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# Molecular Tools for Detection of Plant Pathogenic Fungi and Fungicide Resistance

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## 1. Introduction

Plant pathogenic fungi are the causal agents of the most detrimental diseases in plants, including economically important crops, provoking considerable yield losses worldwide. Fungal pathogens can infect a wide range of plant species or be restricted to one or few host species. Some of them are obligate parasites requiring the presence of the living host to grow and reproduce, but most of them are saprophytic and can survive without the presence of the living plant, in the soil, water or air. Isolates of a fungal species can be differentiated by morphological characteristics, host range (*formae speciales*), pathogenic aggressiveness (pathotypes or races) or their ability to form stable vegetative heterokaryons by fusion between genetically different strains (belonging to the same vegetative compatibility group, VCG).

Detection and accurate identification of plant pathogens is one of the most important strategies for controlling plant diseases to initiate preventive or curative measures. Special interest should be taken in the early detection of pathogens in seeds, mother plants and propagative plant material to avoid the introduction and further spreading of new pathogens in a growing area where it is not present yet. For that reason, the availability of fast, sensitive and accurate methods for detection and identification of fungal pathogens is increasingly necessary to improve disease control decision making. Traditionally, the most prevalent techniques used to identify plant pathogens relied upon culture-based morphological approaches. These methods, however, are often time-consuming, laborious, and require extensive knowledge of classical taxonomy. Other limitations include the difficulty of some species to be cultured *in vitro*, and the inability to accurately quantify the pathogen (Goud & Termorshuizen, 2003). These limitations have led to the development of molecular approaches with improved accuracy and reliability. A high variety of molecular methods have been used to detect, identify and quantify a long list of plant pathogenic fungi. Molecular methods have also been applied to the study of the genetic variability of pathogen populations, and even for the description of new fungal species. In general, these methods are much faster, more specific, more sensitive, and more accurate, and can be performed and interpreted by personnel with no specialized taxonomical expertise. Additionally, these techniques allow the detection and identification of non-culturable

microorganisms, and due to its high degree of specificity, molecular techniques can distinguish closely related organisms at different taxonomic levels. Here, we review the most important tools for molecular detection of plant pathogenic fungi, their applicability, and their implementation in horticultural and agricultural practices.

On the other hand, once the pathogenic fungus is already established in a given crop growing area, the use of synthetic fungicides constitutes the main strategy to control plant diseases, since these compounds act quickly and effectively. The disadvantages of continued use of fungicides are their limited spectrum and the emergence of resistant fungal isolates. This fact leads to many yield losses as control systems are not longer effective. The development of resistance to fungicides in fungal pathogens and the growing public concern over the health and environmental hazards associated with the high level of pesticide have resulted in a significant interest in knowing more about fungal resistance. The emergence of more stringent regulations regarding pesticide residues means that one of the main priorities is to ensure food security by reducing the use of fungi toxics. For this reason, it is important to identify and characterize the mechanisms involved in the emergence of strains resistant to fungicides used for control diseases and to know the molecular methods currently available to detect them.

## **2. Molecular methods for detection of plant pathogenic fungi**

### **2.1 Polymerase Chain Reaction (PCR)**

The polymerase chain reaction (PCR) is the most important and sensitive technique presently available for the detection of plant pathogens. PCR allows the amplification of millions of copies of specific DNA sequences by repeated cycles of denaturation, polymerisation and elongation at different temperatures using specific oligonucleotides (primers), deoxyribonucleotide triphosphates (dNTPs) and a thermostable *Taq* DNA polymerase in the adequate buffer (Mullis & Faloona, 1987). The amplified DNA fragments are visualized by electrophoresis in agarose gel stained with EtBr, SYBR Green or other safer molecule able to intercalate in the double stranded DNA, or alternatively by colorimetric (Mutasa et al., 1996) or fluorometric assays (Fraaije et al., 1999). The presence of a specific DNA band of the expected size indicates the presence of the target pathogen in the sample. Advances in PCR-based methods, such as real-time PCR, allow fast, accurate detection and quantification of plant pathogens in an automated reaction. Main advantages of PCR techniques include high sensitivity, specificity and reliability. Moreover, it is not necessary to isolate the pathogen from the infected material reducing the diagnosis time from weeks to hours, and allowing the detection and identification of non-culturable pathogens. This characteristic has been especially useful in the analysis of symptomless plants. However, the frequent presence of PCR inhibitors in the plant tissues or soil can reduce considerably the sensitivity of the reaction and even result in false negative detection. Many attempts have been carried out to overcome this issue (see below). Another disadvantage of the PCR methodology is the occurrence of false positive results due to the presence of DNA or PCR products (amplicons) contaminants. For this reason, it is advisable to separate physically and temporally pre- and post-PCR analysis. Another failure of PCR-based methods is the inability to discriminate viable from non-viable fungi or fungal structures, which might inform of the real threat for the plant. Development of a prior PCR step involving enrichment culturing (BIO-PCR) (Ozakman &

Schaad, 2003) or amplification of fungal RNA (RT-PCR) (Lee et al., 1989) may solve this problem to some extent. Like this, many PCR variants have been developed to improve sensitivity, specificity, rapidity and throughput, and to allow the quantification of the fungal pathogen in the plant and the environment.

### 2.1.1 Starting material

Collection and preparation of samples is a critical step for the detection of plant pathogenic fungi. The starting material may be symptomatic plant tissue (roots, leaves, stems, flowers, fruits or seeds), soil, water or air. Also, latent infections can be detected on symptomless plants.

In the case of infected plants, the first step consists in the cultivation of the fungi. After surface sterilisation of the plant tissue (e.g. with 1% sodium hypochlorite or 50% hydrogen peroxide) small pieces are transferred to Petri dishes containing an appropriate nutrient medium (e.g. Potato Dextrosa Agar, PDA) supplemented with antibiotics to prevent bacterial contaminants (usually streptomycin), and incubated at required temperature (25-30°C) for pathogen development. If the fungal pathogen has infected deep internal tissues, cutting the plant material to expose core tissues may be necessary. Pure fungal colonies must be obtained either by isolating single spore or single hyphal tips. In the first case, a spore suspension of the fungus is prepared and serial dilutions in sterile water are obtained. An optimal dilution is transferred to Petri dishes containing 2% agar-water and incubated at appropriate temperature to induce spore germination. A single germinating spore is then isolated and a pure colony of the fungus is obtained. To get a single hyphal tip, a small segment of fungal growth is transferred to a new Petri dish containing nutrient medium and incubated at optimum temperature to allow growth of the mycelium. A separate hyphal edge is then transferred to new agar plate to obtain a pure colony of the fungal species. Isolation of the fungus from an infected plant tissue can however obviate the presence in the infected plant of other non-culturable pathogens or fungi having special culture conditions. Competition between distinct fungi can also mask the detection by culturing of an infecting pathogen. For DNA extraction, fungal mycelium must be firstly homogenized in a mortar in the presence of liquid nitrogen. Other homogenisation procedures obviate the use of liquid nitrogen, e.g. grinding the mycelium inside centrifuge tubes with the help of sealed tips or plastic pistils that fit perfectly into the tubes coupled to household drills (González et al., 2008).

Alternatively, total DNA from the plant and the fungi can be isolated together from the infected plant tissue. That allows skipping the fungi culture step, although DNA from different fungal species or strains may be obtained. Homogenisation of the infected plant tissues is usually performed in the presence of liquid nitrogen. Then, an extraction buffer is added to obtain a crude extract. Alternatively, plant material may be introduced into individual plastic bags containing a soft net in the case of tender material (Homex, Bioreba; Stomacher, AES Laboratoire) or a heavy net in the case of dry or harder material such as bark tissue or seeds (PlantPrint Diagnostics). Homogenisation can be made by the help of a manual roller or by the use of special apparatus designed to facilitate the homogenisation (e.g. Homex, Bioreba). As in the case of fungal mycelium, small amounts of plant tissue can be homogenised inside centrifuge tubes using pistils coupled to electric drills in the presence of extraction buffers.

When analysing soil samples, the main focus for phytopathologists is the isolation of DNA from different microorganisms and then, the specific detection and monitoring of the fungus(i) of interest. Classical approach consists of cultivating the soil fungi in different media and screen for the desired pathogen. However, many microorganisms from the soil community can not be isolated by this procedure. An alternative method is to isolate DNA directly from the soil sample without prior culturing. Protocols using enzymatic (e.g. protease, chitinase, glucanase) or mechanical lysis (glass-beads beating, freeze-thawing, vigorous shaking, microwave or grinding in liquid nitrogen) have been reported, but a combination of both procedures seems to be the more effective (Anderson I. C. & Parkin, 2007, Jiang et al., 2011). In the same way, improved protocols for an efficient isolation of DNA from water for detection and monitoring of plant pathogens have been reported (Pereira et al., 2010).

During the homogenisation process, polysaccharides and phenolic compounds from plants or humic and fulvic acids from soils can be released that can inhibit the *Taq* DNA polymerase leading to the occurrence of false negatives (Munford et al., 2006; Tebbe & Vahjen, 1993; Wilson, 1997). This problem may be partially overcome by the use in the extraction buffer of some compounds such as polyvinylpyrrolidone (PVP) or cetyltrimethyl ammonium bromide (CTAB) for plant extracts, and bovine serum albumine (BSA) for soil samples (Anderson I.C. & Cairney, 2004), or by removing inhibitors by the use of spin/vacuum columns. Some PCR variants such as Magnetic Capture-Hybridisation PCR have been developed to remove the presence of PCR inhibitors in plant extracts (see below). In addition, it is increasingly common to use an internal positive control of the PCR reaction either by the amplification of a conserved plant gene (e.g. cytochrome oxidase I, *cox I*) in multiplex (Bilodeau, et al., 2009) or in a parallel assay (Garrido et al., 2009), or by the addition of an exogenous DNA and their corresponding primers to each reaction (Cruz-Pérez et al., 2001).

### 2.1.2 DNA extraction methods

There are no universally validated nucleic-acids extraction protocols for fungi, infected plant material or soil. Many published protocols are available to ensure an efficient and reproducible method for DNA extraction from plants (revised by Demeke and Jenkins, 2010; Biswas and Biswas, 2011); from fungi (Chi et al., 2009; Feng et al., 2010; González-Mendoza et al., 2010; Niu et al., 2008; Zelaya-Molina et al., 2011; Zhang, Y. J. et al., 2010); and from soil (revised by Hirsch et al., 2010).

The use of commercial kits for nucleic acids extraction, either general or specifically designed for plant material, fungi or soil is gaining acceptance because they are easy to use and are able to efficiently remove inhibitory compounds during the purification process. They are generally based on magnetic beads or spin columns, although quicker protocols are also available (e.g. QuickExtract™ Plant DNA Extraction Solution, Epicentre). Automated or semi-automated systems have also been developed to allow the isolation of nucleic acids from different samples, among others, QIAxtractor, QIAgen; 6700 Automated Nucleic Acid Workstation, Life Technologies; Magna Pure LC extraction system, Roche; Solucion m2000, Abbott.

Purification of nucleic acids is labour intensive, costly, time-consuming and not applicable when a large number of samples need to be analysed. Several attempts have been undertaken



to avoid the nucleic acids isolation step. One of them uses few microliters of crude extract loaded and immobilized on small pieces of paper, e.g. FTA cards. A subsequent lysis of the cells in appropriate buffer allows the release of nucleic acids that are fixed in the membrane and protected from degradation. DNA can be stored on dry cards for several years in a dry place at room temperature without decreasing the sensitivity of detection (Smith & Burgoyne, 2004). Moreover, membrane immobilised-DNA is suitable for transportation or mail to other laboratories. Suzuki et al. (2006) reported that nucleic acids recovered from FTA cards could be used for the detection of *Aspergillus oryzae*, releasing the DNA from the fungal tissue by treatment in a microwave oven before application to the membranes. Grund et al. (2010) used FTA cards coupled to PCR for the detection of plant pathogens including oomycetes such as *Phytophthora* and filamentous fungi such as *Fusarium*.

### 2.1.3 Selection of target DNA to amplify

Generally, conserved known genes with enough sequence variation are selected for designing PCR diagnostic assays and performing phylogenetic analysis. The most common region used for these purposes has been the internal transcribed spacer (ITS) region of ribosomal RNA genes. rDNA region consists of multiple copies (up to 200 copies per haploid genome) arranged in tandem repeats comprising the 18S small subunit, the 5.8S, and the 28S large subunit genes separated by internal transcribed spacer regions (ITS1 and ITS2) (Bruns et al., 1991; Liew et al., 1998). This region contains highly conserved areas adequate for genera- or species-consensus primer designing (RNA ribosomal genes), alternate with highly variable areas that allow discrimination over a wide range of taxonomic levels (ITS region) (White et al., 1990). The ITS region is ubiquitous in nature and found in all eukaryotes. In addition, the high copy numbers of rRNA genes in the fungal genome enable a highly sensitive PCR amplification. Furthermore, a large numbers of ribosomal sequences are publicly available in databases, facilitating the validation and the reliability of the detection assays.

Traditionally, molecular identification of plant pathogenic fungi is accomplished by PCR amplification of ITS region followed by either restriction analysis (Durán et al., 2010) or direct sequencing and BLAST searching against GenBank or other databases (White et al., 1990). Identification could be a challenge when using BLAST analysis with ITS sequences because there can be minimal or no differences between some species or, in some cases, intraspecific variation can confuse the boundaries between species (e.g., *P. fragariae* var. *fragariae* and *P. fragariae* var. *rubi* have identical ITS sequences). The sequence analysis of the ITS region has additionally served to proposed new species. Abad et al. (2008) aligned ITS regions of *Phytophthora* spp. associated with root rot from different geographic origins and hosts with GenBank sequences from other *Phytophthora* species and proposed a new isolate *Phytophthora bisheria* sp. nov. Just as, Burgess et al. (2009) also distinguished new and undescribed taxa of *Phytophthora* from natural ecosystems. This alignment analysis has also been used for the identification of new species of *Pythium* (de Cock & Lévesque, 2004; Nechwatal & Mendgen, 2006; Paul, 2006, 2009; Paul et al., 2005; Paul & Bala, 2008).

The ITS region has also been widely used in fungal taxonomy and it is known to show variation between species e.g., between *Pythium ultimum* and *P. helicoides* (Kageyama et al., 2007); *Peronospora arborescens* and *P. cristata* (Landa et al., 2007); *Colletotrichum gloeosporioides* and *C. acutatum* (Kim J. T. et al., 2008), and within species e.g. allowing differentiation of

*Puccinia striiformis* f. sp. *tritici* (Zhao et al., 2007), and distinguishing between weakly and high virulent isolates of *Leptosphaeria maculans* (Xue et al., 1992), or defining anastomosis subgroups of *Rhizoctonia solani* (Budge, 2009; Godoy-Lutz et al., 2008).

The ITS region remains an important locus for molecular identification of fungi. However, as more sequence data is collected from a wider range of fungal isolates, the utility of alternative loci for accurate species identification is increasing. The intergenic spacer sequence (IGS) placed between the 28S and 18S rRNA genes is the region with the greatest amount of sequence variation in rDNA. It is frequently used in PCR-based methods when there are not enough differences available across the ITS. Primers in this region have been designed to detect and identify *Verticillium dahliae* and *V. alboatrum* (Schena et al., 2004) and to distinguish pathogenic and non-pathogenic *Fusarium oxysporum* in tomato (Validov et al., 2011). As another example, Inami et al. (2010) differentiated *Fusarium oxysporum* f. sp. *lycopersici*, and its races using primers and TaqMan-MGB probes based on IGS and avirulent SIX genes.

Other housekeeping genes with higher variability are being more extensively used to develop diagnostics for fungi, including nuclear genes such as  $\beta$ -tubulin (Aroca et al., 2008; Fraaije et al., 2001; Mostert et al., 2006), translation elongation factor 1 alpha (*TEF* 1 $\alpha$ ) (Geiser et al., 2004; Knutsen et al., 2004; Kristensen et al., 2005), calmodulin (Mulè et al., 2004), avirulence genes (Lievens et al., 2009), and mitochondrial genes such as the multicopy *cox* I and *cox* II and their intergenic region (Martin & Tooley, 2003; Nguyen & Seifert, 2008; Seifert et al., 2007). Mating type genes also show high diversity and fast evolutionary rate and could be used for inter- and intra-species differentiation, e.g. Foster et al. (2002) distinguished between the two mating types of *Pyrenopeziza brassicae*. Moreover, Martínez-Espinoza et al. (2003) used mating type genes to specifically detect *Ustilago maydis* in maize cultivars. To enhance the specificity of a diagnostic assay, a combination of multiple diagnostic regions is recommended. Many authors have followed this multi-locus diagnostic strategy, e.g. Collado-Romero et al. (2008) studied the evolutionary relationships among *Verticillium dahliae* vegetative compatibility groups by AFLP fingerprints and sequence analysis of actin,  $\beta$ -tubulin, calmodulin, and histone 3 genes, the ITS region, and a *V. dahliae*-specific sequence; Dixon et al. (2009) demonstrated the host specialisation and phylogenetic diversity of *Corynespora cassiicola* using the ITS region, actine gene and two random hypervariable loci; Glienke et al. (2011) performed sequence analysis of the ITS region and partial *TEF* 1 $\alpha$ , actin and glyceraldehyde-3-phosphate dehydrogenase (GPDH) genes to study the genetic diversity of *Phyllosticta* spp. allowing differentiation of pathogenic and non pathogenic species and describing two new *Phyllosticta* species; Inderbitzin et al. (2010) verified a high species diversity in *Botryosphaeriaceae* species performing phylogenetic analyses based on six loci, including the ITS region and *TEF* 1 $\alpha$ , GPDH, a heat shock protein, histone-3 and  $\beta$ -tubulin genes; Shimomoto et al. (2011) used RAPDs and sequences from  $\beta$ -tubulin, *TEF* 1 $\alpha$ , calmodulin and actin genes to detect pathogenic and genetic variation among isolates of *Corynespora cassiicola*.

#### 2.1.4 Single nucleotide polymorphisms (SNPs)

Closely related pathogens showing different host ranges or pathogenicity often differ in only a single to a few base pairs in target genes commonly used for identification. Therefore, the ability to discriminate single nucleotide polymorphisms (SNPs) should be pursued in any diagnostic assay. Based on the DNA nucleotide sequence difference in the

mitochondrial *cox I* gene, a SNP method has been developed to detect and differentiate isolates of *Phytophthora ramorum* from Europe and those originating in the United States (Kroon et al., 2004). In other experiment, polymorphisms detected in the microsatellite flanking regions of *Phytophthora infestans* allowed the development of a SNP genetic marker system for typing this pathogen (Abbott et al., 2010). Many of the examples described below take advantage of SNPs for designing highly specific detection assays.

### 2.1.5 Design of primers and probes

PCR methods are based on the use of specific oligonucleotides or primers that specifically hybridise with the DNA target and that are required to initiate the synthesis of the new DNA chain. In some real-time PCR methods additional specific oligonucleotides are used, named probes, that hybridise with the target DNA between the two primers. The design of primers and probes is crucial for PCR to be specific and efficient. Primer specificity relies on its sequence, length and GC content, which determines its melting temperature ( $T_m$ , the temperature at which 50% of primer-target duplex are hybridized). DNA amplified fragments (amplicons) size must be short enough to ensure efficiency of the reaction and high sensitivity (Singh and Singh, 1997). Real-time PCR usually requires shorter amplicons than conventional PCR. Balancing of primers concentration is necessary especially in multiplex PCR reactions and in real-time PCR using non-specific fluorescent dyes. Also, formation of hairpin structures or complementarities between primers must be avoided.

The first step for designing primers and probes consists in the alignment of the sequences of interest by the blastn program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) (Altschul et al., 1997) using sequences from the GenBank, EMBL and DDBJ databases. Partial or complete nucleotide sequences of many fungal genes are available at the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/Genbank/>) (Bethesda, MD, USA). Consensus sequences are used to design primers for detection of members of a same genus or species. If the consensus is not possible, degenerate primers can be used, although this may severely affect the overall sensitivity of the PCR reaction. On the other hand, variable sequences are useful for the differentiation of pathogens at lower taxonomic levels and for the analysis of the molecular variability of fungal population in phylogenetic studies.

Software packages for primer and probe design are available, among others Primer Express, Applied Biosystems; LightCycler Probe Design, Roche; Primer Explorer, Eiken Chemical Co.; Beacon Designer, Premier Biosoft International; Primer Premier, Premier Biosoft; Primer Analysis Software, OLIGO; Oligo perfect designer, Life Technologies/Invitrogen; or Primer3 (<http://frodo.wi.mit.edu/primer3/>).

### 2.1.6 PCR-based methods

#### 2.1.6.1 Conventional PCR

Identification of fungal pathogens by conventional PCR may be achieved at different taxonomic levels (genus, species or strain) depending on the specificity of the primers. As recent examples, PCR methods for identification of *Sclerotium rolfsii* (Jeeva et al., 2010) and *Colletotrichum capsici* (Torres-Calzada et al., 2011) have been developed based in specific sequences of the ITS region. Improved variants of PCR have emerged with higher



sensitivity, specificity and throughput and allowing the quantification of fungi in infected plants or environment.

#### 2.1.6.2 Nested-PCR and Cooperational-PCR (Co-PCR)

Nested PCR approach is used when an improvement of the sensitivity and/or specificity of detection is necessary. This method consists in two consecutive rounds of amplification in which two external primers amplify a large amplicon that is then used as a target for a second round of amplification using two internal primers (Porter-Jordan et al., 1990). The two reactions are usually performed in separated tubes involving time and effort and increasing the risks of false positives due to cross contamination. However, some improvements in the relative concentrations of the external and internal primers have permitted to perform the two reactions in a single closed tube supporting high throughput. This method has been widely used for detection and/or further characterisation of numerous fungi (Aroca & Raposo, 2007; Grote et al., 2002; Hong et al., 2010; Ippolito et al., 2002; Langrell et al., 2008; Meng and Wang 2010; Mercado-Blanco et al., 2001; Qin et al., 2011; Wu et al., 2011).

An alternative PCR method that enhances sensitivity and minimise contamination risks is the Co-operational PCR. In Co-PCR a single reaction containing the four primers, one pair internal to other, enhances the production of the longest fragment by the co-operational action of all amplicons (Olmos et al., 2002). Co-PCR is usually coupled with dot blot hybridisation by using a specific probe to enhance the specificity of the detection and provide a sensitivity level similar to nested PCR method. Martos et al. (2011) used this method for sensitive and specific detection of grapevine fungi. In both nested- and Co-PCR methods, the use of external primers can be used for generic amplification and the internal primers for further and more specific characterisation of the amplified product at species or strain level.

