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Review Article

Life style of fungi from Biotrophy to Necrotrophy and Saprotrophy

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ABSTRACT
Plant pathogenic fungi causes' economic menace to crop production throughout the world. On the basis of their life styles they may be classified as biotrophs, hemibiotrophs and necrotrophs. For biotrophs it is mandatory to thrive on living host cell and tissues and often found to secrete a little amount of cell wall degrading enzymes and certain effector molecules for suppressing plant host defense mechanism. Necrotrophs survive on dead host cell and tissues which are killed by them before or during infection. Hemibiotrophs in their early stage of life behave as biotrophs and become necrotrophs on later. This article represents the evolution of biotrophs, interaction of biotrophs, hemibiotrophs and necrotrophs with their host plant and continuum of life styles from biotrophy, through to necrotrophy and ultimately to saprotrophy.
Keywords: *Biotrophs, hemibiotrophs, necrotrophs, evolution, effector*

INTRODUCTION

Plant pathogenic fungi have adopted a variety of life styles, from necrotrophy (see Glossary), actively killing the host (Ottmann *et al*., 2009), to obligate biotrophy showing systemic, almost asymptomatic growth (Ploch and Thines, 2011). Obligate biotrophs can become devastating plant pathogens via reproducing through repeated asexual or sexual cycles. Through sexual recombination or somatic hybridization genetic variation is maintained among the population. Based on their morphology during asexual reproduction, obligate leaf pathogens were historically classified as either rusts or mildews, releasing spores in patches through a ruptured epidermis or form conidiophores on the leaf surface respectively. Since, De Bary's first report of biotrophic infection structures in 1863, a wealth of knowledge has accumulated about rusts and mildews caused by both fungi and oomycetes. The recent sequencing of the most important groups of biotrophic plant pathogens, that cause fungal powdery mildews (Spanu *et al*., 2010) and rusts (Duplessis *et al*., 2011), and oomycete downy mildew (Baxter *et al*., 2010) and white rusts (Kemen *et al*., 2011; Link *et al.*, 2011) represents a new opportunity and dimension to lookinto the life style of these pathogens in an evolutionary context. Here we discuss obligate biotrophy and consequences of this life style; similarities and differences of host penetration; the haustorium as a hallmark of biotroph pathogens; and to what extent horizontal gene transfer (HGT) might have influenced the evolution of rust and mildew like pathogens.

EVOLUTION OF BIOTROPHS

Between 1700 and 1200 million years ago (Mya), the ancestors of fungi and oomycetes diverged (Porter, 2004; Chernikova *et al.*, 2011). Oomycetes belong to the clade of stramenopiles, together with brown algae and diatoms, whereas fungi are members of the opisthokonts together with animals (Baldauf, 2008). It is found that evolution of many stramenopiles (previously part of the chromalveolates) involved secondary endosymbiosis with red algae and possibly even green algae (Kemen *et al.*, 2011; Moustafa *et al.*, 2012). Traces of green- and red-algal genes can be identified within stra-menopile genomes (Kemen *et al.*,

GLOSSARY

Biotrophs: 'Obligate parasites developing on another organism, in a personal relationship with its cytoplasm' (The Dictionary of the Fungi, 2001). 'Growths that develop and replicate in living plant tissue while acquiring nutrients through close interactions with living plant cells' (Latijnhouwers et al., 2003). 'Fungi that have absolute reliance on the host to finish their life-cycle, getting nutrients from living host cells by separation of specific contamination structures called haustoria' (Divon and Fluhr, 2006). 'Pathogens that practice to benefit from living plant tissues and some have built up a close relationship with their host plant' (de Wit, 2007). 'Fungi that don't slaughter their hosts and require living cells for development, co-pick homeostasis in the host to create an advantage for the fungus' (Dickman and de Figueiredo, 2011). 'Parasites that get vitality from living cells and are obligate parasites meaning they can't live without their host, have haustoria, don't secrete abundant lytic enzymes and cause little harm to the host plant' (Kemen and Jones, 2012). 'Pathogens that get nutrients from live host cells and produce effectors to supress the basal plant protection and form appressoria to infect epidermal cells produce cell wall degrading enzymes, hyphae to draw the nutrients, and sporulate without killing host cells' (Pandey *et al.,* 2016). for examples powdery mildews (*Blumeria spp.),* downy mildews, rusts.

