Biological Forum – An International Journal **7**(2): 473-492(2015)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Reliable Detection of the Fungal Pathogen, Molecular Detection and Identification *Fusarium oxysporum*

Khodabakhsh Naroei*and Mohammad Salari**
**M.Sc. Student, Department of Plant Protection, Faculty of Agriculture, University of Zabol, IRAN*
***Associate Professor, Department of Plant Protection, College of Agriculture, University of Zabol, IRAN*

(Corresponding author: Khodabakhsh Naroei)
(Received 29 April, 2015, Accepted 11 May, 2015)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: *Fusarium oxysporum* is a ubiquitous species complex of soil-borne plant pathogens comprising of many different formae speciales, each characterized by a high degree of host specificity. *Fusarium oxysporum* is a ubiquitous inhabitant of soils worldwide and causes diseases such as wilt, yellows and damping-off in different plant species. Rapid and reliable detection of the pathogen is essential for undertaking appropriate and timely disease management measures. The time-consuming and laborious classical detection methods are now being increasingly replaced by culture-independent molecular detection techniques, which are much faster, more specific and sensitive. The molecular techniques like microarrays, whole genome sequencing, DNA barcoding, metagenomics etc. can identify a large number of isolates in a single assay. Some of the emerging tools will also allow complete analysis of developmental processes that are characteristics of the fungus, including the molecular nature of pathogenicity.

INTRODUCTION

Fusarium oxysporum is an economically important soilborne pathogen with worldwide distribution (Santos *et al.,* 2002). The fungus causes vascular wilt in about 80 botanical species by invading epidermal tissues of the root, extends to the vascular bundles, produces mycelia and/or spores in the vessels, and ultimately results in death of the plants (Namiki *et al.,* 1994). Individual pathogenic strain within the species has a limited host range, and strains with similar or identical host range are assigned to intraspecific groups, called form a specialis (Namiki *et al.,* 1994). *Fusarium*, the single most important genus of toxigenic fungi, has had a confusing and unstable taxonomic history. A number of factors, including a lack of clear morphological characters separating species, leading to species concepts that are too broad, together with variation and mutation in culture, have conspired to create taxonomic systems that poorly reflect species diversity. The result of this confusion is the rampant misapplication and inconsistent application of species names to toxigenic and pathogenic isolates. With the recent advent of multilocus phylogenetic methods which allow for the objective identification of species boundaries in the Fungi (Taylor *et al.,* 2000), *Fusarium oxysporum* Schlechtend. Fr. (F.o.) is an important asexual species complex and is well represented among the soil borne fungi in every type of soil all over the world (Burgess 1981). F.o. includes morphologically indistinguishable pathogenic, non-pathogenic and even beneficial strains. The pathogenic strains cause diseases such as vascular wilt, yellows, root rot and damping-off in a wide variety of economically important crops (Beckman and Roberts 1995), while the non-pathogenic strains are defined as the strains for which no host plants have been identified (yet) (Lievens *et al.* 2008). As a species, F.o. probably causes more economic damage to agricultural crops than any other pathogen. In spite of the broad host range of the species as a whole, individual strains usually infect only a single or a few plant species. These individual fungal strains usually show a high level of host specificity and, based on the plant species they can infect, they have been classified into more than 120 formae speciales (Armstrong and Armstrong 1981); for example, F.o. f. sp. cicericauses wilt only in chickpea. However, some formae speciales such as F.o. f.sp. radicis-cucumerinum and F.o. f.sp. radicis-lycopersici have broader host ranges, which, apart from infecting cucumber and tomato respectively, can cause root and stem rot on multiple hosts from different plant families (Lievens *et al.* 2008). Isolates from a particular forma specialis can be further subdivided into physiological races based on cultivar specificity. In addition, based on the ability to form heterokaryons, F.o. strains have been grouped into vegetative compatibility groups (VCGs; Puhalla 1985), and different formae speciales and races may contain multiple VCGs (Katan 1999; Katan and Di Primo 1999).

Thus, with regard to effective management of the pathogen, identification below the species level is essential. Identification of F.o. pathotypes is traditionally based on the combination of diagnostic symptoms on the host and the presence of the fungus in the affected tissues (Baayen *et al.* 2000). However, this classical approach is becoming increasingly challenging because more than one forma specialis may infect a particular host, along with non-pathogenic strains, which are common soil and rhizosphere inhabitants (Edel *et al.* 2000). Genetic differences among F.o. formae speciales have been evaluated through the analyses of pathogenicity, VCG, chromosomal features, rDNA, mtDNA and other molecular markers (Jacobson and Gordon 1990; Puhalla 1985; Katan and Katan 1999; Appel and Gordon 1995; O'Donnell *et al.* 1998; Alves-Santos *et al.* 1999). However, molecular discrimination of F.o. is complicated by the observation that different isolates classified into a single forma specialis may have independent evolutionary (polyphyletic) origins (O'Donnell *et al.* 1998; Baayen *et al.* 2000; Skovgaard *et al.* 2001; Cramer *et al.* 2003), and that isolates that belong to different formae speciales may share a common ancestor (monophyletic origin; Kistler 1997).

Technological advances in molecular detection methods allow fast and accurate detection and quantification of plant pathogens and these are now being applied to practical problems. The information resulting from such experiments could be used to monitor the level of exposure of the crop to pathogen inoculum and to improve disease control by allowing more rational decisions to be made about the choice and the use of fungicides and resistant cultivars.

With all these approaches, implementation of appropriate disease management measures requires timely detection and reliable identification of the pathogen and its races.

Early and reliable detection is crucial for the containment of the disease and implementation of disease control strategies when they are likely to be most effective. In recent years, the increasing use of molecular methods in fungal diagnostics has emerged as a possible answer to the problems associated with existing phenotypic identification systems.

REVIEW OF LITERATURE

Classically, plant pathogenic fungi were characterized by a series of morphological criteria including cultural characteristics on growth media and diagnostic symptoms on the host along with the presence of the fungus in the affected tissues (Baayen *et al.* 2000). However, accurate identification of fungi by visual examination of such morphological criteria is very difficult and erroneous. Moreover, these methods have other major limitations such as, reliance on the ability of the fungus to be cultured, time-consuming and laborious nature of identification process and the requirement for extensive taxonomical knowledge, which complicate timely disease management decisions. Therefore, attempts are being made to replace these methods with molecular identification techniques. As a results, in the last two decades, molecular tools have had a major impact on the identification of plant pathogens. Molecular techniques can avoid many of the drawbacks associated with classical methods of pathogen identification and can also improve our understanding of pathogen detection in different conditions.

Table 1 – Isolates of *Fusarium oxysporum* f. sp. *passiflorae* and *Fusarium oxysporum* f. sp. *cubense*, used on the molecular analysis with Amplified Fragment Length Polymorphism (AFLP) markers.

Number	Isolate	f. sp.	Geographic origin
1	FOC143	*cubense*	Janaúba (MG)
2	FOP001	*passiflorae*	Cruz das Almas (BA)
3	FOP002	*passiflorae*	Cruz das Almas (BA)
4	FOP003	*passiflorae*	Cruz das Almas (BA)
5	FOP004	*passiflorae*	Cruz das Almas (BA)
6	FOP005	*passiflorae*	Cruz das Almas (BA)
7	FOP008	*passiflorae*	Ubaíra (BA)
8	FOP013	*passiflorae*	Ubaíra (BA)
9	FOP022	*passiflorae*	Ubaíra (BA)
10	FOP023	*passiflorae*	Ubaíra (BA)
11	FOP028	*passiflorae*	Ubaíra (BA)
12	FOP057	*passiflorae*	Ubaíra (BA)
13	FOP069	*passiflorae*	Linhares (ES)
14	FOP071	*passiflorae*	Porto Seguro (BA)
15	FOP072	*passiflorae*	Livramento de Nossa Senhora (BA)

In general, these techniques are more specific, sensitive and accurate than traditional methods, and do not demand specialized taxonomical expertise. Today, a wide range of molecular techniques are being applied to accurately identify F.o. isolates (Table 1), of which those based on detection of pathogen DNA or RNA are the most predominant.

A. Identification using anonymous markers

Anonymous marker techniques like restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), etc. have been successfully used for identification of F.o. isolates by several workers.

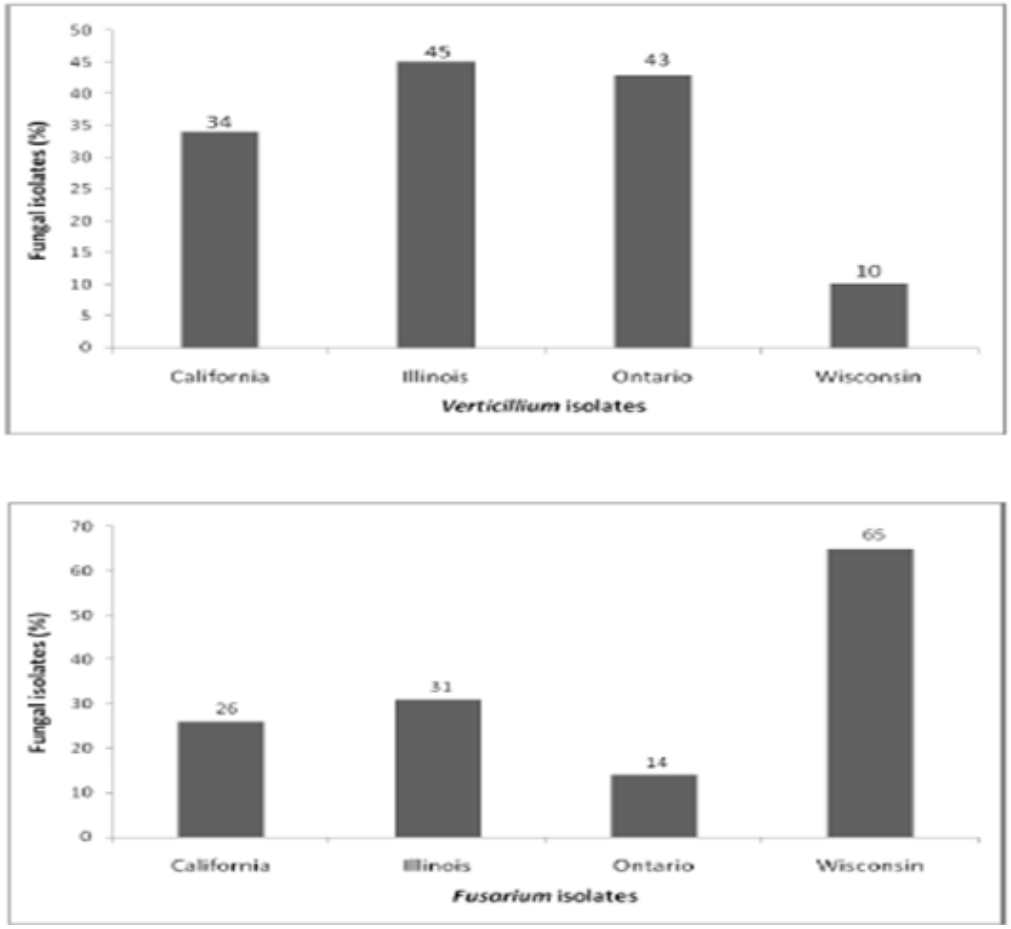


Fig. 1.

Restriction Fragment Length Polymorphism (RFLP). RFLPs have been extensively used to characterize F.o. isolates and VCGs (Flood *et al.* 1992; Manicom and Baayen 1993; Fernandez *et al.* 1994; Mes *et al.* 1994; Appel and Gordon 1995; Baayen *et al.* 1997; Kistler 1997). Baayen *et al.* (1998) screened isolates of F.o. from lily (F.o. f.sp. lilii) for pathogenicity, vegetative compatibility and RFLP patterns, and compared these to reference isolates of the formae speciales gladioli and tulipae. They found that the isolates from Europe and United States shared unique RFLP patterns and belonged to the same VCG. While, RFLP analysis of Fusarium isolates from carnation by Manicom *et al.* (1990) and Manicom and Baayen (1993) showed two major VCGs, each characterized by a distinct RFLP pattern. Similarly, Fernandez *et al.* (1994) used RFLP analysis to identify four ribosomal DNA (rDNA) and seven mitochondrial DNA (mtDNA) haplotypes in F.o. f.sp. *vasinfectum*, the causal organism of cotton wilt. Attitalla *et al.* (2004) evaluated isozyme analysis, mtDNA-RFLP and High performance liquid chromatography (HPLC) to differentiate two morphologically indistinguishable formae speciales of F.o., *lycopersici* and radicis-lycopersici. Although HPLC produced distinct profiles for non-pathogenic and pathogenic isolates, the direct mtDNA-RFLP technique proved to be an efficient diagnostic tool for routine differentiation of lycopersici and radicis-lycopersici isolates.

