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Early Pathogenicity events in Plant Pathogenic Fungi: A Comprehensive Review

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ABSTRACT: The mechanism of pathogenesis in fungi involves use of mechanical forces (formation of appressoria and penetration of the host cuticle and cell wall), chemical weapons including enzymes (cutinases, pectinases, cellulases etc) toxins and growth regulators. Pathogen overcomes different host barriers either by quiescence, detoxification phytoanticipins, phytoalexins, ATP binding cassette (ABC) transporters, suppression of active oxygen species, toxins production and by prevention of senescence cytokinin. Plant pathogenic fungi exhibit a huge variability in their mode of infection, differentiation and function of infection structures and nutritional strategy. Successful penetration of living plant tissue by fungal pathogens is preceded by an exchange of signals between both organisms. Recent mutational approaches revealed the importance of cAMP-dependent signaling pathways for fungal development and virulence on their hosts. Plant pathogenic fungi have developed different lifestyles and modes of interaction with their host plants. Some pathogens synthesize and secrete toxic secondary metabolites at first attempt of colonization, kill their host cells and live on the organic compounds.

Key words: Appresoria, Quiescence, Phytoalexins,Active oxygen species.

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INTRODUCTION

Fungal and bacterial pathogens pass through the external protective layers of the suitable host, gain access to the nutrients that it requires for its own growth, development and is preceded by an exchange of signals between both organisms. The mechanism of pathogenesis in fungi involves use of mechanical forces (formation of appressoria and penetration of the host cuticle and cell wall), chemical weapons including enzymes (cutinases, pectinases, cellulases etc) toxins and growth regulators (Agrios, 2005). Moisture is the key environmental factor in pathogenesis and have role in infection cycle in dispersal, spore germination and plant penetration. Other major factor is the ability of pathogenic fungus to detect and response to host cues, such as chemical signals, electrical stimuli pH and host surface chemistry and surface hardness (Dickinson, 2003; Thanh *et al.* 2017; Sharma and Gautam, 2018). Spore dispersal in fungi is mostly a passive process. On contact with host spore germination signaling starts involving sensing of extracellular carbon sources that controls the breakdown of trehalose tightly regulated cAMP PKA pathways using endogenous energy reserves.

Phenomenon like thigmotrophism (biotrophs), mucilage secretion takes place during elongation of germ tube. Induction and development of receptors starts signaling cascade and the signal transducted through a complex, interlinked network of pathways (Phospolipase, Adenylyl cyclase and MAPK) (Caracuel Rios and Talbot 2007). Physical and chemical mechanism involved in pathogenesis includes factor hydrophobins, melanisation, build up of turgor pressure and CWDE, toxins, phytohormones etc. Pathogen overcomes different host barriers either by quiescence, detoxification phytoanticipins, phytoalexins, ATP binding cassette (ABC) transporters, suppression of active oxygen species, toxins production and by prevention of senescence cytokinin (Ebbole, 2007; Ganbari *et al.* 2015). To cause a disease, a pathogen must find a suitable host plant, pass through the external protective layers of the host, gain access to the nutrients that it requires for its own growth and development. Successful penetration of living plant tissue by fungal pathogens is preceded by an exchange of signals between both organisms. Recent mutational approaches revealed the importance of cAMP-dependent signaling pathways for fungal development and virulence on their hosts.

Plant pathogenic fungi exhibit a huge variability in their mode of infection, differentiation and function of infection structures and nutritional strategy. Plant pathogenic fungi have developed different lifestyles and modes of interaction with their host plants. Some pathogens synthesize and secrete toxic secondary metabolites at first attempt of colonization, kill their host cells and live on the organic compounds. Other fungi, in contrast, live on nutrients provided by the living host over extended time period s and do not produce toxins. These contrasting modes of nutrition are referred to as necrotrophy and biotrophy. However, also combinations of these lifestyles and nutritional strategies exist, and pathogens exhibiting an initial and transient biotrophic, followed by a necrotrophic lifestyle, are called hemibiotrophs.

General mechanisms involved in pathogenesis

(i) Mechanical forces: Formation of appressoria and penetration of the host cuticle and cell wall

(ii) Chemical weapons

(iii) Enzymes**:** cutinases, pectinases, cellulases, hemicellulases, ligninases proteinases, amylases, lipases

(iv) Toxins: non-host specific, host specific

(v) Growth regulators: auxins, gibberellins, cytokinins, ethylene, abscisic acid

(vi) Polysaccharides

PATHOGENICITY EVENTS IN PLANT PATHOGENIC FUNGI

A. Attachment of Pathogen to Host

The interaction of foliar fungal pathogens with plants begins with spore attachment to host surfaces and continues with spore germination, host recognition, formation of infection structures, and penetration of host organs. Active adhesion of fungal spores and infection structures to plant surfaces is regarded as an important mechanism in early pathogenesis. Secreted material like the spore tip mucilage detected on mature spores of the rice blast pathogen, *Magnaporthe grisea*, serves for conidial attachment. It contains proteins and lipids as well as a-1,2-mannose disaccharide linked to an unknown non-carbohydrate substituent. Furthermore, extracellular glycoproteins were associated with attachment and also with fungal cellular differentiation.

