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Olive – *Colletotrichum acutatum*: An Example of Fruit-Fungal Interaction

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1. Introduction

Plants are continuously exposed to an extensive array of environmental biotic and abiotic stresses. Among the first ones, viruses, bacteria, fungi, nematodes and insects are the causal agents of the most serious plant diseases. In some cases the interactions between host and pathogen result in the loss of agricultural yield that is often linked to product quality decrease (Bhadauria et al., 2010; Bray et al., 2000; Montesano et al., 2003; Zipfel, 2008). The best and most effective approach for increasing crop yield is to enhance the production efficiency and to reduce agricultural yield losses due to various plant diseases and several other stress factors. However, plants are under strong evolutionary pressure to maintain surveillance against pathogens. Part of this success is because plants have evolved a variety of sophisticated responses that recognise compounds produced and/or are released by the pathogens (elicitors) and employ these, to trigger defence signalling, normally designated by innate immunity (Montesano et al., 2003; Parker, 2009; Zipfel, 2008). Moreover, to grow in their natural environment, some crop genotypes naturally defend themselves against pathogen infection through the development of a series of morphological, physiological and molecular changes, which are all controlled by functional genomic networks. For instance, the interaction between fungal cell wall and plant surface is the beginning of compatible interaction establishment. Plant cuticle is the region where fungal infection structures differentiated, and the plant/fruit invasion is initiated. The molecular recognition of fungal cell wall may cause stress reactions and activate host’s defence mechanisms. Pathogen stresses are one of the most significant damaging factors that limit the development and consequently, decrease the yield and quality of many crops. Understanding the pathogen infection mechanisms is crucial in an integrated analysis in order to target specialized functions as signalling, defence responses, and cell death, among others. Nowadays, the use of model plants may help to target candidate genes in other crop species, decreasing, therefore, the amount of work required in unknown genomes. This chapter is a review of the state-of-the-art concerning plant-pathogen interaction focused on: (i) plant defence
responses (e.g., levels of host defence, pathogen molecules), (ii) plant defence signalling (e.g., molecular recognition, gene expression), and (iii) *Olea europaea* L. and *Colletotrichum acutatum* strategies in susceptible and resistant olive cultivars.

### 1.1 Compatible and Incompatible Interactions

The resistance in plants has been defined as an incompatible interaction between plant and pathogen. In general, an incompatible interaction involves the plant recognition processes that prevent or retard the pathogen growth, and spread it through plant cells. On the other hand, a compatible interaction is when plant disease occurs, due to an inadequate defence response of the host against the pathogen in terms of timing and intensity (Casado Díaz et al., 2006; Mysore & Ryu, 2004). In many plant species one of the most typical symptoms of defence response is the rapid plant cell death at the infection site, the hypersensitive response (HR), which limits pathogen from spreading to other cells (Dangl et al., 1996; Glazebrook, 2005; Oh et al., 2006). Van Der Plank (1966) reported two categories of disease resistance in plants: vertical and horizontal resistance. Vertical resistance (monogenic or oligogenic) protects hosts against only one pathogen race. This complete resistance is conferred by a few genes, or even it can be based on a single gene pair, i.e., host *R*-gene activation by pathogen avirulence gene (*avr*). The horizontal resistance (polygenic) or incomplete resistance is conditioned by many genes with minor effect. In the review work Mysore & Ryu (2004) proposed that non-host resistance against bacteria, fungi, and oomycetes can be also classified into: (i) type I, which does not result in visible cell death and (ii) type II, in which a hypersensitive response occurs, resulting in cell death at the infection site. The host resistance has been studied intensively in different pathosystem, while non-host resistance remains poorly understood (Oh et al., 2006). However, many aspects related to the gene expression patterns in some host-pathogen interaction have not yet been cleared.