However, although RFLP has been successfully used in many studies to identify Fusarium isolates, due to its labor-intensive nature, elaborate procedure and the need of high amount of DNA (Garcia-Mas *et al.* 2000), it is being replaced by polymerase chain reaction (PCR) based techniques.

PCR allows rapid detection and identification of pathogens and overcomes most of the limitations of classical approaches. It has revolutionized the detection of pathogens and PCR-based methods are now widely used for identification of a variety of pathogens because of its rapid, sensitive and specific nature. Many PCR-based approaches have been reported for identification of F.o. isolates and to study the genetic relationships among them. These fungi have been differentiated using either mycotoxigenic genes, ribosomal DNA, other genes or unique DNA bands from RAPD analysis (reviewed by Edwards *et al.* 2002).

Random Amplified Polymorphic DNA (RAPD). RAPD is a quick and cost-effective method to detect pathogens and study the genetic similarity or diversity among pathogen populations. The technique has been extensively used to analyze genetic diversity among different F.o. formae speciales and races (Grajal-Martin *et al.* 1993; Bentley *et al.* 1994; Kelly *et al.* 1994; Manulis *et al.* 1994; Wright *et al.* 1996). Paavanen-Huhtala *et al.* (1999) analyzed 27 F.o. isolates by RAPD and isozyme patterns; however, all the isolates could only be distinguished from each other by RAPD analysis. Mes *et al.* (1999) screened two races of F.o. f.sp. lycopersicifor vegetative compatibility and characterized them using RAPD analysis, and found that the RAPD profiles coincided with the vegetative compatibility groups.

The RAPD technique has been used to differentiate a collection of isolates into races corresponding to pathogenicity tests in cotton (Assigbetse et al. 1994) and basil (Chiocchetti *et al.* 1999; Chiocchetti 2001), while Jimenez-Gasco *et al.* (2001) identified specific RAPD amplification profiles for Foc races 0, 1B/C, 5, and 6. Using RAPD-generated DNA probes, Wang *et al.* (2001) developed a sensitive and specific method for identifying F.o. f. sp. cucumerinum and F.o. f.sp. luffaeisolates. After RAPD analysis of 13 formae speciales of F.o., specific DNA bands were selected as probes and the forma specialis-specific probes were developed for identification of F.o. f.sp. cucumerinum and F.o. f.sp. luffae isolates by dot blot hybridization.

Lievens *et al.* (2007) developed a robust RAPD marker-based assay to specifically detect and identify the cucumber pathogens F.o. f.sp. cucumerinum and F.o. f.sp. radicis-cucumerinum. Based on the phylogeny of translation elongation factor-1a (TEF-1a), they found that F.o. f.sp. cucumerinum strains were genetically more diverse, while the F.o. f.sp. radicis-cucumerinum strains clustered in a separate clade.

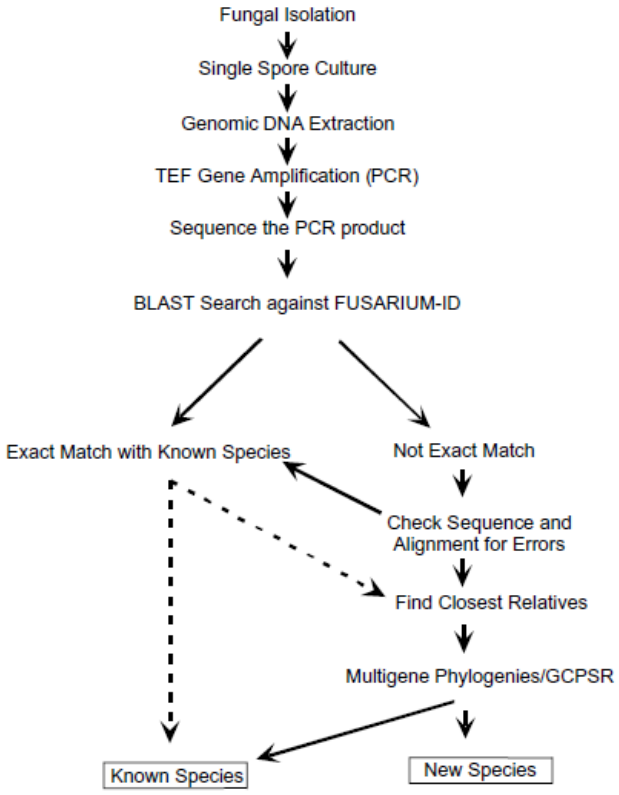


Fig. 2. Flowchart describing the process of using TEF DNA sequence to identify *Fusarium* species.

The developed markers were implemented in an DNA array to enable parallel and sensitive detection and identification of the pathogens in complex samples from diverse origins. However, although the RAPD technique has been successfully used in many studies for detection and identification of F.o. isolates as well as to evaluate the genetic diversity within and among pathogen populations, it suffers from well-known limitations of poor reproducibility and inter-laboratory transferability.

Amplified Fragment Length Polymorphism (AFLP). Amplified Fragment Length Polymorphism (AFLP) markers are extremely powerful because of their high multiplex power, enabling the generation of a large number of bands per gel (amplification). Therefore, it has high potential for the development of specific primers in the identification of isolates. Given these needs and the molecular tools available, this study was conducted to assess the possibility of developing specific fingerprinting within the formae speciales of FOP to be able to trace the presence of these isolates in the main passion fruit-producing regions and to find the different ways of genetic structuring of FOP isolates, one of the principal infectious agents of passion fruit.AFLP has been used in many studies for the analysis of fungal population structure (Majer *et al.* 1996; Gonzalez *et al.* 1998; DeScenzo *et al.* 1999; Purwantara *et al.* 2000; Zeller *et al.* 2000). Genetic variation among pathogenic isolates of F.o. was estimated using AFLP markers by several workers (Baayen *et al.* 2000; Bao *et al.* 2002; Sivaramakrishan *et al.* 2002; Groenewald *et al.* 2006; Stewart *et al.* 2006). Later, the utility, reproducibility and efficiency of AFLP technique led to its broader application in the analysis of population diversity and identification of pathogens (Baayen *et al.* 2000; Abd-Elsalam *et al.* 2002a 2002b; Kiprop *et al.* 2002; Sivaramakrishan *et al.* 2002; Abdel-Satar *et al.* 2003; Leslie *et al.* 2005, Gurjar *et al.* 2009).

Table 2 – Characterization of the AFLP primers by the total number of fragments (NB), number of polymorphic fragments (NBP), polymorphic information content (PIC), marker index (MI) and resolving power of the marker (Rp).

Primer combination	NB	NBP	PIC	MI	Rp
E+CC/M+ATA	29	28	0.19	5.32	7.28
E+CC/M+ATT	28	24	0.17	4.08	5.30
E+CC/M+ATC	22	20	0.18	3.60	4.62
E+CG/M+AAA	55	50	0.24	12.00	16.58
E+CG/M+AAT	54	46	0.18	8.28	10.28
E+CG/M+AAC	40	39	0.21	8.19	10.74
E+CT/M+AAC	39	38	0.18	6.84	8.44
E+CT/M+AAG	43	43	0.25	10.75	15.52
E+CA/M+ATC	50	47	0.15	7.05	8.56
E+CG/M+ATG	26	25	0.17	4.25	5.66
E+CG/M+ATT	42	37	0.16	5.92	9.86
E+CC/M+AAC	38	38	0.21	7.98	11.66
E+CG/M+AAG	39	38	0.18	6.84	8.74
E+CT/M+ATA	30	28	0.28	7.84	12.46
E+CT/M+ATT	45	44	0.21	9.24	11.68
E+CT/M+ATC	32	30	0.16	4.80	6.36
E+CC/M+AAG	53	49	0.18	8.82	12.18
E+CT/M+AAA	61	58	0.21	12.18	15.84
E+CT/M+AAT	49	48	0.17	8.16	9.90
E+CA/M+AAA	51	47	0.23	10.81	14.76
E+CA/M+AAT	54	53	0.24	12.72	17.90
E+CA/M+ATA	34	30	0.20	6.20	8.54
E+AA/M+CTG	36	33	0.15	4.95	5.88
E+AT/M+CTG	43	42	0.21	8.82	11.42
E+AC/M+CTG	57	54	0.23	12.42	16.48
Minimum	22	20	0.15	3.6	4.62
Maximum	61	58	0.28	12.72	17.90
Average	42	40	0.20	7.92	10.66

The technique was used to examine genetic relationships among isolates of F.o. f.sp. vasinfectumby Abd-Elsalam *et al.* (2004) and Wang et al. (2006). While, Gurjar *et al.* (2009) identified two Foc races (1 and 2) based on unique AFLP patterns. Sequence characterization of these race-specific AFLP products revealed significant homologies with metabolically important fungal genes. However, as AFLP is relatively costly and has a rather complicated technical procedure, it is being increasingly replaced by simpler PCR-based methods.

Simple Sequence Repeats (SSR). Microsatellites or simple sequence repeats (SSRs) are composed of tandemly repeated 1-6 bp long units (Tautz, 1989). Microsatellites markers are having a reputation of highly polymorphic, locus specific, easily transferable, and cost-effective molecular markers distributed throughout the genome (Powell *et al.,* 1996).Simple sequence repeats, also known as microsatellites, provide a powerful tool for taxonomic and population genetics studies. They have also been used in fungal studies because of the high resolution that they provide (Bogale *et al.* 2005, 2006; Bayraktar *et al.* 2008). van der Nest *et al.* (2000) used inter-simple sequence repeat (ISSR) and SSR primers (random amplified microsatellites, RAMS) in PCR to develop SSR markers for F.o. In our laboratory at NCL, Barve *et al.* (2001) assessed the genetic variability in Foc populations prevalent in India using 13 oligonucleotide probes and 11 restriction enzymes. Using the distribution of microsatellite repeats, it was found that the races 1 and 4 were closely related as compared to race 2, while race 3 of the pathogen was very distinct. However, as these anonymous marker techniques have several disadvantages, diagnostic DNA fragments identified with these approaches have often been converted into more simple and reliable molecular markers like sequence characterized amplified region (SCAR) or sequence tagged sites (STS). This approach has proven to be effective for the identification of several formae speciales and races of F.o. For example, Kelly *et al.* (1998) developed an in planta PCR method to detect isolates of race 5 of Foc in chickpea. The assay using RAPD-derived SCAR markers specifically identified race 5 of the pathogen from infected chickpea plants.

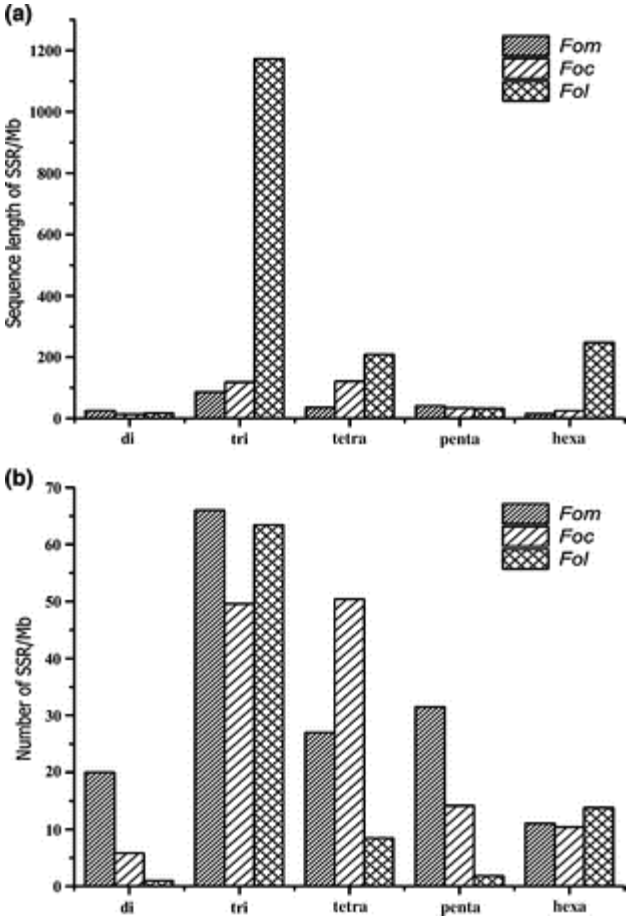


Fig. 3. Graphical representation of relative density (a) and relative abundance (b) of different SSR type found in ESTs and transcripts of *Fusarium oxysporum*.