Pathogens such as mollicutes, fastidious bacteria, protozoa, and most viruses are placed directly into cells of plants by their vectors and, in most cases, they are probably immediately surrounded by cytoplasm, cytoplasmic membranes, and cell walls. However, almost all fungi, bacteria, and parasitic higher plants are first brought into contact with the external surface of plant organs. Before they can penetrate and colonize the host, they must first become attached to the host surface. Attachment takes place through the adhesion of spores and seeds through adhesive materials that vary significantly in composition and with the environmental factors they used to become adhesive. Disruption of adhesion by nontoxic synthetic compounds results in failure of the spores to infect leaves. The propagules of these pathogens have on their surface or at their tips mucilaginous substances consisting of mixtures of water-insoluble polysaccharides, glycoproteins, lipids, and fibrillar materials, which, when moistened, become sticky and help the pathogen to adhere to the plant. In some fungi, hydration of the spore by moist air or dew causes the extrusion of preformed mucilage at the tip of the spore that serves for the immediate adherence of the spore to the hydrophobic plant surface and resistance to removal by flowing water. However, in powdery mildew fungi, which do not require free water for infection, adhesion is accomplished by release of the enzyme cutinase, which makes the plant and spore areas of attachment more hydrophilic and cements the spore to the plant surface. In other cases, propagule adhesion requires on the- spot synthesis of new glycoproteins and it may not reach maximum levels until 30 minutes after contact. In some fungi causing vascular wilts, spores fail to adhere after hydration but become adhesive after they are allowed to respire and to synthesize new proteins. How exactly spores adhere to plant surfaces is not known, but it seems to either involve a very specific interaction of the spore with a host plant surface via lectins, ionic interactions, or hydrophobic contact with the plant cuticle, or involve stimulation of the spore by physical rather than chemical signals. The extracellular matrix surrounding the propagules of many pathogens contains several enzymes, including cutinases, which are expected to play an important role in spore attachment. In any case, the act of attachment often seems necessary for the subsequent transmission of signals for germ tube extension and production of infection structure. It is now clear that many proteins of the fungal cell wall, in addition to their structural role, play an important role in the adhesion of fungi, as well as in the host-surface perception by the fungus.

B. Spore germination and host surface perception **(*Asprgillus nidulans* and *Saccharomyces cerevisae*)**

- Sensing of extracellular carbon source (water or other low molecular weight nutrients)
- Involves non-specific cAMP dependent PKA pathway (sugars and amino acid)

- It controls breakdown of trehalose by activity of trehalase.
- There have been extensive studies in non pathogenic fungi like *Aspergillus nidulans* and *Sccharomyces cervisiae*. It has been shown that the sensing of extra cellular carbon sources controls the breakdown of trehalose by regulating the activity of the enzyme trihalase. Trehalose (alpha D glucopyranosyl alpha D glucopyranoside) accumulates to form up to 15 % of the dry mass of many fungal spores. The breakdown of this trehalase Nth1 in *S. cerevisiae is* tightly regulated by a phosporylation mechanism that involves a cAMP dependent protein kinase (PKA). A similar PKA gene has been identified in the plant pathogen *M. grisea*, which significantly contains the amino acid motif that is proposed to be the target of PKA mediated phosporylation of Nth1. PKA dependent pathway also coordinate changes in the expression of genes encoding components of the protein synthesis machinery during periods of growth resumption or in response to nutritional shifts. This pathway also responds to more specific stimuli of germination. Some examples are presented in Table 1.

Table 1: Host, pathogen/ biotic agent and specific stimuli of germination.

Specific stimuli	Pathogen/ biotic agent	Host
Pisatin	*Nectria haematococca* (*F. solani* f sp. *pis*i)	Pea (in root exudates)
Flavan genictein & Flavanone naringenin	*F. solani* f. sp. *phaseoli*	Bean

However, in model fungi rice physical stimulus and hydration play role in germination and in *M. grisea* hydration play important role in germination. Contact stimulation is clearly an important signal in *B. graminis* f. sp. *Hordei* where elegant studies using silk threads from spiders webs have shown that the point of contact of a conidium with a surface can detect the orientation and emergence of the primary germ tube and subsequent appressorial germ tube.

The conidial response to surface stimulation in *B. graminis* is also immediate and coincident with uptake by the spore of low molecular weight anionic material from the host surface. Conidia may also possess mechanism to prevent germination until such simulation or when in proximity to outer spores'e.g In *M. grisea* lipophilic self inhibition of germination are present in conidial washes and inhibit germination and appressorium development when high concentrations of conidia are placed on surfaces. Germination of all fungal spores requires mobilization of storage reserve s lipids, polyols, and carbohydarted such as trehalose accompanied by polarization and rapid membrane and cellwall biosynthesis during germ tube extension. If appropriate physical and chemical signals are induced resulting in appresorium formation. Germ tube extension and differentiation can occur in response to a number of signals inducing surface hardness, hydrophobilicity, plant signal and surface topography.

GERM-TUBE ELONGATION

A. Thigmotrophism

Once a spore has anchored itself to the plant and the germ tube emerges. The germ tube will grow along the surface on the plant until it receives signals to penetrate. This is a pre-programmed process fuelled by energy reserves from the spore, and most cases, the direction of germ tube growth appears to be an essentially random process. However, there are cases where growth has been shown to be directional and in response to host factors, the best example being the response of rust fungal germ tubes to surface topography in a process known as thigmotrophism**.** Studies using plastic replicas of leaf surfaces have shown that germtubes re-orientate themselves when they contact the ridges between epidermal cells to grow perpendicular to such ridges (Staples *et al.* 1985). This is believed to be a particular advantage for those rusts that grow on cereal leaves, in which the epidermal cells and stomata are arranged in ordered rows, as a means of enhancing the chances of the germ tube locating stomata. The molecular mechanisms involved are unknown, but vesicles accinnulate at the apices of germ tubes in close association with actin filaments and there is a rapid and continuous supply of cell wall precursors to the advancing germ tube tip. In addition, rust germ - tubes differentiate into appressoria in response to the spacing and height of ridges surrounding stomatal lips, a process referred to as thigmotrophism.