#### 2.1.6.3 Multiplex PCR

Multiplex PCR is based on the use of several PCR primers in the same reaction allowing the simultaneous and sensitive detection of different DNA targets, reducing time and cost. This method is useful in plant pathology since plants are usually infected by more than one pathogen. Different fragments specific to the target fungi were simultaneously amplified and identified on the basis of their molecular sizes on agarose gels. Although the efficiency of amplification is strongly influenced by amplicon size (shorter amplicons may be amplified preferentially over longer ones), an accurate and careful design of primers and the optimisation of their relative concentrations are required to overcome this drawback and get an equilibrate detection of all target fungi. Multiplex PCR technique has been used for the simultaneous detection and differentiation of *Podosphaera xanthii* and *Golovinomyces cichoracearum* in sunflower (Chen et al., 2008); for detecting *Phytophthora lateralis* in cedar trees and water samples, including detection of an internal control in the same reaction (Winton & Hansen, 2001); for determining the mating type of the pathogens *Tapesia yallundae* and *T. acuformis* (Dyer et al., 2001); for differentiating two pathotypes of *Verticillium albo-atrum* infecting hop (Radišek et al., 2004) and for distinguishing among eleven taxons of wood decay fungi infecting hardwood trees (Guglielmo et al., 2007). Due to the complexity of the design this technique has recently been replaced by other multiplexing techniques including multiplex real-time PCR (see below).

Another multiplexing method that allows simultaneous detection and identification of multiple oomycetes and fungi in complex plant or environmental samples is the use of ligation detection (LD) system using padlock probes (PLPs). PLPs are long oligonucleotide probes containing asymmetric target complementary regions at their 5' and 3' ends. Padlock probes also incorporate a desthiobiotin moiety for specific capture and release, an internal endonuclease IV cleavage site for linearization, and a unique sequence identifier, the so-called ZipCode, for standardised microarray hybridisation. DNA samples are PCR amplified and subjected to PLP ligation. Under perfectly hybridisation with the target, the PLPs are circularized by enzymatic ligation. Then, the probes are captured with streptavidin-coupled magnetic beads, cut at the internal cleavage site, allowing only the originally ligated PLPs to be visualized by hybridisation on a universal complementary ZipCode microarray. Padlock probes have been used for the simultaneous detection of *Phytophthora cactorum*, *P. nicotianae*, *Pythium ultimum*, *P. aphanidermatum*, *P. undulatum*, *Rhizoctonia solani*, *Fusarium oxysporum* f. sp. *radicis-lycopersici*, *F. solani*, *Myrothecium roridum*, *M. verrucaria*, *Verticillium dahliae* and *V. alboatrum* in samples collected from horticultural water circulation systems in a single assay (van Doorn et al., 2009).

#### 2.1.6.4 Magnetic Capture-Hybridisation (MCH)-PCR

This technique was developed to circumvent the presence of PCR inhibitors in plant extracts. Magnetic beads are coated with a biotinylated oligonucleotide that is specific of a DNA region of the fungus of interest. Hybridisation takes place between the fungal DNA and magnetic beads-oligomer and the conjugate is recovery separated from inhibitory compounds. After the magnetic capture-hybridisation, PCR amplification was carried out using species-specific primers. Langrell & Barbara, (2001) used this method to detect *Nectria galligena* in apple and pear trees.

#### 2.1.6.5 PCR-ELISA

This serological-based PCR method uses forward and reverse primers carrying at their 5' end biotin and an antigenic group (e.g. fluorescein), respectively (Landgraf et al., 1991). PCR amplified DNA can be immobilized on avidin or streptavidin-coated microtiter plates via the biotin moiety of the forward primer and then can be quantified by an ELISA specific for the antigenic group of the reverse primer (e.g. anti-fluorescein antibody detected by colorimetric reactions). PCR-ELISA method is as sensitive as nested PCR. In addition, it does not require electrophoretic separation and/or hybridisation, and can be easily automated. All reactions can be performed in 96-well microtiter plates for mass screening of PCR products making them very suitable for routine diagnostic purposes. This procedure has been used for detection and differentiation of *Didymella bryoniae* from related *Phoma* species in cucurbits (Somai et al., 2002) and for detection of several species of *Phytophthora* and *Pythium* (Bailey et al., 2002).

#### 2.1.6.6 Reverse Transcription (RT)-PCR

An important limitation of molecular methods is the inability to distinguish living or dead fungi or fungal structures. So, results from detection and identification of fungal plant pathogens should be validated by pathogenicity tests. Since mRNA is degraded rapidly in dead cells, the detection of mRNA by RT-PCR is considered an accurate indicator of cell viability (Sheridan et al., 1998). In RT-PCR the RNA is reverse transcribed using the enzyme reverse transcriptase. The resulting cDNA is then amplified using conventional or any other

PCR-based method. RT-PCR has been used to detect viable populations of *Mycosphaerella graminicola* in wheat (Guo et al., 2005). A RT-nested-PCR method was applied for detection of *Oidium neolycopersici* in tomato (Matsuda et al., 2005). Even so, the most frequent application of this technique in phytopathology is the analysis of plant and fungal gene expression during disease development (Yang et al., 2010).

#### 2.1.6.7 *in situ* PCR

This technique allows the amplification of specific gene sequences within intact cells or tissues combining two technologies: PCR and *in situ* hybridisation (ISH) (Long, 1993; Nuovo, 1992). The improved sensitivity of this technique allows the localization of one target copy per cell (Haase et al., 1990; Nuovo et al., 1991). However, background detection is usually high because nonspecific DNA synthesis during *in situ* PCR on tissue sections may occur (Nuovo et al., 1994). In addition, it is a time-consuming technique due to the need for a hybridisation step and technically demanding procedures such as light microscopy. Bindsley et al. (2002) used *in situ* PCR technique to identify *Blumeria graminis* spores and mycelia on barley leaves.

#### 2.1.6.8 PCR-DGGE

This method is mainly applied for the analysis of the genetic diversity of microbial communities without the need of any prior knowledge of the species (Muyzer, 1999; Gothwal et al., 2007; Portillo et al., 2011). DGGE (Denaturing Gradient Gel Electrophoresis) and its variant TGGE (Temperature Gradient Gel Electrophoresis) use chemical gradient such as urea (DGGE) or temperature (TGGE) to denature and separate DNA samples when they are moving across an acrylamide gel. In PCR-DGGE target DNA from plant or environmental samples are firstly amplified by PCR and then subjected to denaturing electrophoresis. Sequence variants of particular fragments migrate at different positions in the denaturing gradient gel, allowing a very sensitive detection of polymorphisms in DNA sequences. In addition, PCR-DGGE primers contain a GC rich tail in their 5' end to improve the detection of small variations (Myers et al., 1985). The bands obtained in the gel can be extracted, cloned or reamplified and sequenced for identification, being even possible to identify constituents that represent only 1% of the total microbial community. These techniques are very suitable for the identification of novel or unknown organisms and the most abundant species can be readily detected.

This method is however time-consuming, poorly reproducible and provides relative information about the abundance of detected species. Interpretation of the results may be difficult since the microheterogeneity present in some target genes may appear as multiple bands in the gel for a single species, leading to an overestimation of the community diversity. Furthermore, fragments with different sequences but similar melting behaviour are not always correctly separated. In other cases, the analysis of complex communities of microorganisms may result in blurred gels due to the large number of bands obtained.

A PCR-DGGE detection tool based in the amplification of the ITS region has been recently applied to detect multiple species of *Phytophthora* from plant material and environmental samples (Rytönen et al., 2011). Other authors have used this technique to compare the structure of fungal communities growing in different conditions or environments, e.g. to study the impact of culture management such as biofumigation, chemifumigation or fertilisation on the relative abundance of soil fungal species (Omirou et al., 2011; Wakelin et al., 2008).

### 2.1.6.9 Real-time PCR

Real-time PCR is currently considered the gold standard method for detection of plant pathogens. This technique allows the monitoring of the reaction during the amplification process by the use of a fluorescent signal that increases proportionally to the number of amplicons generated and to the number of targets present in the sample (Wittwer et al., 1997).

Many are the advantages of real time PCR over conventional PCR, including that this system does not require the use of post PCR processing (electrophoresis, colorimetric reaction or hybridisation), avoiding the risk of carryover contamination and reducing assay labour and material costs. In addition to its improved sensitivity and specificity, this technique allows the accurate quantification of the target pathogen, by interpolating the quantity measured to a standard curve with known amounts of target copies. This quantification characteristic is very useful in phytopathology in order to correlate the amount of fungus in a biological sample with the disease state, or to monitor the progress of the disease in an infected plant (Garrido et al., 2009). In addition, real-time PCR is a high throughput method for the analysis of a large number of samples due to the use of a plate-based system which permits the analysis of 96 or 384 samples at the same time. This characteristic facilitates the robotisation of the nucleic acids extraction and master mix preparation steps, reducing personal and gaining time. Portable real-time PCR machines are emerging as diagnostic tools for *on site* detection under field conditions providing a realistic option to perform molecular tests at the same place of the collection of samples (e.g. portable Cepheid SmartCycler). This is especially interesting in situations where a rapid diagnostic test is needed. Another advantage of real-time PCR is the capability to perform multiplex detection of two or more pathogens in the same reaction (see below).

Real-time RT-PCR chemistry can be based on the use of doubled-stranded DNA binding dyes, such as SYBR Green, specific fluorescent labelled probes such as TaqMan, Molecular Beacons, or Scorpions, or dye-primer based systems, such as hairpin primers or Plexor system.

#### *a. Non-specific fluorescent dyes*

SYBR Green I is a fluorescence intercalating dye with a high affinity for double-stranded DNA. The overall fluorescent signal from a reaction is proportional to the amount of double-stranded DNA (dsDNA) present in the sample, and increase as the target is amplified. The main advantage of the use of intercalator dyes is that no probe is required, which reduces assay setup and running costs. Binding dyes are also attractive because protocols using established primers and PCR conditions can readily be converted to the real-time method. However, intercalating dyes detect accumulation of both specific and non-specific PCR products. So, to assess the specificity of the reaction, it is necessary a further step of melting curve analysis that allows the identification of the PCR product by its  $T_m$ . Additionally, a fine optimisation of primers concentration is crucial to avoid formation of also detected primer-dimers. Quantification of targets using SYBR Green is not very accurate, since the amount of fluorescent signal is proportional to the mass of dsDNA produced in the reaction (amplification of a longer product will generate more signal than a shorter one). Generally, small amplicons must be selected (between 50 and 200 bp) for optimal efficiency. SYBR Green real-time PCR with melting curve analysis has been described as a simple, rapid, and reliable technique for the detection and identification of phytopathogenic fungi even in multiplex assays (Tabla 1).



Pathogen	Real-time chemistry	Reference	Host plant
<i>Botrytis cinerea</i>	SYBR Green	Diguta el al., 2010	Grape
<i>Cladosporium fulvum</i>	SYBR Green	Yan et al., 2008	Tomato
<i>Colletotrichum acutatum</i>	SYBR Green	Samuelian et al., 2011	Grape
<i>Fusarium avenaceum</i>	SYBR Green	Moradi et al., 2010	Wheat
<i>Fusarium culmorum</i>	SYBR Green	Moradi et al., 2010	Wheat
	SYBR Green multiplex	Brandfass & Karlovsky, 2006	Cereals
<i>Fusarium graminearum</i>	SYBR Green	Moradi et al., 2010	Wheat
	SYBR Green multiplex	Brandfass & Karlovsky, 2006	Cereals
<i>Fusarium oxysporum</i>	SYBR Green	Jiménez-Fernández et al., 2010	Chickpea
			Melon
			Pea
			Soil
<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i>	SYBR Green	Abd-Elsalam et al., 2006	Cotton
<i>Fusarium oxysporum</i> f. sp. <i>ciceris</i>	SYBR Green	Jiménez-Fernández et al., 2011	Chickpea
			Soil
<i>Fusarium poae</i>	SYBR Green	Moradi et al., 2010	Wheat
<i>Fusarium verticillioides</i>	SYBR Green	Kurtz et al., 2010	Corn
<i>Greeneria uvicola</i>	SYBR Green	Samuelian et al., 2011	Grape
<i>Macrophomina phaseolina</i>	SYBR Green	Babu et al., 2011	Chickpea
			Soybean
			Pigeon pea
<i>Phoma sclerotioides</i>	SYBR Green	Larsen et al., 2007	Alfalfa
			Wheat
<i>Phoma tracheiphila</i>	SYBR Green	Demontis et al., 2008	Citrus
<i>Phytophthora capsici</i>	SYBR Green	Silvar et al., 2005	Pepper
<i>Phytophthora cryptogea</i>	SYBR Green	Minerdi et al., 2008	Gerbera
<i>Plasmodiophora brassicae</i>	SYBR Green	Sundelin et al., 2010	Oilseed rape
<i>Puccinia horiana</i>	SYBR Green	Alaei et al., 2009	Chrysanthemum
<i>Pythium irregular</i>	SYBR Green	Schroeder et al., 2006	Wheat
			Barley
			Soil
<i>Pythium ultimum</i>	SYBR Green	Schroeder et al., 2006	Wheat
			Barley
			Soil
<i>Rhizoctonia oryzae</i>	SYBR Green	Okubara et al., 2008	Cereals
<i>Rhizoctonia solani</i>	SYBR Green	Okubara et al., 2008	Cereals
<i>Rhynchosporium secalis</i>	SYBR Green	Fountaine et al. 2007	Barley
<i>Sclerotinia sclerotiorum</i>	SYBR Green	Yin et al., 2009	Oilseed rape
<i>Verticillium dahliae</i>	SYBR Green, Plexor	Attallah et al., 2007	Potato
	SYBR Green	Gayoso et al., 2007	Hot pepper
	SYBR Green	Markakis et al., 2009	Olive

Table 1. Examples of SYBR Green real-time PCR assays for detection of plant pathogenic fungi (last 6 years).



### *b. Specific fluorescent labelled probes*

#### *b.1 Hydrolysis probes*

TaqMan chemistry (Heid et al., 1996) is based on the use of an oligonucleotide probe located between the two PCR primers and labelled with a fluorophore covalently attached to the 5'-end (reporter) and a quencher on the 3' end. When the reporter and the quencher are close, the emission of fluorescence is inhibited. After the PCR denaturation step, primers and probes specifically hybridise to the complementary target. The probe is then cleaved by the 5'-3' exonuclease activity of the *Taq* DNA polymerase causing the separation of the fluorophore and the quencher and allowing the reporter dye to emit fluorescence. The fluorescence detected in the real-time PCR thermal cycler is directly proportional to the fluorophore released and the amount of DNA template present in the PCR.

TaqMan probes were designed to increase the specificity of the reaction because detection and accurate quantification require high complementarity with the target sequence. The number of phytopathogenic fungi detected by this method has exponentially increased in recent years. Some examples are detailed in Table 2.

Modified probes have been designed to improve the TaqMan reaction specificity such as MGB probes that include a Minor Groove Binding group at the 3' end raising the *T<sub>m</sub>* of the hybrid. That allows the use of shorter and more specific probes. The high specificity of MGB probes make them very suitable for specific detection of fungal species based on SNPs (Massart et al., 2005). For the same purpose, primers or probes can be synthesized with a lock nucleic acid (LNA), which are modified nucleotides that form methylene bridges after binding to the target DNA (Braasch & Corey, 2001). That provokes the lock of the duplex structure, improving their binding affinity and stability and allowing the use of higher annealing temperatures. LNA primers have been used for the specific detection of *P. ramorum* (Tomlinson et al., 2007) and the multiplex detection of species of *Phytophthora* (Bilodeau et al., 2009). The high specificity of TaqMan probes may compromise however the accuracy of the detection and quantification due to the existence of interstrain variability in the target sequence that may result in failure to detect or underestimation of the amount of DNA targets in the sample.

In addition to its high specificity, one of the advantages of the TaqMan chemistry is that probes can be labeled with different reporter dyes (FAM, VIC, TET, TAMRA, HEX, JOE, ROX, Cy5, Texas Red, etc) which allows multiplexing detection of two or more distinct pathogens in the same reaction increasing throughput (Aroca et al., 2008; Bilodeau et al., 2009). However, for this purpose the synthesis of different probes is required making the multiplex detection analysis more expensive. The types and number of fluorescent labels that can be used depend upon the detection capabilities of the real-time instrument used. Additionally, designing of real-time multiplex assays may be difficult. Special attention must be focus on avoiding primer competition that could strongly drop the levels of specificity and sensitivity. In fact, TaqMan multiplex assays usually show lower detection sensitivity than single reactions.

#### *b.2 Hairpin probes*

Molecular Beacons (MB) (Tyagi & Kramer, 1996) are specific oligonucleotide probes (15-40-mer) flanked by two complementary 5-7-mer arms sequences, with a fluorescent dye covalently attached to the 5' end and a quencher dye at the 3' end. When the molecular beacon is in an unbound state the arms form a stem/loop structure in which the fluorophore

Pathogen	Real-time chemistry	Reference	Host plant
<i>Biscogniauxia mediterranea</i>	TaqMan	Luchi et al., 2005	Oak
<i>Botrytis squamosa</i>	TaqMan	Carisse et al., 2009	Onion
<i>Chalara fraxinea</i>	TaqMan	Ioos et al., 2009	Ash trees
<i>Colletotrichum acutatum</i>	TaqMan	Garrido et al., 2009	Strawberry
<i>Colletotrichum gloesporioides</i>	TaqMan	Garrido et al., 2009	Strawberry
<i>Colletotrichum</i> spp.	TaqMan	Garrido et al., 2009	Strawberry
<i>Discula destructiva</i>	TaqMan	Zhang, N. et al., 2011	Dogwood
<i>Fusarium avenaceum</i>	TaqMan MGB	Kulik et al., 2011	Cereals
<i>Fusarium equiseti</i>	Molecular Beacons	Macía-Vicente et al., 2009	Barley
<i>Fusarium foetens</i>	TaqMan	De Weerd et al., 2006	Begonia
<i>Fusarium graminearum</i>	TaqMan	Demeke et al., 2010	Wheat
			Barley
<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> and its races	TaqMan MGB	Inami et al., 2010	Tomato
<i>Fusarium poae</i>	TaqMan MGB	Kulik et al., 2011	Cereals
<i>Fusarium tricinctum</i>	TaqMan MGB	Kulik et al., 2011	Cereals
<i>Fusicoporia torulosa</i>	Scorpions	Campanile et al., 2008	Oak
<i>Gremmeniella abietina</i>	TaqMan	Børja et al., 2006	Spruce
<i>Guignardia citricarpa</i>	TaqMan	van Gent-Pelzer et al., 2007	Citrus fruit
<i>Macrophomina phaseolina</i>	TaqMan MGB	Babu et al., 2011	Chickpea
			Soybean
			Pigeon pea
<i>Mycosphaerella graminicola</i>	TaqMan MGB	Bearchell et al., 2005	Wheat
<i>Phaeoacremonium aleophilum</i>	Taqman multiplex	Aroca et al., 2008	Grapevine wood
<i>Phaeoacremonium mortoniae</i>	Taqman multiplex	Aroca et al., 2008	Grapevine wood
<i>Phaeoacremonium parasiticum</i>	Taqman multiplex	Aroca et al., 2008	Grapevine wood
<i>Phaeoacremonium viticola</i>	Taqman multiplex	Aroca et al., 2008	Grapevine wood
<i>Phaeospora nodorum</i>	TaqMan MGB	Bearchell et al., 2005	Wheat
<i>Phialophora gregata</i>	TaqMan	Malvick & Impullitti, 2007	Soybean Soil
<i>Phoma tracheiphila</i>	TaqMan	Demontis et al., 2008	Citrus
<i>Phoma tracheiphila</i>	TaqMan	Licciardello et al., 2006	Citrus
<i>Phomopsis</i> sp.	TaqMan	Børja et al., 2006	Spruce
<i>Phytophthora citricola</i>	TaqMan multiplex	Schena et al., 2006	Forest trees
<i>Phytophthora erythroseptica</i>	TaqMan	Nanayakkara et al., 2009	Potato
<i>Phytophthora kernoviae</i>	TaqMan multiplex	Schena et al., 2006	Forest trees

Pathogen	Real-time chemistry	Reference	Host plant
<i>Phytophthora pseudosyringae</i>	TaqMan multiplex	Tooley et al., 2006	Rhododendron and other host species
<i>Phytophthora quercina</i>	TaqMan multiplex	Schena et al., 2006	Forest trees
<i>Phytophthora ramorum</i>	TaqMan multiplex	Bilodeau et al., 2009	Oak
	TaqMan	Hughes et al., 2006	<i>Parrotia persica</i>
	TaqMan multiplex	Schena et al., 2006	Forest trees
	Scorpion and Molecular Beacon	Tomlinson et al., 2007	
	TaqMan multiplex	Tooley et al., 2006	Rhododendron and other host species
<i>Phytophthora</i> spp.	TaqMan	Tomlinson et al., 2005	Rhododendron
	TaqMan multiplex	Bilodeau et al., 2009	Oak
<i>Plasmopara viticola</i>	TaqMan	Valsesia et al., 2005	Grapevine
<i>Pochonia chlamydosporia</i>	Molecular Beacon	Macía-Vicente et al., 2009	Barley
<i>Puccinia coronata</i>	TaqMan	Jackson et al., 2006	Oat
<i>Puccinia graminis</i>	TaqMan	Barnes & Szabo, 2007	Cereals Grasses
<i>Puccinia recondita</i>	TaqMan	Barnes & Szabo, 2007	Cereals Grasses
<i>Puccinia striiformis</i>	TaqMan	Barnes & Szabo, 2007	Cereal Grasses
<i>Puccinia triticina</i>	TaqMan	Barnes & Szabo, 2007	Cereals Grasses
<i>Pyrenophora teres</i>	TaqMan MGB	Leisova et al., 2006	Barley
<i>Pyrenophora teres</i> f. <i>maculata</i>	TaqMan MGB	Leisova et al., 2006	Barley
<i>Pyrenophora teres</i> f. <i>teres</i>	TaqMan MGB	Leisova et al., 2006	Barley
<i>Pythium vexans</i>	TaqMan	Tewoldemedhin et al., 2011	Apple
<i>Rhynchosporium secalis</i>	TaqMan, LNA	Fountaine et al., 2007	Barley
<i>Rosellinia necatrix</i>	Scorpion	Ruano-Rosa et al., 2007	Avocado
<i>Thielaviopsis basicola</i>	TaqMan	Huang & Kang, 2010	Tobacco Soil
<i>Ustilaginoidea virens</i>	TaqMan	Ashizawa et al., 2010	Rice Soil

Table 2. Examples of real-time PCR based on specific fluorescent labelled probes or primers for detection of plant pathogenic fungi (last 6 years).

and the quencher are in close proximity and fluorescence is quenched. When the probe hybridises to the target sequence the complementary arms separate, thus allowing the emission of fluorescence and hence making possible the detection and quantification of the target sequence. Because the stem/loop structure is very thermostable molecular beacons must have a high specificity to hybridise to a target. This makes the chemistry appropriate for the detection of single nucleotide differences in mutation and SNP analyses. Molecular beacons have allowed real-time specific quantification of *Fusarium equiseti* and *Pochonia chlamydosporia* (a nematode parasitic fungus) in barley roots (Macía-Vicente et al., 2009).