Similarly, Jimenez-Gasco and Jimenez-Diaz (2003) sequenced previously identified Foc specific RAPD markers and designed SCAR markers to identify Foc and its four pathogenic races 0, 1A, 5, and 6. The assays were sensitive enough to detect as low as 100 pg of fungal genomic DNA. Based on RAPD analysis, Shimazu *et al.* (2005) developed three sets of STS markers for specific identification of three races of F.o. f.sp. lactucae. These markers were specific to F.o. f.sp. lactucae and did not amplify DNA from isolates of five other F.o. formae speciales as well as other plant pathogenic fungi, bacteria, or plant materials examined in the study.

B. Identification using sequence-specific markers

Although the above-mentioned techniques have been successful in accurately identifying the pathogens in many cases, the markers can be localized anywhere in the genome and often little sequence data are available in public databases for comparison with other sequences. Therefore, extensive screening using a large collection of strains is necessary to validate the robustness of these markers. Lievens *et al.* (2008) have listed specific PCR primers for the detection and identification of several formae speciales and races of F.o. Such markers that are based on specific DNA sequences in the pathogen genomes could be used for pathogen identification as well as for their phylogenetic studies.

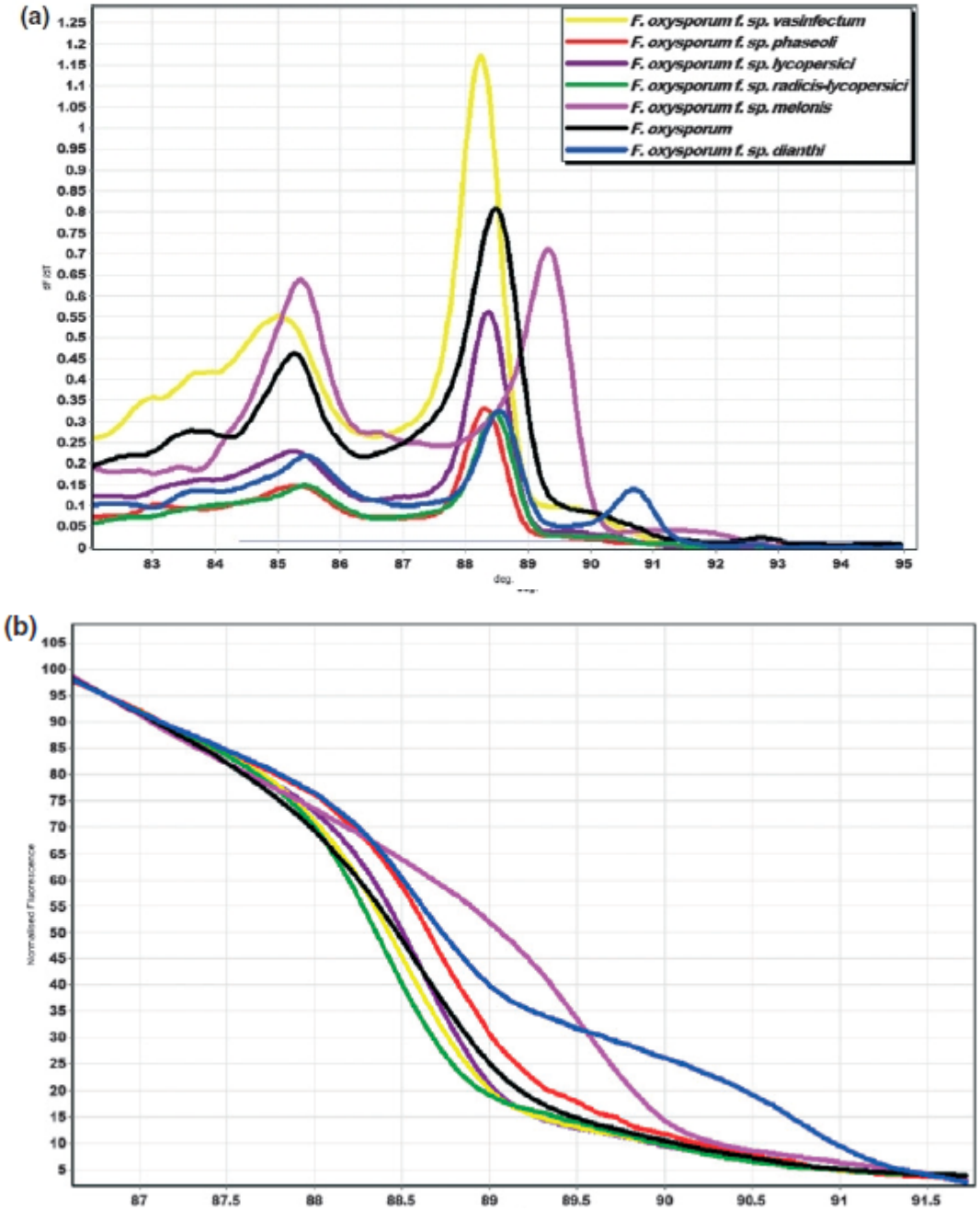
ITS and IGS. The ITS region of rRNA genes is a useful marker for discriminating species because it contains stretches of high sequence conservation, while at the same time, the size of the sequence varies in different Fusarium formae speciales (Suga *et al.,* 2000; Visentin *et al.,* 2010). It has been successfully used before for the identification of *Aspergillus* (Henry *et al.,* 2000) and *Fusarium* (Gurjar *et al.,* 2009) species. The internal transcribed spacer (ITS) and intergenic spacer (IGS) regions of the ribosomal RNA genes possess characteristics that allow pathogen identification (Ward 1994; Appel and Gordon 1995; Waalwijk *et al.* 1996; Edel *et al.* 2000; Bao *et al.* 2002; Singh *et al.* 2006). Bateman *et al.* (1996) used PCR-RFLP of a PCR product consisting of ITS1, 5.8S and ITS2 ribosomal DNAs and eight restriction enzymes to distinguish 18 Fusarium haplotypes, while Edel *et al.* (1997) analyzed further into the 5' end of the 28S rDNA gene to distinguish five Fusarium haplotypes. However, neither of these methods could not distinguish among *F. crookwellense, F. culmorum* and *F. graminearum*, indicating that they might be more closely related. Indeed, Schilling *et al.* (1996) later found that the DNA sequence of ITS1 region from *F. culmorum* and *F. graminearum* was identical.

Additionally, species-specific primers could not be designed due to the minor differences in the ITS2 region of the two Fusarium species. Mishra *et al.* (2003) developed a fluorescent marker based PCR assay for rapid and reliable identification of five toxigenic and pathogenic Fusarium species viz. *Fusarium oxysporum, F. avenaceum, F. culmorum, F. equiseti* and *F. sambucinum*. The method was based on PCR amplification of species-specific DNA fragments using fluorescent oligonucleotide primers designed from ITS region of rDNA.

Similarly, Abd-Elsalam (2003) developed taxon-selective primers based on ITS sequences for quick identification of the *Fusarium* genus, while Abd-Elsalam *et al.* (2006) identified F.o. f.sp. vasinfectum (Fov) using specific primers based on the 16S and 23S rRNA genes. Based on differences in ITS sequences of *Fusarium* and *Mycosphaerella* spp., Zhang *et al.* (2005) developed species-specific PCR assays for rapid and accurate detection of F.o. f.sp. *niveum* and *Mycosphaerella melonis* from isolates of F.o. f.sp. vasinfectum. PCR-RFLP based on the rDNA-IGS region distinguished these isolates from other formae speciales of F.o. Further, they identified single-nucleotide polymorphisms (SNPs) in the 5' portion of the IGS region and developed two specific real-time PCR assays based on these SNPs for absolute quantification of genomic DNA from the isolates obtained from infected cotton tissues as well as soil samples. Similarly, three *Fusarium* species from Dendrobium were characterized by Latiffah *et al.* (2009) using PCR-RFLP of ITS in 5.8S rRNA region. They found that isolates from the same species produced similar PCR-RFLP patterns and UPGMA cluster analysis of the data clearly grouped *Fusarium oxysporum, F.* proliferatum and *F. solani* into separate clusters. Transposons. Mouyna *et al.* (1996) analyzed the South American populations of F.o. f.sp. elaeidis (an oil palm pathogen) and found that they had the palm transposon. They also showed that the palm transposon was present in all the pathogenic isolates, but was absent in all the non-pathogenic isolates, indicating that the pathogenic populations may be marked by the transposon. Similarly, Fernandez *et al.* (1998) designed specific primers for detection of F.o. f.sp. albedinis (the date palm pathogen), based on the sequences of transposable element Fot1. They analyzed a large number of *Fusarium* isolates, including 286 F.o. f.sp. albedinis isolates, 17 other formae speciales, non-pathogenic F.o. isolates and eight other *Fusarium* species and the specific primer amplified a 400-bp fragment only in F.o. f.sp. albedinis.

A diagnostic PCR assay to detect pathogenic F.o. races causing wilt in carnation was developed by Chiocchetti *et al.* (1999). This strategy was based on the genetic characterization of strains using different transposons and cloning and sequencing the regions flanking the insertion sites of these elements, followed by construction of race-specific primers for fast pathogen identification. Using a similar approach, Pasquali *et al.* (2007) developed inter-retrotransposon sequence characterized amplified regions (IR-SCAR) technique to differentiate F.o. f.sp. lactucaerace 1 isolates from other F.o. and F.o. f.sp. lactucaeisolates.

Multiplex PCR.The PCR product is heated at increasing temperatures and the double-stranded PCR product starts 'melting', releasing the intercalated dye.



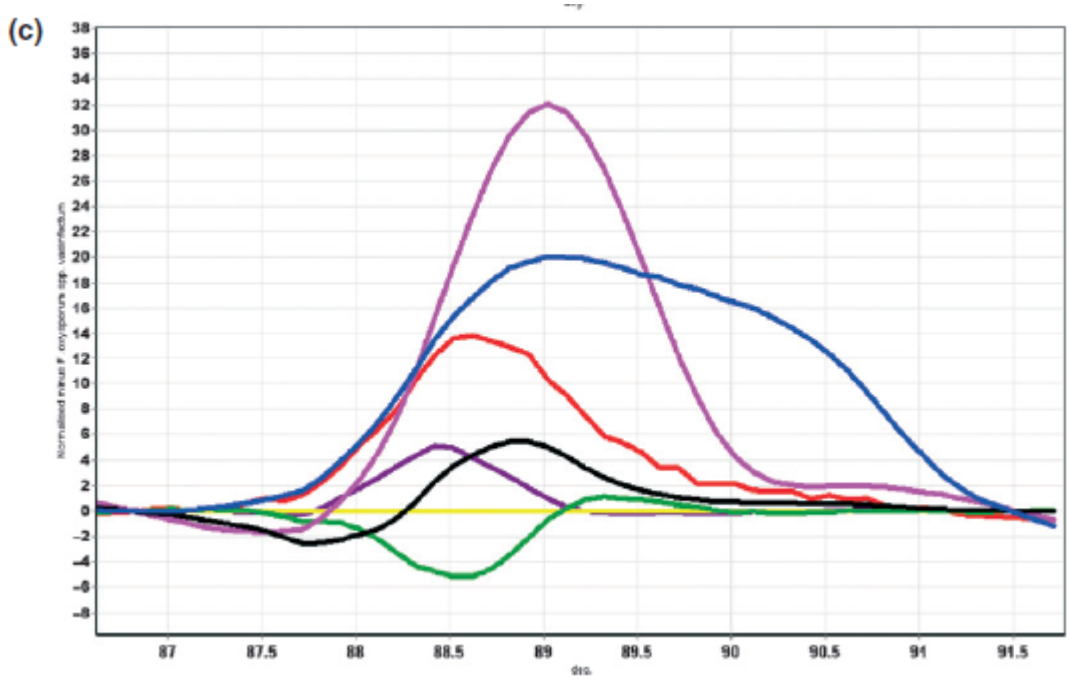


Fig. 4. (a) Conventional melt curves of ITS marker. (b) Genotyping of *Fusarium oxysporum* formae speciales complex using HRM analysis with the universal ITS nuclear marker. (b) Difference graph of seven formae speciales using *F. oxysporum* f. sp. vasinfectum as reference genotype. Assigned genotypes using a cutoff confidence value of 90%. The HRM of all other formae speciales was compared to this control and was resulted as *F. oxysporum* f. sp. vasinfectum at > 90% confidence or as variation if < 90% confidence. Color code table with the formaespeciales used.