### 1.2 Functional genomic

Functional genomics in plant-pathogen interaction involves studies that reveal the complex networks of host stresses perception and signal transduction, leading to the multiple defensive responses to pathogens (Langridge et al., 2006; Sreenivasulu et al., 2007; Vij & Tyagi, 2007). Functional genomics involves the development of global experimental approaches in order to analyse gene function, in contrast to structural genomics, where the entire nucleotide sequence of an organism’s genome is determined (Hieter & Boguski, 1997). The purpose of functional genomics is to understand the genes function, how cells work, how cells form organisms, what goes wrong in disease and how components work together to comprise functioning cells and organisms (Lockhart & Winzeler, 2000). Advances on functional analysis considering genome, proteome and metabolome of an organism, together with the potential of bioinformatics and microscopic tools, enable scientists to assess global gene and, protein expression, and metabolite profiles of some damaged tissues, allowing a better understanding of the plant response mechanisms. The knowledge of complete genomic sequence of different crops is, sometimes the only way to gain access to the entire set of genes. The genome sequencing of the first higher plant, thale cress (*Arabidopsis thaliana*), provides nowadays an excellent model species to study host plant stress responses and to identify target genes for biotechnology applications (Bevan & Walsh,
2005; Zhang et al., 2006). For instance, cDNA from strawberry differently expressed upon challenge with the pathogen Colletotrichum acutatum has recently been isolated, and showed similarity to (At WRKY75) defence genes in Arabidopsis thaliana (Casado-Díaz et al., 2006; Encinas-Villarejo et al., 2009). With the development of high-throughput sequencing technologies, the number of genomes sequenced has been increasing fast. The Oryza sativa L., Triticum aestivum L., Zea mays L., Vitis vinifera L., Glycine max L., and Fragaria vesca L. genome sequencing have been extremely successful in the discovery of new genes. Similarly to plants, the knowledge of the pathogens genome is very important to monitor global changes that occur during plant-fungal interactions. Several fungal genomes have been now sequenced, being some of them extremely important in terms of yield lost in key crops such as Botrytis cinerea (grape grey mould, and other host species), Fusarium graminearum (cereal head blight), Fusarium verticillioides (corn seed rot), Magnaporthe oryzae (rice blast), Mycosphaerella fijiensis (banana black leaf streak), Septoria tritici (wheat leaf blotch), Puccinia graminis (cereal rust), Phytophthora ramorum (sudden oak death) and Phytophthora sojae (soybean stem/root rot) (Bhadauria et al., 2009).

2. Plant defence responses

2.1 Pre and post-invasive levels in plant-pathogen interaction

Disease resistance in plant-pathogen interactions requires sensitive and specific recognition mechanisms for pathogen-derived signals in plants. Plants lack mobile defender cells (like animal antibodies) and a somatically adaptive immune system (Jones & Dangl, 2006; Palma et al., 2009). However, they are equipped, at least, by two levels of defence: a pre-invasive, which is expressed at the cell wall and apoplastic spaces level aiming to prevent pathogens penetration, and a post-invasive, which mediates resistance relatively to pathogen that have successfully penetrated plant cells, and often results in a localized cell death at the infection site.

The physical barriers are the first level of a general plant defence against pathogens invasion, and include waxy cuticular skin layers, the plants’ cell wall and actin cytoskeleton that play a key role in penetration resistance (Dangl & Jones, 2001; Kobayashi & Kobayashi, 2007). The plants’ cell wall is one of the sites where the changes, due to the defence response, can be observed. These can include cell-wall thickening and lignification, papilla formation, phenolic compounds accumulation, phytoalexins and other secondary metabolites, as well transcriptional activation of pathogenesis-related proteins (Anand et al., 2009; Bhadauria et al., 2010; Montesano et al., 2003; Salazar et al., 2007; Shan & Goodwin, 2005; Zipfel, 2008). The papilla structures were observed on blueberry fruits inoculated with Colletotrichum acutatum, and it was formed beneath subcuticular hyphae and represented one of the host defence responses (Wharton & Schilder, 2008). If passive defences, such as the cell wall, is overcome by the pathogen, active defence responses are triggered a long lasting systemic response (systemic acquired resistance, SAR) which confers to the plant resistance against a broad spectrum of pathogens (Thordal-Christensen, 2003).

Primary or basal immune defence response is induced by perception of molecules called pathogen-associated molecular patterns (PAMPs or MAMPs) (Dangl & Jones, 2001; Jeong et al., 2009). The pathogen-associated molecular patterns (PAMPs) are recognised by the plant innate immune systems through receptor proteins called pattern recognition receptors.
(PRRs), and include cold shock protein, flagellin, lipopolysaccharides of Gram-negative bacteria, lipids, elongation factor (EF-Tu), enzyme superoxide dismutase, peptidoglycan, and chitin of fungi (Bent & Mackey, 2007; Chisholm et al., 2006; Jeong et al., 2009; McDowell & Simon, 2008; Rafiqi et al., 2009; Zipfel, 2008). Perception of PAMPs occurs through PRRs located on the cell surface which could activate a chain of intracellular defensive signalling pathways, including the activation of a mitogen-activated protein kinase (MAPK) signalling cascade. When PAMPs are recognised by PRRs the PAMP-triggered immunity (PTI) system can halt microbial growth. In general, the PRRs are constituted by an extracellular leucine-rich repeat (LRR) domain, and an intracellular kinase domain.

