ELSEVIER

British Mycological Society promoting fungal science

journal homepage: www.elsevier.com/locate/mycres

The ITS region as a taxonomic discriminator between *Fusarium verticillioides* and *Fusarium proliferatum*

I. VISENTIN[a], G. TAMIETTI[a], D. VALENTINO[a], E. PORTIS[b], P. KARLOVSKY[c], A. MORETTI[d], F. CARDINALE[e,*]

[a]DiVaPRA, Plant Pathology, University of Turin, via L. da Vinci 44, 10095 Grugliasco (TO), Italy
[b]DiVaPRA, Plant Genetics and Breeding, University of Turin, via L. da Vinci 44, 10095 Grugliasco (TO), Italy
[c]Molecular Phytopathology and Mycotoxin Research Unit, University of Göttingen, Grisebachstrasse 6, D-37077 Göttingen, Germany
[d]ISPA, National Research Council, Via Amendola, 122/O – 70126 Bari (BA), Italy
[e]Dept. of Arboriculture, University of Turin, via L. da Vinci 44, 10095 Grugliasco (TO), Italy

ARTICLE INFO

Article history:
Received 6 April 2009
Received in revised form 15 July 2009
Accepted 16 July 2009
Available online 23 July 2009
Corresponding Editor:
Stephen W. Peterson

Keywords:
Fusarium proliferatum
Fusarium verticillioides
ITS
Maize
PCR

ABSTRACT

The maize pathogens *Fusarium verticillioides* (*Fv*) and *Fusarium proliferatum* (*Fp*) are morphologically very similar to one another, so *Fp* isolates have been often mistaken as *Fusarium moniliforme* (the former name of *Fv*). The only presently accepted morphological discriminator between these species is the presence/absence of polyphialides. Here, a collection of 100 *Fusarium* strains, isolated from infected maize kernels on plants grown in north-western Italy, were assigned as *Fv* or *Fp* on the basis of the presence/absence of polyphialides. This classification was tested on a subset of isolates by sexual crosses, ITS and calmodulin sequencing and AFLP profiling. An ITS-RFLP assay was extended to the full collection and to a number of *Fv* and *Fp* isolates of different geographical origin and hosts. The ITS region is proposed as taxonomically informative for distinguishing between *Fp* and *Fv*.

Introduction

Fusarium verticillioides (*Fv*) (sexual stage *Gibberella moniliformis*) and *Fusarium proliferatum* (*Fp*) [sexual stage *Gibberella intermedia*] are both common as pathogens of maize worldwide, causing [in conjunction with *Fusarium subglutinans*] ear, stalk and root rot (Bottalico 1998; Leslie *et al.* 1990; Nelson *et al.* 1981). They can be particularly aggressive in temperate climates (Munkvold 2003), and maize produced in northern Italy is often heavily contaminated. Infection can be spread by both soil- and seed-borne inoculum, but usually invasion of the growing plant occurs through the silk or the kernels damaged by insects (Munkvold 2003). Ear rot not only reduces grain yield, but also grain quality, since both *Fv* and *Fp* produce mycotoxins within the infected kernel. In particular, significant quantities of the class B fumonisins (FB) are frequently detected in maize grown in northern Italy (Bottalico *et al.* 1989; Moretti *et al.* 1995). Over 20 natural analogues of fumonisins are known, but it is fumonisin B1 (FB1) which is the commonest and most dangerous one, and the one which was associated with severe mycotoxicosis in both domesticate animals and humans (Rheeder *et al.* 2002). Kernels can become heavily contaminated long before harvest, so an understanding of the epidemiology of ear, stalk and root rot is necessary

* Corresponding author. Tel.: +39 011 670 8875; fax: +39 011 236 8875.
E-mail address: francesca.cardinale@unito.it
0953-7562/$ – see front matter © 2009 The British Mycological Society. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.mycres.2009.07.011

before control strategies can be elaborated. The availability of a robust and reliable method to taxonomically identify *Fv* and *Fp* (the major two fumonisin-producing species) is an essential requirement for this purpose.