The rate of dissociation and the complete melting of the PCR product depend on the thermodynamic properties of the product, like the sequence length, the GC content, the complementarity and nearest neighbor of the particular DNA product, which in turn causes a specific change in fluorescence and the observed melting curve during HRM DNA dissociation (Reed and Wittwer, 2004)Multiplex PCR allows simultaneous and sensitive detection of different DNA or RNA targets in a single reaction. It can, therefore, be designed to determine the presence of more than one pathogen in plant material by selectively amplifying specific sequences in two or more of them, or to detect related pathogens on multiple hosts (Louws *et al.* 1999). Simultaneous identification of several plant pathogens by multiplex PCR has been reported by Hamelin *et al.* (1996) and de Haan *et al.* (2000). Demeke *et al.* (2005) developed a species-specific PCR assay for the identification of nine *Fusarium* species viz. avenaceum, acuminatum, crookwellense, culmorum, equiseti, graminearum, poae, pseudograminearum and sporotrichioides in pure mycelial culture. Later, they could also simultaneously and accurately identify *F. culmorum, F. graminearum* and *F. sporotrichioides* using multiplex PCR. If such specific primers are developed for common F.o. formae speciales or physiological races, it would greatly simplify their multiplexed detection and identification for timely disease control (Elnifro *et al.* 2000).

C. Limitations of PCR-based Techniques

Although PCR-based techniques are rapid, highly sensitive and specific, they might suffer from robustness (van der Wolf *et al.* 2001). The failure of PCR amplification to correctly diagnose infected and non-infected plant material has been reported in different comparative assays. Carry-over contamination of amplicons can be responsible for false-positive results and inhibitor components in sample extracts are the main reason for false negatives. Similarly, PCR based techniques (except reverse transcriptase-PCR) can amplify the target DNA sequences from both active and non-active or dead pathogen cells/spores (Malorny *et al.* 2003). Therefore, it may yield false positive results in some cases. Another important limitation of PCR-based identification assays is that the technique is not immediately quantitative. Although it is comparatively easy to quantify the amount of a PCR product produced as a result of a successful PCR amplification, it is difficult to tell the amount of target DNA initially present at the start of the reaction.

This is because the reaction rate is exponential; as a result, slight variations in the amplification procedure can generate different amounts of final product from the same amount of starting material. Although, target DNA can be quantified using competitive PCR (Nicholson *et al.* 1998), this method is labor intensive. However, many of these limitations can be overcome by using modern techniques like real-time PCR and microarrays, which are increasingly being used for routine pathogen identification.

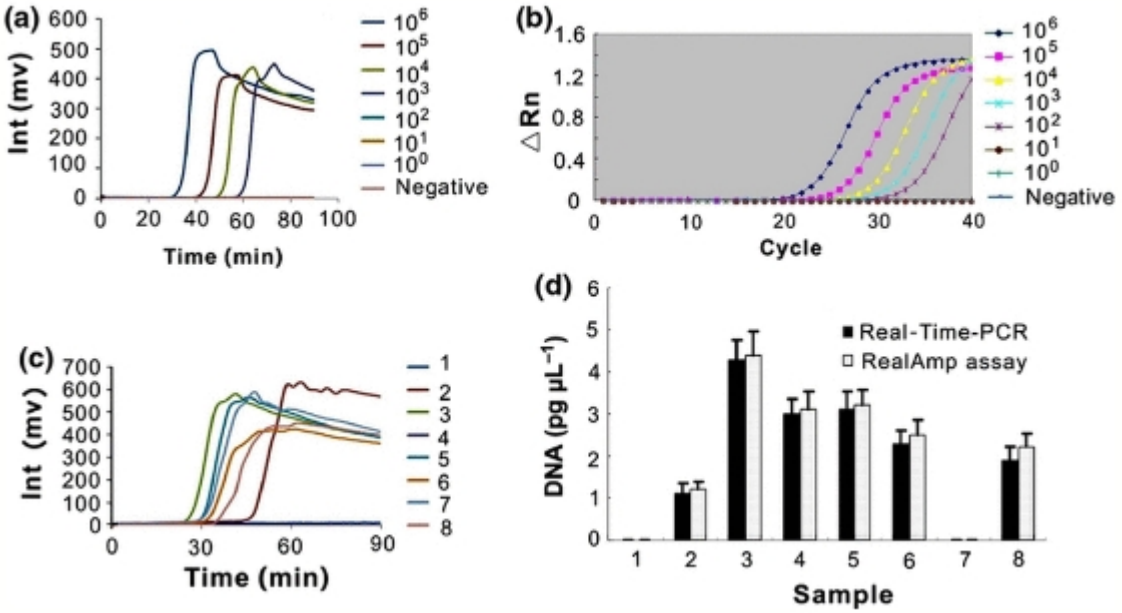


Fig. 5. Quantitative detection of the RealAmp assay in artificially inoculated and partial field soil samples in comparison with real-time PCR. All the samples were tested in triplicate. (a and b) Lanes 1-7 correspond to serial 10-fold dilutions of spores ranging from 10^6 to 10^0 spores g^{-1} artificially inoculated soil. Lane 8 is the negative control. (c) Quantitative detection of partial field soil samples by the Real Amp assay. (d) Statistical comparison of the quantitative results of partial field soil samples between the both assays. (c and d) Lanes 1-2 are the samples collected from the area where watermelon was previously planted. Lanes 3-8 are the samples collected from the watermelon-growing area with *Fusarium* wilt appearance.

RECENT TECHNIQUES FOR IDENTIFICATION OF F.O.

Currently, the detection of plant pathogens is a changing, dynamic and evolving world where established protocols can be modified or optimized only months after having been developed. Accurate and routine pathogen detection requires high levels of specificity, sensitivity, reliability and speed. In this context, specificity can be defined as the capability to detect the pathogen in the absence of false positives and negatives, while sensitivity relates to the lowest number of pathogens reliably detected per assay or sample (Lopez *et al.* 2003). In addition, pathogen quantification is also becoming important, since it serves as a basis for establishing damage thresholds at which a pathogen causes disease, and action thresholds that determine when measures should be taken to limit or prevent losses (Lievens *et al.* 2008). As F.o. is known to survive and remain latent in soil for many years, detection methods of high sensitivity, specificity and reliability are required.

The battery of available techniques and probes for detection of plant pathogens has increased considerably over the last few years. In addition to time benefits, there are great advantages in terms of specificity, sensitivity and reliability with these techniques, as well as, they allow identification of the pathogen camouflaged by other microorganisms. Some of such modern techniques currently used in identification of plant pathogens are discussed below.

A. Real-Time PCR

The RealAmp reaction was conducted as described previously (Peng *et al.*, 2012) with minor modifications and optimization. Recently, the Eiken Chemical Company Ltd (Tokyo, Japan) developed a loop-mediated isothermal amplification (LAMP) method, which is also available for quantification of DNA (Notomi *et al.*, 2000).The real-time PCR technology provides escalating opportunities to identify phytopathogenic fungi and has been used in several studies for detection and identification of various formae speciales of F.o. (Table 2).

It can more accurately quantify the extent of pathogen biomass in the host tissue and, with multiplex formats, enables simultaneous detection of different pathogens (Lievens *et al.* 2003). The main advantage of real-time PCR assay over end-point quantitative PCR is that the amplification products can be monitored as they are accumulated in the exponential phase (Schena *et al.* 2004), thus allowing precise measurement of fungal DNA content in the reaction.

Pasquali *et al.* (2004) developed a real-time PCR assay based on TaqMan chemistry to identify a new group of F.o. f.sp. chrysanthemiisolates highly pathogenic on Paris daisy. They successfully identified infected plants using real-time PCR as early as the fifth day after artificial inoculation, although the plants remained symptomless until the 13th day after inoculation. Zhang *et al.* (2005) used real-time PCR to identify and quantify F.o. f.sp. niveum and *Mycosphaerella melonis* pathogens directly from soil samples. Similarly, Abd-Elsalam *et al.* (2006) used real-time PCR based on the 16S and 23S rRNA genes to detect F.o. f.sp. vasinfectum (Fov) in cotton. The assay detected as low as 200 fg of Fov genomic DNA in infected cotton roots, while no amplification was obtained from other plant structures such as stem and leaf. Lievens *et al.* (2007).developed a robust RAPD marker-based assay to specifically detect and identify the economically important cucumber pathogens F.o. f.sp. *cucumerinum* and F.o. f.sp. radicis-cucumerinum. They used the real-time PCR assay to confirm that the selected RAPD markers for F.o. f.sp. cucumerinum and F.o. f.sp. radicis-cucumerinum represented single copy DNA sequences. Likewise, Zambounis *et al.* (2007) developed two specific real-time PCR-based assays based on the SNPs found in the 5' portion of the rDNA-IGS regions for quantification of genomic DNA of Australian isolates of F.o. f.sp. vasinfectum from infected cotton tissues as well as soil samples.

However, like all other molecular methods based on DNA amplification, a major drawback of the system is that it is unable to distinguish between viable and dead propagules. Similarly, multiplexing in real-time PCR is limited by the number of different fluorescent dyes available. In addition, the initial and running costs of a real-time PCR system are several times more than a normal PCR system. However, considering the many benefits of the real-time PCR technology compared to normal PCR, the use of real-time PCR is still advantageous. Higgins *et al.* (2003) developed a portable real-time PCR instrument for performing diagnostic assays directly in the field. Such rapid real-time PCR diagnosis could result in taking appropriate and timely control measures than possible with traditional methods of pathogen identification.

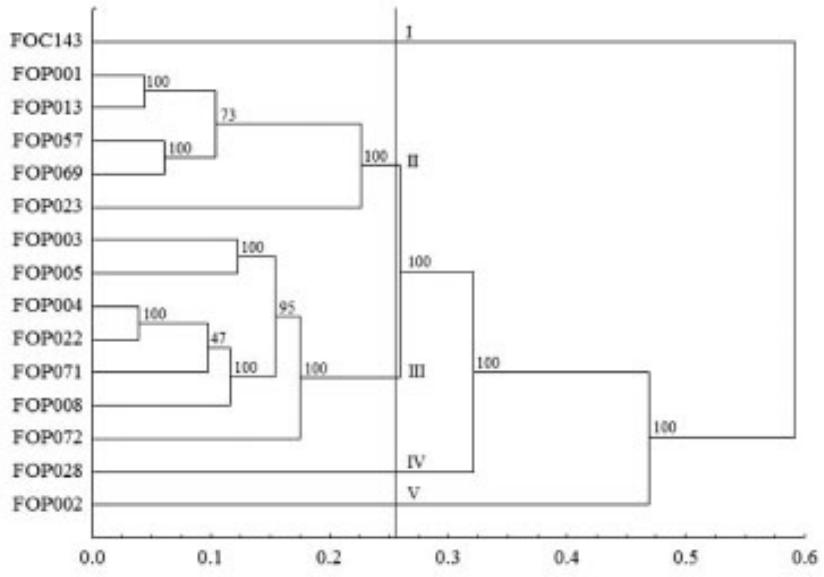


Fig. 6. Using the cutting point defined by the fusion point criteria (Mingotti, 2005), five groups were formed . Isolate FOC143 remained separate from the others in Group I, showing broad molecular differences in relation to those of FOP isolates. Group II consisted of isolates from Cruz das Almas (FOP001), Ubaira (FOP013, FOP023 and FOP057) and Linhares (ES) (19°23'27" S, 40°4'17" W) (FOP069).