Hemibiotrophs: 'Fungi that at first build up a biotrophic relationship with their host however along these lines, the host cells kick the bucket as the contamination continues' (Latijnhouwers *et al.,* 2003). 'Fungi that at first form a relationship with living cells of the host, much like a biotroph, and afterward in the later phases of contamination they become necrotrophic, effectively executing host cells' (Plant Pathology Glossary, 2003). 'Fungi having an underlying time of biotrophy followed by necrotrophic hyphae' (Oliver and Ipcho, 2004). 'Fungi that render its host to a great extent alive while building up itself inside the host tissue with brief biotrophic-like stage and later changing to a necrotrophic way of life' (Divon and Fluhr, 2006). 'Organisms that are parasitic in living tissue for quite a while and afterward keeps on living in dead tissue' (Wiktionary, 2016) e.g. *Magnaporthe grisea, Bipolaris sorokiniana, Phytophthora infestans.*

Necrotrophs: 'Pathogens that execute and feed off the dead tissue. All evident necrotrophic pathogens at first have a biotrophic stage in which they asymptomatically colonize the host tissues' (Spanu *et al.,* 2012). 'Pathogens that cause rapid cell death in has and evoke major molecular reactions from the plant and they have wide host ranges and secrete abundant lytic enzymes and poisons' (Meinhardt *et al.,* 2014) e.g. *Pyrenophoratritici-repentis (*tan spot*), Stagonosporanodorum (septorianodurum* blotch*).*

Endophytes: 'All living beings occupying plant organs that eventually in their life can colonize internal plant tissues without causing apparent harm to the host' (Hyde and Soytong, 2008).

Saprotrophs: Organism that feeds on decaying organic matter or dead tissues' (Spanu *et al.,* 2012).

2011; Moustafa *et al.*, 2012). It is still debated and quite confusing if in stramenopiles that do not contain endosymbionts (i.e., non-photosynthetic stramenopiles), algal genes were acquired by HGT or such genes were derived from ancient endosymbionts that were mostly lost during evolution (Stiller *et al.,* 2009; Maruyama *et al.*, 2009). It is believed that convergent evolution led to filamentous organisms, with the ophistokonts and stramenopiles, (around 1500 Mya, Chernikova *et al.,* 2011) and oomycetes (1000–700 Mya, Bhattacharya *et al.*, 2009), respectively. Filamentous fungi and oomycetes evolved parasitic life styles in aquatic systems, infecting various algae (Li *et al.*, 2010; Vischer *et al.,* 2011). Between 850 and 450 Mya, ancestral fungi diverged into the Glomeromycota, Ascomycota and Basidiomycota (Berbee and Taylor, 2010). During this period of radiation, around 600 Mya, fungi started to colonize terrestrial habitats (Redecker *et al.,* 2000), followed by the first land plants around 450 Mya (Rensing *et al.,* 2008). Early land plants relied on close interactions with fungal and fungal-like organisms to colonize their new habitat (Humphrey *et al.*, 2010).

Fossils dating back about 400 Mya show that plants had already evolved defense mechanisms such as encasement layers around intercellular hyphae to defend themselves from filamentous pathogens (Krings *et al.*, 2007). In the period of about 450 million years of co-evolution between plants and pathogens or symbionts, plants faced high selection pressure to refine their non-self-recognition capabilities in order to detect, defend and survive pathogen pressure (Jones and Dang, 2006) while still maintaining beneficial symbioses. Conversely, pathogens and symbionts has also faced high selection pressure to avoid recognition or to suppress host defense, leading to a nonstop arms race between plant colonizing organisms and their hosts (Boller and He, 2009; Ravens *et al.,* 2011). With the advancement in genome sequencing of current obligate biotrophs are being expected the outcome of such evolution. Biotroph ascomycetes like the pathogen *Blumeria graminis*, causing powdery mildew on barley (*Hordeum vulgare*), or the symbiont *Tuber melanosporum*, the Perigord black truffle, show a substantial genome expansion compared to necrotrophic or saprophytic fungi, primarily due to increased transposable element (TE) abundance (Spanu *et al.,* 2010; Martin *et al.,* 2010). In *B. graminis* an increase in loss of genes required for repeat induced point mutations (RIPs) and TE abundance has been observed and has been discussed as a possible hypothesis (Spanu *et al.,* 2010). RIP renders TEs and other duplications within the genome dysfunctional and therefore inhibits spread of TEs. Lack of RIP has been

