MOLECULAR DETECTION KITS FOR *Fusarium* SPECIES AND *Fusarium oxysporum*

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Fusarium spp. cause diseases in a wide range of host plants. At least 81 of 101 economically important crop plants diseases are caused by *Fusarium*. Several *Fusarium* pathogens cause essentially similar symptoms on different crops such as cortical decay of roots, root rot, wilting, yellowing, rosette and premature death on infected plants (Beckman and Roberts, 1995). Some of them have a narrow range of hosts; others have a very wide range as *F. oxysporum*. *F. oxysporum* is a worldwide, ubiquitous soil-borne fungus that causes *Fusarium* wilt such on oil palm (Rusli *et al*., 2017), tomato (Ignjatov *et al*., 2012) and cotton (Davis *et al*., 2006) (*Figure 1*). There are currently over 150 formae speciales identified to date and each of them has the ability to infect a unique host or set of plant species (O'Donnell and Cigelnik, 1997). The fungus normally causes vascular wilt by root infection and symptoms of the disease include stunting, wilting of yellowed fronds and ultimately results in death of the plants through chronic or acute symptoms (Namiki *et al*.,1994).

Figure 1. a) Fusarium *wilt of oil palm. b) Symptoms of tomato wilt. c) Yellowing and drying of leaves of cotton wilt.*

The detection of these pathogens are imperative for preventing further spread of the pathogen and as a prevention. Early detection of *Fusarium* or *Fusarium oxysporum* by conventional methods is a labour and time-consuming task that requires expertise in fungal taxonomy and chemical analysis. Therefore, diagnostic methods based on the polymerase chain reaction (PCR) is considered ideal as these detection methods are rapid and specific, because the identification of species is made on the basis of genotypic differences, and are highly sensitive, detecting the target DNA molecules in complex mixtures.

OBJECTIVE

Development of an improved and robust detection method by developing *Fusarium* species and *Fusarium oxysporum formae speciales* specific primers with more simple, sensitive protocols and can be introduced for rapid and specific detection of *Fusarium* spp. and *Fusarium oxysporum* ff.spp in diseased tissue, soil, seed and pollen for quarantine purposes of imported materials.

MOLECULAR DIAGNOSTIC PCR KIT FOR *Fusarium* SPECIES SPECIFIC PRIMERS

In order to develop the molecular diagnostic tools, the first step taken was to design genus specific primers. *Fusarium* genus-specific primers (Fusf1 and Fusr1) have been developed based on the sequence variation in the ITS region within the

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Ribosomal DNA gene (*Figure 2*). Ribosomal DNA (rDNA) regions are often used to detect pathogens at the species level because it has both highly conserved and variable regions (Hibbett, 1992).

The specificity of primer pairs Fusf1 and Fusr1 were assessed against forty-two *F. oxysporum* isolates from various hosts, six *Fusarium* spp. isolates, and seven isolates of other fungi were included as controls. Different isolates of *Fusarium* species, irrespective of formae speciales (ff. spp.) and race, were amplified with this primer which yielded a single band on agarose gels with the PCR of ITS region. A band of 220 bp was amplified by Fusf1 and Fusr1. The amplification patterns were always consistently present in all isolates (*Figure 3*).

No amplification product was detected for DNA extracted from the *Trichoderma* isolate and the other out-groups. This is a very significant result as *Trichoderma*, *Verticillium dahliae*, *Neurospora crassa* and *Sclerotinia sclerotiorum* are among the closest genera to *Fusarium* (Wang *et al*., 2009). In order to determine the reliability of the results, all *Fusarium* spp. and the out-groups isolates tested had a positive PCR reaction using the ITS universal primers ITS1/ITS2 (data not shown) and the same procedure was performed using a different thermocycler in a different laboratory and the results were always consistent.