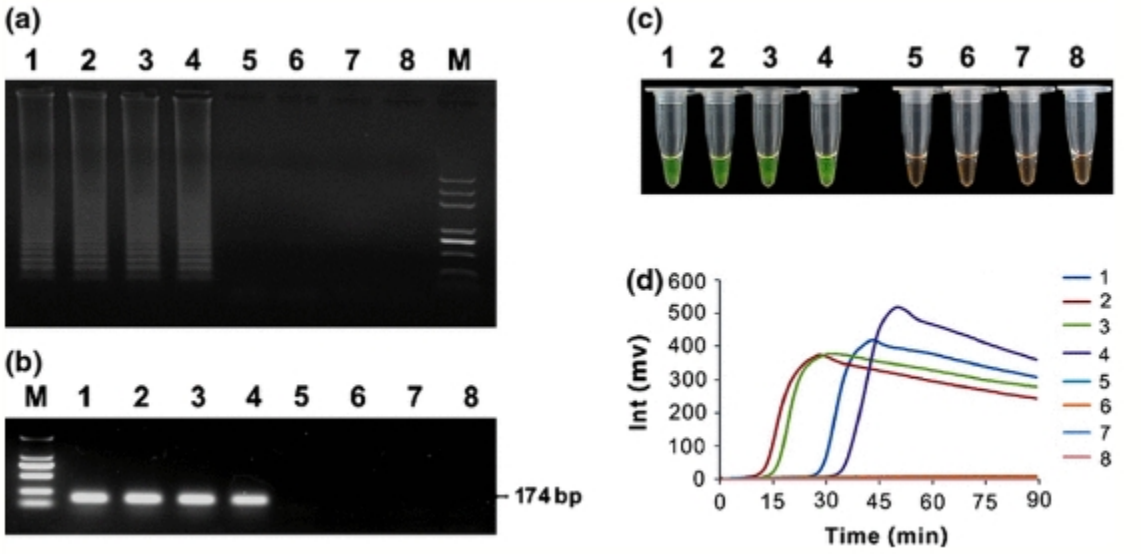


Fig. 7. Specificity test of the real-time fluorescence loop-mediated isothermal amplification assay (RealAmp assay) for the detection of Fon in comparison with conventional PCR. Lanes 1-4, genomic DNAs of *Fusarium oxysporum* f. sp. niveum (Fon) race 0, race 1, race 2, artificially inoculated soil samples, respectively; Lanes 5-8, the DNA of *Mycosphaerella melonis*, *F. oxysporum* f. sp. cucumerium, *F. oxysporum* f. sp. luffae, and *F. oxysporum* f. sp. cubense race 4, respectively; Lane M, Trans2K plus II DNA marker. (a) Agarose gel electrophoresis analysis of Real Amp assay amplicon. (b) Conventional PCR using the specific Fon-1/Fon-2 primer set. (c) Visual detection of the Real Amp amplification products. The original orange colour of SYBR green turned green in the positive reaction mixture. (d) The fluorescence units vs. time graph were plotted automatically by the ESE-Quant Tube Scanner.

B. Microarrays

The application of microarrays in the detection of pathogens in various environments has enabled parallel detection of multiple species in a high throughput format conducive to automation (Small *et al.* 2001; Loy *et al.* 2002). For pathogenic fungi, microarray analysis has great potential to systematically and efficiently identify genes required for infection (Lorenz 2002; Bryant *et al.* 2004). A Magnaporthe grisea array is now commercially available from Agilent Technologies.

A molecular detection system based on DNA array technology was developed by Lievens *et al.* (2003) for rapid and efficient detection of tomato vascular wilt pathogens F.o. f.sp. lycopersici, *Verticillium albo-atrum* and *V. dahliae*. The array was successfully used for sensitive detection of the tomato wilt pathogens from complex substrates like soil, plant tissues and irrigation water as well as samples collected by tomato growers. Similarly, microarray analysis of F.o. f.sp. vasinfectumgenes expressed in planta (McFadden *et al.* 2006) has revealed pathogenic genes in the cotton pathogen. The expression of this gene was also positively correlated with vascular browning, which is a characteristic symptom of Fusarium wilt infection (McFadden *et al.* 2006). Guldener *et al.* (2006) reported the design and validation of the first Affymetrix Gene Chip microarray based on the entire genome of *Fusarium graminearum.*

C. Gene/Genome Sequencing

One of the most robust and informative techniques useful in fungal diagnosis is nucleotide sequencing, where DNA sequence variations can be used to design species-specific primers and/or probes. Sequences of the TEF-1a and the mitochondrial small subunit (mtSSU) ribosomal RNA genes have been valuable in distinguishing species (Baayen *et al.* 2000; O'Donnell *et al.* 2000; Baayen *et al.* 2001; Skovgaard *et al.* 2001). Phylogenetic analysis of TEF-1a data by Gurjar *et al.* (2009) from four Foc races revealed that the race 3 of the pathogen was actually *Fusarium proliferatum* and not *Fusarium oxysporum* as has been considered till now. Similarly, DNA sequences of UTP-ammonia ligase, trichothecene 3-O-acetyltransferase, and a putative reductase (O'Donnell *et al.* 2000) and nitrate reductase, phosphate permease (Skovgaard *et al.* 2001) have also been used successfully to distinguish Fusarium species.

EMERGING TECHNOLOGIES FOR PATHOGEN IDENTIFICATION

A. Next-Generation Sequencing

Among these sequencing platforms, the 454 GS FLX instrument currently has the ability to sequence 400-600 million bp per run (with 400-500 bp individual reads) using the Titanium series reagents (http://www.454.com/). Due to its high accuracy, low cost and long reads compared to the Solexa and Solid systems, many researchers have migrated toward the 454 sequencing platform for a variety of genome projects. As these instruments have the potential of sequencing several microbial genomes in a single run, it is very likely that the genomes of economically important plant pathogens, including various *Fusarium* species, will be shortly available. Indeed, genome sequencing projects of several *Fusarium* species are already in progress. Based on the analysis of these genomes, specific oligonucleotide sequences could be used to design microarray chips, detection probes or PCR primers for high-throughput or multiplexed detection and identification of different F.o. strains.

B. Single-nucleotide polymorphisms

Detection and characterization of single-nucleotide polymorphisms (SNPs) is also one of the promising post-genomics research tools for pathogen identification. This new technology is pushing pathogen identification to its ultimate limit-the single base pair difference.

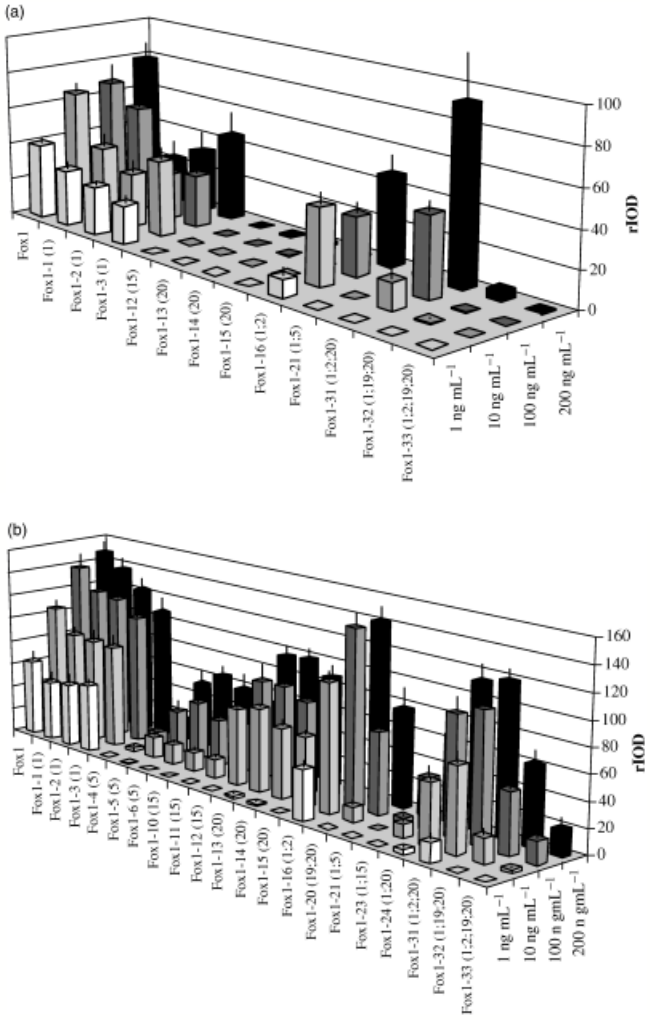


Fig. 8. Discriminatory potential of Fox1-derived mismatch detector oligonucleotides upon hybridization with different amounts of internal transcribed spacer (ITS) II (a) and ITS I-5.8S rRNA gene-ITS II (b) amplicons from *Fusarium oxysporum*. Results are only shown for those oligonucleotides that resulted in detectable hybridization signals. Mismatch positions are indicated following the code of the oligonucleotide. Hybridization signal strength is reported relative to the average integrated optical density of the digoxigenin-labeled reference control (rIOD). Data represent means from three hybridization runs (n=6). Error bars indicate standard errors. Before hybridization, amplicons from different PCR reactions were pooled to minimize variability because of differences in DNA amplification.

It is presumed that many plant pathogenic races or formae speciales differ from their closest relatives by only a few bases in different genes. The next-generation sequencing platforms can rapidly carryout deep sequencing of microbial genomes, enabling quick discovery of SNPs in different pathogenic strains of the microbial species. This will enable designing forma specialis or race-specific cleaved amplified polymorphic sequence (CAPS) or derived cleaved amplified polymorphic sequence (dCAPS) markers for PCR-based identification. CAPS markers result from differential restriction digestion of gene/allele specific PCR products based on the loss or gain of restriction enzyme recognition sites due to the presence of SNPs or insertion/deletion mutations. While, in dCAPS analysis, a restriction enzyme recognition site that includes the SNP is introduced into the PCR product by a primer containing one or more mismatches to template DNA (Neff *et al.* 1998).

C. Metagenomics

It is the study of genomic content of microbial organisms directly in their natural environments, bypassing the need for isolation and culturing of individual species (Chen and Pachter 2005). Hence, metagenomics enables studies of organisms that are not culturable as well as studies of organisms in their natural environment. Using the metagenomics approach, these new sequencing technologies enable researchers to quickly and affordably identify the organisms present in a complex sample (such as soil, irrigation water or plant tissues) without any prior knowledge. Such metagenomics approach to pathogen identification should facilitate quick identification and quantification of a range of pathogens present in the sample and enable undertaking appropriate disease control strategies well before the pathogen populations rich damage thresholds. The 454 GS FLX System is very suitable for metagenomics as the system's long reads help in accurate identification of pathogenic strains present in the sample. Researchers often use the platform for counting gene tags to analyze the relative abundance of different microbial species in different samples.

POTENTIAL LIMITATIONS OF MOLECULAR IDENTIFICATION TECHNIQUES

PCR could amplify DNA from both active and non-active or dead pathogen cells/spores. Therefore, it may yield false positive results in some cases. Similarly, false negatives can be attributed in standard PCR protocols due to the presence of compounds that inhibit the polymerases, degradation of the DNA target sequence, or reagent problems (Louws *et al.* 1999). Likewise, although microarray is the most suitable technique for multiplexed detection of many isolates of F.o. and other pathogens in a single assay, currently microarrays are expensive for routine application. Moreover, additional work is needed to address the challenges of working with environmental samples where contaminants may interfere with DNA hybridization and affect the performance of microarrays. Similarly, the lack of adequate sequence information can hamper the development of reliable molecular diagnostic assays. Moreover, techniques like DNA barcoding are presently unable to differentiate pathogenic strains from non-pathogenic ones that belong to the same microbial species. Hence, if no molecular markers are available to distinguish the pathogenic subspecies, pathogenicity test is the only way to determine whether or not a given isolate is pathogenic on a specific crop or variety.

Although technically feasible and potentially invaluable, large sequencing studies still face significant challenges. Foremost among the challenges is analyzing the tremendous amounts of data generated (Nelson 2003). It is relatively easy to characterize genes and genomes of a well-studied and easily cultivated microbe, however, it would be a the daunting task to understand the genomics of unknown or uncultured microbes or whole environmental genomes revealed by metagenomics approaches. For example, Tringe *et al.* (2005) could assemble as many as 150,000 sequence reads into contiguous sequences spanning only 1% of a soil metagenome.