suggested to be a potential advantage for pathogens, because it could speed up evolution due to genome re-arrangements after TE insertions and gene duplications (Oliver and Greene, 2009). Abundant transposons have also been identified in the genome of the hemibiotroph oomycete *Phytophthora infestans*, where gene sparse regions rich in TEs and repeats have been identified as parts of the pathogen genome where effector genes for host pathogen interaction are found (Haas *et al.*, 2009). Although *P. infestans* most likely lacks RIP, TEs and genes are likely to be silenced by RNA interference (RNAi) (Vetukuri *et al.*, 2011), an important defense mechanism against viruses that leads to degradation of viral RNA (Obbard *et al.*, 2009). Fast reorganization of genome due to gene duplication and TEs might be one mechanism by which some pathogens can speed up their habitation to hosts but paradoxically in other pathogens, like the causal agent of blackleg disease on Brassica crops (*Leptospaeria maculans*), RIP promotes rapid sequence diversification that likely promotes pathogen adaptation (Rouxel *et al.*, 2011). Although some biotroph basidiomycetes, such as rust fungi show an increase in genome size compared to non-pathogenic basidiomycetes (Dupleesis *et al.*, 2011), they have a lower proportion of TEs than biotroph ascomycetes. A similar trend of observation in size differences can be seen in oomycetes also. While the downy mildew pathogen of *Arabidopsis thaliana*, *Hyaloperonospora arabidopsidis*, has an increased genome size correlated with many TEs (Baxter *et al.*, 2010), the genomes of oomycetes that cause white rust on *A. thaliana* (*Albugo laibachii*), and white rust of Brassica crops (*Albugo candida*) are significantly smaller. For the biotroph smut fungus on corn (*Ustilago maydis*) neither RIP nor any other mechanism to inhibit spread of TEs has been identified, yet the genome is small with low numbers of TEs (Kamper *et al.*, 2006). So we can say that sequencing genomes of different taxa revealed different mechanisms that might be due to genome size and effector evolution. Comparing genomes of organisms with rust like phenotypes to those with mildew like phenotypes within their respective phylogenetic group of fungi or oomycetes, reveals that a smaller genome is more often found in rust-like than mildew-like organisms. An exception might be the causal agent of soybean rust (*Phakopsora pachyrhizi*) with an estimated genome size of more than 700 Mbp (http://www.osti.gov/bridge/servlets/ purl/860744-7ClNx8/860744.pdf). As very few species have been sequenced so far and genome size estimates need to be validated, such findings can only suggest early indications, but will influence thinking about which organisms should be sequenced next. Comparative genomics between fungi and oomycetes might reveal new mechanisms for effector evolution and for regulation of TEs and gene duplications within these genomes. Obligate biotrophy, life styles from parasitism to mutualism. Although the first plant fungal interactions may have been symbiotic (Humphrey *et al.*, 2010) it is debatable if pathogenic interactions that we observe today evolved from mutualistic interactions or if each evolved independently. Phylogenetic studies suggest that pathogenicity and there-fore biotrophy evolved independently in different phylogenetic clades of fungi and oomycetes (Thines and Kamun, 2010; Mclaughlin *et al.*, 2009), excluding the hypothesis of a common ancestor of biotrophic pathogens. The availability of genomes of pathogen and symbionts such as the ectomycorrhizal basidiomycetes *Laccaria bicolor* (Martin *et al.*, 2008) or the ascomycete *T. melanosporum* (Martin *et al.*, 2010) enables us to investigate these important evolutionary questions. The genome sequence of *L. bicolor* (Martin *et al.*, 2008), revealed a significant number of genes conserved with the biotroph corn smut fungus *U. maydis* and a significant reduction in cell wall degrading enzymes like cellulases (Martin and Selose, 2008) that might suggest a conserved mode of host cell penetration or recognition avoidance between symbionts and pathogens. Electron microscopy on rust fungi reveals anclose interaction between host and pathogen mediated by haustoria, highly differentiated hyphae that penetrate the cell wall, but stay separated from the host cytoplasm by a differentiated plant plasma membrane (Mendgen *et al.*, 1991). With genome sequences of obligate biotroph organisms available it became apparent that biotrophic organisms show loss of biosynthetic pathways (Spanu *et al.*, 2010; Baxter *et al.*, 2011; Kemen *et al.*, 2011). We can therefore hypothesize that obligate biotrophic pathogens depend on their host, because they lack the ability to synthesize important metabolites (Spanu *et al.*, 2010; Baxter *et al.*, 2011; Kemen *et al.*, 2011). It is therefore not surprising that most attempts to grow and propagate obligate biotroph pathogens on complex culture media have failed (Voeglee and Mendgen, 2011) and if growth was observed in rare cases, growth was extremely impaired (Williams, 1984). A most common property of biotrophs is their ability to make host cells susceptible to pathogens that are otherwise not capable of growing on these hosts (Cooper *et al.*, 2008, Health 1983, Lyngkjaer and Carver 1999), suggesting highly effective host defense suppression. It has been proposed that there are certain effectors molecules that enable pathogens to grow in intimate interactions with their hosts permitting acquisition of sugars, amino acids and cofactors (Raffaele *et al.*, 2010). This leads to relaxed selection pressure in the biotroph pathogen to maintain biosynthetic pathways present in host plants and therefore relaxes selection against gene loss and host-