The second molecular level of plants defence (post-invasive) occurs after the effectors pathogen invasion. The effectors secreted by pathogens into host cells are recognised by intracellular nucleotide-binding (NB)-LRR receptors which induce effector-triggered immunity (ETI). Successful pathogens have evolved effector molecules that target virulence effector proteins (Avr) that can overcome host defensive pathways. In a dynamic co-evolution between plants and pathogens, some plants have evolved disease resistance proteins (R) to recognise these effectors directly or indirectly, and activate an effective immune response like activation of localised cell death at the pathogen infection sites, called the hypersensitive response (HR) (Kim et al., 2008; Lindeberg & Collmer, 2009; Zipfel, 2008). The gene-for-gene resistance model, proposed by Flor (1971), requires that Avr-protein recognise the corresponding R-protein, which is accompanied by localised cell death. This effect triggers a cascade of signal transduction events that include rapid ion fluxes, extracellular oxidative burst, changes of phosphorylation status, induction of salicylic acid, and localised transcription reprogramming at the infection site (Dangl & Jones, 2001; Dangl & McDowell, 2006; Kim et al., 2008; Palma et al., 2009). Although they have been documented in others pathosystem in the olive fruits infected by Colletotrichum acutatum no reports considering these events have been made. New perspectives on plant defence signal transduction mechanisms have been given by the discovery of novel intracellular perception of pathogen effector proteins. The events of pathogen effectors recognition are mediated by plant NB-LRR proteins and allows resistance defence response to pathogens (Dodds & Rathjen, 2010). The plant NB-LRR proteins are able to recognise pathogen effectors through diverse pathways: (i) direct recognition, (ii) guard and decoy models, or by (iii) bait-and-switch model, and translate these interactions into a defence response (Collier & Moffett, 2009; Dangl & Jones, 2001; Dodds & Rathjen, 2010; Rafiqi et al., 2009; Van der Hoorn & Kamour, 2008). All models illustrated the diversity of perception mechanisms employed by plants to detect the broad variety of pathogen effectors, and activating plant defence responses to infection. In pathogen and plant direct recognition, the effectors triggers immune signalling by physical binding to the NB-LRR receptor. The LRR domain is considered the major determinant of perception and recognition specificity (Rafiqi et al., 2009). The guard and decoy models report a modification on an accessory protein which is then recognised by NB-LRR receptor. In bait-and-switch model the effector interacts with an accessory protein associated with NB-LRR, and then a recognition mechanism occurs between the effector and NB-LRR protein in order to trigger signalling. However, none of these models are completely understood. Thus we face the need to expand our knowledge on how plant immune receptors are activated by effector recognition and how the resistance signal is triggered.
2.2 Plant defence signaling molecules

Various signalling molecules mediate the expression of pathogenesis-related proteins, which can interfere with the plant resistant to a pathogen attack. The defence protein products include peroxidase, polyphenol oxidase, which catalyzes the formation of lignin and phenylalanine ammonia-lyase, involved in phytoalexin and phenolics biosynthesis (Salazar et al., 2007). Some pathogenesis-related proteins such as chitinase, and β-1,3 glucanase have potential antifungal activity which degrade the fungal cell wall and cause fungal cells lyses. Co-induction of chitinases, peroxidases, γ-thionins and β-1,3-glucanases gene expression, during pathogen infection, has been described in several plants, including wheat, strawberry, potato, soybean, maize, tobacco, tomato, bean, and pea, among others (Bettini et al., 1998; Casado-Díaz et al., 2006; Cheong et al., 2000; Lambais & Mehdy, 1998; Li et al., 2001; Liu et al., 2010; Petruzzelli et al., 1999; Vogelsang & Barz, 1993). Defence responses can also be mediated by endogenous signalling molecules such as salicylic acid, jasmonic acid, and ethylene (Encinas-Villarejo et al., 2009; Mysore & Ryu, 2004). Plant hormones have been reported to have a role in induce plant defence responses, operating in two major defence pathways in plants, depending on salicylic acid or jasmonic acid and ethylene, and conferring resistance to different pathogens (de Vos et al., 2005; Dempsey et al., 1999; Jones & Dangl, 2006; Métraux, 2001). Salicylic acid confers resistance to host plants, especially against biotrophs and hemibiotrophs pathogens, whereas jasmonic acid and ethylene signalling contributes to resistance against necrotrophs pathogens (Ausbubel, 2005; Chisholm et al., 2006; Glazebrook, 2005). An incompatible interaction has been reported (Lee et al., 2009), where salicylic acid protects unripe fruit of pepper against Colletotrichum gloeosporioides infection through the inhibition of appressorium development. Genes encoding ethylene and jasmonic acid biosyntheses and indole-3-acetic acid regulation were found to be highly induced in citrus flowers during Colletotrichum acutatum infection (Lahey et al., 2004; Li et al., 2003).