The growth of the rust's germ tube across the leaf's surface towards stomata is technically termed thigmotropism or more colloquially contact sensing, which is simply movement in response to the physical stimulus or topography of the leaf's surface. The germ tube grows in response to this topography until it runs out of endogenous nutrient reserves (Hoch & Staples 1987) or until it finds the stomatal guard cells surrounding a stoma (Brand & Gow 2009), which stick up on the order of micrometers from the otherwise constant surface topography.

Upon recognition of the guard cells, a section of hypha swells with protoplasm and forms an appressorium directly over the stoma and an infection peg penetrates the stomatal aperture.

Next a substomatal vesicle forms from the infection peg and continues to elongate into the substomatal cavity, from which haustorial mother cells are formed and penetrate cell walls, after which septate haustoria invaginate cell membranes to retrieve nutrients (Dean 1997, Hoch and Staples, 1987). The second and third steps in this infection process – thigmotropism of germ tubes and differentiation/formation of appressoria – offer a critical point of attack for phytopathologists studying host resistance.

B. Induction of appressorial development

There is increasing evidence that particular physical and chemical characteristics of plant surfaces induce appressorium development, including hydrophohicity, surface hardness, chemical composition, cutin monomers and wax polar lipids. Together, these cues relay the presence of a conducive environment and cause developmental changes in the fungus. Most studies involving development and pathogenicity related gene of fungus have been tagged and isolated using restricted enzyme mediated insertion (REMI). e.g: *PTH11* from *M. grisea*. Based on analogies of mammalian system involvement of intregrins for sensing surface topography and signaling (Tucker *et al.* 2001). Integrins are transmembrane receptors which bind to actin microfilament of the cytoskeleton on their cytoplasmic sides and matrix glycoproteins such as vitronetin and fibrobectin on their extra cellular sides. Perception of environment signals through transmembrane receptor results in conformational change in the protein and interactions with heterotrimeric G protein inside the cell. PTH 11 encodes a putative transmembrane protein located in the plasmalemma.

C. Role of Hydophobins in Germ Tube Elongation

Fungal hydophohins are small proteins of 96-187 amino acids that contain eight cysteine residues arranged in a defined pattern. They are secreted by fungi and undergo polymerisation in response to air-water or hydrophobic surface interfaces. Once polymerised, they form rodlet layers as structural components of spores and hyphal walls that provide a hydrophobic surface presumably as protection against desiccation. Hydrophobins may also play a role in spore dispersal and their abundance and conservation among fungal species indicates that they perform a number of other functions in development.

One of the best- studied hydrophobins is that encoded by the MPG1 gene *M. grisea*. During appressorial formation, the *MPGI* hydrophobin self-assembles at the rice leaf surface providing a layer upon which subsequent appressorium development occurs. *mpg1* mutants are reduced in their ability to form appressoria, although these mutants can be complemented by hydrophohins from other fungi expressed under control of the MPG1 promoter in *M. grisea.* Models have recentlv been proposed in which the *MPG1* protein acts as part of a mechanosensory pathway in the developing appressorium that regulates the developmental pathway. However, such a mechansim is not universal to fungi, since there is no evidence for hydrophohins or rodlet formation in*B. cinerea*, whilst a number of hydophobins have been found in *C. fulvum*, which does not form appressoria. In the fungus causing Dutch elm disease, *Ophiostoma novo-ulmi*, the hydrophobin cerato-ulmin has a different function as a phytotoxtn and transfomation of the Cu gene encoding this hydrophohin into the non-pathogenic fungus *O. quercus*results in its conversion into a virulent pathogen because of this phytotoxicity.

D. Role of MPG1 Gene

During appressorial formation, the MPGI hydrophobin self-assembles at the rice leaf surface providing a layer upon which subsequent appressorium development occurs. *M. grisea* mutants in *Mpg1* hyphae lacking rodlets and were defective in appressorium formation and host infection. Inability of the germ tubes to firmly attach to the hydrophobic plant cuticle and to appropriately sense surface features that leads to appressorium differentiation. *MPG1* act as primer for action of hydrophilic molecules, allowing strong attachment of the germ tube tip prior to appressorium differentiation.Class II hydrophobin Mhp1 was also found to be involved in hyphal surface hydrophobicity and for pathogenesis mhp1 mutants exhibited pleiotropic effects on fungal morphogenesis (Kim *et al*., 2005)

E. Appresorium development: Melanisation

DHN-melanin is a dark fungal pigment produced by polymerisation of l, 8-dihydroxynaphthalene. This monomer is obtained through a series of reactions involving polyketide synthesis in which joining and cyclisation of five acetate molecules occurs, followcd by four subsequent steps alternating reduction and dehydration reactions. In *M. grisea*, three melanin biosynthetic genes have been identified, ALB1, RSY1 and BUF I. which encode a polyketide synthase, a seytalonc dehydratase and a poly-hydroxynaphthalene reductase respectively, and a second napthol reductase gene has also been identified. The role of melanin in the appressorium cell wall is to retard the efflux of glycerol from the appressorium, allowing hydrostatic turgor to build up. Melanin reduces the pore size in the appressorial wall lo less than l nm, which allows water but no larger molecules to pass and retard efflux of glycerol resulting in generation of very high turgor pressure (De Jong *et al.* 1997).