dependence (Kemen *et al.*, 2011). Independent loss due to effective host adaptation is further supported by the observation that not all obligate biotroph pathogens have lost the same biosynthetic pathways. For example *A. laibachii* and *B. graminis* lost the ability to synthesize vitamin B (Spanu *et al.*, 2010; Kemen *et al.*, 2011) while *Uromyces fabae*, the causal agent of bean rust retained these enzymes (Sohn *et al.*, 2000).

HORIZONTAL GENE TRANSFER

Similarities in virulence and lifestyle between biotroph fungi and oomycetes have long been thought to be a result of convergent evolution, with HGT playing a minor role (Latin *et al.*, 2003). HGT was first revealed between prokaryotes and nematodes, fungi or oomycetes and revealed that genes encoding for secondary metabolites have been transferred (Schmitt and Lumbsch 2009; Danchin *et al.*, 2010). Some of these pathways, such as the lipopolysaccharide biosynthesis that has been identified in oomycetes (Whittaker, 2009) might even change surface properties and pathogen recognition and therefore influence host range. However, oomycetes and other fungi belonging to two different eukaryotic kingdoms, there is evidence for gene transfer from fungi to oomycetes (Richards *et al.*, 2006). On the basis of these findings it has become apparent that HGT has a very great impact on pathogenicity and life style in oomycetes than previously expected (Richards *et al.*, 2011). In particular, a majority of genes acquired by HGT, such as numerous putative secreted lipases and mono- or di-oxygenases, are involved in either attacking or feeding on plant tissue (Richards *et al.*, 2011). In the oomycete *H. arabidopsidi*s, 21 putative fungal-derived genes were identified, with 13 gene products potentially secreted. These points towards a major impact of HGT on the secre-tome and effector complement of biotroph pathogens (Richards *et al.*, 2011). Within the white rust genome, only one fungal- derived gene has so far been identified. This gene shows close homology to a fungal rust protein with unknown function (Richards *et al.*, 2011). We speculate that the reason for not identifying more genes transferred from fungi into oomycete rusts is either the under representation of pathogenic basidiomycetes in the datasets or the lack of a successful integration of genes into the oomycete rust genome by horizontal transfer. The capacity of biotrophs to suppress defence to pathogens that would otherwise not be able to grow on the same host promotes growth of different biotrophs in close proximity. These intimate interactions might promote gene flow not only within species of populations, but also between species of different family. Not much about the exact mechanism of HGT between species is still unclear (Silva *et al.*, 2004).

EVOLUTIONARY PROCESS TO OBLIGATE PARASITISM

- Specialization of haustoria, expansion of effectors and suppressing host defenses.
- Loss of cellular apparatus, expansion of genome size and loss of functional genes.
- Loss of biosynthetic pathway and absolute dependence on the host.

