Changes in Effectors

With the development of new computational tools for predicting effectors, researchers have focused their attention on how factors such as genome structure and gene transfer impact fungal effector composition. Fungal effector composition seems to be driven by the evolutionary arms race between effector recognition by plant R-proteins, however, where they are located within the genome and how they are transferred from one isolate to another can significantly influence fungal effector repertoire. Interestingly, plant-associated microbes continually evolve new effectors to retain or improve their ability to cause disease and to minimize detection by a plant host. Plants are also compelled to maintain disease resistance and improve their ability to detect pathogen effectors by developing new R-gene allelic varations. The antagonistic cycles of evolution between pathogen effectors and plant R-proteins is described as the co-evolutionary "arms-race" or zigzag model (Jones and Dangl, 2006; Dodds and Rathjen, 2010; Tyler and Rouxel, 2013).
An ongoing change in gene alleles especially effector genes, provides plant pathogens with the ability to evade detection while optimizing or maintaining virulence. To ensure survival, pathogens may have to evolve new effectors to successfully colonize and infect a new host target. Adaptation to hosts is believed to be enabled by compartmentalization of effector genes within the genome. Plant pathogenic fungi and oomycetes contain gene-sparse genomic regions that are exceedingly enriched in repetitive elements and putative effector genes (Raffaele and Kamoun, 2012).

Conclusions and Future Prospects

A short overview of the putative roles of effectors from necrotrophic, ectomycorrhizal and biotrophic fungi and different types of interaction with their hosts is depicted here. Much additional research is

required to confirm or reject the proposed models as they are in part speculative. With the rapid advances in novel sophisticated and efficient sequencing platforms, many fungal genomes will be sequenced in the near future and, with parallel advances in bioinformatics tools, this will speed up the discovery of novel fungal effectors by comparative genomics. However, so far, homology among fungal effectors is limited. For most fungal effectors, a function in virulence can be investigated experimentally by gene disruption, gene silencing or overexpression, and it is expected that the number play a role in the suppression of PTI induced by fungal PAMPs. The discovery of more fungal PAMPs is anticipated and will enrich our understanding of the evolution of the plant defense system and the interplay between PTI and ETI. The finding that some fungal effector genes reside on pathogenicity islands is intriguing and it will be interesting to determine how these islands have arisen during evolution. The elucidation of the mechanisms of translocation and the identification of interactors of fungal effectors in host cells remain major challenge for the future. Future research on effectors of mycorrhizal fungi and of necrotrophic and biotrophic fungal pathogens will reveal whether they represent similar biological functions but only differ in their degree of co-evolution.

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