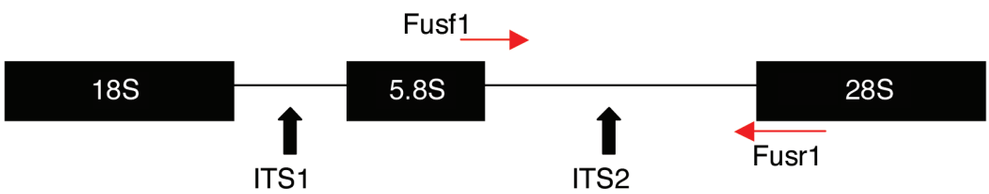
The detection of *Fusarium* spp. specific DNA is based on Polymerase Chain Reactions (PCR) providing a simple, reliable and rapid result for the detection of *Fusarium* spp. The kit components comprised of specific Primer Mix, Reagents Mix, *Fusarium* Positive Control and Negative Control for 96 reactions. All reagents can be stored for one year at -20°C without showing any reduction in performance.

MOLECULAR DIAGNOSTIC PCR KIT FOR *Fusarium* OXYSPORUM SPECIFIC PRIMERS

Specific primers for *F. oxysporum* were designed using the Translation Elongation Factor 1-α (TEF) gene. TEF gene encodes an essential part of the protein translation machinery, has high phylogenetic utility because it is highly informative at the species level in *Fusarium* and non-orthologous copies of the gene have not been detected in the genus (Geiser *et al*., 2004).

Forward primer Foxy F2 and reverse primer EF 2 emerged as the best potential candidate for species-specific primers (*Figure 4*).

The primers were designed to operate at high annealing temperatures tested using gradient PCR thus preventing the co-amplification of the non-specific DNA targets. The species-specific primer pair Foxy F2/ EF2 was able to amplify a DNA fragment of approximately 280 bp of all *F. oxysporum* spp. isolates tested (*Figure 5*). No amplification was observed for *F. fujikuroi*, *F.*



Source: White *et al.* (1990).

Figure 2. ITS gene region of the rDNA gene.

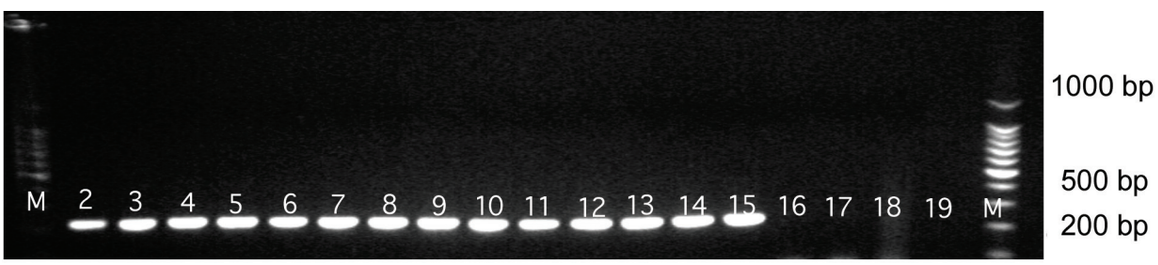
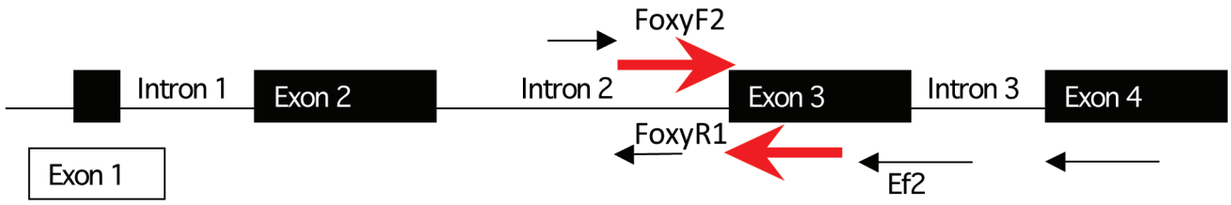


Figure 3. *Polymerase chain reaction (PCR) amplification of DNA from isolates of* F. graminearum *(lane 2),* F. oxysporum *f.sp.* pisi *(lane 3-4),* F. oxysporum *f.sp.* lycopersici *(lane 5-6),* F. oxysporum *f. sp.* vasinfectum *(lane 7),* F. oxysporum *f.sp.* tulipae *(lane 8),* F. oxysporum *f.sp.* phaseoli *(lane 9),* F. oxysporum *f.sp.* narcissi *(lane 10),* F. oxysporum *f.sp.* cubense *(lane 11-12),* F. oxysporum *f.sp.* elaeidis *(13-15),* Trichoderma *sp. (lane 16),* Verticillium *sp. (lane 17),* Aspergillus *sp. (lane 18) and* Sclerotinia sclerotiorum *(lane 19).*



Source: Geiser *et al.* (2004).