Specialization of haustoria, expansion of effectors and suppressing host defenses:

Fossils show that close interactions between fungi and plants already involved host-cell-embedded arbuscules more than 400 Mya (Remy *et al.*, 1994). Arbuscules are effectively haustoria of symbiotic mycorrhizal fungi that penetrate through the plant cell wall, but stay separated from the host by the invaginated plant plasma membrane, evenafter branching and secondary growth within host cells. This membrane (the peri-arbuscular membrane) shows highly differentiated micro-domains containing symbiosis-specific proteins that are not present in normal plant plasma membranes, indicating specialization (Pumplin and Harrison, 2009). Comparable to arbuscules, haustoria of pathogenic fungi, rustfungi and mildews show secondary growth of haustoria within the host cell, whereas oomycetes haustoria do not shows such growth. Haustoria of biotrophic pathogens stay separated from the host cytoplasm by a membrane throughout their life. This membrane is called the extra haustorial membrane (EHMe).

Effectors are pathogen proteins, secreted in host apoplast or delivered into host cytoplasm to alter host response and suppression of host defense against invading pathogen e.g. *Ustilagomaydis –Hum 2, Hum-3, Pep-1, Magnaportheoryzae – AvrPita, PWL 1, PWL 2.* Effectors (red dots) are secreted into the apoplast, including the extra haustorial matrix, and must cross the extra haustorial membrane (a modified host plasma membrane) before entering the plant cytoplasm, where they may target host proteins to manipulate host metabolism, or can be recognized by host resistance proteins, resulting in the triggering of the host defense response.

A further task of haustoria is the delivery of effector proteins that accumulate within the EHM prior to crossing the EHMe into the host (Kemen *et al.*, 2005; Rafiqi *et al.*, 2010). The binding of effector proteins to

inositol-3-phosphate has been reported (Kale *et al.*, 2010; Yaeno *et al.*, 2011) and suggested as a mechanism involved in the transfer (Kale *et al.*, 2010) though the presence of phosphatidyl inositol-3-phosphatein the EHMe is still not proven. Additional mechanismsmay also occur such as tyrosine-O-sulphate dependent translocation which has been shown for an effector from the fish pathogen *Saprolegnia parasitica* (Wawra *et al.*, 2012). Effectors of different pathogens convergently evolved to target common hubs in the plant immune system (Mukhtar *et al.*, 2011), so a differential inter-atomic network analysis between rusts and mildews is perhaps most likely to reveal why rust fungiare more effective in suppressing defence than mildews and how this is correlated with their lifestyle. In summary, haustoria share many features, but thereare significant differences in intracellular growth between fungi and oomycetes. In terms of defense suppression, there is a significant difference between the causal agentsof mildews and rusts that might result from their penetration mechanism, but common core effectors cannot be excluded.

Loss of cellular apparatus, expansion of genome size and loss of functional genes:

It is being suggested that there is loss of proteins associated with zoospore formation and motility. Lack of adherent cysts is found that normally develop from zoospores during infection. In due course of time, the loss of flagellated spore stage, when fungi left aquatic systems to terrestrial habitats. *Example: Hyaloperonospora arabidopsidis* lost the ability to produce motile zoospores (*Albugo laibachii – white blister rust).* The **gene** encoding the flagellar internal arm dynein 1 substantial chain α is missing.

Gene losses in powdery mildews; Reduced number of genes devoted to secondary metabolism and genes encoding cellulose or hemicellulose degrading enzymes in *Blumeria, U. maydis* and *Puccinia graminis*, also possess reduced enzyme systems for degradation of the plant cell wall (cellulose, xylan, or pectin degrading enzymes). The most unexpected finding in sequencing the obligate biotrophic powdery mildew and rust fungi is the increase in size compared with close relatives that are either non pathogens or non biotrophs. In the oomycetes, this isn't the situation: **Obligate** biotrophs *H. arabidopsidis* and *A. laibachii* have genomes that are both altogether littler than those of related *Phytophthora spp*. larger genomes are not accompanied by larger numbers of structural genes. One possible explanation for this conundrum is that any increase is invariably the result of increased proliferation of transposable elements detectable as repetitive DNA, and that transposable elements can determine genetically heritable variation independently and additionally to sexual recombination (Biemont and Vieira, 2006). An increase in genetic polymorphism may have given competitive advantage to pathogens that need to adapt constantly to changes in host immunity brought about by natural selection in a prototypical Red Queen evolutionary scenario (Van, 1973). These advantages may be particularly acute in organisms such as the powdery mildews and the rusts, which rely on a rapid succession of asexual generations during their epidemics and thus miss out on the power of sexually induced recombination when it is most needed. There are alternative ways of generating variation. *M. graminicola* may have depended on the introgression of a dispensome as a vault of variation. The smuts have advanced a decreased genome size, however are obligate sexual organisms: Each round of contamination requires mating. At every generation, meiotic recombination has the capability of generating variation. Repetitive DNA is effectively excluded by the homologous recombination mechanisms, which are exceptionally developed toward eliminating repetitive DNA (Holliday, 2004). This makes their genomes highly streamlined. In the oomycetes, where there is no correlation between genome size and biotrophy, the effector genes and other genes involved in pathogenicity appear to be specifically contained in hyper variable areas of the genome (Hass *et al*., 2009; Raffaele *et al*., 2010).