CONCLUSIONS

As these new molecular technologies gain wide acceptance, routine detection, identification and monitoring of plant pathogens should become more common in plant pathology. Microarray chips are now being fabricated with oligonucleotides that are either synthesized directly on a solid surface or are microspotted. Similarly, the next generation sequencing technologies like 454 and SOLiD can sequence several microbial genomes in a single run. If the complete DNA sequence of plant pathogens is known, oligonucleotides specific for a pathogen can be designed and a single high-density microarray chip could accommodate oligonucleotides specific for a large number of pathogens. In the next few years, complete genome sequences of many pathogenic strains of F.o. are likely to become available and these will help to design PCR primers or probes very specific to the pathogen strains enabling accurate identification of the strains even if camouflaged by other pathogens. For example, if each microarray chip contained oligonucleotides specific for each of the known formae speciales and races of F.o., it would be possible to have multiplex detection of all these pathogens in one experiment even from complex substrates like soil, plant tissues and irrigation water.

With such high-throughput technologies, integration of more strains into the detection systems of F.o. should become possible and identification of pathogens is likely to become an easier task. However, these should be observed as management tools, to be used in combination with the knowledge of the crop and understanding of the biology of the different formae speciales and the ecology of the diseases. In this respect, the increasing availability of full-genome sequences of many plant pathogens including formae speciales of F.o. is a welcome development. With the availability of affordable and portable real-time PCR instruments (Higgins *et al.* 2003) and simpler protocols, molecular-based diagnosis of crop diseases is becoming a field reality. Routine diagnosis of many crop diseases is now possible in one day or less by the recent innovative technologies. This, coupled with high throughput that reduces the cost per sample, should make these assays more attractive for use in crop protection. A combination of DNA microarrays with other genomic methods will certainly accelerate the efforts to characterize the function of unknown stretches of the fungal genomes. The resulting database will allow complete analysis of developmental processes that are characteristics of the fungus, including the molecular nature of pathogenicity.

REFERENCES

Abd-Elsalam KA (2003). Non-gel based techniques for plant pathogen genotyping. *Acta Microbiol Polon.* **52**: 329-341.

Abd-Elsalam KA, Asran-Amal A, Schnieder F, Migheli Q Verreet JA (2006). Molecular detection of *Fusarium oxysporum* f.sp. vasinfectumin cotton roots by PCR and real-time PCR assay. *J Plant Dis Protect.,* **113**: 14-19.

Abd-Elsalam KA, Khalil MS, Aly AA Asran-Amal A (2002a). Genetic diversity among *Fusarium oxysporum* f.sp. vasinfectumisolates revealed by UP-PCR and AFLP markers. *Phytopathologia Medit.,* **41**: 1-7.

Abd-Elsalam KA, Khalil MS, Aly AA, Asran-Amal A (2002b). Population analysis of *Fusarium* spp. *Phytomedizin* **3**: 18-19.

Abd-Elsalam KA, Omar MR, Migheli Q, Nirenberg HI (2004). Genetic characterization of *Fusarium oxysporum* f.sp. vasinfectumisolates by random amplification of polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP). *J Plant Dis Protect.,* **111**: 534-544.

Abdel-Satar MA, Khalil MS, Mohmed IN, Abd-Elsalam KA, Verreet JA (2003) Molecular phylogeny of Fusarium species by AFLP fingerprint. *Afr J Biotechnol.,* **2**: 51-55.

Abo K, Klein KK, Edel-Hermann V, Gautheron N, Traore D, Steinberg C (2005) High genetic diversity among strains of *Fusarium oxysporum* f.sp. vasinfectumfrom cotton in Ivory Coast. *Phytopathology,* **95**: 1391-1396.

Alves-Santos FM, Benito EP, Eslava AP, Diaz-Minguez JM (1999). Genetic diversity of *Fusarium oxysporum* strains from common bean fields in Spain. *Appl Environ Microbiol.,* **65**: 3335-3340.

Alves-Santos FM, Ramos B García-Sánchez MA, Eslava AP, Díaz-Mínguez JM (2002). A DNA-based procedure for in planta detection of *Fusarium oxysporum* f.sp. *phaseoli*. *Phytopathology,* **92**: 237-244.

Aoki T and O'Donnell K (1999). Morphological and molecular characterization of *Fusarium pseudograminearum* sp nov., formerly recognized as the Group 1 population of *F. graminearum*. *Mycologia,* **91**: 597-609.

Aoki T and O'Donnell KL (1998). Fusarium kyushuense sp. nov. from Japan. *Mycoscience*, **39**: 1-6.

Aoki T, O'Donnell K, Homma Y and Lattanzi AR (2003). Sudden-death syndrome of soybean is caused by two morphologically and phylogenetically distinct species within the *Fusarium solani* species complex - F. virguliforme in North America and *F. tucumaniae* in South America. *Mycologia,* **95**: 660-684.

Appel DJ, Gordon TR (1995). Intraspecific variation within populations of *Fusarium oxysporum* based on RFLP analysis of the intergenic spacer region of the rDNA. *Exp Mycol.,* **19**: 120-128.

Armstrong GM, Armstrong JK (1981). Formae specialis and races of *Fusarium oxysporum* causing wilt diseases. In: Fusarium: Diseuses, Biology and Taxonomy, Pennsylvania State University Press, University Park (US) pp 391-399.

Assigbetse KB, Fernandez D, Dubois MP, Geiger JP (1994). Differentiation of *Fusarium oxysporum* f.sp. vasinfectumraces on cotton by random amplified polymorphic DNA (RAPD) analysis. *Phytopathology,* **84**: 622-626.

Attitalla IH, Fatehi J, Levenfors J, Brishammar S (2004). A rapid molecular method for differentiating two special forms (lycopersici and radicis-lycopersici) of Fusarium oxysporum. *Mycol Res.,* **108**: 787-794.

Baayen RP, Forch MG, Waalwijk C, Bonants PJM, Loffler HJM, Roebroeck EJA (1998). Pathogenic, genetic, and molecular characterization of *Fusarium oxysporum* f.sp. lilii. *Eur J Plant Pathol.,* **104**: 887-894.

Baayen RP, O'Donnell K, Bonants PJM, Cigelnik E, Kroon LPNM, Roebroeck EJA, Waalwijk, C (2000). Gene genealogies and AFLP analyses within the *Fusarium oxysporum* complex identify monopbyletic and non-monophyletic formae specialis causing wilt and rot disease. *Phytopathology,* **90**: 891-900.

Baayen RP, O'Donnell K, Breeuwsma S, Geiser DM, Waalwijk C. (2001). Molecular relationships of fungi within the Fusarium redolens-F hostae clade. *Phytopathology,* **91**: 1037-1044.

Baayen RP, van Dreven F, Krijger MC, Waalwijk C (1997). Genetic diversity in *Fusarium oxysporum* f.sp. dianthiand Fusarium redolens f.sp. dianthi. *Eur J Plant Pathol.,* **103**: 395-408.

Bao JR, Fravel DR, O'Neill NR, Lazarovits G, van Berkum P (2002). Genetic analysis of pathogenic and non-pathogenic *Fusarium oxysporum* from tomato plant. *Can J Bot.*, **80**: 271-279.

Barve MP, Haware MP, Sainani MN, Ranjekar PK, Gupta VS (2001). Potential of microsatellites to distinguish four races of *Fusarium oxysporum* f.sp.ciceriprevalent in India. *Theor Appl Genet* **102**: 138-147.

Bateman GL, Kwasna H, Ward E (1996). Relationship among *Fusarium* spp estimated by comparing restriction fragment length polymorphisms in polymerase chain reaction-amplified nuclear rDNA. *Can J Microbiol.*, **42**: 1232-1240.

Bayraktar H, Dolar FS, Maden S (2008). Use of RAPD and ISSR markers in detection of genetic variation and population structure among *Fusarium oxysporum* f.sp. cicerisisolates on chickpea in Turkey. *J Phytopathol* **156**: 146-154.

Beckman CH, Roberts EM, (1995). On the nature and genetic basis for resistance and tolerance to wilt diseases of plants. *Adv Bot Res.,* **21**: 35- 77.

Bentley S, Pegg KG, Dale JL (1994). Optimization of RAPD-fingerprinting 1 to analyze genetic variation within populations of *Fusarium oxysporum* f.sp. cubense. *J Phytopathol*., **142**: 64-78.

Bentley S, Pegg KG, Dale JL (1995). Genetic variation among a world-wide collection of isolates of *Fusarium oxysporum* f.sp.cubenseanalyzed by RAPD-PCR fingerprinting. *Mycol Res.*, **11**: 1378-1384.

Bodker L, Lewis BG, Coddington, A (1993). The occurrence of a new genetic variant of *Fusarium oxysporum* f.sp. pisi. *Plant Pathol.*, **42**: 833-838.

Boehm EWA, Ploetz RC, Kistler HC (1994). Statistical analysis of electrophoretic karyotype variation among vegetative compatibility groups of *Fusarium oxysporum* f.sp.cubense. *Mol Plant-Microbe Interact* **7**: 196-207.

Bogale M, Wingfield BD, Wingfield MJ, Steenkamp ET (2005) Simple sequence repeat markers for species in the *Fusarium oxysporum* complex. *Mol Ecol Notes.*, **5**: 622-624.

Bogale M, Wingfield BD, Wingfield MJ, Steenkamp ET (2006) Characterization of *Fusarium oxysporum* isolates from Ethiopia using AFLP, SSR and DNA sequence analyses. *Fungal Div.*, **23**: 51-66 .

Bogale M, Wingfield BD, Wingfield MJ, Steenkamp ET (2007) Species-specific primers for *Fusarium redolens* and a PCR-RFLP technique to distinguish among three clades of *Fusarium oxysporum*. *FEMS Microbiol Lett* **271**: 27-32.

Bosland PW, Williams PH (1987). An evaluation of *Fusarium oxysporum* from crucifers based on pathogenicity, isozyme polymorphism, vegetative compatibility, and geographic origin. *Can J Bot.,* **65**: 2067-2073.

Bryant P, Venter A, Robins-Browne D, Curtis R (2004). Chips with everything: DNA microarrays in infectious diseases. *Lancet Infect Dis.*, **4**: 100-111.

Burgess LW (1981). General ecology of the Fusaria In: Nelson, PE, Toussoun, TA, Cook, RJ, Eds, Fusarium: Diseases, Biology and Taxonomy. Pennsylvania State University Press, University Park, Pennsylvania, USA, pp 225-235.

Chen K, Pachter L (2005). Bioinformatics for whole-genome shotgun sequencing of microbial communities. *PLoS Comp Biol*., **1**: 24-23.

Chiocchetti A (2001). PCR Detection of *Fusarium oxysporum* f sp basilici on Basil. *Plant Disease.* **85**: 607-611.

Chiocchetti A, Ghignone S, Minuto A, Gullino M L, Garibaldi A, Migheli Q (1999). Identification of *Fusarium oxysporum* f.sp. basilica isolated from soil, basil seed, and plants by RAPD analysis. *Plant Dis.,* **83**: 576-581.

Cho SW, Mitchell A, Regier JC, Mitter C, Poole RW, Friedlander TP and Zhao SW (1995). A highly conserved nuclear gene for low-level phylogenetics - Elongation Factor- 1-Alpha recovers morphology-based tree for Heliothine moths. *Molecular Biology and Evolution*, **12**: 650-656.

Cramer RA, Byrne PF, Brick MA, Panella L, Wickliffe E, Schwartz HF (2003). Characterization of *Fusarium oxysporum* isolates from common bean and sugar beet using pathogenicity assays and random amplified polymorphic DNA markers. *J. Phytopathol.,* **151**: 352-360.

de Haan LAM, Numansen A, Roebroeck EJA, van Doorn J (2000). PCR detection of *Fusarium oxysporum* f.sp. gladiolirace 1, causal agent of Gladiolus yellows disease, from infected corms. *Plant Pathol.,* **49**: 89-100.

Demeke T, Clear RM, Patrick SK, Gaba D, (2005). Species-specific PCR based assays for the detection of Fusarium species and a comparison with the whole seed agar plate method and trichothecene analysis. *Intl J Food Microbiol* **103**: 271-284.

DeScenzo RA, Engel SR, Gomez G, Jackson EL, Munkvold GP, Weller J, Irelan NA (1999). Genetic analysis of Eutypa strains from California supports the presence of two pathogenic species. *Phytopathology*, **89**: 884-893.

Edel V, Steinberg C, Gautheron N, Alabouvette C (1997). Evaluation of restriction analysis of polymerase chain reaction (PCR)- amplified ribosomal DNA for the identification of *Fusarium* species. *Mycol Res*., **101**: 179-187.