Scorpions® are bifunctional molecules in which an upstream hairpin probe is covalently linked to a downstream primer sequence (Whitcombe et al., 1999). The hairpin probe contains a fluorophore at the 5' end and a quencher at the 3' end. The loop portion of the scorpion probe is complementary to the target sequence. During the amplification reaction the probe becomes attached to the target region synthesized in the first PCR cycle. Following the second cycle of denaturation and annealing, the probe and the target hybridise resulting in separation of the fluorophore from the quencher and an increase in the fluorescence emitted. Improvement of Scorpions sensitivity has been achieved by placing the quencher in a separate oligonucleotide (Scorpions bi-probes) allowing greater separation of fluorophore and quencher and giving stronger signals. As with all dye-probe based methods, Scorpion probes follow strict design considerations for secondary structure and primer sequence to ensure that a secondary reaction will not compete with the correct probing event. Scorpion technology has been used for the detection of *Rosellinia necatrix* in roots of different plant host species and soils (Ruano-Rosa et al., 2007; Schena & Ippolito, 2003), and for the detection of *Fuscoporia torulosa* in holm oaks (Campanile et al., 2008).

### c. Specific fluorescent labelled primers

Unlike other real-time chemistries, in which the incorporation of the fluorescent dye increases with the increasing number of copies of the DNA target, Plexor system measures the decrease of the fluorescence over time by the quenching of the dye. One of the two primers is labelled with a fluorescent dye and modified with methylisocytosine (isodC) residue at the 5' end, whereas the other primer is not. The dabcyI-iso dGTP (iso-dG) present in the real-time PCR reaction cocktail is incorporated at the position complementary to the iso-dC label acting as a quencher and reducing the fluorescence over time. A quantitative real-time using Plexor primers has been developed for the detection and quantification of *Verticillium dahliae* in potato (Atallah et al., 2007).

## 2.2 Isothermal amplification methods

An efficient and cost-effective alternative to PCR is the possibility of isothermal amplification that does not require thermocycler apparatus. Loop-Mediated Isothermal Amplification (LAMP) (Notomi et al., 2000) uses a set of six oligonucleotide primers with eight binding sites hybridizing specifically to different regions of a target gene, and a thermophilic DNA polymerase from *Geobacillus stearothermophilus* for DNA amplification. This technique can specifically amplify the DNA target using only a heated block in less than 1 hour. Amplification products can be detected directly by visual inspection in vials using SYBR Green I, or by measuring the increased turbidity (due to the production of large

amounts of magnesium pyrophosphate), as well as by electrophoresis on agarose gel. LAMP method is very suitable for field testing and potentially valuable to laboratories without PCR facilities. This isothermal method has been applied for the rapid detection of *Fusarium graminearum* in contaminated wheat seeds (Abd-Elsalam et al., 2011) and for the detection of *Phytophthora ramorum* and *P. kernoviae* in field samples (Tomlinson et al., 2007, 2010).

## 2.3 Fingerprinting

Fingerprinting approaches allow the screening of random regions of the fungal genome for identifying species-specific sequences when conserved genes have not enough variation to successfully identify species (McCartney et al., 2003). Fingerprinting analyses are generally used to study the phylogenetic structure of fungal populations. However, these techniques have been also useful for identifying specific sequences used for the detection of fungi at very low taxonomic level, and even for differentiate strains of the same species with different host range, virulence, compatibility group or mating type.

### 2.3.1 Restriction fragment length polymorphism (RFLP)

RFLP involves restriction enzyme digestion of the pathogen DNA, followed by separation of the fragments by electrophoresis in agarose or polyacrilamide gels to detect differences in the size of DNA fragments. Polymorphisms in the restriction enzyme cleavage sites are used to distinguish fungal species. Although DNA restriction profile can be directly observed by staining the gels, Southern blot analysis is usually necessary. DNA must be transferred to adequate membranes and hybridised with an appropriate probe. However, the Southern blot technique is laborious, and requires large amounts of undegraded DNA. RFLPs have been largely used for the study of the diversity of micorrhizal and soil fungal communities (Thies, 2007; Kim Y. T. et al., 2010; Martínez-García et al., 2011). Although used for differentiation of pathogenic fungi (Hyakumachi et al., 2005) this early technique has been progressively supplanted by other fingerprint techniques based in PCR.

PCR-RFLP combines the amplification of a target region with the further digestion of the PCR products obtained. PCR primers specific to the genus *Phytophthora* were used to amplify and further digest the resulting amplicons yielding a specific restriction pattern of 27 different *Phytophthora* species (Drenth et al., 2006). PCR-RFLP analysis of the ITS region demonstrated the presence of different anastomosis group (AG) within isolates of *Rhizoctonia solani* (Pannecoucque & Höfte, 2009); It also allowed the differentiation of pathogenic and non pathogenic strains of *Pythium myriotolum* (Gómez-Alpizar et al., 2011). In other cases, the analysis of the ITS region by this technique failed in differentiating closely related species (e.g., clade 1c species such as *Phytophthora infestans* and *P. mirabilis*) (Grünwald et al., 2011).

### 2.3.2 Random Amplified Polymorphic DNA (RAPD)

RAPD analyses rely on PCR amplification of the pathogen genome with short arbitrary sequences (usually decamers) that are used as primers. These primers are probably able to find distinct complementary sequences in the genome producing specific banding patterns. The resulting PCR fragments are then separated by electrophoresis to obtain fingerprints



that may distinguish fungal species varieties or strains (Welsh & McClelland, 1990; Williams et al., 1990). Some of the specific DNA fragments detected in a profile may be cut out of the gel and sequenced to obtain a SCAR (Sequence-characterized amplified region), into which specific primers can be designed for a more precise PCR detection. SCAR primers have been used for instance to specifically identify *Phytophthora cactorum* (Causin et al., 2005), *Fusarium subglutinans* (Zaccaro et al., 2007) and *Guignardia citricarpa* (Stringari et al., 2009) in infected plant material; to distinguish among several *formae speciales* of *Fusarium oxysporum* (Lievens et al., 2008); to differentiate the bioherbicidal strain of *Sclerotinia minor* from like organisms (Pan et al., 2010); and to establish two different groups in *Gaeumannomyces graminis* var. *tritici* (Daval et al., 2010).

RAPD results are also useful for the analysis of the genetic diversity among populations. Fingerprints are scored for the presence (1) or absence (0) of bands of various molecular weight sizes in the form of binary matrices. Data are analyzed to obtain statistic coefficients among the isolates that are then clustered to generate dendrograms. RAPDs have been used to analyze the genetic diversity among different species and races of *Fusarium* spp. (Lievens et al., 2007; Arici & Koc 2010) and different pathotypes of *Elsinoë* spp. (Hyun et al., 2009). This technique has also been applied to differentiate fungi isolates according to their host plant (Midorikawa et al., 2008), enzyme production profiles (Saldanha et al., 2007) or geographical origin and chemotypes (Zheng et al., 2009).

The RAPD technique is rapid, inexpensive and does not require any prior knowledge of the DNA sequence of the target organism. Results obtained from RAPD profiles are easy to interpret because they are based on amplification or non amplification of specific DNA sequences. In addition, RAPD analyses can be carried out on large numbers of isolates without the need for abundant quantities of high-quality DNA (Nayaka et al., 2011). Disadvantages of this technique include poor reproducibility between laboratories, and the inability to differentiate non-homologous co-migrating bands. In addition, RAPDs are dominant markers so, they cannot measure the genetic diversity affected by the number of alleles at a locus, nor differentiate homozygotes and heterozygotes individuals. This is not an issue with haploid fungi, but it can be a problem with many basidiomycetes and oomycetes that are heterokaryons, diploids or polyploids (Fourie et al., 2011).

### 2.3.3 Amplified fragment length polymorphism (AFLP)

AFLP analysis (Vos et al., 1995) consists in the use of restriction enzymes to digest total genomic DNA followed by ligation of restriction half-site specific adaptors to all restriction fragments. Then, a selective amplification of these restriction fragments is performed with PCR primers that have in their 3' end the corresponding adaptor sequence and selective bases. The band pattern of the amplified fragments is visualized on denaturing polyacrilamide gels. The AFLP technology has the capability to amplify between 50 and 100 fragments at one time and to detect various polymorphisms in different genomic regions simultaneously. It is also highly sensitive and reproducible. As with other fingerprinting techniques, no prior sequence information is needed for amplification (Meudt & Clarke 2007). The disadvantages of AFLPs are that they require high molecular weight DNA, more technical expertise than RAPDs (ligations, restriction enzyme digestions, and polyacrilamide gels), and that AFLP analyses suffer the same analytical limitations of RAPDs (McDonald et al., 1997).

Depending on the primers used and on the reaction conditions, random amplification of fungal genomes produces genetic polymorphisms specific at the genus, species or strain levels (Liu et al., 2009). As a result, AFLP has been used to differentiate fungal isolates at several taxonomic levels e.g. to distinguish *Cladosporium fulvum* from *Pyrenopeziza brassicae* species (Majer et al., 1996), *Aspergillus carbonarius* from *A. ochraceus* (Schmidt et al., 2004), and *Colletotrichum gossypii* from *C. gossypii* var. *cephalosporioides* (Silva et al., 2005); also to differentiate *Monilinia laxa* that infect apple trees from isolates infecting other host plants (Gril et al., 2008); and to separate non-pathogenic strains of *Fusarium oxysporum* from those of *F. commune* (Stewart et al., 2006). AFLP markers have also been used to construct genetic linkage maps e.g. of *Phytophthora infestans* (Van der Lee et al., 1997). Specific AFLP bands may also be used for SCAR markers development used in PCR-based diagnostic tests. Using SCAR markers Cipriani et al. (2009) could distinguish isolates of *Fusarium oxysporum* that specifically infect the weed *Orobancha ramosa*. AFLP profiles have also been widely used for the phylogenetic analysis of *Fusarium oxysporum* complex (Baayen et al., 2000; Fourie et al., 2011; Groenewald et al., 2006).

### 2.3.4 Microsatellites

Microsatellites, also known as simple sequence repeats (SSRs) or short tandem repeats (STRs), are motifs of one to six nucleotides repeated several times in all eukaryotic genomes (generally in non-coding regions). These nucleotide units can differ in repeat number among individuals and their distribution in the genome is almost random. Using primers flanking such variable regions PCR products of different lengths can be obtained. So, the microsatellites are highly versatile genetic markers that have been widely exploited for DNA fingerprinting. The advantages of SSRs are that they are multiallelic, codominant, highly polymorphic and several thousand potentially polymorphic markers are available. Moreover, it is possible the analysis of samples with limited DNA amounts or degraded DNA with high reproducibility. The microsatellites have a high mutation rate and are able to gain and lose repeat units by DNA-replication slippage, a mutation mechanism that is specific to tandemly repeated sequences (Schlötterer, 2000). This characteristic can create difficulties for populations-genetic analyses. Other drawbacks of the SSRs include the requirement of a prior knowledge of the DNA sequences of the flanking regions and their cost and low throughput because of difficulties for automation and data management. Moreover, a high number of microsatellite loci are necessary for a reliable phylogenetic reconstruction. However, the next-generation sequencing technologies and multiplexing microsatellites solve, in part, these problems.

Microsatellites have been used for the study of the genetic diversity of plant pathogenic fungi within species e.g. *Ascochyta rabiei* (Bayraktar et al., 2007), *Ceratocystis fimbriata* (Rizatto et al., 2010), *Macrophomina phaseolina* (Jana et al., 2005), *Puccinia graminis* and *P. trititica* (Szabo, 2007; Szabo & Kolmer, 2007), *Sclerotinia subarctica* and *S. sclerotiorum* (Winton et al., 2007); and for genetic map construction, e.g. Zheng et al. (2008) constructed a genetic map of *Magnaporthe grisea* consisting of 176 SSR markers. In other experiment, microsatellite markers specific for *Phytophthora ramorum* were employed to distinguish between A1 and A2 mating types isolates of this pathogen from two different geographic origins (Prospero et al., 2004).

To reduce the cost of developing microsatellites a novel technique has emerged based on sequence tagged microsatellites (STMs). Each STM is amplified by PCR using a single

primer specific to the conserved DNA sequence flanking the microsatellite repeat in combination with a universal primer that anchors to the 5'-ends of the microsatellites (Hayden et al., 2002). STMs have been developed for the plant pathogens *Rhynchosporium secalis* (Keipfer et al., 2006) and *Pyrenophora teres* (Keipfer et al., 2007).

## 2.4 DNA hybridisation technology

The use of Southern blot or dot blot hybridisation techniques using selected probes from DNA libraries was a strategy for the identification of plant pathogens prior to the introduction of PCR-based methods with greater sensitivity, simplicity and speed (Takamatsu et al., 1998; Levesque et al., 1998; Xu et al., 1999). Nevertheless, new and revolutionary methods based in hybridisation have been recently developed for detection and differentiation of phytopathogenic fungi:

### 2.4.1 DNA arrays

A DNA array is a collection of species-specific oligonucleotides or cDNAs (known as probes) immobilized on a solid support that is subjected to hybridisation with a labelled target DNA. Macroarrays are membrane-based arrays containing spotted samples of 300 µm in diameter or more. Microarrays uses higher density chips such as glass or silicon, or microscopic beads in where thousands of sample spots (less than 200 µm in diameter) are immobilised via robotisation. The target DNA is a labelled PCR fragment, amplified with universal primers, spanning a genomic region that includes species-specific sequences. Probe-target hybridisation is usually detected and quantified by detection of fluorophore-, silver-, or chemiluminescence-labeled targets and the relative abundance of nucleic acids sequences in the target can be determined. DNA micro- and macro-arrays are generally used for gene expression profiling but are also powerful tools for identification and differentiation of plant pathogens (Anderson N. et al., 2006; Lievens & Thomma, 2005). Currently, it is one of the most suitable techniques to detect and quantify multiple pathogens present in a sample (plant, soil, or water) in a single assay (Lievens et al., 2005a). The specificity of the DNA array technology allows an accurate SNP detection. This characteristic is crucial for diagnostic application since closely related pathogens may differ in only a single base pair polymorphism for a target gene. Specificity of the assay depends on the number and position of the mismatch(es) in the oligonucleotide probes, the oligonucleotide sequence and the length of the amplicon target. For instance, mismatches at the 3' end of the oligonucleotide must be avoided and center mismatches are usually the most discriminatory sites (Lievens et al., 2006). Furthermore, using longer amplicons as targets increases the sensitivity but decreases the specificity of the array hybridisation. For improving specificity and robustness, the use of multiple oligonucleotides for a single pathogen and the use of multiple diagnostic regions are desirable. One of the main drawbacks of this technique is however, the lack of sensitivity. To reach sensitive detections, PCR amplification before array hybridisation is required, biasing the results through the species that are more represented in the sample.

This technology has been applied for detecting oomycete plant pathogens by using specific oligonucleotides designed on the ITS region (Anderson N. et al., 2006; Izzo & Mazzola, 2009). Another ITS and rRNA genes-based microarrays allowed the multiple detection and quantification of tomato pathogens (*Verticillium*, *Fusarium*, *Pythium* and *Rhizoctonia*)

confirmed by real-time PCR analysis (Lievens et al., 2005b), the monitoring of *Phytophthora* species diversity in soil and water samples (Chimento et al., 2005), and the identification and differentiation of toxin producing and non-producing *Fusarium* species in cereal grains (Nicolaisen et al., 2005). Using a *cox I* high density oligonucleotide microarray Chen et al. (2009) could identify *Penicillium* species. Moreover, Lievens et al. (2007) could detect and differentiate *F. oxysporum* f. sp. *cucumerinum* and *F. oxysporum* f. sp. *radicis-cucumerinum* pathogens by a DNA array containing genus-, species- and *forma specialis*-specific oligonucleotides.

Additionally, A DNA array for simultaneous detection of over 40 different plant pathogenic soilborne fungi and 10 bacteria that frequently occur in greenhouse crops has been developed. This array, called DNA Multiscan® (<http://www.dnamultiscan.com/>), is routinely used worldwide by companies that offer disease diagnostic services and advice to commercial growers.

## 2.5 Sequencing

As discussed above, morphological characteristics are not always enough to identify a pathogen. One of the most direct approaches to do that consists in the PCR amplification of a target gene with universal primers, followed by sequencing and comparison with the available publicly databases. In addition, new fungal species have been described by using sequencing approaches. However, the use of sequence databases to identify organisms based on DNA similarity may have some pitfalls including erroneous and incomplete sequences, sequences associated with misidentified organisms, the inability to easily change or update data, and problems associated with defining species boundaries, all of them leading to erroneous interpretation of search results. An effort for generating and archiving high quality data by the researchers community should be the remedy of this drawback (Kang et al., 2010). Other limitation of sequencing as diagnostic tool is the need to sequence more than one locus for the robustness of the result, and the impractical of this method in cases when rapid results are needed such as for the control or eradication of serious plant disease outbreaks. Nevertheless, the increase of sequencing capacity and the decrease of costs have allowed the accumulation of a high numbers of fungal sequences in publicly accessible sequence databases, and sequences of selected genes have been widely used for the identification of specific pathogens and the development of sequence-based diagnostic methods.

### 2.5.1 Massive sequencing techniques

The Sanger sequencing method has been partially supplanted by several “next-generation” sequencing technologies able to produce a high number of short sequences from multiple organisms in short time. Massive sequencing technologies offer dramatic increases in cost-effective sequence throughput, having a tremendous impact on genomic research. They have been used for standard sequencing applications, such as genome sequencing and resequencing, and quantification of sequence variation. The next-generation technologies commercially available today include the 454 GS20 pyrosequencing-based instruments (Roche Applied Science), the Solexa 1G analyzer (Illumina, Inc.), and the SOLiD instrument (Applied Biosystems).

Pyrosequencing is a DNA sequencing technology based on the sequencing-by-synthesis principle. The technique is built on a four enzyme real-time monitoring of DNA synthesis by



chemiluminescence and a fifth protein, SSB, which can be included to enhance the quality of the obtained sequences and thereby prolong the read length. The detection system is based on the pyrophosphate released when a nucleotide is introduced in the DNA strand. Thereby, the signal can be quantitatively connected to the number of bases added (Ahmadian et al., 2006). The pyrosequencing principle is used by the 454 platform, the first next-generation sequencing technology released to the market by Roche Applied Science. 454 technology is based in emulsion PCR (Tawfik et al., 1998), which uses fixing adapter-ligated-DNA fragments to streptavidin beads in water-in-oil emulsion droplets. In each droplet the DNA fixed to these beads is then amplified by PCR producing about  $10^7$  copies of a unique DNA template per bead. Each DNA-bound bead is placed into a  $\sim 29 \mu\text{m}$  well on a PicoTiterPlate, a fiber optic chip, and analyzed using a pyrosequencing reaction. The use of the picotiter plate allows hundreds of thousands of pyrosequencing reactions to be carried out in parallel, massively increasing the sequencing throughput. 454 platform is capable of generating 80–120 Mb of sequence in 200 to 300 bp reads in a 4 h run. This technology enables a rapid and accurate quantification of sequence variation, including mutation detection, SNP genotyping, estimation of allele frequency and gene copy number, allelic imbalance and methylation status. Pyrosequencing can be applied to any DNA source, including degraded or low-quality DNA. Disadvantages are short read lengths, which may be problematic for sequence assembly particularly in areas associated with sequence repeats, the need for expensive biotinylated primers, and the inability to accurately detect variants within long ( $\sim 5$  or  $6$  bp) homopolymer stretches. In addition, multiplexing, while possible, is difficult to design.

The pyrosequencing technology has not been widely applied for the control of fungal plant diseases yet. However, Nunes et al. (2011) applied 454 sequencing technology to elucidate and characterize the small RNA transcriptome (15 - 40 nt) of mycelia and appressoria of *Magnaporthe oryzae*. Thus, they propose that a better understanding of key small RNA players in *M. oryzae* pathogenesis-related processes may illuminate alternative strategies to engineer plants capable of modifying the *M. oryzae* small transcriptome, and suppress disease development in an effective manner. Another application of this new sequencing technology is the rapid generation of genomic information to identify putative single-nucleotide polymorphisms (SNPs) to be used for population genetic, evolutionary, and phylogeographic studies on non-model organisms. Thus, Broders et al. (2011) described the sequencing, assembly and discovery of SNPs from the plant fungal pathogen *Ophiognomonia clavigignenti-juglandacearum*, for which virtually no sequence information was previously available. Moreover, Malausa et al. (2011) described a high-throughput method for isolating microsatellite markers based on coupling multiplex microsatellite enrichment and 454 pyrosequencing in different organisms, such as *Phytophthora alni* subsp. *uniformis*.

The principle of the Illumina/Solexa system is also based on sequencing-by-synthesis chemistry, with novel reversible terminator nucleotides for the four bases each labeled with a different fluorescent dye, and a special DNA polymerase enzyme able to incorporate them (Ansorge, 2010). In the ABI SOLiD (Sequencing by Oligo Ligation and Detection), the sequence extension reaction is not carried out by polymerases but rather by ligases. In the sequencing-by-ligation process, a sequencing primer is hybridized to single-stranded copies of the library molecules to be sequenced. (Kircher & Kelso, 2010). These two above mentioned systems have not been currently used in studies on plant pathogenic fungi.



### 2.5.2 DNA barcoding

DNA barcoding is a taxonomic method that uses a short genetic marker in the DNA to identify an organism as belonging to a particular species. It has facilitated the description of numerous new species and the characterisation of species complexes. Current fungal species identification platforms are available: *Fusarium*-ID was created as a simple, web-accessible BLAST server that consisted of sequences of the *TEF 1*  $\alpha$  gene from representative species of *Fusarium* (Geiser et al., 2004). Sequences of multiple marker loci from almost all known *Fusarium* species have been progressively included for supporting strain identification and phylogenetic analyses. Two additional platforms have been constructed: the *Fusarium* Comparative Genomics Platform (FCGP), which keeps five genomes from four species, supports genome browsing and analysis, and shows computed characteristics of multiple gene families and functional groups; and the *Fusarium* Community Platform (FCP), an online research and education forum. All together, these platforms form the Cyber infrastructure for *Fusarium* (CiF; <http://www.fusariumdb.org/>) (Park, B. et al., 2011).