The gene losses observed in a large number of the (obligate) biotrophs are then an outcome of dynamic retro transposition as observed in the regions of the *H. arabidopsidis* genome that are syntenic with the flagellum-related genes of zoosporicoomycetes (Kemen et al., 2011). These losses are at first tolerated in plant pathogens that no longer require certain functions (e.g., inorganic nitrogen uptake) but are essentially irreversible, in a manner very close to that described by Dollo's law of irreversibility (Marshall *et al*., 1934). Once these losses include loss of the regulatory network capacity to adapt metabolism in the absence of a host (in axenic culture), biotrophs become obligate. This hypothesis now needs to be tested.

Loss of nitrate, nitrite reductase and nitrate transporter from the syntenic region of *Hyaloperonospora arabidopsidis from Phytopthora spp.* Similar gene loss also reported in rust fungi *Melampsora populinalarici* and *Puccinia graminis f. sp. tritici.* Powdery mildew fungi *Blumeria graminis*, *Erysiphepisi* and *Golovino mycesorontii.* Likewise loss of sulfite reductase in rusts was also reported in rust fungi *Melampsora populina larici* and *Puccinia graminis f.sp. tritici. (*Spanu *et al.*, 2010)

Loss of biosynthetic pathway and absolute dependence on the host:

The absence of various genes encoding enzymes and transporter required for the biosynthesis of various metabolites may also be the reason for this extraordinary biological compatibility. (de Wit, 2007; Meadows, 2011; Kemen and Jones, 2012; Delaye *et al.*, 2013; Guzman and Heil, 2014). The fungus *Albugo laibachii* the causal agent of white rust and *Hyaloperonospora arabidopsidis* causing downy mildew disease have lost the genes which codes for nitrogen and sulfur biosynthetic pathways (Meadows, 2011). Powdery mildew causing fungi such as *Blumeria spp.* have lost the genes encoding enzymes for anaerobic fermentation (pyruvate decarboxylase, alcohol dehydrogenase), biosynthesis of glycerol from glycolytic intermediates and biosynthesis of nitrate and thiamine (Spanu *et al.*, 2012; Spanu *et al.*, 2010) state that barley powdery mildew, *Blumeria graminis* and two other powdery mildew species, *Erysiphe pisi* causing powdery mildew on *Pisum sativum* and *Golovinomyces orontii* causing powdery mildew on *Arabidopsis thaliana*), have lost the genes encoding enzymes for primary and secondary metabolism, carbohydrate-active enzymes, and transporters. These missing genes are referred to as 'missing ascomycete core genes' because they are found in other ascomycetes. Through various research it has been found that these missing genes are expressed in hemibiotrophic *Colletotrichum higginsianum* during its biotrophic phase (Spanu *et al.*, 2010). This suggests that, the lack or absence of 'missing ascomycete core genes' is must for biotrophs for e.g. fungi causing powdery mildew disease, but it is not so for hemibiotrophic fungi.