Both *Fv* and *Fp* belong to the *Gibberella fujikuroi* species complex, in which at least eleven different mating populations (MPs) (i.e. biological species) have been identified (Leslie & Summerell 2006). Among the species in the *G. fujikuroi* complex which are pathogenic on maize, *Fv* and *Fp* can be distinguished by their microconidial chains (not formed by *F. subglutinans*), and it is the presence of polyphialides which differentiates *Fp* from *Fv* (Nirenberg & O'Donnell 1998). As sexual crosses are both labour- and time-consuming to generate, species diagnosis relies currently on morphological traits, supported by the DNA sequence of several genes, most often, tubulin and calmodulin (O'Donnell *et al.* 1998). Although a number of criteria show *Fv* and *Fp* to be true evolutionary species (Taylor *et al.* 2000), the morphological distinction between *Fp* and *Fv* is rather fine and requires trained personnel to recognize, while DNA sequencing can be expensive if large numbers of strains require identification. PCR-based genotyping offers a more cost-effective means of molecular diagnosis. Primer pairs which should specifically amplify DNA from *Fv* have been designed based on sequence variation within either the intergenic spacer (IGS) region of the ribosomal locus (VERT1–VERT2) (Patiño *et al.* 2004) or the calmodulin gene (VER1–VER2) (Mulé *et al.* 2004). Similarly, it has been possible to design the *Fp*-specific primers Fp3F–Fp4R (IGS) (Jurado *et al.* 2006) and PRO1–PRO2 (calmodulin) (Mulé *et al.* 2004) (Table 1). An important issue in the context of PCR-based species diagnostics is amplification specificity, especially where the fungal strains to be tested have been isolated from an environment which was not sampled during the process of primer design. The intention of the present research was to classify *Fusarium* strains in a collection of isolates from maize samples grown in Piedmont (north-western Italy) using morphological, biological and molecular tools, and to develop robust PCR primers to distinguish between *Fv* and *Fp* as a complement to the primers already available.

Table 1 – Primer sequences used for genotyping *Fusarium* spp.

Primer pairs	Target sequence	Amplicon size	Specificity	Reference
	Heat shock protein			
FUS1	5′-cttggtcatgggccagtcaagac-3′	1600	*Fusarium moniliforme*	Murillo *et al.* (1998)
FUS2	5′-cacagtcacatagcattgctagcc-3′			
	IGS			
VERT1	5′-gtcagaatccatgccagaacg-3′	800	*Fusarium verticillioides*	Patiño *et al.* (2004)
VERT2	5′-cacccgcagcaatccatcag-3′			
	IGS			
Fp3F	5′-cggccaccagaggatgtg-3′	230	*Fusarium proliferatum*	Jurado *et al.* (2006)
Fp4R	5′-caacacgaatcgcttcctgac-3′			
	Calmodulin			
VER1	5′-cttcctgcgatgtttctcc-3′	578	*F. verticillioides*	Mulé *et al.* (2004)
VER2	5′-aattggccattggtattatatatcta-3′			
	Calmodulin			
PRO1	5′-ctttccgccaagtttcttc-3′	585	*F. proliferatum*	Mulé *et al.* (2004)
PRO2	5′-tgtcagtaactcgacgttgttg-3′			
	Calmodulin			
CL1	5′-gartwcaaggaggccttctc-3′	670	*Fusarium* spp.	O′Donnell *et al.* (1998)
CL2A	5′-ttttgcatcatgagttggac-3′			
	ITS			
ITS1	5′-tccgtaggtgaacctgcgg-3′	620	All fungal species	White *et al.* (1990)
ITS4	5′-tcctccgcttattgatatgc-3′			
	ITS			
verITS-F	5′-aaatcgcgttccccaaattga-3′	172	*F. verticillioides*	This work, White *et al.* (1990)
ITS4	5′-tcctccgcttattgatatgc-3′			
	ITS			
ITS1	5′-tccgtaggtgaacctgcgg-3′	390	*F. proliferatum*	This work, White *et al.* (1990)
proITS-R	5′-gcttgccgcaagggctcgc-3′			
	MAT1			
fusALPHArev	5′-ggartaracyttagcaatyagggc-3′	200	*Fusaria* species with *Calonectria*, *Gibberella* and *Nectria* teleomorphs	Kerenyi *et al.* (2004)
fusALPHAfor	5′-cgccctctkaaygscttcatg-3′			
	MAT2			
fusHMGfor	5′-tgggcggtactggtartcrgg-3′	260	*Fusaria* species with *Calonectria*, *Gibberella* and *Nectria* teleomorphs	Kerenyi *et al.* (2004)
fusHMGrev	5′-cgacctcccaaygcytacat-3′			