2.3 Colletotrichum spp. as pathogenic fungi of Olea europaea L.

Fungi are the causal agents of most serious disease and are one of the pathogens that are able to breach the intact surfaces of hosts, rapidly establishing infections that can result in significant agricultural yield loss (Bhadauria et al., 2010). Colletotrichum species comprises a diverse range of important plant pathogenic fungi that cause pre- and postharvest crop losses worldwide. Colletotrichum acutatum and Colletotrichum gloeosporioides have been reported as causal agents of olive anthracnose, which is a major disease of cultivated olive orchards. The Olea europaea L. was domesticated in the Mediterranean region where it has a huge eco-social role. However, due to its importance as a crop it has spread to other Mediterranean climates worldwide (Gutiérrez & Ponti, 2009). Like other crops, olive is susceptible to a large number of diseases some of which are causing considerable damage to the olive orchards worldwide. Without the support of pest-control chemicals, such as fungicides or herbicides, crops are increasingly exposed to a range of biotic attacks. As for Olea europaea L. one of the main problems, concerning long-term cultivation, is the fungal contamination such as Colletotrichum acutatum, Colletotrichum gloeosporioides, Fusarium oleagineum, Phytophthora megasperma, Rhizoctonia, Verticillium dahliae, Pseudomonas syringae pv. savastanoi, and the pests such as Bactrocera oleae, and Prays oleae (Sergeeva & Spooner-Hart, 2009; Tsitsipis et al., 2009).

Olive anthracnose was reported for the first time in Portugal in 1899 by Almeida (1899) which classified the Gloeosporium olivarum as the causal agent of olive disease. In 1957, Von
Arx reported that *Colletotrichum gloeosporioides* was the species responsible for olive anthracnose. Latter, Simmonds introduced *Colletotrichum acutatum* in 1965 as one of the pathogen responsible for olive anthracnose. Research focused in host–pathogen interactions developed in Greece, Italy, and Spain has provided new insights into olive disease identification, and *Colletotrichum gloeosporioides* (originally: *Gloeosporium olivarum*) was reported as the primary cause of olive anthracnose (Mateo-Sagasta, 1968; Zachos & Makris, 1963). It has also been very difficult to discriminate between *Colletotrichum acutatum* and *Colletotrichum gloeosporioides* by traditional taxonomical methods. Morphologically, *Colletotrichum acutatum* and *Colletotrichum gloeosporioides* are very similar because of their host range overlapping and the wide variability found among their pathotypes. Considerable progress has been made in *Colletotrichum* species identification. In Portugal, Talhinhas et al. (2005) using molecular approaches, and Carvalho et al. (2003) with Potato dextrose agar enzyme linked immunosorbent assay (PDA-ELISA) reported that *Colletotrichum acutatum* is the predominate species in olive orchards, and consequently responsible for olive anthracnose. Nowadays, *Colletotrichum acutatum* is the dominant species in both olive growing regions: Alentejo in Portugal, and Andalusia in Spain (Moral et al., 2009; Talhinhas et al., 2005, 2009). Recently, molecular tools such as random amplification of polymorphic DNA (RAPD), species-specific primers for *Colletotrichum acutatum* isolates using internal transcribed spacer (ITS) region of rDNA sequences, and other regions of the genome have been effectively used to clearly discriminate *Colletotrichum acutatum* from *Colletotrichum gloeosporioides* and to identify genetically distinct subgroups of *Colletotrichum acutatum* (Peres et al., 2005; Talhinhas et al., 2005, 2011). Other characters, such as growth rates and sensitivity to benomyl fungicide, have been helpful to differentiate between *Colletotrichum acutatum* and *Colletotrichum gloeosporioides* (Peres et al., 2005).