Therefore water influx occurs as osmotically active solutes accumulate. Melanin biosynthesis appears to play a similar role in appressorium-mediated penetration by *C. lagenarimn*, and genes encoding polyketide synthase (PKSl), polyhydroxynaphthalene reductase (THRI) and seylalone dehydratase (SCDI) have been isolated and shown to be required for appressorium mediated penetration. However, similar genes identified in *Alternaria alternata* for 5 regulating melanin production are not involved in appressorial melanisation and penetration, indicating that this is not a universal strategy.

F. Appresorium development: Turgor pressure

Spores of many fungi can germinate in water with no requirement for exogenous nutrients during appressorium morphogenesis. Energy for process if therefore derived entirely from storage compounds within the spores. In *M.grisea*, Glycogen, trehalose and lipids are the major carbohydrate stored in dormant conidia and are the most likely candidate to support the energy requirement for germ tube emergence and appressorium development (Wang *et al.*, 2005). During trehalose breakdown during germtube formation glycerol levels increase to maximum at 48h after conidial germination. Potential source of glycerol would be the disappearance of glycogen granules in appresorium during turgor generation. As a result glycerol concentration reaches to 3.2M and that generates turgor pressure of 5.8 Mpa that is translated into mechanical force allowing penetration peg to rupture plant cuticle.

G. Regulation of G-Protein Activity: signaling

Different subunits of G proteins have been isolated from various fungi. It regulates fundamental events of fungi such as mating, pathogenicity and virulence. Trimeric G protein consists of three protein subunits: alpha, beta and Gamma, induces an exchange of GDP for GTP on the G protein α subunit and dissociation of the α subunit from the $\beta\gamma$ heterodimer. The GTP-α subunit complex mediates intracellular signaling either indirectly by acting on effector molecules such as adenylyl cyclise (AC) or phospholipase C (PLC), or directly by regulating ion channel or kinase function. Targeted disruption of these subunits in fungus *Cryphonectria parasitica* causing chestnut blight as resulted in loss of virulence. G proteins are important intermediate in a number of signal pathway involving protein kinases (Xu *et al*. 2000).

In the phospholipase C pathway, the result is calmodulin mediated activation of a Ca^{2+} dependent protein kinase and calodulin genes have been shown to by induced n *Colletotrichum* species when conidia contact a hard surface. Inhibitors of calcium channels and calmodulin have been found to block induction of thigmotrohism in rust. Calmodulin gene induces by *Colletotrichum* species when conidia contacts hard surface. Inhibition of calcium channels and calmodulin blocks induction of thigmo differentiation in rust (Kronstad *et al.*, 1998).

In second signal transduction pathway the G protein interact with adenylyl cyclase to result in cAMP generation and protein kinase A activation. Genes encoding the catalytic and regulatory subunits of PKAs have been isolated from *M. grisea* and targeted deletions of these have shown a clear delay in appresorial formation and the ability to cause disease.

The third G protein mediated pathway involves mitogen activated protein kinases (MAPK) cascades, which are serine / threonnine kinases that differentially alter the phosphorylation status of transcription factors in the nucleus and alter gene expression. Three MAPK genes have been isolated from *M. grisea*, although two have been confirmed for their role in pathogenicity. PMK1 has been shown to be functional through its ability to complement mutations in the yeast FUS3/ KSS1 MAPK. Pmk 1 mutants in *M. grisea* are unable to differentiate appresoria although they do show normal vegetative growth. Consequently mutants of second *MAPK* gene i.e. *MPS1* for penetration and sporulation are able to form appresoria but cannot penetrate and grow invasively in the host plant. *MAPK* genes have been isolated from different fungi including *Colletotrichum* spp., *Botrytis ceneria*, *Cochliobolus heterostrophus*, *Pyrenophora teres* and *Fusarium oxysporium*.

H. MAPK signaling during infection-related development of M. grisea

Mitogen-activated protein kinases (MAPKs) co-ordinate diverse cellular programs in eukaryotic cells in response to environmental signals (Raman and Cobb, 2003) and in *Saccharomyces cerevisiae*, for instance, five MAPK signal transduction pathways have been extensively studied. In *M. grisea* three distinct MAPK pathways have been identified: PMK1 (pathogenicity MAP kinase), MPS1 (MAP kinase for penetration and sporulation) and OSM1 (osmoregulation MAP kinase) that are homologous to *S. cerevisiae* FUS3/ KSS1, SLT2 and HOG1, respectively. MAPK mediated signalling pathways have now been directly implicated in regulating infection-related development in numerous phytopathogenic fungi, in which functional homologues of the *M. grisea* PMK1 MAPK have been analysed, highlighting the conservation of MAPK signalling as a regulatory component of fungal pathogenicity. In *M. grisea*, the Pmk1 MAPK pathway has been elucidated in considerably greater detail in the past two years.