For *Phytophthora* identification two web-based databases have been created: (i) *Phytophthora* Database (<http://www.phytophthoradb.org/>) based in nine loci sequences including the ITS region and the 5' portion of the large subunit of rRNA genes; nuclear genes encoding 60S ribosomal protein L10,  $\beta$ -tubulin, enolase, heat shock protein 90, TigA fusion protein, and TEF 1 $\alpha$ ; the mitochondrial gene *cox II* and spacer region between *cox I* and *cox II* genes (Park, J. et al., 2008); and (ii) *Phytophthora*-ID (<http://phytophthora-id.org/>) based on sequences of the ITS and the *cox I* and *cox II* spacer regions (Grunwald et al., 2011). Additional web-based databases are available including UNITE (<http://unite.ut.ee/index.php>), an ITS database supporting the identification of ectomycorrhizal fungi (Koljalg et al., 2005); TrichOKey (<http://www.isth.info/tools/molkey/index.php>), a database supporting the identification of *Hypocrea* and *Trichoderma* species (Druzhinina et al., 2005); and BOLD (<http://www.boldsystems.org/>) containing ITS and *cox I* databases from oomycetes (Ratnasingham et al., 2007; Robideau et al., 2011). Consortium for the Barcode of Life (CBOL) is an international collaborative effort which aims to use DNA barcoding to generate a unique genetic barcode for every species of life on earth. The *cox I* mitochondrial gene is emerging as the standard barcode region for eukariotes.

## 3. Fungicide resistance

Despite extensive fungicide use in the previous 90 years, resistance emerged as a practical problem as recently as 1970. The incidence of resistance has been restricted largely to systemic fungicides that operate to biochemical targets (single-site inhibitors). These included several of major groups of fungicides: sterol demethylation, bezimidazoles, pyrimidines, phenylamines, dicarboximides, carboxanilides and morpholines.

The resistance to toxic compounds is a genetic adaptation of the fungus to one or more fungicides that leads to a reduction in sensitivity to these compounds. This phenomenon, described as genotypic or acquired resistance, is found in numerous fungi that have been sensitive to the fungicide prior to exposure. Fungicide resistance can be acquired by single mutations in genes of the pathogen or by increasing the frequency of subpopulations that are naturally less sensitive. We have to differentiate between resistance and natural or intrinsic insensitivity of species that are not sensitive to the action of these compounds (Delp & Bekker,

1985; Brent, 1995). The term “lower sensitivity” is used in practical situations where there is a decreased sensitivity to a fungicide without an effect on field performance. The term “field resistance” is used when both the level of resistance and frequency of resistant strains are high and coincident, resulting in noticeable decline of field performance (Hewit, 1998).

It is to be expected that the evolutionary and dynamic progress of selection should eventually produce fungi that are resistant to fungicides. Therefore fungicide resistance is variable, with fungi developing resistance to some fungicides more rapidly than to others and in some cases no resistance had been reported after long periods of fungicide use.

In fungicide resistance, we should consider how pathogens have the ability to evolve resistant and how fungicides varies their susceptibility to resistance. Hence, resistance risk is determined by the target pathogen and selected fungicide.

Populations of fungi are so diverse that mechanisms of resistance may be present within the population before the fungicide is applied. The rate of resistance development in a population depends on the resultant mechanism of resistance such as how resistance characters are inherited, the epidemiology of the fungus, the environment, and the persistence of selective pressure.

### 3.1. Mechanisms of fungicide resistance

Fungicide resistance can be conferred by various mechanisms including: (I) an altered target site, which reduces the binding of the fungicide; (II) the synthesis of an alternative enzyme capable of substituting the target enzyme; (III) the overproduction of the fungicide target; (IV) an active efflux or reduced uptake of the fungicide; and (V) a metabolic breakdown of the fungicide. In addition, some unrecognized mechanisms could also be responsible for fungicide resistance.

#### 3.1.1 The reduction of intracellular concentration of antifungal compound

Currently, the most widespread hypothesis to explain the reduced levels of toxic products in the cell is based on active efflux of these compounds by ABC transporters (ATP binding cassette) and MFS (Major Facilitator Superfamily) (Hayashi et al., 2001, 2002a, 2002b, 2003; Stergiopoulos et al., 2002a, 2002b; Stergiopoulos & de Waard, 2002, Vermeulen et al., 2001). ABC and MFS transporters are the most studied so far and have been described in many fungi such as *Aspergillus nidulans*, *Botrytis cinerea*, *Mycosphaerella graminicola*, *Magnaporthe grisea*, *Penicillium digitatum*, etc (Andrade et al., 2000a, 2000b; del Sorbo et al., 2000; Nakaune, 2001; Schoonbeek et al., 2003; Stergiopoulos et al., 2002a; Stergiopoulos & de Waard, 2002; Vermeulen et al., 2001; Yoder and Turgeon 2001; Zwiers et al., 2003). Its function is to prevent or reduce the accumulation of compounds and therefore to avoid or minimize their toxic action (Bauer et al., 1999, Pao et al., 1998). ABC transporters comprise a large family of proteins and are located outside the plasma membrane or within the cell in intracellular compartments as vacuoles, endoplasmic reticulum, peroxisomes and mitochondria. They can carry a wide variety of toxic against the gradient (Del Sorbo et al., 2000; Theodoulou, 2000). ABC transporters include systems both capture and removal, generally showing activity on a wide range of substrates (fungicides, drugs, alkaloids, lipids, peptides, sterols, flavonoids, sugars, etc), but there are specific transporters for substrates (Bauer et al., 1999, Del Sorbo et al., 2000).

The overexpression of ABC and MFS genes plays an essential role in the resistance of chemically unrelated phenomenon described as multidrug resistance or MDR drugs (Del Sorbo et al., 2000; White, 1997). This phenomenon has been observed in a wide variety of organisms and can be a real threat to the effective control of fungal pathogens (Fling et al., 1991). In phytopathogenic fungi, these transporters can also be a virulence factor in providing protection against defense compounds produced by the plant or mediating the secretion of host-specific toxins. Also play a major role in determining the baseline sensitivity to fungicides and other antifungal agents. (De Waard, 1997; Stergiopoulos et al., 2003a, 2003b).

In *P. digitatum*, causal agent of citrus green rotten, four ABC transporters have been identified so far. ABC transporters PMR1 (Hamamoto et al., 2001b; Nakaune et al., 1998) and PMR5 (Nakaune, 2001; Nakaune et al., 2002) have been studied previously. Disruption of PMR1 in sensitive and resistant strains results in an increased sensitivity to DMIs and other compounds (Nakaune et al., 1998; Nakaune et al., 2002). However, the introduction of resistant strains from PMR1 restores the resistance while the introduction of PMR1 from sensitive strains does not have the same effect and does not restore the resistance. This suggests that although PMR1 plays an important role in the sensitivity of *P. digitatum* against DMIs alone does not explain the differences between sensitive and resistant strains (Hamamoto et al., 2001). Another of the genes studied is PMR5. This gene has highly homologous to PMR1, and also to *atrB* from *Aspergillus nidulans* and *BcAtrB* from *Botrytis cinerea* (Schoonbeek et al., 2003), however, is strongly induced by benzimidazoles, resveratrol and other compounds, but not for DMIs. This shows the different substrate specificity of both proteins and may play an important role in providing protection against natural or synthetic toxic compounds (Nakaune et al., 2002).

Sequence analysis in all four ABC transporter genes in several sensitive and resistant strains revealed no mutations in PMR1, PMR3 and PMR4, and point mutations only were observed in both the promoter and coding regions of PMR5 in multiple resistant strains (TBZ- and DMI-resistant) (Sánchez-Torres & Tuset, 2011). But no explanation was ascertained for the absence of sequence changes relating to fungicide resistance in the other ABC transporters, particularly in the PMR1 gene given that transcription of PMR1 has proven to be strongly activated in the presence of different fungicides (Hamamoto et al., 2001b; Nakaune, 2001; Nakaune et al., 1998).

To date, MFS transporters have been described in several fungi, e.g. *Botrytis cinerea* shows a broad spectrum of resistance to different fungicides and their expression has been induced by many of them, particularly noteworthy *Bcmfs4* induction in the presence of strobilurin (trifloxiestrobin) (Hayashi et al., 2002a, 2002b, 2003; Schoonbeek et al., 2003; Vermeulen et al., 2001). All this means that these transporters are potential candidates for the study of factors involved in resistance based on active efflux of these toxic compounds since they have remarkably broad substrate specificity although they can also transport specific compounds.

Recently, five different MFS transporters have been identified and characterized in the postharvest phytopathogenic fungus *Penicillium digitatum* (PdMFS1-PdMFS5). Sequence analysis of these five genes revealed different genomic structure and although all genes seem to be implicated in pathogenicity, only 2 out of five MFS transporters confirmed to be involved in fungicide resistance (Sánchez-Torres et al., submitted). Therefore, the most recent thought for fungicide resistance based on active efflux of these toxic compounds is

now discussed. These results suggest that many genes could be involved in the mechanisms conferring fungicide resistance to phytopathogenic fungi and some are fungicide-dependent.

From a practical standpoint, the fact that ABC and MFS transporters determine the baseline sensitivity to fungicides, are responsible for MDR and can act as virulence factors implies that these carriers are an attractive target for chemical control. In this context, inhibitors of these transporters could improve the effectiveness of control and reduce the virulence of fungal pathogens.

### 3.1.2 Changes in binding target that causes a reduced affinity of the compound fungicide

The most extent mechanism to confer DMI resistance involved mutations of *CYP51* gene, the target enzyme of DMIs fungicides and has been described for a large number of pathogens such as *Botrytis cinerea* (Albertini et al., 2002, Albertini & Leroux, 2004), a substitution of Phe for Tyr at position 136 (Y136F) was found in *Uncinula necator* (Délye et al., 1997) and also in *Erisiphe graminis* f. sp. *hordei* (Délye et al., 1998). Two single nucleotide mutations of *CYP51* resulting in amino acid substitutions Y136F and K147Q in *Blumeria graminis* were also found (Wyand & Brown, 2005). Different mutations were also found in *Tapesia* sp (Albertini et al., 2003), *Penicillium italicum* (Joseph-Horne & Hollomon, 1997), *Ustilago maydis* (Butters et al., 2000) and *Blumeriella jaapii* (Ma et al., 2006)

Similarly, mutations have been described in the cytochrome b gene that lead to change of its corresponding protein G143A, conferring resistance to strobilurin (Avila-Adame & Koller, 2003a, 2003b; Gisi et al., 2000, Zheng et al., 2000, Zhang, Z. et al., 2009) or the amino acid substitution in the  $\beta$ -tubulin target protein involved in development of resistance to benzimidazoles in *Botrytis cinerea* (Banno, 2008), *Venturia inaequalis* and *Penicillium italicum* (Koenraad et al., 1992), *Monilinia fructicola* (Ma et al., 2003a), *M. laxa* (Ma & Michailides, 2005), *P. expansum* (Baraldi et al., 2003) and *P. digitatum* (Sánchez-Torres & Tuset, 2011).

### 3.1.3 Over-expression of the target of union of fungicide

P45014DM increased by over-expression of the *CYP51* gene has been described as a mechanism of resistance to azoles. In *Penicillium digitatum* a unique 126-bp sequence in the promoter region of *CYP51* was tandem repeated five times in resistant isolates and was present only one in sensitive isolates. This provided a quick and easy method to detect DMI-resistant strains of *P. digitatum* (Hamamoto et al., 2001). Insertions in the promoter were also found in *Blumeriella jaapii* (Ma et al., 2006), *Venturia inaequalis* (Schnabel & Jones 2001) and in *Monilinia fructicola* (Luo et al., 2008) and recently another insertion of 199-bp was found in *P. digitatum* *CYP51A* gene (Ghosop et al., 2007) and *PdCYP51B* gene (Sun et al., 2011; Sánchez-Torres et al., submitted) leading to resistant phenotypes.

### 3.1.4 Compensation of the toxic effects of the fungicide by altered biosynthetic or metabolic pathway

This phenomenon has been described both in the case of DMIs because they exert their toxic effect by depletion of ergosterol and the accumulation of C14 methylated precursors and in the case of strobilurins since the presence of AOX (alternative oxidase) allows the use an



alternative route in mitochondrial respiration (Gisi et al., 2000; Schnabel et al., 2001; Wood & Hollomon, 2003).

### 3.2 Molecular detection of fungicide resistance in phytopathogenic fungi

The procedures for detecting fungicide resistance using conventional methods are labor-intensive and time-consuming if large numbers of isolates have to be tested. Advances in molecular biology have provided new opportunities for rapidly detecting fungicide resistant genotype once the mechanisms of resistance have been elucidated at a molecular level. Several molecular techniques, such as PCR, PCR-restriction fragment length polymorphism (PCR-RFLP), allele specific PCR, allele-specific real-time PCR and massive sequencing techniques (all above described) have been used successfully to detect fungicide-resistant genotypes of several plant pathogens. Many examples of these techniques have been reported.

#### 3.2.1 PCR detection of DMI-resistant isolates

PCR has revolutionized molecular biology and diagnostics and has become a fast tool for detecting fungicide-resistant pathogens. In *Penicillium digitatum*, the DMI resistance resulted from over-expression of the *PdCYP51* genes driven by a tandem repeat of five copies of a 126 transcriptional enhancer (Hamamoto et al., 2000), or insertion of 199-bp in the promoter region of *PdCYP51A* (Gosoph et al., 2007) or by the presence of 199-bp enhancer in the promoter region of *PdCYP51B* (Sun et al., 2011). Based on the DNA sequence of the *PdCYP51*-genes, PCR primers have been developed which are able to distinguish not only between sensitive and resistant DMI strains but allow identification of molecular mechanism that takes place (Hamamoto et al., 2001; Gosoph et al., 2007; Sánchez-Torres & Tuset, 2011; Sun et al., 2011).

#### 3.2.2 PCR-RFLP and primer-introduced restriction analysis PCR (PIRA-PCR)

PCR amplification followed by restriction enzyme analysis (PCR-RFLP) is a general technique to detect a point mutation that alters a restriction enzyme site. This method has been used to rapidly detect benzimidazole-resistant isolates of *Monilinia laxa* from stone fruit and almond crops in California (Ma & Michailides, 2005) and can also detect azoxystrobin-resistant isolates of *Alternaria alternata*, *A. tenuissima*, and *A. arborescens* within a few hours (Ma et al., 2003b). Since the PCR-RFLP is an easy and rapid technique, these assays have been developed for detecting benzimidazole resistance in *Botrytis cinerea* (Luck & Gillings, 1995), *Cladobotryum dendroides* (McKay et al., 1998), *Helminthosporium solani* (Cunha & Rizzo, 2003; McKay & Cooke, 1997), and for detecting strobilurin resistance in *Blumeria graminis* f.sp. *hordei* (Baumler et al., 2003), *Erysiphe graminis* f. sp. *tritici* (Sierotzki et al., 2000), and *Podosphaera fusca* (Ishii et al., 2001).

Although PCR-RFLP is a simple method for detection of point mutations, some target DNA fragments may not contain a restriction endonuclease recognition sequence at the site of point mutation. Thus, primer-introduced restriction analysis PCR (PIRA-PCR) has become a useful method to create diagnostic artificial RFLPs (Haliassos et al., 1989). A PIRA-PCR assay was developed for detecting carpropamid resistant *Magnaporthe grisea* (Kaku et al., 2003).



### 3.2.3 Allele-specific PCR and quantitative allele-specific real-time PCR

Allele-specific PCR is another simple and rapid method for detecting point mutations. Usually, one of two PCR primers used in an allele specific amplification is designed to amplify preferentially one allele by matching the desired allele and mismatching the other allele at the 3' end of primer.

Allele-specific PCR assays have been developed for detecting benzimidazole-low resistant isolates of *Monilinia laxa* (Ma & Michailides, 2005), and azoxystrobin-resistant isolates of *Alternaria alternata*, *A. tenuissima*, and *A. arborascens* (Ma & Michailides, 2004). Additionally, allele-specific PCR assays have been developed for the rapid detection of strobilurin-resistant isolates of *Blumeria graminis* f. sp. *tritici* (Fraaije et al., 2002) and *Mycosphaerella fijiensis* (Gisi et al., 2002), and DMI-resistant isolates of *Erysiphe graminis* f. sp. *hordei* (Dèlye et al., 1997).

Real-time PCR technique can be used to quantitatively determine the amount of target DNA in a sample. An allele-specific real-time PCR assay has been used to follow the dynamics of *Qol* resistant allele A143 in field populations of *Blumeria graminis* f. sp. *tritici* before and after fungicide application (Fraaije et al., 2002). A real-time PCR assay to rapidly detect azoxystrobin-resistant *Alternaria* has been developed in California pistachio orchards (Ma & Michailides, 2005). Additionally, a real-time PCR using the *Alternaria* specific PCR primer pair can quantify both resistant and sensitive alleles in the same tested samples, thereby enabling a rapid determination of frequencies of the azoxystrobin-resistant allele in *Alternaria* populations.

## 4. Conclusion

Advances in the development of molecular methods, especially PCR technology have provided diagnostic laboratories with powerful tools for detection and identification of phytopathogenic fungi. Molecular techniques have also contributed to elucidate the phenotypic and genetic structure within species and the complexity of plant and environment fungal populations. New technologies and improved methods with reduced cost and improved speed, throughput, multiplexing, accuracy and sensitivity have emerged as an essential strategy for the control of plant fungal diseases. These advances have been complemented by the development of new nucleic acids extraction methods, increased automation, reliable internal controls, multiplexing assays, *online* information and *on site* molecular diagnostics. Nevertheless, molecular diagnostic tools should be complemented with other techniques, either traditional culture-based methods or the newly emerged proteomic, a promising tool for providing information about pathogenicity and virulence factors that will open up new possibilities for crop disease diagnosis and crop protection.

On the other hand, fungicides continue to play a key role in strategies for the control of diseases in crops, and the development of resistance in the target pathogens is a continuing risk. This fact leads to many losses as control systems are not longer effective. Therefore, the better understanding on mechanisms developed during fungicide resistance is essential for a better management of chemical control, environment and human health.

Great advances have been made in the development of molecular methods to identify and monitor resistance of plant pathogens to fungicides. The highly sensitive methods can

improve our ability of studying the evolution of fungicide resistance at the population level. Molecular techniques can be also developed based on the different fungicide mechanisms to rapidly detect resistant isolates. Furthermore, a timely detection of resistance levels in populations of phytopathogenic fungi in a field would help growers make proper decisions on resistance management programs to control plant diseases.

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## 6. References

- Abad, Z.G.; Abad, J.A.; Coffey, M.D.; Oudemans, P.V.; Man, W.A.; de Gruyter, H.; Cunnington, J. & Louws, F.J. (2008). *Phytophthora bishetia* sp nov., a new species identified in isolates from the Rosaceous raspberry, rose and strawberry in three continents. *Mycologia*, Vol.100, No.1, pp. 99-110, ISSN 0027-5514
- Abbott, C.L.; Gilmore, S.R.; Lewis, C.T.; Chapados, J.T.; Peters, R.D.; Platt, H.W.; Coffey, M.D. & Lévesque, C.A. (2010). Development of a SNP genetic marker system based on variation in microsatellite flanking regions of *Phytophthora infestans*. *Canadian Journal of Plant Pathology*, Vol.4, No.32, pp. 440-457, ISSN 0706-0661
- Abd-Elsalam, K.; Bahkali, A.; Moslem, M.; Amin, O.E. & Niessen, L. (2011). An optimized protocol for DNA extraction from wheat seeds and Loop-Mediated Isothermal Amplification (LAMP) to detect *Fusarium graminearum* contamination of wheat grain. *International Journal of Molecular Sciences*, Vol.12, No.6, pp. 3459-3472, ISSN 1422-0067
- Abd-Elsalam, K.A.; Asran-Amal, A.; Schnieder, F.; Migheli, Q. & Verreet, J.-A. (2006). Molecular detection of *Fusarium oxysporum* f. sp. *vasinfectum* in cotton roots by PCR and real-time PCR assay. *Journal of Plant Diseases and Protection*, Vol.113, No.1, pp. 14-19, ISSN 1861-3829
- Ahmadian, A.; Ehn, M. & Hober, S. (2006). Pyrosequencing: History, biochemistry and future. *Clinica Chimica Acta*, Vol.363, No.1-2, pp. 83-94, ISSN 0009-8981
- Alaei, H.; Baeyen, S.; Maes, M.; Hofte, M. & Heungens, K. (2009). Molecular detection of *Puccinia horiana* in *Chrysanthemum x morifolium* through conventional and real-time PCR. *Journal of Microbiological Methods*, Vol.76, No.2, pp. 136-145, ISSN 0167-7012
- Albertini, C.; Gredt, M. & Leroux, P. (2003). Polymorphism of the 14<sub>a</sub> demethylase gene (CYP51) in the cereal eyespot fungi *Tapesia acuformis* and *Tapesia yallundae*. *European Journal of Plant Pathology*, Vol.109, pp. 117-128, ISSN 0929-1873
- Albertini, C. & Leroux, P. (2004). A *Botrytis cinerea* putative 3-keto reductase gene (ERG27) that is homologous to the mammalian 17 beta-hydroxysteroid dehydrogenase type 7 gene (17 beta-HSD7). *European Journal of Plant Pathology* Vol.110, No.7, pp.723-733, ISSN: 0929-1873
- Albertini, C.; Thebaud G.; Fournier, E. & Leroux P. (2002). Eburicol 14<sub>a</sub>-demethylase gene (CYP51) polymorphism and speciation in *Botrytis cinerea*. *Mycological Research*, Vol.106, pp. 1171-1178, ISSN 0953-7562