Besides olive crop, *Colletotrichum acutatum* is a major constraint in global food production as it causes many of the world’s most devastating diseases in cereals, grasses, legumes, vegetables, perennial crops and a number of fruit trees (Bailey et al., 1992; Peres et al., 2005; Wharton & Diéguez-Uribondo, 2004). This species can infect all plant surfaces, but favours the young leaves, small branches and fruits of herbaceous species growing in a humid microclimate (Peres et al., 2005; Wharton & Diéguez-Uribondo, 2004). In different regions of Portugal the *Colletotrichum acutatum* severely affects olive orchards leading to a decrease in olive oil production and quality, which compromises the protected denomination of origin (PDO) of Portuguese olive oils (Fig. 1). The *Colletotrichum acutatum* can affect up to 100% of the fruit on a olive tree during humid (or rain) autumns where susceptible olive cultivars are grown (Casado-Díaz et al., 2006; Freeman et al., 2002; Garrido et al., 2008; Peres et al., 2005; Talhinhas et al., 2011; Trapero & Blanco, 2008). Moreover, a poor quality and low stability olive oil is obtained from olives harvested in areas affected by anthracnose, presenting alterations in oil color (red), high acidity and other typical organoleptic characteristics (Carvalho et al., 2006; Moral et al., 2008; Talhinhas et al., 2009, 2011) (Fig.1).

In Portugal the olive oil production employs more than 400 000 people and the average yield is about 42 000 ton/year. The area occupied by olive trees is more than 340 thousand of hectares. In the last ten years, an expansion of olive tree planting area by Portuguese farmers was observed. This was as a result of an increasing of 2.5% in the demand of olives in the world. During the same period of time, in Portugal, the consume of olive oil was recuperated from 3.3 Kg to 7.0 Kg per capita. Beyond the improvement of national production/consume of
Fig. 1. Olive fruits infected by *Colletotrichum acutatum*. (a) Olive fruits without pathogen infection; (b) olive fruits with *Colletotrichum acutatum* infection, and (c) 192 hours after inoculation olive fruit is completely destroyed by pathogen infection.

Olive oil, there was also an increase in exportations of about 19%. Nationally, the prevalent olive cultivar is ‘Galega’ that gives to olive oil a good specificity when compared with olive oils from others cultivars. This characteristic is unique in the world and for that reason it is a national imperative to preserve ‘this heritage’ at all cost. However, this cultivar has a disease resistant problem, related to its high susceptibility to olive anthracnose caused by the *Colletotrichum acutatum*, known in Portugal as ‘gafa’ disease. This disease is very aggressive and is one of the main constraints affecting both the Portuguese olive oil production and unique characteristics. The *Colletotrichum* species are known to produce enzymes that degrade carbohydrates and thus dissolve plant cell walls (e.g., polygalacturonases, pectin lyases and proteases) and hydrolyze fruit cuticles (Wharton & Diéguez-Uribeondo, 2004). In a response, several plants have evolved inhibitor proteins (PGIPs) that specifically recognise and inhibit fungal polygalacturonases (Mehli et al., 2004). Recently, a new report concerning the reduction of *Colletotrichum acutatum* infection by a polygalacturonase inhibitor protein extracted from apple, has been provided (Gregori et al., 2008).

The penetration of the plant tissue is always a crucial event for plant-pathogen interaction and the success of colonization depends on the ability of the pathogen to retrieve the nutrients from the host. In some host-pathogen interactions, such as olive fruits and pepper, the fruit index maturation may compromise the success of colonization (Lee et al., 2009; Moral et al., 2008). The resistance of immature fruits to colonization by *Colletotrichum* species can be related with the sugar content which is a non-suitable substrate to fulfil the nutritional and energy requirements of the pathogen (Wharton & Diéguez-Uribeondo, 2004). Additionally, there are a large number of secondary metabolites such as alkaloids, tannins, phenols and resins, which create a hostile and toxic environment for pathogens growth due to their anti-microbial activity (Dixon, 2001). Preformed fungi toxic compounds in unripe avocado fruit were reported to inhibit the *Colletotrichum gloeosporioides* growth...
Phytoalexins have also been identified in *Capsicum annum* L. anthracnose, caused by *Colletotrichum capsici* and *Glomerella cingulata*. There are few reports about phytoalexins production after *Colletotrichum* species infection. The most recently, an evidence of phytoalexins production was observed on unripe blueberry fruits inoculated with *Colletotrichum acutatum* (Wharton & Diéguez-Uribeondo, 2004).