Gene replacement mutants lacking PMK1 are non pathogenic, do not form appressoria and fail to cause blast lesions on rice plants, even when inoculated directly into plant tissue. A MAPK kinase (MAPKK), Mst7, and a MAPKK kinase (MAPKKK), Mst11, activate Pmk1 in *M. grisea* and Dmst11 and Dmst7 mutants are defective in appressorium formation and are also non-pathogenic (Zhao *et al.* 2005). Expression of a dominant active form of Mst7 restores appressorium formation to a Dmst11 mutant, consistent with Mst7 acting downstream of Mst11 (Zhao *et al.*, 2005). Mst7 has also been shown to be responsible for Pmk1 phosphorylation. Mst7 and Pmk1 appear to interact physically during appressorium development, on the basis of bi-molecular fluorescence complementation and co-immunoprecipitation studies. Deletion of a MAPK-docking site on Mst7 eliminates this interaction and also prevents appressorium formation. The Mst11 MAPKKK contains a sterile a-motif (SAM) domain for protein–protein interactions and associates with the SAM-containingMst50 protein, which may function as the adaptor or scaffold protein for the Mst11-Mst7 signalling module. Deletion of MST50 gene abolishes appressorium formation and pathogenicity (Park *et al.*, 2006). Mst50 also contains a Ras-association domain in its C-terminus and can interact with two *M. grisea* Ras proteins, encoded by RAS1 and RAS2, in addition to Cdc42 and Mgb1, in a yeast two-hybrid assay. Each of these proteins may therefore transmit distinct environmental or developmental signals and interact with Mst50 to regulate appressorium development, acting upstream of the Mst11-Mst7-Pmk1 cascade. The thigmotropic response to the rice leafsurface may bemediated by heterotrimeric G-proteins and associated GPCRs, and a novel Rgs1 regulator of Ga subunits has recently been identified.

Cell wall degrading enzymes (CWDEs). CWDE usually play only an additional role in the penetration. In addition to specialized infection structures by some pathogenic fungi produces certain cell wall degrading enzymes (CWDE) that digest plant cell wall components, or combine both strategies (Park *et al* 2008). In fact, many pathogenic fungi rely mainly on the production of CWDE to enter plant tissue. A large set of CWDE is generated primarily by necrotrophic pathogens because the infection strategy of biotrophs is to keep plant tissue alive (Spanu *et al.*, 2010). Synthesis of CWDE is dependent on the pH of plant cell sap. e.g. *Fusarium moniliforme* produces more polygalacturonases in infected tomato tissues which have acidic cell sap (pH 6.4), while the secretion of pectate lyase is increased during the infection of cauliflower, whose cell sap is alkaline (pH 7.7). Pectinases are the amongst the first CWDEs secreted by plant pathogens upon contact with plants, and in the largest amount. The pectin matrix consisting of homogalacturonan and rhamnogalacturonan with side chains of arabinans, xylano and arabinogalactans is in both the cellwall and middle lamella between cells in plants. The main pectinase enzymes produced by fungi are endo and exo polygalacturonases (PGs) that use hydrolytic cleavage and the pectate lyases (PLs) that use hydrolytic cleavage and formation of a double bond in one of the resultant galacturonate residues. Pectin lyases, polymethylgalacturonases, pectin methylesterases and rhmno galacturonase may also be produced by some fungi. In many cases, particularly for the pectinase, cellulases and cutinases there are multiple genes encoding enzymes with the same function indicating significant redundancy. *e.g. N. haematcocca* there are four functional pectate lyases, *B. cinerea* has five endo-PGs and *Cochliobolus carbonum*and *M. gresia* have atleast four xylanases each. Whilst this may allow the fungus greater flexibility in its pathogenicity it hampers gene kockout experiments aimed at determination a role in penetration. As a result fungus shows greater flexibility in its pathogenicity.

CWDE comprise xylanases degrading hemicelulose, exopolygalacturonases and pectin methylesterases digesting pectin polymers, endoglucanases acting on cellulose, and polysaccharide deacetylases which cleave acetyl substituents in polysaccharide components. Importantly, varied effectiveness of CWDE on cell walls of different plant species is significantly dependent on the pH of plant cell sap. This is consistent with the maximum activity of these enzymes in a given pH. Penetration of plant cell by appressoria of *B. cinerea* that do not possess thick melanin layer is accomplished by the release of hydrolytic enzymes that digest the host cell wall rather than by physical force. *Ustilago maydis*, a biotrophic pathogen of maize is a good example. It penetrates the host cell wall using unmelanized appressoria which in all probability do not operate *via* turgor pressure. Consequently, *U. maydis* depends on hydrolytic enzymes. Examples of some pathogens and their cell wall degrading enzymes are presented in Table 2.

Table 2: Examples of some pathogens and their cell wall degrading enzymes.

Sr. no.	Pathogen	Cell Wall Degrading Enzyme
1.	*N. haematocca*	Four pectate lyases
2.	*B. cinerea*	Five endo PGs
3.	*Cochliobolus carbonum*	Four xylanases
4.	*M. grisea*	Four xylanases

ROLE OF EFFECTOR PROTEINS IN PATHOGENICITY

A. In Oomycetes and fungi

Oomycetes and fungi can secrete a variety of effector proteins intointracellular and intercellular spaces.The oomycetes form a phylogenetically distinct group of eukaryotic microorganisms that includes some of the most notorious pathogens of plants (Kamoun, 2003). Among these, members of the genus *Phytophthora* cause enormous economic losses on crop species as well as environmental damage in natural ecosystems. *Phytophthora* and downy mildews establish intimate associations with plants and typically require living host cells to complete their infection cycle, a process known as biotrophy. An emerging view on oomycete pathogenesis is that the oomycetes accomplish parasitic colonization of plants by reprogramming the defense circuitry of host cells through an array of disease effector proteins. Two classes of effectors target distinct sites in the host plant: apoplastic effectors are secreted into the plant extracellular space, where they interact with extracellular targets and surface receptors; and cytoplasmic effectors are translocated inside the plant cell presumably through specialized structures like infection vesicles and haustoria that invaginate inside living host cells**.** Effectors are defined as molecules that manipulate host cell structure and function, thereby facilitating infection (virulence factors or toxins) and/or triggering defense responses (avirulence factors or elicitors). The majority of the oomycete effectors described here, including the avirulence (AVR) proteins, are so far known only by their ability to activate defense responses and innate immunity.But, these AVReffectors and some of the other defense elicitors have virulence functions of unknown nature.