To obtain nutrients from the cells of host plants, many biotrophic fungi have evolved specialized hyphae haustoria. Haustoria are differentiated or specialized hyphae with spherical or lobed structures that penetrate the leaf mesophyl cell wall and grow adjacent to the plasma membrane, without entering into the cytoplasm (Latijnhouwers *et al.*, 2003; de Wit, 2007; Kemenand Jones, 2012; Delaye *et al.*, 2013; Kabbage *et al.*, 2015). Both *et al.* (2005) found that, in *Blumeria graminis* (powdery mildew of barley) haustoria take up glucose from the epidermal cells of plant to synthesize glycogen for the formation of conidia. In addition, some biotrophs have extra-haustorial membranes that separate the haustorium from the plant cytoplasm (Latijnhouwers *et al.*, 2003; Horbach *et al.*, 2011; Kemenand Jones, 2012). This type of nutrient absorbing system is important to acquire essential nutrients from the cytoplasm for nutrients enrich micro-environment (nutrient sink) between the haustorium and the host cell membrane (de Wit, 2007; Delaye *et al.*, 2013). Some endophytes such as *Disculaumbrinella* and *Rhabdoclineparkeri* however, produce haustoria during the early death of infected cells (Delaye *et al.*, 2013). For the colonization, fitness and assimilation of nutrient by fungi, the production of haustoria is very essential (Parniske, 2000; Divon and Fluhr, 2006; Kemen and Jones, 2012).

INTERACTIONS BETWEEN BIOTROPHS AND HOST PLANTS

Biotrophs have a close relationship with their host plants (Both *et al.*, 2005; de Wit, 2007; Gao *et al.*, 2010; Delaye *et al.*, 2013). Generally, a biotrophic fungus such as rust or powdery mildew pathogen are unable to grow in auxenic cultures or in lab and requires a living host to complete their life cycles (Parniske, 2000; Both *et al.*, 2005; Meadows, 2011). They have limited ability to synthesize cell wall degrading enzymes (Kabbage *et al.*, 2015). The fungi *Blumeria graminis* causing powdery mildew disease in barley has reduced production of carbohydrate active enzymes which is responsible for plant cell wall degradation (Spanu *et al.*, 2010). These fungi also produce various types of secondary metabolites which work as pathogenicity factors. Polyketide synthetases, modular non ribosomal peptide synthetases, terpenecyclases, and dimethyl allyl diphosphate tryptophan synthases are some of the key enzymes involved in the biosynthesis of secondary metabolites in fungi. Only two enzymes are however recorded from *Blumeria graminis* namely polyketide synthases and modular non ribosomal peptide synthetases (Spanu *et al.*, 2010). These facts indicate that biotrophic fungi have lost many genes for pathogenesis. These characters might be important keys to maintain long term interactions with living host plant cells, while avoiding detection of the fungus as a pathogen by the host plant (Micali *et al.*, 2008; Kemen and Jones, 2012).

INTERACTIONS BETWEEN HEMIBIOTROPHS AND HOST PLANTS

In contrast to biotrophs, hemibiotrophs have dual life-styles. They first establish biotrophic relationships with their hosts and subsequently switch to necrotrophic relationships (Oliver and Ipcho, 2004; Divon and Fluhr, 2006; Krola *et al.*, 2015). GAL4 like transcriptional activators in hemibiotrophs encoded by the CLTA1 gene are involved in reprogramming host cell metabolism and thereby switch the life-style from biotrophic to necrotrophic (Oliver and Ipcho, 2004; Krola *et al.*, 2015). The initial biotrophic lifestyle of

hemibiotrophs causes minimum damage to the plant tissues, while the fungus obtains nutrients from living plant tissues (Latijnhouwers *et al.*, 2003). Generally all hemibiotrophic fungi develop haustoria, but some also produce intracellular hyphae to absorb nutrients from the host cytoplasm (Oliver and Ipcho, 2004; Divon and Fluhr, 2006). Even though, the hemibiotrophic life-style later breaks down host cell walls through secretion of cell wall degrading enzymes and the fungi survive on the released nutrients (Latijnhouwers *et al.*, 2003; Kabbage *et al.*, 2015). They also produce extracellular hyphae between the host cells to facilitate nutrient assimilation (Latijnhouwers *et al.*, 2003; Oliver and Ipcho, 2004).