Fig. 2. The *Colletotrichum acutatum* life cycle on *Olea europaea* L. fruits. All *Colletotrichum* spp. produce acervuli with abundant spores that are rain-splashed and serve as an inoculum source for futures infections. Spore germinates to form appressoria, and quiescent infections can be established on fruits, small branches and/or leaves. Long biotrophic phase on unripe fruits are observed during spring, and summer. Necrotrophic phase appears primarily on ripe fruits and then on leaves and branches (adapted from Peres et al., 2005; Trapero & Blanco, 2008).

### 2.3.1 *Colletotrichum acutatum* life cycle on olive fruits

The early events of basic pathogenicity and susceptible interactions between olive cultivars and *Colletotrichum acutatum* as well as lifestyle of the *Colletotrichum* species are
still not understood, and therefore needs to be explored. The *Colletotrichum* species life cycle comprises a teleomorph (sexual) and an anamorph (asexual) stages. However, in many plant-fungal systems asexual phase has still not been found, even on economically important crops like olive tree. The *Colletotrichum* species life cycle is summarized in Fig. 2. In general, the sexual stage accounts for the fungus genetic variability while the asexual is responsible for fungal spore formation and appressorium development. The *Colletotrichum* spores are essential for the fungus dispersal (Peres et al., 2005; Wharton & Diéguez-Uribeondo, 2004). Specific structures or developmental stages, such as acervuli formation, spore germination and appressorium formation are distinguished during the *Colletotrichum* species life cycle.

### 2.4 *Colletotrichum acutatum* and olive fruits interaction

To establish a compatible interaction with a host, the fungal pathogen does not only need to overcome host physical barriers, but also they have to establish a feeding relationship with the host. The early events in *Colletotrichum* spp. infection process are very similar among the different hosts. Frequently, the host-pathogen interaction includes (i) spore adhesion to the host surface, (ii) spore germination, (iii) appressorium development and (iv) fungal growth through the colonization of the hosts' tissues (Fig. 3). Spores are embedded in a matrix of moist hydrophilic mucilaginous material including glycoproteins, lipids and polysaccharides. These compounds are essential not only for spore adhesion, but also for their protection against desiccation, physical damage, and host defence responses. After spore germinate a short germ tube is generated which finally differentiate into an appressorium with a penetration pore (Fig. 3).

The appressorium with penetration pore are a penetration structure that is formed after germ tube germination, and allows many pathogens to enter into the host cells. Pathogens may force their way through plant surfaces by different means; some take advantage of natural doors, such as stomata or lenticels; some others enter through wounds or directly through cuticle (Bailey et al., 1992; Gomes et al., 2009; Jong & Ackerveken, 2009). In order to infect plants, fungal pathogens have developed a wide variety of infection strategies: (i) *Necrotrophs*, pathogen kill the host and feed from the cell contents; (ii) *Biotrophs*, require a living host to complete their life cycle; and (iii) *Hemibiotrophs*, act as both biotrophs and necrotrophs at different stages of infection. Most of the *Colletotrichum* species are hemibiotrophs with different spans of their biotrophic phase, and may also undergo a period of quiescence in order to overcome resistance mechanisms (Gomes et al., 2009; Peres et al., 2005). Two types of interaction between *Colletotrichum* species and their hosts have been reported: intercellular hemibiotrophy and subcuticular intramural necrotrophy (Gomes et al., 2009; O’Connell et al., 2000; Perfect et al., 1999) (Fig. 4).