Green Island. The term 'green island' has been used to describe a ring or spot of living green plant (generally leaf) tissue centred on a site of pathogen infection, which is surrounded by yellow, senescing tissue (Bushnell, 1967). This phenomenon was first described in the 19 th Century, when Cornu (1881) noted that the sites of infection by several obligate biotrophic fungal pathogens (causing powdery mildews and rusts) and oomycete pathogens (causing downy mildews) were associated with pigment retention. In the early 20th Century, the group of fungi that induced green island formation was expanded to include both biotrophic and necrotrophic fungal pathogens (Rice, 1927). However, there are also reports of 'green islands' associated with virus infection (e.g. Atkinson & Matthews, 1967), treatment of leaves with toxins produced by necrotrophic fungal pathogens (*e.g*. Bunkers & Strobel, 1991) and infestation by insects (e.g. leaf miners; Engelbrecht, 1968). The term green island was first used to describe an area of living, green tissue surrounding a site of infection by an obligately biotrophic fungal pathogen, differentiated from neighbouring yellowing, senescent tissue. In leaves infected by obligate biotrophs such as rust and powdery mildew pathogens, green islands are areas where senescence is retarded, photosynthetic activity is maintained and polyamines accumulate. Areas, in which both host and pathogen cells are alive, be termed green bionissia. A range of biotrophic/hemibiotrophic fungi and leaf-mining insects produce cytokinins and it has been suggested that this cytokinin secretion may be responsible for the green island formation. Indeed, localised cytokinin accumulation may be a common mechanism responsible for green island formation in interactions of plants with biotrophic fungi, viruses and insects. Models have been developed to study if green island formation is pathogen-mediated or host-mediated. They suggest that green bionissia on leaves infected by biotrophic fungal pathogens represent zones of host tissue, altered physiologically to allow the pathogen maximum access to nutrients early in the interaction, thus supporting early sporulation and increasing pathogen fitness. They lead to the suggestion that green islands are 'red herrings', representing no more than the consequence of the infection process and discrete changes in leaf senescence.

(i) Green island formation

Green islands are typically associated with infection of host tissues by fungal pathogens which are obligate biotrophs and may also be associated with biotrophic stages of host colonisation by hemibiotrophic fungal pathogens. However, it is questionable whether 'green islands' formed during colonisation by nectrophic fungal pathogens conform to the classic definition of a green island. In this definition, green islands are composed of living host tissues surrounding a site of infection by a biotrophic pathogen such as *Puccinia striiformis*, cause of yellow rust on barley. There are numerous examples of green islands associated with infection of host tissues (often leaves) by biotrophic fungal and oomycete pathogens that cause mildews or rusts on hosts such as wheat, barley, brassicas and faba beans. A common feature of all these green island host-pathogen interactions is that both the host and pathogen are alive. Since such obligate biotrophic fungal pathogens derive their energy for growth and reproduction (sporulation) only from living host cells, this arrangement is mutually beneficial. For such obligate biotrophic pathogens, which possess haustoria for specialised feeding to cause minimal damage to host tissues and cannot grow on dead host tissues or artificial media, it is advantageous to maintain the host alive to provide a longer period for production of new spores. Thus, green islands are typically observed during the later stages of disease development, when sites of pathogen infection remain green whilst surrounding host tissues are yellowing as they senesce and die.

Usually green islands are observed under natural conditions only when disease epidemics are not severe and the density of pathogen pustules is low. Thus, for obligate biotrophic fungi, green islands are associated with compatible host pathogen interactions (Bushnell, 1967). Environmental influences affect the development of green islands incompatible host-pathogen interactions; for example theydevelop when leaf senescence is accelerated by low light intensity, darkness or leaf detachment (Bushnell, 1967).

(ii) Common mechanism for green island formation

Not all greenislands, in leaves infected with pathogenic fungi, are the same. Thus, it is possible to distinguish between green bionissia, where both host and pathogen cells are alive, and green necronissia, where pathogen cells are alive but host cells are dead or dying. Green bionissia are associated with interactions between plants and biotrophic fungal pathogens like powdery mildews and rusts, certain hemibiotrophic fungi (e.g. *P. brassicae*) and certain insects (e.g. leaf miners). Green necronissia are formed in interactions with necrotrophic fungi like *D. gigantea*, *P. teres* and *H. carbonum*, and certain hemibiotrophic fungi (e.g. *R. secalis*). The dark green islands associated with virus infection do not fit easily into this scheme, since although the host cells are alive, the virus is generally absent. Given the differences in the pattern of green island formation in the various interactions, it seems unlikely that a common mechanism is responsible for formation of all green islands. It is possible however, that common, but separate, mechanisms are responsible for the formation of green bionissia and green necronissia. Murphy *et al.* (1997) and Cooper and Ashby (1998) distinguish between biotrophic/hemibiotrophic fungi and necrotrophic fungi: the former produced and secreted cytokinins, while the latter did not. In deed, Ashby (2000) suggested a link between obligate parasitism and cytokinin production. In various insect-plant interactions, especially those involving leaf mining and gall formation, evidence supports a role for cytokinins in green island formation and the source of the cytokinin is the insect larvae (Mapes and Davies, 2001b). The only biotic interaction examined in this review for which no data on cytokinins and green island formationexist is virus infection. Yet there are dark green islands onvirus-infected plants. These contain few, if any, virus particles (Moore *et al.* 2001) and contain substances capable of inhibiting virus replication. Interestingly, exogenous cytokinin inhibits virus replication in plants.