- Altschul, S.F.; Madden, T.L.; Schaffer, A.A.; Zhang, J.H.; Zhang, Z.; Miller, W. & Lipman, D.J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, Vol.25, pp. 3389-3402
- Anderson, I.C. & Cairney, J.W.G. (2004). Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. *Environmental Microbiology*, Vol.6, No. 8, pp. 769-779
- Anderson, I.C. & Parkin, P.I. (2007). Detection of active soil fungi by RT-PCR amplification of precursor rRNA molecules. *Journal of Microbiological Methods*, Vol.68, pp. 248-253
- Anderson, N.; Szemes, M.; O'Brien, P.; De Weerd, M.; Schoen, C.; Boender, P. & Bonants, P. (2006). Use of hybridization melting kinetics for detecting *Phytophthora* species using three-dimensional microarrays: demonstration of a novel concept for the differentiation of detection targets. *Mycological Research*, Vol.110, pp. 664-671
- Andrade, A.C.; Del Sorbo, G.; Van Nistelrooy, J.G.M. & de Waard, M.A. (2000a). The ABC transporter AtrB from *Aspergillus nidulans* is involved in resistance to all major classes of fungicides and natural toxic compounds. *Microbiology U.K.*, Vol.146, pp. 1987-1997, ISSN 1350-0872
- Andrade, A.C.; Van Nistelrooy, J.G.M.; Peery, R.B.; Skatrud, P.L. & de Waard M.A. (2000b). ABC transporters from *Aspergillus nidulans* are involved in protection against cytotoxic agents and antibiotic production. *Molecular General Genetics*, Vol.263, pp. 966-977, ISSN 0026-8925
- Ansorge, W.J. (2010). Novel Next-Generation DNA Sequencing Techniques for Ultra High-Throughput Applications in Bio-Medicine, In: *Molecular Diagnostics* (Second Edition), G.P. Patrinos and W.J. Ansorge, pp. 365-378, Elsevier, ISBN 978-0-12-374537-8
- Arici, S.E. & Koc N.K. (2010). RAPD-PCR analysis of genetic variation among isolates of *Fusarium graminearum* and *Fusarium culmorum* from wheat in Adana Turkey. *Pakistan Journal of Biological Sciences*, Vol.13, No. 3, pp. 138-142, ISSN 1028-8880
- Aroca, A. & Raposo, R. (2007). PCR-based strategy to detect and identify species of *Phaeoacremonium* causing grapevine diseases. *Applied and Environmental Microbiology*, Vol.73, No.9, pp. 2911-2918, ISSN 0099-2240
- Aroca, A.; Raposo, R. & Lunello, P. (2008). A biomarker for the identification of four *Phaeoacremonium* species using the beta-tubulin gene as the target sequence. *Applied Microbiology and Biotechnology*, Vol.80, No.6, pp. 1131-1140
- Ashizawa, T.; Takahashi, M.; Moriwaki, J. & Hirayae, K. (2010). Quantification of the rice false smut pathogen *Ustilaginoidea virens* from soil in Japan using real-time PCR. *European Journal of Plant Pathology*, Vol.128, No.2, pp. 221-232, ISSN 0929-1873
- Attallah, Z.K.; Bae, J.; Jansky, S.H.; Rouse, D.I. & Stevenson, W.R. (2007). Multiplex real-time quantitative PCR to detect and quantify *Verticillium dahliae* colonization in potato lines that differ in response to *Verticillium* wilt. *Phytopathology*, Vol.97, No.7, pp. 865-872
- Avila-Adame, C. & Koller, W. (2003a). Characterization of spontaneous mutants of *Magnaporthe grisea* expressing stable resistance to the Qo-inhibiting fungicide azoxystrobin. *Current Genetics*, Vol.42, pp. 332-338, ISSN 0172-8083
- Avila-Adame, C. & Koller, W. (2003b). Impact of alternative respiration and target-site mutations on responses of germinating conidia of *Magnaporthe grisea* to Qo-inhibiting fungicides. *Pest Management Science*, Vol.59, pp. 303-309, ISSN 1526-498X

- Baayen, R.P.; O'Donnell, K.; Bonants, P.J.M.; Cigelnik, E.; Kroon, L.P.N.M.; Roebroeck, E.J.A. & Waalwijk, C. (2000). Gene genealogies and AFLP analyses in the *Fusarium oxysporum* complex identify monophyletic and nonmonophyletic formae speciales causing wilt and rot disease. *Phytopathology*, Vol.90, No.8, pp. 891-900, ISSN 0031-949X
- Babu, B.K.; Mesapogu, S.; Sharma A.; Somasani, S.R. & Arora, D.K. (2011). Quantitative real-time PCR assay for rapid detection of plant and human pathogenic *Macrophomina phaseolina* from field and environmental samples. *Mycologia* Vol.103, No.3, pp.466-473, ISSN: 0027-5514
- Bailey, A.M.; Mitchell, D.J.; Manjunath, K.L.; Nolasco, G. & Niblett, C.L. (2002). Identification to the species level of the plant pathogens *Phytophthora* and *Pythium* by using unique sequences of the ITS1 region of ribosomal DNA as capture probes for PCR Elisa. *Fems Microbiology Letters*, Vol.207, No.2, pp. 153-158, ISSN 0378-1097
- Banno, S.; Fukumori, F.; Ichiishi, A.; Okada, K.; Uekusa, H.; Kimura, M. & Fujimura, M. (2008). Genotyping of benzimidazole-resistant and dicarboximide-resistant mutations in *Botrytis cinerea* using real-time polymerase chain reaction assays. *Phytopathology*, Vol.98, pp. 397-404, ISSN 0031-949X
- Baraldi, E.; Mari, M.; Chierici, E.; Pondrelli, M.; Bertollini, P. & Pratella, G.C. (2003). Studies of thiabendazole resistance of *Penicillium expansum* of pears: pathogenic fitness and genetic characterization. *Plant Pathology*, Vol.52, pp. 362-370, ISSN 0032-0862
- Barnes, C.W. & Szabo, L.J. (2007). Detection and identification of four common rust pathogens of cereals and grasses using real-time polymerase chain reaction. *Phytopathology*, Vol.97, No.6, pp. 717-727, ISSN 0031-949X
- Bauer, B.E.; Wolfger, H. & Kuchler, K. (1999). Inventory and function of yeast ABC proteins: about sex, stress, pleiotropic drug and heavy metal resistance. *Biochimistry Biophysic Acta*, Vol.1461, No.2, pp. 217-236, ISSN 0005-2736
- Baumler, S.; Felsenstein, F.G. & Schwarz, G. (2003). CAPS and DHPLC analysis of a single nucleotide polymorphism in the cytochrome b gene conferring resistance to strobilurin in field isolates of *Blumeria graminis* f. sp. *hordei*. *Journal of Phytopathology*, Vol.151, pp. 149-152, ISSN 0931-1785
- Bayraktar, H.; Dolar, F.S. & Tor, M. (2007). Determination of genetic diversity within *Ascochyta rabiei* (pass.) labr., the cause of ascochyta blight of chickpea in Turkey. *Journal of Plant Pathology*, Vol.89, No.3, pp. 341-347, ISSN 1125-4653
- Bearchell, S.J.; Fraaije, B.A.; Shaw, M.W. & Fitt, B.D.L. (2005). Wheat archive links long-term fungal pathogen population dynamics to air pollution. *Proceedings of the National Academy of sciences of the United States of America*, Vol.102, No.15, pp. 5438-5442, ISSN 0027-8424
- Bilodeau, G.J.; Pelletier, G.; Pelletier, F.; Lévesque, C.A. & Hamelin, R.C. (2009). Multiplex real-time polymerase chain reaction (PCR) for detection of *Phytophthora ramorum*, the causal agent of sudden oak death. *Canadian Journal of Plant Pathology*, Vol.31, No.2, pp. 195-210, ISSN 0706-0661
- Bindislev, L.; Oliver, R.P. & Johansen, B. (2002). *In situ* PCR for detection and identification of fungal species. *Mycological Research*, Vol.106, No.3, pp. 277-279, ISSN 0953-7562
- Biswas, K. & Biswas, R.A. (2011). Modified method to isolate genomic DNA from plants without liquid nitrogen. *Current Science*, Vol.100, No.11, pp. 1622-1624, ISSN 0011-3891



- BOLD, Barcode of Life Database Systems: (<http://www.boldsystems.org>)
- Børja, I.; Solheim, H.; Hietala, A.M. & Fossdal, C.G. (2006). Etiology and real-time polymerase chain reaction-based detection of *Gremmeniella*- and *Phomopsis*-associated disease in Norway spruce seedlings. *Phytopathology*, Vol.96, No.12, pp. 1305-1314, ISSN 0031-949X
- Braasch, D.A. & Corey, D.R. (2001) Locked nucleic acid (LNA): fine-tuning the recognition of DNA and RNA. *Chemistry & Biology*, Vol.8, No.1, pp. 1-7, ISSN 1074-5521
- Brandfass, C. & Karlovsky, P. (2006). Simultaneous detection of *Fusarium culmorum* and *F. graminearum* in plant material by duplex PCR with melting curve analysis. *BMC Microbiology*, Vol.6, No.4, ISSN 1471-2180
- Brent, K.J. (1995). *Fungicide resistance in crop pathogen: How can it be managed?*. FRAC Monograph N° 1 Global Crop Protection Federation, ISBN 90-72398-07-6, Brussels
- Broders, K.D.; Woeste, K.E.; SanMiguel, P.J.; Westerman, R.P. & Boland, G.J. (2011). Discovery of single-nucleotide polymorphisms (SNPs) in the uncharacterized genome of the ascomycete *Ophiognomonia clavignenti-juglandacearum* from 454 sequence data. *Molecular Ecology Resources*, Vol.11, No.4, pp. 693-702, ISSN 1755-098X
- Bruns T.D., White T.J. & Taylor J.W. (1991). Fungal molecular systematics. *Annual Review of Ecology and Systematics*, Vol.22, pp. 525-564.
- Budge, G.E.; Shaw, M. W.; Colyer, A.; Pietravalle, S. & Boonham, N. Molecular tools to investigate *Rhizoctonia solani* distribution in soil. *Plant Pathology*, Vol.58, No.6, pp. 1071-1080, ISSN 0032-0862
- Burgess, T.I.; Webster, J.L.; Ciampini, J.A.; White, D.; Hardy, G.E.S. & Stukely, M.J.C. (2009). Re-evaluation of *Phytophthora* species isolated during 30 years of vegetation health surveys in western Australia using molecular techniques. *Plant Disease*, Vol.93, No.3, pp. 215-223, ISSN 0191-2917
- Butters, J.A.; Zhou, M. & Hollomon, D.W. (2000). The mechanism of resistance to sterol 14<sub>α</sub>-demethylation inhibitors in a mutant (Erg40) of *Ustilago maydis*. *Pest Management Science*, Vol.56, pp. 257-263, ISSN 1526-498X
- Campanile, G.; Schena L. & Luisi, N. (2008). Real-time PCR identification and detection of *Fuscoporia torulosa* in *Quercus ilex*. *Plant Pathology*, Vol.57, No.1, pp. 76-83, ISSN 0032-0862
- Carisse, O.; Tremblay, D.M.; Lévesque, C.A.; Gindro, K.; Ward, P. & Houde, A. (2009). Development of a TaqMan real-time PCR assay for quantification of airborne conidia of *Botrytis squamosa* and management of Botrytis leaf blight of onion. *Phytopathology*, Vol.99, No.11, pp. 1273-1280, ISSN 0031-949X
- Causin, R.; Scopel, C.; Grendene, A. & Montecchio L. (2005). An improved method for the detection of *Phytophthora cactorum* (L.C.) Schröeter in infected plant tissues using SCAR markers. *Journal of Plant Pathology*, Vol.87, No.1, pp. 25-35, ISSN 1125-4653
- Chen, R.S.; Chu, C.; Cheng, C.W.; Chen W.Y. & Tsay J.G. (2008). Differentiation of two powdery mildews of sunflower (*Helianthus annuus*) by a PCR-mediated method based on ITS sequences. *European Journal of Plant Pathology*, Vol.121, No.1, pp. 1-8, ISSN 0929-1873
- Chen, W.; Seifert, K.A. & Lévesque, C.A. (2009). A high density COX1 barcode oligonucleotide array for identification and detection of species of *Penicillium*

- subgenus *Penicillium*. *Molecular Ecology Resources*, Vol.9 (Suppl. 1), pp. 114-129, ISSN 1755-098X
- Chi, M.-H.; Park, S.-Y. & Lee, Y.-H. (2009). A quick and safe method for fungal DNA extraction. *Plant Pathology Journal*, Vol.25, No.1, pp. 108-111, ISSN 1598-2254
- Chimento, A.; Scibetta, S.; Schena, L.; Cacciola, S.O. & Cooke, D.E.L. (2005). A new method for the monitoring of *Phytophthora* diversity in soil and water. *Journal of Plant Pathology*, Vol.87, pp. 290
- CiF, Cyber infrastructure for *Fusarium* : (<http://www.fusariumdb.org>)
- Cipriani, M.G.; Stea, G.; Moretti, A.; Altomare, C.; Mulè, G. & Vurro, M. (2009). Development of a PCR-based assay for the detection of *Fusarium oxysporum* strain FT2, a potential mycoherbicide of *Orobanche ramosa*. *Biological Control*, Vol.50, No.1, pp. 78-84, ISSN 1049-9644
- Collado-Romero, M.; Mercado-Blanco, J.; Olivares-Garcia, C. & Jimenez-Diaz, R. M. (2008). Phylogenetic analysis of *Verticillium dahliae* vegetative compatibility groups. *Phytopathology*, Vol.98, No.9, pp. 1019-1028, ISSN 0031-949X
- Cruz-Perez, P.; Buttner, M.P. & Stetzenbach, L. D. 2001. Detection and quantitation of *Aspergillus fumigatus* in pure culture using polymerase chain reaction. *Molecular and Cellular Probes*, Vol.15, No.2, pp. 81-88, ISSN 0890-8508
- Cunha, M.G. & Rizzo, D.M. (2003). Development of fungicide cross resistance in *Helminthosporium solani* population from California. *Plant Disease*, Vol.87, No.7, pp. 798-803, ISSN 0191-2917
- Daval, S.; Lebreton L.; Gazengel K.; Guillermin-Erckelboudt A.-Y. & Sarniguet A. (2010). Genetic evidence for differentiation of *Gaeumannomyces graminis* var. *tritici* into two major groups. *Plant Pathology*, Vol.59, No. 1, pp. 165-178, ISSN 0032-0862
- de Cock, A.W.A.M. & Lévesque, A. (2004). New species of *Pythium* and *Phytophthora*. *Studies in Mycology*, Vol.50, Special Issue, pp. 481-487, ISSN 0166-0616
- De Waard, M.A. (1997). Significance of ABC transporters in fungicide sensitivity and resistance. *Pesticide Science*, Vol.51, pp. 271-275, ISSN 0031-613X
- de Weerd, M.; Zijlstra, C.; van Brouwershaven, I.R.; van Leeuwen, G.C.M.; de Gruyter, J. & Kox, L.F.F. (2006). Molecular detection of *Fusarium foetens* in Begonia. *Journal of Phytopathology*, Vol.154, No.11-12, pp. 694-700, ISSN 0931-1785
- Del Sorbo, G.; Schoonbeek, H. & de Waard, M.A. (2000). Fungal transporters involved in efflux of natural toxic compounds and fungicides. *Fungal Genetics Biology*, Vol.30, pp. 1-15, ISSN 1087-1845
- Delp, C.J. & Dekker, J. (1985). Fungicide resistance: definitions and use of terms. *EPPO Bulletin*, Vol.15, pp. 333-335
- Dèlye, C.; Laigret, F. & Coster, M.F. (1997). A mutation in 14  $\alpha$  demethylase gene of *Uncinula necator* that correlates with resistance to a sterol biosynthesis inhibitor. *Applied Environmental Microbiology*, Vol.63, pp. 2966-2970, ISSN 0099-2240
- Demeke, T. & Jenkins, G.R. (2010). Influence of DNA extraction methods, PCR inhibitors and quantification methods on real-time PCR assay of biotechnology-derived traits. *Analytical and Bioanalytical Chemistry*, Vol.396, No.6, pp. 1977-1990, ISSN 1618-2642
- Demeke, T.; Gräfenhan, T.; Clear, R.M.; Phan, A.; Ratnayaka, I.; Chapados, J.T.; Patrick, S.K.; Gaba, D.; Seifert, K.A. & Lévesque, C.A. (2010). Development of a specific TaqMan® real-time PCR assay for quantification of *Fusarium graminearum* clade 7 and comparison of fungal biomass determined by PCR with deoxynivalenol

- content in wheat and barley. *International Journal of Food Microbiology*, Vol.141, No.1-2, pp. 45-50, ISSN 0168-1605
- Demontis, M.A.; Cacciola, S.O.; Orru, M.; Balmas, V.; Chessa, V.; Maserti, B.E.; Mascia, L.; Raudino, F.; Quirico, G.M.D.S.L. & Migheli, Q. (2008). Development of real-time PCR systems based on SYBR (R) Green I and TaqMan (R) technologies for specific quantitative detection of *Phoma tracheiphila* in infected Citrus. *European Journal of Plant Pathology*, Vol.120, No.4, pp. 339-351, ISSN 0929-1873
- DNA Multiscan®: (<http://www.DNAMultiscan.com>)
- Diguta, C. F.; Rousseaux, S.; Weidmann, S.; Bretin, N.; Vincent, B.; Guilloux-Benatier, M. & Alexandre, H. (2010). Development of a qPCR assay for specific quantification of *Botrytis cinerea* on grapes. *Fems Microbiology Letters*, Vol.313, No.1, pp. 81-87, ISSN 0378-1097
- Dixon, L.J.; Schlub, R.L.; Pernezny, K. & Datnoff, L.E. (2009). Host specialization and phylogenetic diversity of *Corynespora cassiicola*. *Phytopathology*, Vol.99, No.9, pp. 1015-1027, ISSN 0031-949X
- Drenth, A.; Wagals, G.; Smith, B.; Sendall, B.; O'Dwyer, C.; Irvine, G. & Irwin, J.A.G. (2006). Development of a DNA-based method for the detection and identification of *Phytophthora* species. *Australasian Plant Pathology*, Vol.35, No.2, pp. 147-159, ISSN 0815-3191
- Druzhinina, I. S.; Kopchinskiya, A. G.; Komoja, M.; Bissett, J.; Szakacs, G. & Kubicek, C.P. (2005). An oligonucleotide barcode for species identification in *Trichoderma* and *Hypocrea*. *Fungal Genetics and Biology*, Vol.42, No.10, pp. 813-828, ISSN 1087-1845
- Durán, A.; Gryzenhout, M.; Drenth, A.; Slippers, B.; Ahumada, R.; Wingfield, B.D. & Wingfield, M.J. (2010). AFLP analysis reveals a clonal population of *Phytophthora pinifolia* in Chile. *Fungal Biology*, Vol.114, No.9, pp. 746-752, ISSN 1878-6146
- Dyer, P.S.; Furneaux, P.A.; Douhan, G. & Murray, T.D. (2001). A multiplex PCR test for determination of mating type applied to the plant pathogens *Tapesia yallundae* and *Tapesia acuformis*. *Fungal Genetics and Biology*, Vol.33, No.3, pp. 173-180, ISSN 1087-1845
- Feng, J.; Hwang, R.; Chang, K. F.; Hwang, S. F.; Strelkov, S. E.; Gossen, B. D. & Zhou, Q. (2010). An inexpensive method for extraction of genomic DNA from fungal mycelia. *Canadian Journal of Plant Pathology*, Vol.32, No.3, pp. 396-401, ISSN 0706-0661
- Fling, M.E.; Kopf, J.; Tamarkin, A.; Gorman, J.A.; Smith, H.A. & Koltin, Y. (1991). Analysis of a *Candida albicans* gene encodes a novel mechanism for resistance to benomyl and methotrexate. *Molecular General Genetics*, Vol.227, pp. 318-329, ISSN 0026-8925
- Foster, S.J.; Ashby, A.M. & Fitt, B.D.L. (2002). Improved PCR-based assays for pre-symptomatic diagnosis of light leaf spot and determination of mating type of *Pyrenopeziza brassicae* on winter oilseed rape. *European Journal of Plant Pathology*, Vol.108, No.4, pp. 379-383, ISSN 0929-1873
- Fountaine, J.A.; Shaw, M.W.; Napier, B.; Ward, E. & Fraaije, B. A. (2007). Application of real-time and multiplex polymerase chain reaction assays to study leaf blotch epidemics in barley. *Phytopathology*, Vol.97, No.3, pp. 297-303, ISSN 0031-949X
- Fourie, G.; Steenkamp, E.T.; Ploetz, R.C.; Gordon, T.R. & Viljoen, A. (2011). Current status of the taxonomic position of *Fusarium oxysporum* formae *specialis cubense* within the

- Fusarium oxysporum* complex. *Infection Genetics and Evolution*, Vol.11, No.3, pp. 533-542, ISSN 1567-1348
- Fraaije, B.A.; Butters, J.A.; Coelho, J.M.; Johes, D.R. & Hollomon, D.W. (2002). Following the dynamics of strobilurin resistance in *Blumeria graminis* f. sp. *tritici* using quantitative allele-specific realtime PCR measurements with the fluorescent dye SYBR green I. *Plant Pathology*, Vol.51, pp. 45-54, ISSN 0032-0862
- Fraaije, B.A.; Lovell, D.J.; Coelho, J.M.; Baldwin, S. & Hollomon, D.W. (2001). PCR-based assays to assess wheat varietal resistance to blotch (*Septoria tritici* and *Stagonospora nodorum*) and rust (*Puccinia striiformis* and *Puccinia recondita*) diseases. *European Journal of Plant Pathology*, Vol.107, No.9, pp. 905-917, ISSN 0929-1873
- Fraaije, B.A., Lovell, D.J., Rohel, E.A. & Hollomon, D.W. (1999). Rapid detection and diagnosis of *Septoria tritici* epidemics in wheat using a polymerase chain reaction PicoGreen assay. *Journal of Applied Microbiology*, Vol.86, No.4, pp. 701-708, ISSN 1364-5072
- Garrido, C.; Carbú, M.; Fernández-Acero, F.J.; Boonham, N.; Colyer, A.; Cantoral, J.M. & Budge G. (2009). Development of protocols for detection of *Colletotrichum acutatum* and monitoring of strawberry anthracnose using real-time PCR. *Plant Pathology*, Vol.58, No.1, pp. 43-51, ISSN 0032-0862
- Gayoso, C.; de Ilárduya, O.M.; Pomar, F. & de Cáceres, F.M. (2007). Assessment of real-time PCR as a method for determining the presence of *Verticillium dahliae* in different *Solanaceae* cultivars. *European Journal of Plant Pathology*, Vol.118, No.3, pp. 199-209, ISSN 0929-1873
- Geiser, D. M.; Jiménez-Gasco, M.; Kang, S.; Makalowska, I.; Veeraraghavan, N.; Ward, T.J.; Zhang, N.; Kuldau, G.A. & O'Donnell, K. (2004). *Fusarium-ID* v.1.0: A DNA sequence database for identifying *Fusarium*. *European Journal of Plant Pathology*, Vol.110, No.5-6, pp. 473-479, ISSN 0929-1873
- Ghosoph, J.M.; Schmidt, L.S.; Margosan, D.A. & Smilanick, J.L. (2007). Imazalil resistance linked to a unique insertion sequence in the PdCYP51 promoter region of *Penicillium digitatum*. *Postharvest Biology Technology*, Vol.44, pp. 9-18, ISSN 0925-5214
- Gisi, U.; Chin, K.M.; Knapova, G.; Küng Färber, R.; Mohr, U.; Parisi, S.; Sierotzki, H. & Steinfeld, U. (2000). Recent development in elucidating modes of resistance to phenylamide, DMI and strobilurin fungicides. *Crop Protection*, Vol.19, pp. 863-872, ISSN 0261-2194
- Gisi, U.; Sierotzki, H.; Cook, A. & McCaffery, A. (2002). Mechanisms influencing the evolution of resistance to Qo inhibitor fungicides. *Pest Management Science*, Vol.58, pp. 859-867, ISSN 1526-498X
- Glienke, C.; Pereira, O.L.; Stringari, D.; Fabris, J.; Kava-Cordeiro, V.; Galli-Terasawa, L.; Cunnington, J.; Shivas, R.G.; Groenewald, J.Z. & Crous, P.W. (2011). Endophytic and pathogenic *Phyllosticta* species, with reference to those associated with Citrus Black Spot. *Persoonia*, Vol.26, pp. 47-56, ISSN 0031-5850
- Godoy-Lutz, G.; Kuniyaga, S.; Steadman, J. R. & Powers, K. (2008). Phylogenetic analysis of *Rhizoctonia solani* subgroups associated with web blight symptoms on common bean based on ITS-5.8S rDNA. *Journal of General Plant Pathology*, Vol.74, No.1, pp. 32-40, ISSN 1345-2630