Edel V, Steinberg C, Gautheron N, Alabouvette C (2000). Ribosomal DNA-targeted oligonucleotide probe and PCR assay specific for *Fusarium oxysporum*. *Mycol Res.*, **104**: 518-526.

Edwards SG, O'callaghan J, Dobson ADW (2002). PCR-based detection and quantification of mycotoxigenic fungi. Mycol Res 106: 1005-1025.

Elias KS, Schneider RW (1991). Vegetative compatibility groups in *Fusarium oxysporum* f.sp. lycopersici. *Phytopathology*, **81**: 159-162.

Elias KS, Schneider RW (1992). Genetic diversity within and among races and vegetative compatibility groups of *Fusarium oxysporum* f.sp. *lycopersicias* determined by isozyme analysis. *Phytopathology*, **82**: 1421-1427.

Elias KS, Zamir D, Lichtman-Pleban T, Katan, T (1993). Population: structure of *Fusarium oxysporum* f.sp. lycopersici: restriction fragment length polymorphisms provide genetic evidence that vegetative compatibility group is an indicator of evolutionary origin. *Mol Plant-Microbe Interact.*, **6**: 565-572.

Elnifro EM, Ashshi AM, Cooper RJ, Klapper PE (2000). Multiplex PCR: optimization and application in diagnostic virology. *Clin Microbiol Rev.*, **13**: 559-570.

Fessehaie A, De Boer SH and Levesque CA (2003). An oligonucleotide array for the identification and differentiation of bacteria pathogenic on potato. *Phytopathology*, **93**: 262-269. 478.

Fernandez D, Assigbetse KB, Dubois MP, Geiger, JP (1994). Molecular characterization of races and vegetative compatibility groups in *Fusarium oxysporum* f.sp.vasinfectum. *Appl Environ Microbiol.*, **60**: 4039-4046.

Fernandez D, Ouinten M, Tantaoui A, Geiger JP, Daboussi MJ, Langin T (1998). Fot1 insertions in the *Fusarium oxysporum* f sp albedinis genome provide diagnostic PCR targets for detection of the date palm pathogen. *Appl Environ Microbiol.*, **64**: 633-636.

Fernandez D, Ouinten M, Tantaoui A, Lourd M, Geiger JP (1995) Population genetic structure of *Fusarium oxysporum* f.sp. albedinis (Abstr). *Fungal Genet Newsl*, **42A**: 34.

Fernandez D, Tantaoui A (1994). Random amplified polymorphic DNA (RAPD) analysis: a tool for rapid characterization of *Fusarium oxysporum* f.sp. albedinisisolates. *Phytopathol Mediterr*, **33**: 223-229.

Flood J, Whitehead DS, Cooper RM (1992). Vegetative compatibility and DNA polymorphisms in *Fusarium oxysporum* f.sp. elaeidisand their relationship to isolate virulence and origin. *Physiol Mol Plant Pathol.*, **41**: 201-215.

Garcia-Mas J, Oliver M, G´omez-Paniagua H, de Vicente, MC (2000). Comparing AFLP, RAPD and AFLP markers for measuring genetic diversity in melon. Theor Appl Genet 101: 860-864.

Gerlach KS, Bentley S, Moore NY, Pegg KG, Aitken, AB (2000). Characterisation of Australian isolates of *Fusarium oxysporum* f.sp. cubenseby DNA fingerprinting analysis. *Aus J Agri Res.*, **51**: 945-953.

Gonzalez M, Rodriguez MEZ, Jacabo JL, Hernandez F, Acosta J, Martinez O, Simpson J (1998). Characterization of Mexican isolates of *Colletotrichum lindemuthianum* by using differential cultivars and molecular markers. *Phytopathology*, **88**: 292-299.

Grajal-Martin MJ, Simon CJ, Muehlbauer FJ (1993). Use of random amplified polymorphic DNA (RAPD) to characterize race 2 of *Fusarium oxysporum* f.sp. pisi. *Phytopathology*, **83**: 612-614.

Groenewald S, Berg NVD, Marasas WFO, Viljoen A (2006). The application of high-throughput AFLP's in assessing genetic diversity in *Fusarium oxysporum* f.sp. cubense. *Mycol Res.*, **110**: 297-305.

Guldener U, Seong KU, Boddu J, Cho S, Trail F, Xu JR, Adam G, Mewes HW, Muehlbauer FJ, Kistler HC (2006) Development of a *Fusarium graminearum* Affymetrix GeneChip for profiling fungal gene expression in vitro and in planta. *Fungal Gen Biol.*, **43**: 316-325.

Gurjar G, Barve M, Giri A, Gupta V (2009) Identification of Indian pathogenic races of *Fusarium oxysporum* f.sp. ciceriswith gene specific, ITS and random markers. *Mycologia*, **101**: 480-491.

Hajibabaei M, Janzen DH, Burns JM, Hallwachs W, Hebert PDN (2006) DNA barcodes distinguish species of tropical Lepidoptera. *Proc Natl Acad Sci USA*, **103**: 968-971.

Hamelin RC, Berube P, Gignac M, Bourassa M (1996) Identification of root rot fungi in nursery seedlings by nested multiplex PCR. *Appl Environ Microbiol.*, **62**: 4026-4031.

Hebert PDN, Cywinska A, Ball SL, de Waard JR (2003). Biological identifications through DNA barcodes. *Proc R Soc Lond B Biol Sci.*, **270**: 313-322.

Higgins JA, Nasarabadi S, Karns JS, Shelton DR, Cooper M, Gbakima A, Koopman RP (2003) A handheld real time thermal cycler for bacterial pathogen detection. *Biosens Bioelectr.*, **18**: 1115-1123.

Hirano, Y Arie, T (2006). PCR-based differentiation of *Fusarium oxysporum* f.sp. lycopersiciand radicis-lycopersici and races of *F. oxysporum* f.sp. lycopersici. *J Genet Plant Pathol.*, **72**: 273-283.

Hirota N, Hashiba T, Yoshida H, Kikumoto T, Ehara Y (1992). Detection and properties of plasmid-like DNA in isolates from twentythree formae specialis of Fusarium oxysporum. *Ann Phytopathol Soc Jpn* **58**: 386-392.

Honnareddy N, Dubey SC (2006). Pathogenic and molecular characterization of Indian isolates of *Fusarium oxysporum* f.sp. ciceriscausing chickpea wilt. *Curr Sci.*, **91**: 661-666.

Jacobson DJ, Gordon TR (1990). Variability of mitochondrial DNA as an indicator of relationships between populations of *Fusarium oxysporum* f sp melonis. *Mycol Res.*, **94**: 734-744 .

Jimenez-Gasco MD, Perz-Artes E, Jimenez-Diaz RM (2001). Identification of pathogenic races 0, 1B/C, 5, and 6 of *Fusarium oxysporum* f.sp. ciceris with random amplified polymorphic DNA (RAPD). *Eur J Plant Pathol* **107**: 237- 248.

Jimenez-Gasco MM, Jimenez-Diaz RM (2003). Development of a specific polymerase chain reaction-based assay for the identification of *Fusarium oxysporum* f.sp.cicerisand its pathogenic races 0, IA, 5, and 6. *Phytopathology*, **93**: 200-209.

Katan T (1999). Current status of vegetative compatibility groups in *Fusarium oxysporum*. *Phytoparasitica*, **27**: 51-64.

Katan T, Di Primo P (1999). Current status of vegetative compatibility groups in *Fusarium oxysporum*. *Phytoparasitica*, **27**: 273-277.

Katan T, Zamir D, Sarfatti M, Katan J (1991). Vegetative compatibility groups and subgroups in *Fusarium oxysporum* f.sp. radicis-lycopersici. *Phytopathology*, **81**: 255-262.

Kelly A, Alcala-Jimenez AR, Bainbridge BW, Heale JB, Perez-Artes E, Jimenez-Diaz RM (1994). Use of genetic fingerprinting and random amplified polymorphic DNA to characterize pathotypes of *Fusarium oxysporum* f.sp.cicerisinfecting chickpea. *Phytopathology*, **84**: 1293-1298.

Kelly AG, Bainbridge BW, Heale JB, Perez-Artes E, Jimenez-Diaz RM (1998). In planta-polymerase-chain-reaction detection of the wilt-inducing pathotype of *Fusarium oxysporum* f.sp. cicerisin chickpea (*Cicer arietinum* L). *Physiol Mol Plant Pathol.*, **52**: 397-409.

Kim DH, Martyn RD, Magill CW (1993). Chromosomal polymorphism in *Fusarium oxysporum* f.sp. *niveum*. *Phytopathology*, **83**: 1209-1216.

Kiprop EK, Baudoin JP, Mwang'ombe AN, Kimani PM, Mergeai G, Maquet A (2002). Characterization of Kenyan isolates of *Fusarium udum* from pigeonpea [*Cajanus cajan* (L) Mill sp] by cultural characteristics, aggressiveness and AFLP analysis. *J Phytopathology*, **150**: 517-527.

Kistler HC (1997). Genetic diversity in the plant-pathogenic fungus *Fusarium oxysporum*. *Phytopathology*, **87**: 474-479.

Kistler HC, Benny U (1989). The mitochondrial genome of Fusarium oxysporum. *Plasmid*, **22**: 86-89.

Kistler HC, Bosland PW, Benny U, Leong S, Williams PH (1987). Relatedness of strains of *Fusarium oxysporum* from crucifers measured by examination of mitochondrial and ribosomal DNA. *Phytopathology*, **77**: 1289-1293.

Kistler HC, Momol EA, Benny U (1991) Repetitive genomic sequences for determining relatedness among strains of *Fusarium oxysporum*. *Phytopathology*, **81**: 331-336.

Koenig RL, Kistler HC, Ploetz RC (1993). Restriction fragment length polymorphism analysis of *Fusarium oxysporum* f.sp. cubense. *In: 6th Int Congr Plant Pathol National Research Council of Canada, Ottawa*, pp 158.

Latiffah Z, Nur-Hayati MZ, Baharuddin S, Maziah Z (2009). Identification and Pathogenicity of Fusarium species associated with root rot and stem rot of Dendrobium. *Asian J Plant Pathol.*, **3**: 14-21.

Leslie JF, Zeller KA, Lamprecht SC, Rheeder JP, Marasas WFO (2005). Toxicity, pathogenicity, and genetic differentiation of five species of Fusarium from sorghum and millet. *Phytopathology*, **95**: 275-283.

Lévesque CA (2001) Molecular methods for detection of plant pathogens-What is the future? *Can J Plant Pathol.*, **24**: 333-336.

Lievens B, Brouwer M, Vanachter ACRC, Lévesque CA, Cammue BPA, Thomma BPHJ (2003). Design and development of a DNA array for rapid detection and identification of multiple tomato vascular wilt pathogens. *FEMS Microbiol Lett.*, **223**: 113-122.

Lievens B, Claes L, Vakalounakis DJ, Vanachter ACRC, Thomma BPHJ (2007). A robust identification and detection assay to discriminate the cucumber pathogens *Fusarium oxysporum* f.sp. cucumerinumand f.sp. radicis-cucumerinum. *Environ Microbiol.*, **9**: 2145-2161 .

Lievens B, Rep M, Thomma BPHJ (2008). Recent developments in the molecular discrimination of formae speciales of *Fusarium oxysporum*. *Pest Manag Sci.*, **64**: 781-788

Lopez MM, Bertolini E, Olmos A, Caruso P, Gorris MT, Llop P, Penyalver R, Cambra M (2003). Innovative tools for detection of plant pathogenic viruses and bacteria. *Int Microbiol*., **6**: 233-243.

Lorenz MC (2002) Genomic approaches to fungal pathogenicity. *Curr Opin Microbiol*., **5**: 372-378.

Louws FJ, Rademaker JLW, Brujin FJ (1999). The three Ds of PCR-based genomic analysis of phytobacteria: diversity, detection, and disease diagnosis. *Ann Rev Phytopathol* **37**: 81-125.

Loy A, Lehner A, Lee N, Adamczyk J, Meier H, Ernst J, Schleifer K, Wagner M (2002). Oligonucleotide arrays for 16S rRNA genebased detection of all recognized lineages of sulfate-reducing Prokaryotes in the environment. *Appl Environ Microbiol.*, **68**: 5064-5081.