In intercellular hemibiotrophic infections, a symptomless biotrophic phase is followed by a destructive necrotrophic one, during which symptoms become apparent. Examples of pathogens employing this infection strategy are *Colletotrichum graminicola* Politis & Wheeler, 1973). *Colletotrichum gloeosporioides* (Ogle et al., 1990), and *Colletotrichum lindemuthianum* (Mercer et al., 1975; O’Connell et al., 1985). In the subcuticular intramural infection strategy, rather than penetrating the epidermal cell wall, the fungus grows under the cuticle and within the periclinal and anticlinal walls of epidermal cells (Arroyo et al., 2007; O’Connell et
al., 2000; Wharton & Diéguez-Uribeondo, 2004). Colletotrichum phomoides (Bailey et al., 1992), Colletotrichum capsici (Pring et al., 1995) and Colletotrichum circinans (Bailey et al., 1992) behave in this manner. On olive fruits, a combination of both strategies has been reported, and results in intra- and intercellular colonization of fruits (Gomes et al., 2009) (Fig. 4). In this pathosystem Colletotrichum acutatum used hemibiotrophy and subcuticular colonization strategies to colonize ‘Galega’ that is Colletotrichum acutatum susceptible olive cultivar and ‘Picual’ that is Colletotrichum acutatum resistant olive cultivar.

Fig. 3. Colletotrichum acutatum infection structures in olive fruits. (a) Massive adhesion of fusiform spore on the susceptible cultivar (Galega) was observed at 48 hours after inoculation (hai). (b) Germinated and ungerminated spore on the fruit surface 48 hai; a penetration pore has differentiated under a mature appressorium and internal light spot (ILS) was observed at 72 hai. (c) Conidia (with a septum at the equatorial zone (arrow)) developed a germ tube at the end of which a pigmented fluorescing appressorium differentiated within 72 hai. (d) The development of one or more secondary conidia simultaneously was observed at 48 hai. Scale bar represents 20 µm in panel (a) 10 µm in panels (b) and (d) and 5 µm in panel (e) CL - host cuticle; MS - mesocarp; C - conidium; A - appressorium; ILS - internal light spot; PP - penetration pore; GT - germ tubes; SP - septum; NC - new conidial formation.
Fig. 4. Intracellular hemibiotrophic-like infection structures in infected olive fruit. Penetration of epidermal cells and fungal development inside the host were observed. (a) The host membrane (arrow) encloses a globose infection vesicle without damaging the host plasma membrane. (b) and (c) Subcuticular infection vesicles were observed in susceptible ‘Galega’ (b) and resistant ‘Picual’ (c) olive fruit. During the early events of infection, hyphae grow inside the host cell wall of ‘Galega’ without penetrating the lumen. (c) Development of hyphae with internodes are marked with arrows. Scale bar represents 10 µm in all panels. HM - host membrane; GIV - globose infection vesicle; I - internodes; SCH - subcuticular hypha.

3. Conclusion

Functional genomics studies in species like *Olea europaea* L., where no sequence data are available, has a major constraint, which limits the screening of potentially transcript-derived fragments (TDFs) and their function. The identification and the role of genes involved in olive polygenic resistance is complex and remain unknown. Current knowledge about *Colletotrichum acutatum*-olive interaction, in susceptible and resistant cultivars and its functional genomic impact on plant resistance is not yet sufficient to provide solid explanations. Data for model plants may direct strategies that are transferable to crop systems, even when there is a lack of sequencing information related to the crop under study, once they may provide guidelines to which direction studies may be conducted. Furthermore, *Colletotrichum acutatum*-strawberry (pathosystem model for *Colletotrichum acutatum* - plant interaction) interactions are still poorly understood making the task even
more difficult in olive. The identification of resistance genes is essential not only to understand the basis of olive anthracnose disease but also to identify novel fungicide targets and, in the long term, environmental and human safe fungicides.

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Modern plant breeding is considered a discipline originating from the science of genetics. It is a complex subject, involving the use of many interdisciplinary modern sciences and technologies that became art, science and business. Revolutionary developments in plant genetics and genomics and coupling plant "omics" achievements with advances on computer science and informatics, as well as laboratory robotics further resulted in unprecedented developments in modern plant breeding, enriching the traditional breeding practices with precise, fast, efficient and cost-effective breeding tools and approaches. The objective of this Plant Breeding book is to present some of the recent advances of 21st century plant breeding, exemplifying novel views, approaches, research efforts, achievements, challenges and perspectives in breeding of some crop species. The book chapters have presented the latest advances and comprehensive information on selected topics that will enhance the reader's knowledge of contemporary plant breeding.

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