- Gómez-Alpizar, L.; Saalau, E.; Picado I.; Tambong, J.T. & Saborio, F. (2011). A PCR-RFLP assay for identification and detection of *Pythium myriotylum*, causal agent of the cocoyam root rot disease. *Letters in Applied Microbiology*, Vol.52, No.3, pp. 185-192, ISSN 0266-8254
- González, C.; Noda, J.; Espino, J.J & Brito, N. (2008). Drill-assisted genomic DNA extraction from *Botrytis cinerea*. *Biotechnology Letters*, Vol.30, No.11, pp. 1989-1992, ISSN 0141-5492
- González-Mendoza, D.; Argumedo-Delira, R.; Morales-Trejo, A.; Pulido-Herrera, A.; Cervantes-Díaz, L.; Grimaldo-Juarez, O. & Alarcon, A. (2010). A rapid method for isolation of total DNA from pathogenic filamentous plant fungi. *Genetics and Molecular Research*, Vol.9, No.1, pp. 162-166, ISSN 1676-5680
- Goud, J.C. & Termorshuizen A.J. (2003) Quality of methods to quantify microsclerotia of *Verticillium dahliae* in soil. *European Journal of Plant Pathology*, Vol.109, No.6, pp. 523-534, ISSN 0929-1873
- Gril, T.; Celar, F.; Munda, A.; Javornik, B. & Jakse, J. (2008). AFLP analysis of intraspecific variation between *Monilinia laxa* isolates from different hosts. *Plant Disease*, Vol.92, No.12, pp.1616-1624, ISSN 0191-2917
- Groenewald, S.; Van Den Berg, N.; Marasas, W.F.O. & Viljoen, A. (2006). The application of high-throughput AFLPs in assessing genetic diversity in *Fusarium oxysporum* f.sp. *cubense*. *Mycological Research*, Vol.110, pp. 297-305, ISSN 0953-7562
- Grote, D.; Olmos, A.; Kofoet, J.J.; Tuset, E.; Bertolini, E. & Cambra, M. (2002). Specific and sensitive detection of *Phytophthora nicotianae* by simple and nested-PCR. *European Journal of Plant Pathology*, Vol.108, No.3, pp. 197-207, ISSN 0929-1873
- Grund, E.; Darissa, O. & Adam, G. (2010). Application of FTA (R) cards to sample microbial plant pathogens for PCR and RT-PCR. *Journal of Phytopathology*, Vol.158, No.11-12, pp. 750-757, ISSN 0931-1785
- Grünwald, N.J.; Martin, F.N.; Larsen, M.M.; Sullivan, C.M.; Press, C.M.; Coffey, M.D.; Hansen, E.M. & Parke, J.L. (2011). *Phytophthora-ID.org*: A sequence-based *Phytophthora* identification tool. *Plant Disease*, Vol.95, No.3, pp. 337-342, ISSN 0191-2917
- Guglielmo, F.; Bergemann, S.E.; Gonthier, P.; Nicolotti, G. & Garbelotto, M. (2007). A multiplex PCR-based method for the detection and early identification of wood rotting fungi in standing trees. *Journal of Applied Microbiology*, Vol.103, pp. 1490-1507, ISSN 1364-5072
- Guo, J.R.; Schnieder, F.; Beyer, M. & Verreet, J.A. (2005). Rapid detection of *Mycosphaerella graminicola* in wheat using reverse transcription-PCR assay. *Journal of Phytopathology*, Vol.153, No.11-12, pp. 674-679, ISSN 0931-1785
- Haase, A.T.; Retzel, E.F. & Staskus, K.A. (1990). Amplification and detection of lentiviral DNA inside cells. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.87, No.13, pp. 4971-4975, ISSN 0027-8424
- Hamamoto, H.; Hasegawa, K.; Nakaune, R.; Lee, Y.J.; Makizumi, Y.; Akutsu, K. & Hibi T. (2000). Tandem repeat of a transcription enhancer upstream of the sterol 14  $\alpha$  demethylase gene (CYP51) in *Penicillium digitatum*. *Applied Environmental Microbiology*, Vol.66, pp. 3421-3426, ISSN 099-2240
- Hamamoto, H.; Nawata, O.; Hasegawa, K.; Nakaune, R.; Lee, Y.J.; Makizumi, Y.; Akutsu, K. & Hibi T. (2001). The role of the ABC transporter gene *PMR1* in demethylation

- inhibitor resistance in *Penicillium digitatum*. *Pest Biochemical Physiology*, Vol.70, pp. 19-26, ISSN 0048-3575
- Hayashi, K.; Schoonbek, H.J. & de Waard, M.A. (2002a). *Bcmfs1*. A novel major facilitator superfamily transporter from *Botrytis cinerea* provides tolerance towards the natural toxic compounds camptothecin and cercosporin and towards fungicides. *Applied Environmental Microbiology*, Vol.68, pp. 4996-5004, ISSN 0099-2240
- Hayashi, K.; Schoonbeek, H.J. & de Waard, M.A. (2002b). Expression of the ABC transporter *BcatrD* from *Botrytis cinerea* reduces sensitivity to sterol demethylation inhibitor fungicides. *Pest Biochemical Physiology*, Vol.73, pp. 110-121, ISSN 0048-3575
- Hayashi, K.; Schoonbeek, H.J. & de Waard MA. (2003). Modulators of membrane drug transporters potentiate the activity of the DMI fungicide oxpoconazole against *Botrytis cinerea*. *Pest Managment Science*, Vol.59, pp. 294-302, ISSN 1526-498X
- Hayashi, K.; Schoonbeek, H.J.; Sugiura, H. & de Waard, M.A. (2001). Multidrug resistance in *Botrytis cinerea* associated with decreased accumulation of the azole fungicide oxpoconazole and increased transcription of the ABC transporter gene *BcatrD*. *Pest Biochemical Physiology*, Vol.70, pp. 168-179, ISSN 0048-3575
- Heid, C.A.; Stevens, J.; Livak, K.J. & Williams, P.M. (1996). Real time quantitative PCR. *Genome Research*, Vol.6, pp. 986-994
- Hewit, H. G. (1998). *Fungicides in crop protection*. CAB Intenational. University Press, ISBN 0-85199-201-3, Cambridge. UK.
- Hirsch, P.R.; Mauchline, T.H. & Clark, I.M. (2010). Culture-independent molecular techniques for soil microbial ecology. *Soil Biology & Biochemistry*, Vol.42, pp. 878-887, ISSN 0038-0717
- Hong, S.Y.; Kang, M.R.; Cho, E.J.; Kim, H.K. & Yun, S.H. (2010). Specific PCR detection of four quarantine *Fusarium* Species in Korea. *Plant Pathology Journal*, Vol.26, No.4, pp. 409-416, ISSN 1598-2254
- Huang, J. & Kang, Z. (2010). Detection of *Thielaviopsis basicola* in soil with real-time quantitative PCR assays. *Microbiological Research*, Vol.165, No.5, pp. 411-417, ISSN 0944-5013
- Hughes, K.J.D.; Giltrap, P.M.; Barton, V.C.; Hobden E.; Tomilson, J.A. & Barber, P. (2006). On-site real-time PCR detection of *Phytophthora ramorum* causing dieback of *Parrotia persica* in the UK. *Plant Pathology*, Vol.55, No.6, pp. 813-813, ISSN 0032-0862
- Hyakumachi, M.; Priyatmojo, A.; Kubota, M. & Fukui, H. (2005). New anastomosis groups, AG-T and AG-U, of binucleate *Rhizoctonia* spp. causing root and stem rot of cut-flower and miniature roses. *Phytopathology*, Vol.95, No.7, pp. 784-792, ISSN 0031-949X
- Hyun, J. W.; Yi, S. H.; MacKenzie, S. J.; Timmer, L. W.; Kim, K. S.; Kang, S. K.; Kwon, H. M. & Lim, H. C. (2009). Pathotypes and genetic relationship of worldwide collections of *Elsinoe* spp. causing scab diseases of citrus. *Phytopathology*, Vol.99, No.6, pp. 721-728, ISSN 0031-949X
- Inami, K.; Yoshioka, C.; Hirano, Y.; Kawabe, M.; Tsushima, S.; Teraoka, T. & Arie, T. (2010). Real-time PCR for differential determination of the tomato wilt fungus, *Fusarium oxysporum* f. sp *lycopersici*, and its races. *Journal of General Plant Pathology*, Vol.76, No.2, pp.116-121, ISSN 1345-2630

- Ioos, R.; Kowalski, T.; Husson, C. & Holdenrieder, O. (2009). Rapid in planta detection of *Chalara fraxinea* by a real-time PCR assay using a dual-labelled probe. *European Journal of Plant Pathology*, Vol.125, No.2, pp. 329-335, ISSN 0929-1873
- Ippolito, A.; Schena, L. & Nigro, F. (2002). Detection of *Phytophthora nicotianae* and *P. citrophthora* in citrus roots and soils by nested PCR. *European Journal of Plant Pathology*, Vol.108, No.9, pp. 855-868, ISSN 0929-1873
- Ishii, H.; Fraaije, B.A.; Sugiyama, T.; Noguchi, K.; Nishimura, K.; Takeda, T.; Amano, T. & Hollomon, D.W. (2001). Occurrence and molecular characterization of strobilurin resistance in cucumber powdery mildew and downy mildew. *Phytopathology*, Vol.91, pp. 1166-1171, ISSN 0031-949
- Izzo, A.D. & Mazzola, M. (2009). Hybridization of an ITS-based macroarray with ITS community probes for characterization of complex communities of fungi and fungal-like protists. *Mycological Research*, Vol.113, pp. 802-812, ISSN 0953-7562
- Jackson, E.W.; Avant, J.B.; Overturf, K.E. & Bonman, J.M. (2006). A quantitative assay of *Puccinia coronata* f. sp. *avenae* DNA in *Avena sativa*. *Plant Disease*, Vol.90, No.5, pp. 629-636, ISSN 0191-2917
- Jana, T.; Sharma, T.R. & Singh, N.K. (2005). SSR-based detection of genetic variability in the charcoal root rot pathogen *Macrophomina phaseolina*. *Mycological Research*, Vol.109, Part 1, pp. 81-86, ISSN 0953-7562
- Jeeva, M.L.; Mishra, A.K.; Vidyadharan, P.; Misra, R.S. & Hegde, V. (2010). A species-specific polymerase chain reaction assay for rapid and sensitive detection of *Sclerotium rolfsii*. *Australasian Plant Pathology*, Vol.39, No.6, pp. 517-523, ISSN 0815-3191
- Jiang, Y.X.; Wu, J.G.; Yu, K.Q.; Ai, C.X.; Zou, F. & Zhou, H.W. (2011) Integrated lysis procedures reduce extraction biases of microbial DNA from mangrove sediment. *Journal of Bioscience and Bioengineering*, Vol.111, No.2, pp. 153-157, ISSN 1389-1723
- Jiménez-Fernández, D.; Montes-Borrego, M.; Navas-Cortés, J.A.; Jiménez-Díaz, R.M. & Landa, B.B. (2010). Identification and quantification of *Fusarium oxysporum* in planta and soil by means of an improved specific and quantitative PCR assay. *Applied Soil Ecology*, Vol.46, No.3, pp. 372-382, ISSN 0929-1393
- Jiménez-Fernández D.; Montes-Borrego M.; Jimenez-Diaz R.M.; Navas-Cortes, J.A. & Landa, B.B. (2011). In planta and soil quantification of *Fusarium oxysporum* f. sp. *ciceris* and evaluation of *Fusarium* wilt resistance in chickpea with a newly developed quantitative polymerase chain reaction assay. *Phytopathology* Vol. 101, No.2, pp. 250-262, ISSN: 0031-949X
- Joseph-Horne, T. & Hollomon, D.W. (1997). Molecular mechanisms of azole resistance. *FEMS Microbiology Letters*, Vol.149, pp. 141-149
- Justesen, A.F.; Hansen, H.J.; Pinnschmidt, H.O. (2008). Quantification of *Pyrenophora graminea* in barley seed using real-time PCR. *European Journal of Plant Pathology*, Vol.122, No.2, pp. 253-263, ISSN 0929-1873
- Kageyama, K.; Senda, M.; Asano, T.; Suga, H. & Ishiguro, K. (2007). Intra-isolate heterogeneity of the ITS region of rDNA in *Pythium helicoides*. *Mycological Research*, Vol.111, pp. 416-423, ISSN 0953-7562
- Kaku, K.; Takagaki, M.; Shimizu, T. & Nagayama, K. (2003). Diagnosis of dehydratase inhibitors in melanin biosynthesis inhibitor (MBID) resistance by primer-introduced restriction enzyme analysis in scytalonedehydratase gene of *Magnaporthe grisea*. *Pest Management Science*, Vol.59, pp. 843-846, ISSN 1526-498X

- Kang, S.; Mansfield, M.A.; Park, B.; Geiser, D.M.; Ivors, K.L.; Coffey, M.D.; Grünwald, N.J.; Martin, F.N.; Lévesque, C.A. & Blair, J.E. (2010). The promise and pitfalls of sequence-based identification of plant-pathogenic fungi and oomycetes. *Phytopathology*, Vol.100, No.8, pp. 732-737, ISSN 0031-949X
- Keiper, F.J.; Capio, E.; Grcic, M. & Wallwork, H. (2007). Development of sequence tagged microsatellites for the barley net blotch pathogen *Pyrenophora teres*. *Molecular Ecology Notes*, Vol.7, No.4, pp. 664-666, ISSN 1471-8278
- Keiper, F.J.; Hayden, M.J. & Wallwork, H. (2006) Development of sequence tagged microsatellites for the barley scald pathogen *Rhynchosporium secalis*. *Molecular Ecology Notes*, Vol.6, No.4, pp. 543-546, ISSN 1471-8278
- Kim, J.T.; Park, S.Y.; Choi, W.; Lee, Y.H. & Kim H.T. (2008). Characterization of *Colletotrichum* isolates causing anthracnose of pepper in Korea. *Plant Pathology Journal*, Vol.24, No.1, pp. 17-23, ISSN 1598-2254
- Kim, Y.T.; Cho, M.; Jeong, J.Y.; Lee, H.B. & Kim, S.B. (2010). Application of Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis to monitor effect of biocontrol agents on rhizosphere microbial community of hot pepper (*Capsicum annuum* L.). *Journal of Microbiology*, Vol.48, No.5, pp. 566-572, ISSN 1225-8873
- Kircher, M. & Kelso, J. (2010). High-throughput DNA sequencing - concepts and limitations. *Bioessays*, Vol.32, No. 6, pp. 524-536
- Knutsen, A.K.; Torp, M. & Holst-Jensen, A. (2004). Phylogenetic analyses of the *Fusarium poae*, *Fusarium sporotrichioides* and *Fusarium langsethiae* species complex based on partial sequences of the translation elongation factor-1 alpha gene. *International Journal of Food Microbiology*, Vol.95, No.3, pp. 287- 295, ISSN 0168-1605
- Koenraadt, H.; Somerville, S.C.; Jones, A.L. (1992). Characterization of mutations in the beta-tubulin gene of benomyl-resistant field strains of *Venturia inaequalis* and other plant pathogenic fungi. *Phytopathology*, Vol.82, pp. 1348-1354, ISSN 0031-949X
- Koljalg, U.; Larsson, K.-H.; Abarenkov, K.; Nilsson, R.H.; Alexander, I.J.; Eberhardt, U.; Erland, S.; Hoiland, K.; Kjoller, R.; Larsson, E.; Pennanen, T.; Sen, R.; Taylor, A.F.S., Tedersoo, L., Vralstad, T., & Ursing, B.M. (2005). UNITE: A database providing web-based methods for the molecular identification of ectomycorrhizal fungi. *New Phytologist*. Vol.166, No.3, pp. 1063-1068, ISSN 0028-646X
- Kristensen, R.; Torp, M.; Kosiak, B. & Holst-Jensen, A. (2005). Phylogeny and toxigenic potential is correlated in *Fusarium* species as revealed by partial translation elongation factor 1 alpha gene sequences. *Mycological Research*, Vol.109, No.2, pp. 173-186, ISSN 0953-7562
- Kroon, L.P.N.M.; Verstappen, E.C.P.; Kox, L.F.F.; Flier, W.G. & Bonants, P. (2004). A rapid diagnostic test to distinguish between American and European populations of *Phytophthora ramorum* *Phytopathology*, Vol.94, No.6, pp. 613-620, ISSN 0031-949X
- Kulik, T.; Jestoi, M. & Okorski, A. (2011). Development of TaqMan assays for the quantitative detection of *Fusarium avenaceum*/*Fusarium tricinctum* and *Fusarium poae* esyn1 genotypes from cereal grain. *FEMS Microbiology Letters*, Vol.314, No.1, pp. 49-56, ISSN 0378-1097
- Kurtz, B.; Karlovsky, P. & Vidal, S. (2010). Interaction between western corn rootworm (Coleoptera: Chrysomelidae) larvae and root-infecting *Fusarium verticillioides*. *Environmental Entomology*, Vol.39, No.5, pp. 1532-1538, ISSN 0046-225X



- Landa, B.B.; Montes-Borrego, M.; Munoz-Ledesma, F.J. & Jimenez-Diaz, R.M. (2007). Phylogenetic analysis of downy mildew pathogens of opium poppy and PCR-Based in planta and seed detection of *Peronospora arborescens*. *Phytopathology*, Vol.97, No.11, pp. 1380-1390, ISSN 0031-949X
- Landgraf, A., Reckmann, B. & Pingoud, A. (1991). Direct analysis of polymerase chain-reaction products using enzyme-linked-immunosorbent-assay techniques. *Analytical Biochemistry*, Vol.198, pp. 86-91, ISSN 0003-2697
- Langrell, S.R.H. & Barbara, D.J. (2001). Magnetic capture hybridization for improved PCR detection of *Nectria galligena* from lignified apple extracts. *Plant Molecular Biology Reporter*, Vol.19, No.1, pp. 5-11, ISSN 0735-9640
- Langrell, S.R.H.; Glen, M. & Alfenas, A.C. (2008). Molecular diagnosis of *Puccinia psidii* (guava rust) - a quarantine threat to Australian eucalypt and *Myrtaceae* biodiversity. *Plant Pathology*, Vol.57, No.4, pp. 687-701, ISSN 0032-0862
- Larsen, J.E.; Hollingsworth, C.R.; Flor, J.; Dornbusch, M.R.; Simpson, N.L. & Samac, D.A. (2007). Distribution of *Phoma sclerotoides* on alfalfa and winter wheat crops in the north central United States. *Plant Disease*, Vol.91, No.5, pp. 551-558, ISSN 0191-2917
- Lee, M.S.; LeMaistre, A.; Kantarjian, H.M.; Talpaz, M.; Freireich, E.J.; Trujillo, J.M. & Stass, S.A. (1989). Detection of two alternative bcr/abl mRNA junctions and minimal residual disease in Philadelphia chromosome positive chronic myelogenous leukemia by polymerase chain reaction. *Blood*, Vol.73, No.8, pp. 2165-2170, ISSN 0006-4971
- Leisova, L.; Minarikova, V.; Kucera, L. & Ovesna, J. (2006). Quantification of *Pyrenophora teres* in infected barley leaves using real-time PCR. *Journal of Microbiological Methods*, Vol.67, No.3, pp. 446-455, ISSN 0167-7012
- Lévesque, C.A.; Harlton, C.E. & de Cock, A.W.A.M. (1998). Identification of some oomycetes by reverse dot-blot hybridization. *Phytopathology*, Vol.88, No.3, pp. 213-222, ISSN 0031-949X
- Licciardello, G.; Grasso, F. M.; Bella, P.; Cirvilleri, G.; Grimaldi, V. & Catara, V. (2006). Identification and detection of *Phoma tracheiphila*, causal agent of citrus mal secco disease, by real-time polymerase chain reaction. *Plant Disease*, Vol.90, No.12, pp. 1523-1530, ISSN 0191-2917
- Lievens, B. & Thomma B.P.H.J., (2005). Recent developments in pathogen detection arrays: implications for fungal plant pathogens and use in practice. *Phytopathology*, Vol.95, No.12, pp. 1374-1380, ISSN 0031-949X
- Lievens, B.; Brouwer, M.; Vanachter, A.C.R.C.; Levesque, C.A.; Cammue, B.P.A. & Thomma B.P.H.J. (2005b). Quantitative assessment of phytopathogenic fungi in various substrates using a DNA macroarray. *Environmental Microbiology*, Vol.7, No.11, pp. 1698-1710, ISSN 1462-2912
- Lievens, B.; Claes, L.; Vakalounakis, D.J., Vanachter, A.C.R.C. & Thomma, B.P.H.J. (2007). A robust identification and detection assay to discriminate the cucumber pathogens *Fusarium oxysporum* f. sp *cucumerinum* and f. sp *radicis-cucumerinum*. *Environmental Microbiology*, Vol.9, No.9, pp. 2145-2161, ISSN 1462-2912
- Lievens, B.; Claes, L.; Vanachter, A.C.R.C.; Cammue, B.P.A. & Thomma, B.P.H.J. (2006). Detecting single nucleotide polymorphisms using DNA arrays for plant pathogen diagnosis. *FEMS Microbiology Letters* Vol.255, No.1, pp.129-139, ISSN 0378-1097