MacConaill L, Meyerson M (2008). Adding pathogens by genomic subtraction. *Nat Genet.*, **40**: 380-382

Majer D, Mithen R, Lewis B, Vos P, Oliver RP (1996). The use of AFLP fingerprinting for the detection of genetic variation in fungi. *Mycol Res.*, **100**: 1107-1111.

Malorny B, Tassios PT, Radstrom P, Cook N, Wagner M, Hoorfar J (2003). Standardization of diagnostic PCR for the detection of foodborne pathogens. *Intl J Food Microbiol.*, **83**: 39- 48.

Manicom BQ, Baayen RP (1993). Restriction fragment length polymorphisms in *Fusarium oxysporum* f.sp. dianthiand other fusaria from *Dianthus* species. *Plant Pathol.*, **42**: 851-857 .

Manicom BQ, Bar-Joseph M, Kotze JM, Becker MM (1990). A restriction fragment length polymorphism probe relating vegetative compatibility groups and pathogenicity in *Fusarium oxysporum* f.sp. dianthi. *Phytopathology*, **80:** 336-339

Manulis S, Kogan N, Reuven M, Ben-Yephet Y (1994). Use of the RAPD technique for identification of *Fusarium oxysporum* f.sp. dianthifrom carnation. Phytopathology 84: 98-101.

McFadden HG, Wilson IW, Chapple RM, Dowd C (2006). Fusarium wilt (*Fusarium oxysporum* f.sp. vasinfectum) genes expressed during infection of cotton (*Gossypium hirsutum*). *Mol Plant Pathol.*, **7:** 87-101.

Mes JJ, van Doom J, Roebroeck EJA, van Egmond E, van Aartrijk J, Boonekamp PM (1994). Restriction fragment length polymorphisms, races and vegetative compatibility groups within a worldwide collection of *Fusarium oxysporum* f.sp. gladioli. *Plant Pathol.*, **43**: 362-370.

Mes JJ, Weststeijn EA, Herlaar F, Lambalk JJM, Wijbrandi J, Haring MA, Cornelissen BJC (1999). Biological and molecular characterization of *Fusarium oxysporum* f.sp. lycopersicidivides race 1 isolates into separate virulence groups. *Phytopathology*, **89**: 156-160

Miao VPW (1990). Using karyotype variability to investigate the origins and relatedness of isolates of *Fusarium oxysporum* f.sp. cubense. In: Fusarium Wilt of Banana Ploetz, R C ed *The American Phytopathological Society*, St Paul, MN pp 55-62.

Min XJ, Hickey DA (2007). Assessing the effect of varying sequence length on DNA barcoding of fungi. *Mol Ecol Notes* **7**: 365-373.

Mishra PK, Fox RTV, Culham A (2003). Development of a PCR-based assay for rapid and reliable identification of pathogenic *Fusaria*. *FEMS Microbiol Lett.,* **218**: 329-332.

Mouyna I, Renard JL, Brygoo Y (1996). DNA polymorphism among *Fusarium oxysporum* f.sp. elaeidispopulations from oil palm, using a repeated and dispersed sequence "Palm". *Curr Genet* **30**: 174 - 180.

Mule 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 Microbiol Lett* **230**: 235-240.

Namiki F, Shiomi T, Kayamura T, Tsuge T (1994). Characterization of the formae specialis of *Fusarium oxysporum* causing wilts of cucurbits by DNA fingerprinting with nuclear repetitive DNA sequences. *Appl Environ Microbiol.,* **60**: 2684-2691.

Neff M, Neff J, Chory J, Pepper A (1998). dCAPS, a simple technique for the genetic analysis of single nucleotide polymorphisms: experimental applications in Arabidopsis thaliana genetics. *Plant J.*, **14**: 387-392.

Nelson KE (2003). The future of microbial genomics. Environ Microbiol 5:1223-1225

Nicholson P, Simpson DR, Weston G, Rezanoor HN, Lees AK, Parry DW, Joyce D (1998). Detection and quantification of Fusarium culmorum and Fusarium graminearum in cereals using PCR assays. *Physiol Mol Plant Pathol.*, **53**: 17-37.

O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC (1998). Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondria1 gene genealogies. *Proc Natl Acad Sci USA* **95**: 2044-2049.

O'Donnell K, Kistler HC, Tacke BK, Casper HH (2000). Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of Fusarium graminearum, the fungus causing wheat scab. *Proc Natl Acad Sci USA* **97**: 7905-7910 .

Paavanen-Huhtala S, Hyvonen J, Bulat SA, Yli-Mattila T (1999). RAPD-PCR, isozyme, rDNA RFLP and rDNA sequence analyses in identification of Finnish *Fusarium oxysporum* isolates. *Mycol Res.*, **103**: 625 - 634 .

Pasquali M, Dematheis F, Gullino ML, Garibaldi A (2007). Identification of race 1 of *Fusarium oxysporum* f.sp.lactucaeon lettuce by inter-retrotransposon sequence-characterized amplified region technique. *Phytopathology,* **97**: 987-996.

Pasquali M, Marena L, Fiora E, Piatti P, Gullino ML, Garibaldi A (2004). Real-time PCR for the identification of a highly pathogenic group of *Fusarium oxysporum* f.sp. *chrysanthemion Argyranthemum frutescens* L. *J Plant Pathol*., **86**: 51-57 .

Pasquali M, Piatti P, Gullino ML, Garibaldi A (2006). Development of a Real-time Polymerase Chain Reaction for the Detection of *Fusarium oxysporum* f.sp. *basilicifrom* basil seed and roots. *J Phytopathol*., **154**: 632-636.

Perez-Artes E, Roncero MIG, Diaz RMJ (1995). Restriction fragment length polymorphism analysis of the mitochondrial DNA of *Fusarium oxysporum* f.sp.ciceri. *J Phytopathol.,* **143**: 105-109 .

Ploetz RC (1990). Variability in *Fusarium oxysporum* f.sp.cubense. *Can J Bot.,* **68**: 1357-1363

Puhalla JE (1985). Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. *Can J Bot* **63:** 179-183.

Purwantara A, Barrins JM, Cozijnsen AJ, Ades PK, Howlet BJ (2000). Genetic diversity of the Leptosphaeria maculans species complex from Australia, Europe and North America using amplified fragment length polymorphism analysis. *Mycol Res.*, **104**: 772-781.

Santos FMA, Ramos B, Sanchez MAG, Eslava AP & Minguez JMD (2002). A DNA based procedure for in planta detection of *Fusarium oxysporum* f. sp. phaseoli. *Phytopathology,* **92**: 237-244.

Schena L, Franco N, Ippolito A, Gallitelli D (2004). Real-time quantitative PCR: a new technology to detect and study phytopathogenic and antagonistic fungi. *Eur J Plant Pathol.*, **110**: 893-908

Schilling AG, Möller EM, Geiger HH (1996). Polymerase chain reaction-based assays for species-specific detection of *Fusarium culmorum, F graminearum,* and *F avenaceum. Phytopathology*, **86**: 515-522.

Schloss PD, Handelsman J (2005). Metagenomics for studying unculturable microorganisms: Cutting the Gordian knot. *Genome Biol.*, **6**: 229.

Seifert KA, Samson RA, Dewaard JR, Houbraken J, Levesque CA, Moncalvo JM, Louis-Seize G, Hebert PD (2007). Prospects for fungus identification using CO1 DNA barcodes, with Penicillium as a test case. *Proc Natl Acad Sci USA* **104**: 3901-3906.

Shimazu J, Yamauchi N, Hibi T, Satou M, Horiuchi S, Shirakawa T (2005). Development of sequence tagged site markers to identify races of *Fusarium oxysporum* f.sp. lactucae. *J Gen Plant Pathol.,* **71**: 183-189.

Singh BP, Saikia R, Yadav M, Singh R, Chauhan VS, Arora DK (2006). Molecular characterization of *Fusarium oxysporum* f.sp. cicericausing wilt of chickpea. *Afr J Biotechnol.,* **5**: 497-502.

Sivaramakrishan S, Kannan S, Singh SD (2002). Genetic variability of *Fusarium* wilt pathogen isolates of chickpea (*Cicer arietinum* L) assessed by molecular markers. *Mycopathologia* **155**: 171-178.

Skovgaard K, Nirenberg HI, O'Donnell K, Rosendahl S (2001). Evolution of *Fusarium oxysporum* f.sp. vasinfectumraces inferred from multigene genealogies. *Phytopathology*, **91**: 1231-1237.

Small J, Call D, Brockmn F, Straub T, Chandler D (2001). Direct detection of 16S rRNA in soil extracts by using oligonucleotide microarrays. *Appl Environ Microbiol.*, **67**: 4708-4716

Stewart JE, Kim MS, James RL, Dumroese RK, Klopfenstein NB (2006). Molecular characterization of *Fusarium oxysporum* and Fusarium commune isolates from a conifer nursery. *Phytopathology*, **96**: 1124-1133.

Tringe SG, von Mering C, Kobayashi A, Salamov AA, Chen KN, Chang HW *et al.* (2005). Comparative metagenomics of microbial communities. *Science*, **22**: 554-557

van der Does HC, Lievens B, Claes L, Houterman PM, Cornelissen BJC, Rep M (2008). The presence of a virulence locus discriminates *Fusarium oxysporum* isolates causing tomato wilt from other isolates. *Environ Microbiol* **10**: 1475-1485.

van der Nest MA, Steenkamp ET, Wingfield BD, Wingfield MJ (2000). Development of simple sequence repeat (SSR) markers in Eucalyptus from amplified inter-simple sequence repeats (ISSR). *Plant Breed.*, **119**: 433-436.

van der Wolf JM, van Beckhoven JRCM, Bonanats PJM, Schoen CD (2001). New technologies for sensitive and specific routine detection of plant pathogenic bacteria. In: de Boer, SH Ed Plant pathogenic bacteria Kluwer, Dordrecht, pp 75-77.

Waalwijk C, De Koning JRA, Baayen RP, Gams W (1996). Discordant groupings of Fusarium spp from sections Elegans, Liseola and Dlaminia based on ribosomal ITS1 and ITS2 sequences. M*ycologia* **88**: 316-328.

Wang B, Brubaker CL, Tate W, Woods MJ, Matheson BA, Burdon JJ (2006). Genetic variation and population structure of Fusarium oxysporum f.sp. vasinfectumin Australia. *Plant Pathol.*, **55**: 746-755.

Wang PH, Lo1 HS, Yeh Y (2001). Identification of F o cucumerinum and F o luffae by RAPD-generated DNA probes. *Lett Appl Microbiol.*, **33**: 397-401.

Ward E (1994). Use of polymerase chain reaction for identifying plant pathogens.Blakemen, J P Williamson, B (Eds) CAB International Oxon, UK.

Whitehead DS, Coddington A, Lewis BG (1992). Classification of races by DNA polymorphism analysis and vegetative compatibility grouping in *Fusarium oxysporum* f.sp. pisi. *Physiol Mol Plant Pathol* **41**: 295-305.

Woudt LP, Neuvel A, Sikkema A, van Grinsven MQJM, de Milliano WAJ, Campbell CL, Leslie JF (1995) Genetic variation in *Fusarium oxysporum* from cyclamen. *Phytopathology*, **85**: 1348-1355.

Wright KGF, Guest DI, Wimalajeewa DLS, Van Heeswijck R (1996). Characterisation of *Fusarium oxysporum* isolated from carnation based on pathogenicity, vegetative compatibility and random amplified polymorphic DNA (RAPD) assay. *Eur J Plant Pathol.*, **102**: 451-457 .

Zambounis AG, Paplomatas E, Tsaftaris AS (2007) Intergenic spacer-RFLP analysis and direct quantification of Australian *Fusarium oxysporum* f sp vasinfectum isolates from soil and infected cotton tissues. *Plant Dis* **91**: 1564-1573

Zeller KA, Jurgenson JE, El-Assiuty EM, Leslie JF (2000) Isozyme and amplified fragment length polymorphisms (AFLPs) from Cephalosporium maydis in Egypt. *Phytoparasitica* **28**: 121-130.

Zhang Z, Zhang J, Wang Y, Zheng X (2005) Molecular detection of *Fusarium oxysporum* f.sp. niveum and *Mycosphaerella melonis* in infected plant tissues and soil. *FEMS Microbiol Lett.*, **249**: 39-47.