INTERACTIONS BETWEEN NECROTROPHS AND HOST PLANTS

Fungal diseases pose constant threats to the global economyand food safety. Being the largest group of plant fungal pathogens, necrotrophic fungi cause heavy crop losses all over the world. The molecular mechanisms of the interaction between necrotrophic fungi and plants are complex and involve sophisticated recognition and signaling networks. From the recent studies on necrotrophic fungi, the roles of phytotoxin and proteinaceous effectors pathogen-associated molecular patterns (PAMPs) and small RNAs has been comprehended. We also consider the functions of damage-associated molecular patterns (DAMPs), the receptor-like protein kinase BIK1, and epigenetic regulationin plant immunity to necrotrophic fungi. Toxin effectors from necrotrophic fungi can target a host's central signal regulator to trigger R gene-mediated resistance and to thereby increase host susceptibility to attack by necrotrophic fungi. Chitin, PGs, SCFE1, and other PAMP effectors secreted by necrotrophic fungi can be recognized by RLPs or RLKs, and such recognition triggers a series of PTI responses. Although necrotrophic fungi can secrete enzymes thatdegrade the host cell wall, some of the degradation products, i.e., DAMPs, act as elicitors that trigger host immune responses. By binding to the host RNAi machinery, small RNAs delivered by necrotrophic fungi into host cells can act as virulenceeffectors that suppress host immune responses. Although PAMPs/DAMPs are initially recognized by distinct upstream PRRs, the immune signaling pathways triggeredby those PRRs may converge on a central regulator like BIK1 and SOBIR1. By regulating the expression of defense genes, epigeneticmodifications, including DNA methylation and histonemodifications, play important roles in plant immunity to necrotrophic fungi.

SAPROPHYTIC FUNGI

The word saprophytic (sapro - rotten material, phyte - plant) is misnomer when fungi are considered, since fungi are not plants (this term was used before, when fungi were considered to be members of the plant kingdom). It would be better to say saprotrophic fungi. Saprotrophic fungi are those fungi that have an extracellular digestion mechanism for putrefying organic matter (originating from dead or decaying organisms) and in this way absorbing the essential nutrients for their growth and reproduction. Many fungal species are saprobes, in fact every species which is not a parasite or symbiont (which, in contrary, obtain nutrients from other living). Oyster mushrooms (*Pleurotus ostreatus*), shiitake mushrooms (*Lentinu laedodes*), Rhizopus, Mucor, Aspergillus, Penicillium, Agaricus, Morchella etc. are the examples ofsaprophytic fungi.

CONCLUSIONS

Biotrophy is a pervasive trait that evolved independently in plantpathogenic fungi and oomycetes. Ever expanding reservoirs of effectors will require us to locate the most essential targets for genetic resistance or chemical controls.Genes that are not necessary for growth on a plant disappeared, butwe still do not know what lost functions make some of these pathogens obligate. The evolution of biotrophs associated with expansion of genome with transposons and loss of genes coding for several nutrient metabolism and toxin production. The journey of evolution, they gained ways to overcome host defenses and losing the ability to make nutrients. Pathogen lost the ability of several metabolisms (N, S and thiamine), reduced expression secondary metabolism and gained several host-defense modification effectors resulting in biotrophic life style. The role of effector proteins in sustaining biotrophic interaction with the host cell and mechanism of manipulating host physiology need to be explored. Rapidly evolving effectors due to transposable elements and genome compartmentalization become serious threat to agro-eco system.Understanding the mechanisms of virulence is instrumental for designing management strategies.

The degree to which fungal and oomycetes rusts show similarities during host invasion, host colonization and asexual sporulation is not restricted to morphological features. Similarities can also be seen within the genome structure and the proteome. It is questionable whether these similarities are only due to convergent evolution and unknown to what extent HGT has contributed to similarities such as nutrient uptake and virulence mechanisms and what are the ways in which pathogens colonize their hosts. Rare transfer of genes from fungi to oomycetes is now clear, whereas gene

transfer from oomycetes to fungi remains to be demonstrated. It may be hypothesize that host

penetration through stomata might have an evolutionary advantage over direct penetration in terms of avoiding recognition. A high degree of gene loss in powdery mildews compared to other biotroph pathogens may reduce their capacity for adaptation even further. Sequencing further genomes will help to enable further and more robust insights into genome evolution of obligate biotroph pathogens.

From the above discussion it may be concluded that fungi exhibited continuum of life style from biotrophy to necrotrophy and ultimately to saprotrophy. However, it is still confusing and difficult task to place each and every fungus in a clear cut boundary. For better understanding of the life style more cytological, molecular studies, more genome sequencing and a better investigation of host pathogen interaction is required.

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