- Lievens, B.; Grauwet, T.J.M.A.; Cammue, B.P.A. & Thomma B.P.H.J. (2005a). Recent developments in diagnostics of plant pathogens: a review. *Recent Research Developments in Microbiology*, Vol.9, pp. 57-79
- Lievens, B.; Rep, M. & Thomma, B.P.H.J. (2008). Recent developments in the molecular discrimination of *formae speciales* of *Fusarium oxysporum*. *Pest Management Science*, Vol.64, No.8, pp. 781-788, ISSN 1526-498X
- Lievens, B.; Van Baarlen P.; Verreth C.; Van Kerckhove, S.; Rep, M. & Thomma, B.P.H.J. (2009). Evolutionary relationships between *Fusarium oxysporum* f. sp *lycopersici* and *F. oxysporum* f. sp *radicis-lycopersici* isolates inferred from mating type, elongation factor-1 alpha and exopolygalacturonase sequences. *Mycological Research*, Vol.113, pp. 1181-1191, Part: 10, ISSN 0953-7562
- Liew, E.C.Y.; MacLean, D.J. & Irwin, J.A.G. (1998). Specific PCR based detection of *Phytophthora medicaginis* using the intergenic spacer region of the ribosomal DNA. *Mycological Research*, Vol.102, pp. 73-80, ISSN 0953-7562
- Liu, J.H.; Gao L.; Liu T.G. & Chen W.Q. (2009). Development of a sequence-characterized amplified region marker for diagnosis of dwarf bunt of wheat and detection of *Tilletia controversa* Kühn. *Letters in Applied Microbiology*, Vol.49, No.2, pp. 235-240, ISSN 0266-8254
- Long, A.A. (1998). In-situ polymerase chain reaction: foundation of the technology and today's options. *European Journal of Histochemistry*, Vol.42, pp. 101-109, ISSN 1121-760X
- Luchi, N.; Capretti, P.; Pinzani, P.; Orlando, C. & Pazzagli, M. (2005). Real-time PCR detection of *Biscogniauxia mediterranea* in symptomless oak tissue. *Letters in Applied Microbiology*, Vol.41, No.1, pp. 61-68, ISSN 0266-8254
- Luck, J.E. & Gillings, M.R. (1995). Rapid identification of benomyl resistant strains of *Botrytis cinerea* using the polymerase chain reaction. *Mycological Research*, Vol.99, pp. 1483-1488, ISSN 0953-7562
- Luo, C.; Cox, K.D.; Amiri, A. & Schnabel, G. (2008). Occurrence and detection of the DMResistance-associated genetic element 'Mona' in *Monilinia fructicola*. *Plant Disease*, Vol.92, pp. 1099-1103, ISSN 0191-2917
- Ma, Z. & Michailides, T.J. (2004). An allele-specific PCR assay for detecting strobilurin-resistant *Alternaria* isolates from pistachio in California. *Journal of Phytopathology*, Vol.152, pp. 118-121, ISSN 0931-1785
- Ma, Z. & Michailides, T.J. (2005). Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. *Crop Protection*, Vol.24, pp. 853-863, ISSN 0261-2194
- Ma, Z.; Felts, D. & Michailides, T.J. (2003b). Resistance to azoxystrobin in *Alternaria* isolates from pistachio in California. *Pesticide Biochemical Physiology*, Vol.77, No.2, pp. 66-74, ISSN 0048-3575
- Ma, Z.; Proffer, T.J.; Jacobs J.L. & Sundin G.W. (2006). Overexpression of the 14 $\alpha$ -demethylase target gene (CYP51) mediates fungicide resistance in *Blumeriella jaapii*. *Applied Environmental Microbiology*, Vol.72, pp. 2581-2585, ISSN 0099-2240
- Ma, Z.; Yoshimura, M. & Michailides, T.J. (2003a). Identification and characterization of benzimidazole resistance in *Monilinia fructicola* from stone fruit orchards in California. *Applied Environmental Microbiology*, Vol.69, pp. 7145-7152, ISSN 0099-2240

- Macia-Vicente, J.G.; Jansson, H.B.; Talbot, N.J. & Lopez-Llorca, L.V. (2009). Real-time PCR quantification and live-cell imaging of endophytic colonization of barley (*Hordeum vulgare*) roots by *Fusarium equiseti* and *Pochonia chlamydosporia*. *New Phytologist*, Vol.182, No.1, pp. 213-228, ISSN 0028-646X
- Malausa, T.; Andre, G.; Meglecz, E.; Blanquart, H.; Duthoy, S.; Costedoat, C.; Dubut, V.; Pech, N.; Castagnone-Sereno, P.; Delye, C.; Feau, N.; Frey, P.; Gauthier, P.; Guillemaud, T.; Hazard, L.; Le Corre, V.; Lung-Escarmant, B.; Male, P.J.G.; Ferreira, S.; Martin, J.F. (2011). High-throughput microsatellite isolation through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries. *Molecular Ecology Resources*, Vol.11, No.4, pp. 638-644, ISSN 1755-098X
- Malvick, D.K. & Impullitti, A.E. (2007). Detection and quantification of *Phialophora gregata* in soybean and soil samples with a quantitative, real-time PCR assay. *Plant Disease*, Vol.91, No.6, pp. 736-742, ISSN 0191-2917
- Markakis, E.A.; Tjamos, S.E.; Antoniou, P.P.; Paplomatas, E.J. & Tjamos, E.C. (2009). Symptom development, pathogen isolation and real-time QPCR quantification as factors for evaluating the resistance of olive cultivars to *Verticillium* pathotypes. *European Journal of Plant Pathology*, Vol.124, No.4, pp. 603-611, ISSN 0929-1873
- Martin, F.N. & Tooley, P.W. (2003). Phylogenetic relationships among *Phytophthora* species inferred from sequence analysis of mitochondrially encoded cytochrome oxidase I and II genes. *Mycologia*, Vol.95, No.2, pp. 269-284, ISSN 0027-5514
- Martínez-Espinoza, A.D.; León-Ramírez, C.G.; Singh, N. & Ruiz-Herrera, J. (2003). Use of PCR to detect infection of differentially susceptible maize cultivars using *Ustilago maydis* strains of variable virulence. *International Microbiology*, Vol.6, No.2, pp. 117-120, ISSN 1139-6709
- Martínez-García, L.B.; Armas, C.; Miranda, J.D.; Padilla, F.M. & Pugnaire, F.I. (2011). Shrubs influence arbuscular mycorrhizal fungi communities in a semi-arid environment. *Soil Biology & Biochemistry*, Vol.43, No.3, pp. 682-689, ISSN 0038-0717
- Martos, S.; Torres, E.; El Bakali, M.A.; Raposo, R.; Gramaje, D.; Armengol, J. & Luque, J. (2011) Co-operational PCR coupled with Dot Blot Hybridization for the detection of *Phaeomoniella chlamydospora* on infected grapevine wood. *Journal of Phytopathology*, Vol.159, No.4, pp. 247-254, ISSN 0931-1785
- Massart, S.; De Clercq, D.; Salmon, M.; Dickburt, C. & Jijakli, M.H. (2005). Development of real-time PCR using Minor Groove Binding probe to monitor the biological control agent *Candida oleophila* (strain O). *Journal of Microbiological Methods*, Vol.60, No.1, pp. 73-82, ISSN 0167-7012
- Matsuda, Y.; Sameshima, T.; Moriura, N.; Inoue, K.; Nonomura, T.; Kakutani, K.; Nishimura, H.; Kusakari, S.; Tamamatsu, S. & Toyoda, H. (2005). Identification of individual powdery mildew fungi infecting leaves and direct detection of gene expression of single conidium by polymerase chain reaction. *Phytopathology*, Vol.95, No.10, pp. 1137-1143, ISSN 0031-949X
- McCartney, H.A.; Foster, S.J.; Fraaije, B.A. & Ward, E. (2003). Molecular diagnostics for fungal plant pathogens. *Pest Management Science*, Vol.59, No.2, pp. 129-142, ISSN 1526-498X
- McDonald, B.A. (1997). The population genetics of fungi: tools and techniques. *Phytopathology*, Vol.87, No. pp. 448-453, ISSN 0031-949X

- McKay, G.J. & Cooke, L.R. (1997). A PCR-based method to characterize and identify benzimidazole resistance in *Helminthosporium solani*. *FEMS Microbiology Letters*, Vol.152, pp. 371–378, ISSN 0378-1097
- McKay, G.J.; Egan, D.; Morris, E. & Brown, A.E. (1998). Identification of benzimidazole resistance in *Cladobotryum dendroides* using a PCR-based method. *Mycological Research*, Vol.102, pp. 671–676, ISSN 0953-7562
- Meng, J. & Wang, Y. (2010). Rapid detection of *Phytophthora nicotianae* in infected tobacco tissue and soil samples based on its *Ypt1* gene. *Journal of Phytopathology*, Vol.158, No.1, pp. 1–7, ISSN 0931-1785
- Mercado-Blanco, J.; Rodríguez-Jurado, D.; Pérez-Artés, E. & Jiménez-Díaz, R.M. (2001). Detection of the nondefoliating pathotype of *Verticillium dahliae* in infected olive plants by nested PCR. *Plant Pathology*, Vol.50, No. 5, pp. 609–619
- Meudt, H.M. & Clarke, A.C. (2007). Almost forgotten or latest practice? AFLP applications, analyses and advances. *Trends in Plant Science*. Vol.12, No.3, pp. 106–117, ISSN 1360-1385
- Midorikawa, G.E.O.; Pinheiro, M.R.R.; Vidigal, B.S.; Arruda, M.C.; Costa, F.F.; Pappas, G.J. Jr.; Ribeiro, S. G.; Freire, F. & Miller, R.N.G. (2008). Characterization of *Aspergillus flavus* strains from Brazilian Brazil nuts and cashew by RAPD and ribosomal DNA analysis. *Letters in Applied Microbiology*, Vol.47, No.1, pp. 12–18, ISSN 0266-8254
- Minerdi, D.; Moretti M.; Li Y.; Gaggero, L.; Garibaldi, A. & Gullino, M.L. (2008). Conventional PCR and real time quantitative PCR detection of *Phytophthora cryptogea* on *Gerbera jamesonii*. *European Journal of Plant Pathology*, Vol.122, No.2, pp. 227–237, ISSN 0929-1873
- Moradi, M.; Oerke, E.-C.; Steiner, U.; Tesfaye, D.; Schellander, K. & Dehne, H.-W. (2010). Microbiological and Sybr®Green Real-Time PCR detection of major *Fusarium* Head Blight pathogens on wheat ears. *Microbiology*, Vol.79, No.5, pp. 646–654, ISSN 0026-2617
- Mostert, L.; Groenewald, J.Z.; Summerbell, R.C.; Gams, W. & Crous, P.W. (2006). Taxonomy and pathology of *Togninia* (Diaporthales) and its *Phaeoacremonium* anamorphs. *Studies in Mycology*, Vol.54, pp. 1–113, ISSN 0166-0616
- Mulè, G.; Susca, A.; Stea, G. & Moretti, A. (2004). Specific detection of the toxigenic species *Fusarium proliferatum* and *F. oxysporum* from asparagus plants using primers based on calmodulin gene sequences. *FEMS Microbiology Letters*. Vol.230, No.2, pp. 235–240
- Mullis, K.B. & Faloona, F.A. (1987). Specific synthesis of DNA in vitro via a polymerase-catalyzed chain-reaction. *Methods in Enzymology*, Vol.155, pp. 335–350
- Mumford, R.; Boonham, N.; Tomlinson, J. & Barker, I. (2006). Advances in molecular phytodiagnostics-new solutions for old problems. *European Journal of Plant Pathology*, Vol.116, pp. 1–19, ISSN 0929-1873
- Nakaune, R. (2001). ABC transporter genes involved in multidrug resistance in *Penicillium digitatum*. *Journal of General Plant Pathology*, Vol.67, No.3, pp. 251
- Nakaune, R.; Adachi, K.; Nawata, O.; Tomiyama, M.; Akutsu, K. & Hibi, T. (1998). A novel ATP-binding cassette transporter involved in multidrug resistance in phytopathogenic fungus *Penicillium digitatum*. *Applied Environmental Microbiology*, Vol.64, pp. 3983–3988, ISSN 0099-2240



- Nakaune, R.; Hamamoto, H.; Imada, J. & Akutsu, K. (2002). A novel ABC transporter gene, *PMR5*, is involved in multidrug resistance in the phytopathogenic fungus *Penicillium digitatum*. *Molecular Genetics and Genomics*, Vol.267, pp. 179-185, ISSN 1617-4615
- Nanayakkara, U.N.; Singh, M.; Al-Mugharabi, K.I. & Peters, R.D. (2009). Detection of *Phytophthora erythroseptica* in above-ground potato tissues, progeny tubers, stolons and crop debris using PCR techniques. *American Journal of Potato Research*, Vol.86, No.3, pp. 239-245, ISSN 1099-209X
- Nayaka, S.C.; Wulff, E.G.; Udayashankar, A.C.; Nandini, B.P.; Niranjana, S.R.; Mortensen, C.N. & Prakash, H.S. (2011). Prospects of molecular markers in *Fusarium* species diversity. *Applied Microbiology and Biotechnology*. Vol.90, No.5, pp. 1625-1639, ISSN 0175-7598
- NCBI, National Center for Biotechnology Information:  
(<http://www.ncbi.nlm.nih.gov/Genbank/>)
- Nechwatal, J. & Mendgen, K. (2006). *Pythium litorale* sp nov., a new species from the littoral of Lake Constance, Germany *Fems Microbiology Letters*, Vol.255, No.1, pp. 96-101, ISSN 0378-1097
- Nguyen, H.D.T. & Seifert, K.A. (2008). Description and DNA barcoding of three new species of *Leohumicola* from South Africa and the United States. *Persoonia*, Vol.21, pp. 57-69, ISSN 0031-5850
- Nicolaisen, M.; Justesen, A.F.; Thrane, U.; Skouboe, P. & Holmstrom, K. (2005). An oligonucleotide microarray for the identification and differentiation of trichothecene producing and nonproducing *Fusarium* species occurring on cereal grain. *Journal of Microbiological Methods*, Vol.62, No. 1, pp. 57-69, ISSN 0167-7012
- Niu, C.; Kebede, H.; Auld, D.L.; Woodward, J.E.; Burow, G. & Wright R.J. (2008). A safe inexpensive method to isolate high quality plant and fungal DNA in an open laboratory environment. *African Journal of Biotechnology*, Vol.7, No.16, pp. 2818-2822, ISSN 1684-5315
- Notomi, T.; Okayama, H.; Masubuchi, H.; Yonekawa, T.; Watanabe, K.; Amino, N. & Hase, T. (2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research*, Vol.28, No. 12, Article Number: e63, ISSN 0305-1048
- Nunes, C.C.; Gowda, M.; Sailsbery, J.; Xue, M.F.; Chen, F.; Brown, D.E.; Oh, Y.; Mitchell, T.K. & Dean, R.A. (2011). Diverse and tissue-enriched small RNAs in the plant pathogenic fungus, *Magnaporthe oryzae*. *BMC Genomics*, Vol.12, Article Number: 288, ISSN 1471-2164
- Nuovo, G.J. (1992). *PCR in situ hybridization: Protocols and applications*. Raven Press, ISBN 0881679402, New York
- Nuovo, G.J.; MacConnell, P. & Gallery, F. (1994). Analysis of nonspecific DNA synthesis during in situ PCR and solution-phase PCR. *Genome Research*, Vol.4, pp. 89-96
- Nuovo, G.J., MacConnell, P.; Forde, A. & Delvenne, P. (1991). Detection of human papillomavirus DNA in formalin fixed tissues by in situ hybridization after amplification by the polymerase chain reaction. *American Journal of Pathology*, Vol.139, No.4, pp. 847-854, ISSN 0002-9440
- Okubara, P.A.; Schroeder, K.L. & Paulitz, T.C. (2008). Identification and quantification of *Rhizoctonia solani* and *R. oryzae* using real-time polymerase chain reaction. *Phytopathology*, Vol.98, No.7, pp. 837-847, ISSN 0031-949X

- Olmos, A.; Bertolini, E. & Cambra, M. (2002). Simultaneous and co-operational amplification (Co-PCR): a new concept for detection of plant viruses. *Journal of Virological Methods*, Vol.106, No.1, pp. 51-59, ISSN 0166-0934
- Omirou, M.; Rousidou, C.; Bekris, F.; Papadopoulou, K.K.; Menkissoglou-Spiroudi, U.; Ehaliotis, C. & Karpouzas, D.G. (2011). The impact of biofumigation and chemical fumigation methods on the structure and function of the soil microbial community. *Microbial Ecology*, Vol.61, No.1, pp. 201-213, ISSN 0095-3628
- Ozakman, M. & Schaad, N.W. (2003). A real-time BIO-PCR assay for detection of *Ralstonia solanacearum* race 3, biovar 2, in asymptomatic potato tubers. *Canadian Journal of Plant Pathology*, Vol.25, No.3, pp. 232-239, ISSN 0706-0661
- Pan, L.; Ash, G.J.; Ahn, B. & Watson, A.K. (2010). Development of strain specific molecular markers for the *Sclerotinia minor* bioherbicide strain IMI 344141. *Biocontrol Science and Technology*, Vol.20, No.9, pp. 939-959, ISSN 0958-3157 print/ISSN 1360-0478 online
- Pannecoucq, J. & Hofte M. (2009). Detection of rDNA ITS polymorphism in *Rhizoctonia solani* AG 2-1 isolates. *Mycologia*, Vol.101, No. 1, pp. 26-33, ISSN 0027-5514
- Pao, S.S.; Paulsen, I.T. & Saier MH Jr. (1998). Major facilitator superfamily. *Microbiology Molecular Biology Review*, Vol.62, pp. 1-34, ISSN 1092-2172
- Park, B.; Park, J.; Cheon, K.-C.; Choi, J.; Jung, K.; Kim, D.; Lee, Y.H.; Ward, T.J.; O'Donnell, K.; Geiser, D.M. & Kang, S. (2011). Cyber infrastructure for Fusarium: three integrated platforms supporting strain identification, phylogenetics, comparative genomics and knowledge sharing. *Nucleic Acids Research* Vol.39, Supplement. 1, pp. D640-D646, ISSN 0305-1048
- Park, J.; Park, B.; Veeraraghavan, N.; Blair, J.E.; Geiser, D.M.; Isard, S.; Mansfield, M.A.; Nikolaeva, E.; Park, S.-Y.; Russo, J.; Kim, S.H.; Greene, M.; Ivors, K.L.; Balci, Y.; Peiman, M.; Erwin, D.C.; Coffey, M.D.; Jung, K.; Lee, Y.-H.; Rossman, A.; Farr, D.; Cline, E.; Grünwald, N.J.; Luster, D.G.; Schrandt, J.; Martin, F.; Ribeiro, O.K.; Makalowska, I. & Kang, S. (2008). *Phytophthora* database: forensic database supporting the identification and monitoring of *Phytophthora*. *Plant Disease*, Vol.92, No.6, pp. 966-972, ISSN 0191-2917
- Paul, B. (2006). *Pythium apiculatum* sp. nov. isolated from Burgundian vineyards: morphology, taxonomy, ITS region of its rRNA, and comparison with related species. *FEMS Microbiology Letters*, Vol.263, No.2, pp. 194-199, ISSN 0378-1097
- Paul, B. (2009). *Pythium burgundicum* sp. nov. isolated from soil samples taken in French vineyards. *FEMS Microbiology Letters*, Vol.301, No. , pp. 109-114, ISSN 0378-1097
- Paul, B. & Bala, K. (2008). A new species of *Pythium* with inflated sporangia and coiled antheridia, isolated from India. *FEMS Microbiology Letters*, Vol.282, No.2, pp. 251-257, ISSN 0378-1097
- Paul, B., Bala, K., Gognies, S. & Belarbi, A. (2005). Morphological and molecular taxonomy of *Pythium longisporangium* sp. nov. isolated from the Burgundian region of France. *FEMS Microbiology Letters*, Vol.246, No.2, pp. 207-212, ISSN 0378-1097
- Pereira, V.J.; Fernandes, D.; Carvalho, G.; Benoliel, M.J.; Romao, M.V.S. & Crespo, M.T.B. (2010). Assessment of the presence and dynamics of fungi in drinking water sources using cultural and molecular methods. *Water Research*, Vol.44, No.17, Special Issue: SI, pp. 4850-4859, ISSN 0043-1354
- Phytophthora Database: (<http://www.phytophthoradb.org>)

Phytophthora-ID: (<http://phytophthora-id.org>)

- Porterjordan, K.; Rosenberg, E.I.; Keiser, J.F.; Gross, J.D.; Ross, A.M.; Nasim, S. & Garrett, C.T. (1990). Nested polymerase chain reaction assay for the detection of cytomegalovirus overcomes false positives caused by contamination with fragmented DNA. *Journal of Medical Virology*, Vol.30, No.4, pp. 85-91, ISSN 0146-6615
- Portillo, M.C.; Villahermosa, D.; Corzo, A. & González, J.M. (2011). Microbial community fingerprinting by differential display-denaturing gradient gel electrophoresis. *Applied and Environmental Microbiology*, Vol.77, No.1, pp. 351-354, ISSN 0099-2240
- Primer3: (<http://frodo.wi.mit.edu/primer3>)
- Prospero, S.; Black, J.A. & Winton, L.M. (2004). Isolation and characterization of microsatellite markers in *Phytophthora ramorum*, the causal agent of sudden oak death. *Molecular Ecology Notes*, Vol.4, No.4, pp. 672-674, ISSN 1471-8278
- Qin, L.; Fu, Y.; Xie, J.; Cheng, J.; Jiang, D.; Li, G. & Huang, J. (2011). A nested-PCR method for rapid detection of *Sclerotinia sclerotiorum* on petals of oilseed rape (*Brassica napus*). *Plant Pathology*, Vol.60, No.2, pp. 271-277, ISSN 0032-0862
- Saldanha, R.L.; García, J.E.; Dekker, R.F.H.; Vilas-Boas, L.A. & Barbosa, A.M. (2007). Genetic diversity among *Botryosphaeria* isolates and their correlation with cell wall-lytic enzyme production. *Brazilian Journal of Microbiology*, Vol.38, No.2, pp. 259-264, ISSN 1517-8382
- Samuelian, S.K.; Greer, L.A.; Savocchia, S. & Steel, C.C. (2011). Detection and monitoring of *Greeneria uvicola* and *Colletotrichum acutatum* development on grapevines by real-time PCR. *Plant Disease*, Vol.95, No.3, pp. 298-303, ISSN 0191-2917
- Sánchez-Torres, P. & Tuset, J.J. (2011). Molecular insights into fungicide resistance in sensitive and resistant *Penicillium digitatum* strains infecting citrus. *Postharvest Biology and Technology*, Vol.59, No.2, pp. 159-165, ISSN 0925-5214
- Schena, L. & Ippolito, A. (2003). Rapid and sensitive detection of *Rosellinia necatrix* in roots and soils by real time Scorpion-PCR. *Journal of Plant Pathology*, Vol.85, No.1, pp. 15-25, ISSN 1125-4653
- Schena, L.; Hughes K.J.D. & Cooke D.E.L. (2006). Detection and quantification of *P. ramorum*, *P. kernoviae*, *P. citricola* and *P. quercina* in symptomatic leaves by multiplex realtime PCR. *Molecular Plant Pathology*, Vol.7, No.5, pp. 365-379, ISSN 1464-6722
- Schena, L., Nigro, F., Ippolito, A. & Gallitelli, D. (2004). Real-time quantitative PCR: a new technology to detect and study phytopathogenic and antagonistic fungi. *European Journal of Plant Pathology*, Vol.110, No.9, pp. 893-908, ISSN 0929-1873
- Schmidt, H.; Taniwaki, M.H.; Vogel, R.F. & Niessen, L. (2004) Utilization of AFLP markers for PCR-based identification of *Aspergillus carbonarius* and indication of its presence in green coffee samples. *Journal of Applied Microbiology* Vol.97, No.5, pp.899-909, ISSN 1364-5072
- Schnabel, G. & Jones, A.L. (2001). The 14 $\alpha$ -demethylase (CYP51A1) gene is overexpressed in *Venturia inaequalis* strains resistant to myclobutanil. *Phytopathology*, Vol.91, pp. 102-110, ISSN 0031-949X
- Schnabel, G.; Dait, Q. & Paradkar, M.R. (2001). Cloning and expression analysis of the ATP-binding cassette transporter gene *MFABC1* and the alternative oxidase gene *MfAOX1* from *Monilinia fructicola*. *Pest Management Science*, Vol.59, pp. 1143-1151, ISSN 1526-498X