Overcoming host barriers

(a) Quiescence

In some tissues, *B. cinerea* causes long-lasting quiescent infections (Prusky, 1996), in which no symptoms are discernible at first. Prominent examples are described in soft fruit such as strawberry, raspberry and grape. In these hosts, *B. cinerea* predominantly infects the host flowers and resides quiescent in the developing fruit tissue, often for several weeks. Fungal growth resumes at the onset of fruit ripening. It has been postulated that high levels of fungitoxic or fungistatic compounds (phytoalexins) in immature fruits contribute to grey mould quiescence. The level of these compounds decreases during the ripening process concomitant with fungal outgrowth. Attempts have been undertaken to increase the levels of antifungal compounds or to prevent their degradation during ripening. The level of the stilbene phytoalexin resveratrol in grapes is correlated with grey mould resistance. The effect of over-expressing stilbene synthase genes from *Vitis* in transgenic plants on resistance towards *B. cinerea* was evaluated. A significant, partial resistance was obtained in tobacco but not in tomato. Besides phytoalexins, immature fruits usually contain high levels of proteinaceous inhibitors of fungal cell wall degrading enzymes, the PolyGalacturonase Inhibiting Proteins (PGIPs) and their level decreases during ripening .In view of the significant role that polygalacturonases play in the infection (see below), efforts to produce transgenic plants overexpressing PGIPs have been undertaken to obtain resistance towards *B. cinerea*. Indeed high constitutive expression of a heterologous PGIP gene in tomato and an endogenous PGIP gene in Arabidopsis resulted in an increased resistance to *B. cinerea* (Ferrari *et al*., 2003). One of the considerations in this strategy is that PGIPs have a differential activity towards individual 82 fungal endoPGs. This makes it relevant to choose PGIPs that are most potent against the *B. cinerea* endoPG isozymes that are important in virulence.

(b) Detoxification of phytoanticipins

Pathogens have enzymes to degrade constitutive antimicrobial compound of plant. This ability to produce enzymes determines their host range. Examples:

i) Presence of avenacinase in oat colonizing isolates of *Gaeumannomyces graminis.*

ii) Avenacinase is not producing in wheat colonizing isolates.

iii*)* Enzyme has been cloned avinacinase transgenic with disruptive gene losses infectivity in oats.

iv*)* Avenacin deficient oatvarieties are susceptible to such transgenic.

In an experiment it was found that fungal ability to grow on green tomato fruit having high tomatine increases on addition of tomatinase gene to *Nectria haematococco* and addition to *C. fulum* also increases fungal virulence. In addition to detoxification of phytoanticipins through enzymic hydrolysis, the resultant breakdown products induces a signal transduction mechanism in the plants that in suppression of defense response.

Overcoming host barriers: Detoxification of phytoalexins

Most fungal pathogens detoxify best studied pisatin (Isoflavinoid phytoalexin) by pisatin demethyl enzyme (pda/cyp57). Enzyme is involved in degradative and biosynthetic reactions and is best studied in *N. haematococca.* Pisatin demethyl enzyme (pda/cyp57) coded by Cyp gene. Pisatin in plant itself act as primary stimulator of *Cyp* gene during pea pathogenesis. Acts by a 35 bp pisatin responsive element present in the the transcriptional control region of the gene. Also *Pda1* gene of fungus have role of phytoalexin detoxification. Mono oxidase gene (MPK gene) detoxifies medicarpin and maakian in chickpea produces in response to *N. haematococca. B. cinerea* Lacase that detoxify the grape stilbene phytoalexin resveratol. It is also produces a glutathione S-transferase enzyme that may be involved in conjugating toxic compounds to glutathione, thereby removing potentially fungitoxic compounds.

(c) ATP binding cassette (ABC) transporters

An alternative to detoxification of antimicrobial compounds is to transport them out of the cell and the main class of transporter protein in fungi involved in protection against plant defence compounds are of ATP- binding cassette (ABC) type. ABC transporters are transmembrane protein that utilizes the energy of adenosine triphosphate (ATP) hydrolysis to carry out translocation of antimicrobial compounds out of across cell membranes.

In *M. grisea* ABC transporters has been identified by insertional mutation. Screened mutants although pentrate but die during initial colonization *i.e.* susceptibility to plant defense product. The rice phytoalexin sakuranetin can induce expression of the ABC1 gene, although it is not certain whether it is this or other antifungal compounds that are the main substrate for transport A number of ABC transport have been identified in *Gibberella pulicaris* a necrotrophic potato pathogen and *B. cineria* where one of these, BcatrB is induced by the grapevine phytoalexin resvertrol. Disruption of these genes result in isolates with a lower virulence grapevines. Both these and ABC1 transport mechanism from *M. gresia* are strongly induced by azole and phenylpyrrole fungicides. Fungi utilize their ABC transport system to export fungicides and other toxic compounds as part fungicide and multidrug resistance.

(d) Suppression of active oxygen species

Fungi produce suppressors of plant defense responses. In *Mycosphaerella pinodes* infected pea showed increased susceptibility even to non pathogenic fungi. The reason for this is disturbed signal transduction pathway in plants,

(i) Suppression of host defense by producion of specific glycoprotein takes place.

(ii) Delay in production of PR proteins (PAL and chalone synthase) by host.