- Schoonbeek, H.-J.; van Nistelrooy, J.G.M. & de Waard, M.A. (2003). Functional analysis of ABC transporter genes from *Botrytis cinerea* identifies *BcatrB* as a transporter of eugenol. *European Journal of Plant Pathology*, Vol.109, pp. 1003-1011, ISSN 0929-1873
- Schroeder, K.L.; Okubara, P.A.; Tambong, J.T.; Levesque, C.A. & Paulitz, T.C. (2006). Identification and quantification of pathogenic *Pythium* spp. from soils in eastern Washington using real-time polymerase chain reaction. *Phytopathology*, Vol.96, No.6, pp. 637-647, ISSN 0031-949X
- Seifert, K.A.; Samson, R.A.; deWaard, J.R.; Houbroken, J.; Lévesque C.A.; Moncalvo, J.-M.; Louis-Seize, G. & Hebert P.D.N. (2007). Prospects for fungus identification using CO1 DNA barcodes, with *Penicillium* as a test case. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.104, No.10, pp. 3901-3906, ISSN 0027-8424
- Sheridan, G.E.C.; Masters, C.I.; Shallcross, J.A. & Mackey, B.M. (1998). Detection of mRNA by reverse transcription-PCR as an indicator of viability in *Escherichia coli* cells. *Applied Environmental Microbiology*, Vol.64, No.4, pp. 1313-1318, ISSN 0099-2240
- Shimomoto, Y.; Sato, T.; Hojo, H.; Morita, Y.; Takeuchi, S.; Mizumoto, H.; Kiba, A. & Hikichi, Y. (2011). Pathogenic and genetic variation among isolates of *Corynespora cassiicola* in Japan. *Plant Pathology*, Vol.60, No.2, pp. 253-260, ISSN 0032-0862
- Sierotzki, H.; Wulschleger, J. & Gisi, U. (2000). Point mutation in cytochrome b gene conferring resistance to strobilurin fungicides in *Erysiphe graminis* f. sp. *tritici* field isolates. *Pesticide Biochemical Physiology*, Vol.68, pp. 107-112, ISSN 0048-3575
- Silvar, C.; Díaz, J. & Merino, F. (2005). Real-time polymerase chain reaction quantification of *Phytophthora capsici* in different pepper genotypes. *Phytopathology*, Vol.95, No.12, pp. 1423-1429, ISSN 0031-949X
- Singh, M. & Singh, R.P. (1997). Potato virus Y detection: Sensitivity of RT-PCR depends on the size of fragment amplified. *Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie*, Vol.19, pp. 149-155, ISSN 0706-0661
- Smith, L.M. & Burgoyne, L.A. (2004). Collecting, archiving and processing DNA from wildlife samples using FTA® databasing paper. *BMC Ecology*, 4:4
- Somai, B.M.; Keinath, A.P. & Dean, R.A. (2002). Development of PCR-ELISA detection and differentiation of *Didymella bryoniae* from related *Phoma* species. *Plant Disease*, Vol.86, No.7, pp. 710-716, ISSN 0191-2917
- Stergiopoulos, I. & de Waard, M.A. (2002). Activity of azole fungicides and ABC transporters modulators on *Mycosphaerella graminicola*. *Journal of Phytopathology*, Vol.150, pp. 313-320, ISSN 0931-1785
- Stergiopoulos, I.; Gielkens, M.M.C.; Goodall, S.D.; Venema, K. & de Waard, M.A. (2002b). Molecular cloning and characterization of three new ATP-binding cassette transporter genes from the wheat pathogen *Mycosphaerella graminicola*. *Gene*, Vol.289, pp. 141-149, ISSN 0378-1119
- Stergiopoulos, I.; van Nistelrooy, J.G.; Kema, G.H. & de Waard, M.A. (2003b). Multiple mechanisms account for variation in base-line sensitivity to azole fungicides in field isolates of *Mycosphaerella graminicola*. *Pest Management Science*, Vol.59, pp. 1333-1343, ISSN 1526-498X
- Stergiopoulos, I.; Zwiers, L.-H. & de Waard, M.A. (2002a). Secretion of natural and synthetic toxic compounds from filamentous fungi by membrane transporters of the ATP-



- binding cassette and major facilitator superfamily. *European Journal of Plant Pathology*, Vol.108, pp. 719-734, ISSN 0929-1873
- Stergiopoulos, I.; Zwiers, L.-H. & de Waard, M.A. (2003a). The ABC transporter MgAtr4 is a virulence factor of *Mycosphaerella graminicola* that effects colonisation of substomatal cavities in wheat leaves. *Molecular Plant-Microbe Interaction*, Vol.16, pp. 689-698, ISSN 0894-0282
- Stewart, J.E.; Kim, M.-S.; James, R.L.; Dumroese, R.K. & Klopfenstein, N.B. (2006). Molecular characterization of *Fusarium oxysporum* and *Fusarium commune* isolates from a conifer nursery. *Phytopathology*, Vol.96, pp. 1124-1133, ISSN 0031-949X
- Stringari, D.; Glienke, C.; de Christo, D.; Maccheroni, W. & de Azevedo, J.L. (2009). High molecular diversity of the fungus *Guignardia citricarpa* and *Guignardia mangiferae* and new primers for the diagnosis of the Citrus Black Spot. *Brazilian archives of biology and technology*, Vol.52, No.5, pp. 1063-1073, ISSN 1516-8913
- Sun, X.; Wang, J.; Feng, D.; Ma, Z. & Li, H. (2011). PdCYP51B, a new putative sterol 14 $\alpha$ -demethylase gene of *Penicillium digitatum* involved in resistance to imazalil and other fungicides inhibiting ergosterol synthesis. *Applied Microbiology and Biotechnology*, Vol.91, No.4, pp. 1107-1119, ISSN 0175-7598
- Sundelin, T.; Christensen, C.B.; Larsen, J.; Møller, K.; Lübeck, M.; Bødker, L. & Jensen, B. (2010). In planta quantification of *Plasmodiophora brassicae* using signature fatty acids and real-time PCR. *Plant Disease*, Vol.94, No.4, pp. 432-438, ISSN 0191-2917
- Suzuki, S.; Taketani, H.; Kusumoto, K.I. & Kashiwagi, Y. (2006). High-throughput genotyping of filamentous fungus *Aspergillus oryzae* based on colony direct polymerase chain reaction. *Journal of Bioscience and Bioengineering*, Vol.102, No.6, pp. 572-574, ISSN 1389-1723
- Takamatsu, S.; Nakano, M.; Yokota, H. & Kunoh, H. (1998). Detection of *Rhizoctonia solani* AG-2-2-IV, the causal agent of large patch of Zoysiagrass, using plasmid DNA as probe. *Annals of the Phytopathological Society of Japan*, Vol.64, No.5, pp. 451-457
- Tawfik, D.S. & Griffiths, A.D. (1998). Man-made cell-like compartments for molecular evolution, *Nature Biotechnology*, Vol.16, No.7, pp. 652-656, ISSN 1087-0156
- Tebbe, C.C. & Vahjen, W. (1993). Interference of humic acids and dna extracted directly from soil in detection and transformation of recombinant-DNA from bacteria and a yeast. *Applied and Environmental Microbiology* Vol.59, No.8, pp. 2657-2665, ISSN 0099-2240
- Tewoldemedhin, Y.T.; Mazzola, M.; Botha W.J.; Spies, C.F.J. & McLeod, A. (2011). Characterization of fungi (*Fusarium* and *Rhizoctonia*) and oomycetes (*Phytophthora* and *Pythium*) associated with apple orchards in South Africa. *European Journal of Plant Pathology*, Vol.130, No 2, pp. 215-229, ISSN 0929-1873
- Theodoulou, F.L. (2000). Plant ABC transporters. *Biochimica Biophysica Acta*, Vol.1465, pp. 79-103, ISSN 0005-2736
- Thies, J.E. (2007). Soil microbial community analysis using terminal restriction fragment length polymorphisms. *Soil Science Society of America Journal*, Vol.71, No.2, pp. 579-591, ISSN 0361-5995
- Tomlinson J. A.; Barker I. & Boonham N. (2007). Faster, simpler, more-specific methods for improved molecular detection of *Phytophthora ramorum* in the field. *Applied and Environmental Microbiology* Vol.73, No.12, pp. 4040-4047, ISSN 0099-2240

- Tomlinson, J.A.; Boonham, N.; Hughes, K.J.D.; Griffen, R.L. & Barker, I. (2005). On-site DNA extraction and real-time PCR for detection of *Phytophthora ramorum* in the field. *Applied and Environmental Microbiology* Vol.71, No.11, pp. 6702-6710, ISSN 0099-2240
- Tomlinson J.A.; Dickinson M.J. & Boonham N. (2010). Rapid detection of *Phytophthora ramorum* and *P. kernoviae* by two-minute DNA extraction followed by isothermal amplification and amplicon detection by generic lateral flow device. *Phytopathology* Vol.100, No.2, pp, 143-149, ISSN 0031-949X
- Tooley, P.W.; Martin, F.N.; Carras, M.M. & Frederick, R.D. (2006). Real-time fluorescent polymerase chain reaction detection of *Phytophthora ramorum* and *Phytophthora pseudosyringae* using mitochondrial gene regions. *Phytopathology*, Vol.96, No.4, pp. 336-345, ISSN 0031-949X
- Torres-Calzada, C.; Tapia-Tussell, R.; Quijano-Ramayo, A.; Martin-Mex, R.; Rojas-Herrera, R.; Higuera-Ciapara, I.; Perez-Brito, D. (2011). A species-specific polymerase chain reaction assay for rapid and sensitive detection of *Colletotrichum capsici*. *Molecular Biotechnology*, Vol.49, No.1, pp. 48-55, ISSN 1073-6085
- TrichOKey, Molecular Barcode: (<http://www.isth.info/tools/molkey/index.php>)
- Tyagi, S. & Kramer, F.R. (1996). Molecular beacons: Probes that fluoresce upon hybridization. *Nature Biotechnology*, Vol.14, No.3, pp. 303-308, ISSN 1087-0156
- UNITE, A molecular database for identification of fungi: (<http://unite.ut.ee/index.php>)
- Validov, S.Z.; Kamilova F.D. & Lugtenberg B.J.J. (2011). Monitoring of pathogenic and non-pathogenic *Fusarium oxysporum* strains during tomato plant infection. *Microbial Biotechnology*, Vol.4, No.1, pp. 82-88, ISSN 1751-7907
- Valsesia, G.; Gobbin, D.; Patocchi, A.; Vecchione, A.; Pertot, I. & Gessler, C. (2005). Development of a high-throughput method for quantification of *Plasmopara viticola* DNA in grapevine leaves by means of quantitative real-time polymerase chain reaction. *Phytopathology*, Vol.95, No.6, pp. 672-678, ISSN 0031-949X
- van Doorn, R.; Slawiak, M.; Szemes, M.; Dulleman, A.M.; Bonants, P.; Kowalchuk, G.A. & Schoen, C.D. (2009). Robust detection and identification of multiple oomycetes and fungi in environmental samples by using a novel cleavable padlock probe-based ligation detection assay. *Applied and Environmental Microbiology*, Vol.75, No.12, pp. 4185-4193, ISSN 0099-2240
- van Gent-Pelzer, M.P.E.; van Brouwershaven, I.R.; Kox, L.F.F. & Bonants, P.J.M. (2007). A TaqMan PCR method for routine diagnosis of the quarantine fungus *Guignardia citricarpa* on citrus fruit. *Journal of Phytopathology*, Vol.155, No.6, pp. 357-363, ISSN 0931-1785
- VanderLee, T.; DeWitte, I.; Drenth, A.; Alfonso, C. & Govers, F. (1997). AFLP linkage map of the oomycete *Phytophthora infestans*. *Fungal Genetics and Biology*, Vol.21, No.3, pp. 278-291, ISSN 1087-1845
- Vermeulen, T.; Schoonbeek, H. & de Waard, M.A. (2001). The ABC transporter BcatrB from *Botrytis cinerea* is a determinant of the activity of the phenylpyrrole fungicide fludioxonil. *Pest Management Science*, Vol.57, pp. 393-402, ISSN 1526-498X
- Vos, P.; Hogers, R.; Bleeker, M.; Reijans, M.; Vandele, T.; Hornes, M.; Frijters, A.; Pot, J.; Peleman, J.; Kuiper, M. & Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, Vol.23, No.21, pp. 4407-14, ISSN 0305-1048

- Wakelin S.A.; Warren, R.A.; Kong, L. & Harvey, P.R. (2008). Management factors affecting size and structure of soil *Fusarium* communities under irrigated maize in Australia. *Applied Soil Ecology*, Vol.39, pp. 201-209, ISSN 0929-1393
- Welsh, J. & McClelland, M. (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research*, Vol.18, No.24, pp. 7213-7218, ISSN 0305-1048
- Whitcombe, D.; Kelly, S.; Mann, J.; Theaker, J.; Jones, C. & Little, S. (1999). Scorpions (TM) primers - a novel method for use in single-tube genotyping. *American Journal of Human Genetics*, Vol.65, No.4, pp. A412-A412, Meeting Abstract: 2333, ISSN 0002-9297
- White, T. (1997). Increased mRNA levels of *ERG16*, *CDR*, and *MDR1* correlate with increases in azole resistance in *Candida albicans* isolates from a patient infected with human immunodeficiency virus. *Antimicrobe Agents Chemotherapy*, Vol.41, pp. 1482-1487
- White, T.J.; Bruns, T.; Lee, S. & Taylor, J.W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: A Guide to Methods and Applications*, Innis, M.A.; Gelfand, D.H.; Sninsky, J.J. & White, T.J. Academic Press, Inc., New York. pp. 315-322
- Williams, J.G.K.; Kubelik, A.R.; Livak, K.J.; Rafalski, J.A. & Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*, Vol.18, No.22, pp. 6531-6535, ISSN 0305-1048
- Wilson, I.G. (1997) Inhibition and facilitation of nucleic acid amplification. *Applied and Environmental Microbiology* Vol.63, No.10, pp. 3741-3751, ISSN 0099-2240
- Winton, L.M. & Hansen, E.M. (2001). Molecular diagnosis of *Phytophthora lateralis* in trees, water, and foliage baits using multiplex polymerase chain reaction. *Forest Pathology*, Vol.31, No.5, pp. 275-283, ISSN 1437-4781
- Winton, L.M.; Krohn, A.L. & Leiner, R.H. (2007). Microsatellite markers for *Sclerotinia subarctica* nom. prov., a new vegetable pathogen of the High North. *Molecular Ecology Notes*, Vol.7, No.6, pp. 1077-1079, ISSN 1471-8278
- Wittwer, C.T.; Herrmann, M.G.; Moss, A.A. & Rasmussen, R.P. (1997). Continuous fluorescence monitoring of rapid cycle DNA amplification. *Biotechniques*, Vol.22, No.1, pp. 130-138, ISSN 0736-6205
- Wood, P.M. & Hollomon, D.W. (2003). A critical evaluation of the role of alternative oxidase in the performance of strobilurin and related fungicides acting at the Qo site of complex III. *Pest Management Science*, Vol.59, pp. 499-511, ISSN 1526-498X
- Wu, C.P.; Chen, G.Y.; Li, B.; Su, H.; An, Y.L.; Zhen, S.Z. & Ye, J.R. (2011). Rapid and accurate detection of *Ceratocystis fagacearum* from stained wood and soil by nested and real-time PCR. *Forest Pathology*, Vol.41, No.1, pp. 15-21, ISSN 1437-4781
- Wyand, R.A. & Brown, J.K.M. (2005). Sequence variation in the *CYP51* gene of *Blumeria graminis* associated with resistance to sterol demethylase inhibiting fungicides. *Fungal Genetics Biology*, Vol.42, pp. 726-765, ISSN 1087-1845
- Xu, M.L.; Melchinger, A.E. & Lübberstedt, T. (1999). Species-specific detection of the maize pathogens *Sporisorium reilianum* and *Ustilago maydis* by dot-blot hybridization and PCR-based assays. *Plant Disease*, Vol.83, No.4, pp. 390-395, ISSN 0191-2917
- Xue, B; Goodwin, P.H. & Annis, S.L. (1992). Pathotype identification of *Leptosphaeria maculans* with PCR and oligonucleotide primers from ribosomal internal transcribed spacer sequences. *Physiological and Molecular Plant Pathology*, Vol.41, No.3, pp. 179-188, ISSN 0885-5765

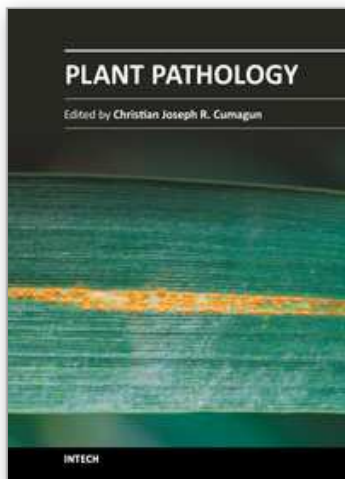
- Yan, L.; Zhang, C.; Ding, L. & Ma, Z. (2008). Development of a real-time PCR assay for the detection of *Cladosporium fulvum* in tomato leaves. *Journal of Applied Microbiology*, Vol.104, No.5, pp. 1417-1424, ISSN 1364-5072
- Yang F.; Jensen J. D.; Svensson B.; Jorgensen, H. J. L.; Collinge, D.B. & Finnie, C. (2010). Analysis of early events in the interaction between *Fusarium graminearum* and the susceptible barley (*Hordeum vulgare*) cultivar Scarlett. *Proteomics*, Vol.10, No.21, pp. 3748-3755, ISSN 1615-9853
- Yin, Y.; Ding, L.; Liu, X.; Yang, J. & Ma, Z. (2009). Detection of *Sclerotinia sclerotiorum* in planta by a real-time PCR assay. *Journal of Phytopathology*, Vol.157, No.7-8, pp. 465-469, ISSN 0931-1785
- Yoder, O.C. & Turgeon, B. (2001). Fungal genomics and pathogenicity. *Current Opinion in Plant Biology*, Vol.4, pp. 315-321, ISSN 1369-5266
- Zaccaro, R. P.; Carareto-Alves, L.M.; Travençolo, R.F.; Wickert, E. & Lemos, E.G.M. (2007). Use of molecular marker SCAR in the identification of *Fusarium subglutinans*, causal agent of mango malformation. *Revista brasileira de fruticultura*, Vol.29, No.3, pp. 563-570, ISSN 0100-2945
- Zelaya-Molina L.X.; Ortega M.A. & Dorrance A.E. (2011). Easy and efficient protocol for oomycete DNA extraction suitable for population genetic analysis. *Biotechnology Letters*, Vol.33, No.4, pp. 715-720, ISSN 0141-5492
- Zhang, N.; Tantarini, A.; Miller, S.; Eng, A. & Salvatore, N. (2011). TaqMan real-time PCR method for detection of *Discula destructiva* that causes dogwood anthracnose in Europe and North America. *European Journal of Plant Pathology*, Vol.130, No.4, pp. 551-558, ISSN 0929-1873
- Zhang, Y.J.; Zhang, S.; Liu, X.Z.; Wen, H.A. & Wang, M. (2010). A simple method of genomic DNA extraction suitable for analysis of bulk fungal strains. *Letters in Applied Microbiology*, Vol.51, No.1, pp. 114-118, ISSN 0266-8254
- Zhang, Z.; Zhu, Z.; Ma, Z., & Li, H. (2009). A molecular mechanism of azoxystrobin resistance in *Penicillium digitatum* UV mutants and a PCR-based assay for detection of azoxystrobin-resistant strains in packing- or store-house isolates. *International Journal of Food Microbiology*, Vol.131, pp. 157-161, ISSN 0168-1605
- Zhao, J.; Wang, X. J.; Chen, C. Q.; Huang, L. L. & Kang, Z.S. (2007). A PCR-Based assay for detection of *Puccinia striiformis* f. sp. *tritici* in wheat. *Plant Disease*, Vol.91, No.12, pp. 1969-1974, ISSN 0191-2917
- Zheng, D.; Olaya, G. & Koller, W. (2000). Characterization of laboratory mutants of *Venturia inaequalis* resistant to the strobilurin-related fungicide kresoxim-methyl. *Current Genetics*, Vol.38, pp. 148-155, ISSN 0172-8083
- Zheng, P.H.; Meng, X.Y.; Yu, Z.X.; Wu, Y.; Bao, Y.L.; Yu, C.L. & Li, Y.X. (2009). Genetic and phytochemical analysis of *Armillaria mellea* by RAPD, ISSR, and HPLC. *Analytical Letters*, Vol.42, No.10, pp. 1479-1494, ISSN 0003-2719
- Zheng, Y.; Zhang, G.; Lin, F.C.; Wang, Z.H.; Jin, G.L.; Yang, L.; Wang, Y.; Chen, X.; Xu, Z.H.; Zhao, X.Q.; Wang, H.K.; Lu, J.P.; Lu, G.D. & Wu, W.R. (2008). Development of microsatellite markers and construction of genetic map in rice blast pathogen *Magnaporthe grisea*. *Fungal Genetics and Biology*, Vol.45, No.10, pp. 1340-1347, ISSN 1087-1845



Zwiers, L-H.; Stergiopoulos, I.; Gielkens, M.M.C.; Goodall, S.D. & de Waard, M.A. (2003). ABC transporters of the wheat pathogen *Mycosphaerella graminicola* function as protectants against biotic and xenobiotic toxic compounds. *Molecular Genetics and Genomics*, Vol.269, pp. 499-507, ISSN 1617-4615

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