(iii) In order to scavenge AOS by plants, fungi secrete superoxide dismutase (SOD) and catalase to convert them into less active products. *e.g: S. sclerotiorum* secretes oxalic acid that interferes with signal transduction in plants as mean to suppressing oxidative burst (Heiser *et al*. 1998).

(e) Avoidance of recognition

Fungi use some mechanisms which lead to non recognition of fungal infection structure.In absence of such recognition these recognition there is no host defense activation. Requirement of $CgDN_3$ gene (Nitrogen starvation induced peptides) in *C. gloeosporioides* to avert hypersensitive response of plants. As mutant form normal appressoria but did not infect and reproduce in plants.

Alternate stratergies used by fungal pathogens includes:

(i) *C. lindemuthianum Clh1* gene switches on during initial biotrophic phase and off during necrotrophic phase.

(ii) Same pathogen foms intracellular hyphae than haustoria.

(iii) Protein coded by *Clh1* gene is believed to coat the hyphae to form pseduo cell wall to avoid recognition in beans.

(iv) Rust fungi deacylates chitin (a well known ellicitor) and coats infection structures with chitosan.

Role of toxins

Some pathogens produce phytotoxin which kill plant cells, preventing them from responding in a co-ordinated manner to resist infection. Phytotoxins can be specific or nonspecific: Pathogen- derived molecules. In Race T of *C. heterostrophus* (Southern corn leaf blight), contains two genes polyketide synthase PKS1 and decarboxylase(DEC1) are required for toxin biosynthesis.

Mechanism of action:

T-toxin causes pores in the mitochondria and results in leakage of small molecule causing cessation of ATP synthesis and utimately lead to host cell death (Wolper *et al.* 2002). HC toxin genes is surrounded by two copies of *tox* genes encodes toxins which not only secreted in plants but it also protect the fungus from its effects by MFS transporter mechanism.

Alternaria alternata (AAl toxins) cause death by three ways:

(i) Affecting host plasma membranes to cause potassium and electrolyte leak.

(ii) Affecting uncoupling phosphorylation

(iii) Decreasing photosynthetic mitochondria and carbon dioxide fixation.

Host non- selective toxins

The symptoms caused in plants by host nonselective toxin are often very similar being chlorotic or wilted. This result from the toxin inducing ROS in plants causing membrane breakdown and nutrient leakage (e.g Cercosporin).

These toxins are produced by a number of phytopathogens including *Cladosporium* spp. and *A. alternata*. These toxins are photodynamically active pigment that induces lipid peroxidation in plant cell. They are secreted from fungus *via* MFS transporters and gene encoded toxin. In *Nectria haematococca* production of non selective toxin work through light dependent pathway to form ROS resulting in lipid and membrane damage formation in plant cells by light dependent pathways.

Biotrophy

Use stealth by avoiding recognition by plants therefore non activation of defence genes. The generalized structure of fungal haustoria essentially consists of a tubular neck connected to a haustorial body that invaginates into the host cell. The neck is surrounded by an electron dense neckband that bridges the plant and fungal plasma membranes and appears to act as a seal to prevent the flow of solutes from the extrahaustorial matrix to the apoplast. The Main haustorial body is surrounded by modified fungal wall, extrahaustoria matrix, and extrahaustorial membrane that are derived from plant plasma membrane, but is thicker than normal membranes richer in carbohydrates and appears to lack ATPase activity. Through these structures nutrient and signal between plant and pathogen pass. H+- ATPase plays crucial role in active nutrient transfer. e. g. Rust fungi (Table 3).

Table 3: The growth habits of some common biotrophic fungi.

Epiphytic	
Non haustorial	*Myriogenospora*(tangle top on grass)
Haustorial	*Erysiphae, Blumeria, Uncinula*(Powdery mildew)
Endophytic	
Intercellular hypae (Non haustorial)	*Taphrina deformans*(Peach leaf curl), *Cladosporium fulvum* (tomato leaf curl)
Inter and intra cellular hyphae	*Puccinia* (Monokaryotic form) Rust *Claviceps pupurua* (Ergot), *Ustilago maydis* (maize smut), mycorrhizal fungi (mutualistic)
Intercellular hyphae/ haustorial	*Puccinia* dikaryotic rust, *Uromyces, Bremia* downy mildews, *Pernospora* (downy mildews), Phytophthora *infestance, Albugo candida*
Intracellular hyphae	*Colletotrichum* (anthracnose)

Haustoria function

Different genes and gene product that are specifically associated with haustoria have been identified by isolating haustoria from infected plants and raising antibodies to their constitutive proteins, or making cDNA library of the genes that are expressed in them. Plasma membrane H+ ATPase are believed to play cruicial role in active nutrient transfer.

In Rust fungi currently Hexose transport model through haustoria invokes invertase encoded by fungus that is secreted into the haustoria matrix to converts sucrose into hexoses, glucose and fructose (Mendgen *et al.,* 2000). Mannitol, sorbitol and trehalose are the main glucose storage compounds in fungi and ATPase coupled hexose transporter in the haustoria plasma membrane in combination with hexitol dehydrogenases, transport and convert the hexoses into these. However increase invertase activity during infection by *Albugio candida* in arabidosis with increased level of a host invertase mRNA so it may be that their roles for fungal and host encoded invertases in different interactions.

ATPase coupled transporter are also present in the membrane to transport essential aminoacids for fungi, possibly those that the fungus is unable to synthesise. In some fungi like *C. lindemuthianum* where pathogen switches to necrotrophic infection from biotrophic phase and insertion mutagenesis has reaveled a zinc cluster family transcriptional activator enoded by the *CLTA1* gene that appears to control this switch.

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