Figure 4. Map of the TEF gene region in Fusarium *used in FUSARIUM-ID, with primer locations.*

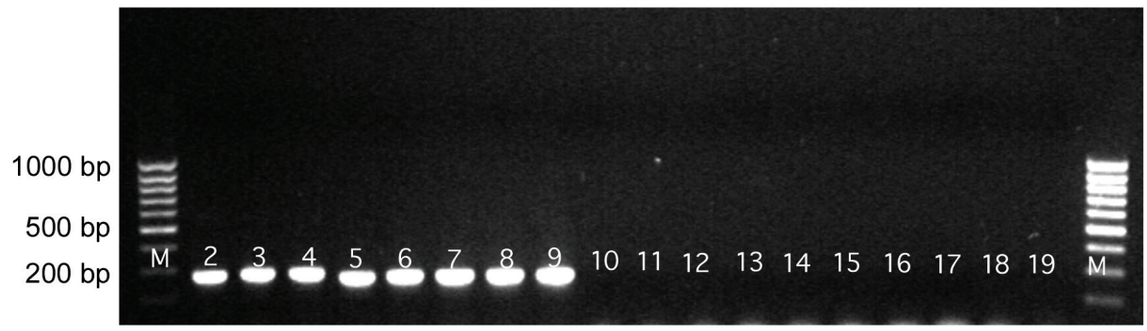


Figure 5. Polymerase chain reaction (PCR) amplification of DNA from isolates of F. oxysporum *f.sp.* elaeidis *(lane 2),* F. oxysporum *f.sp.* pisi *(lane 3-4),* F. oxysporum *f.sp.* lycopersici *(lane 5-6),* F. oxysporum *f. sp.* vasinfectum *(lane 7),* F. oxysporum *f.sp.* tulipae *(lane 8),* F. oxysporum *f.sp.* phaseoli *(lane 9),* F. graminearum *(lane 10),* F. culmorum *(lane 11),* F. fujikuroi *(lane 12),* F. redolens *(lane 13),* F. foetens *(lane 14),* F. phaseoli *(lane 15)* Trichoderma *sp. (lane 16),* V. dahliae *(lane 17),* Aspergillus *sp. (lane 18) and* Sclerotinia sclerotiorum *(lane 19). The PCR amplification was done at 53°C annealing temperature using primer pair Foxy F2 and EF2.*

redolens, *F. culmorum* and *F. foetens* DNA. The fact that these primers did not amplify *F. foetens* and *F. redolens* provides a strong indication as to the reliability of the primers as they are among the closest *Fusarium* species to *F. oxysporum* (O'Donnell *et al*., 2008; Cheng *et al*., 2008). The primers also consistently did not amplify any other tested fungi including *Trichoderma*.

The *Fusarium oxysporum* spp primers in this PCR diagnostic kit are very specific for the genes of a pathogen, and amplification will occur only in diseased plants. The kit components comprised of specific Primer Mix, Reagents Mix, *Fusarium oxysporum* Positive Control and Negative Control for 96 reactions. This rapid test is designed to provide quick, reliable results to enable faster decision making and prevent the spread of disease for efficient crop disease management, and environmental protection. All reagents can be stored for one year at -20°C without showing any reduction in performance.

COST

RM 50* per screening (inclusive of one positive and negative control reactions). *The price are subject to change.

BENEFITS

Molecular approaches are essential tools for identification within the *Fusarium* complex. These technologies offer *Fusarium* genus-specific diagnostic kit that can amplify *Fusarium* spp. from various hosts and origins as well as excluding the closest out-groups to *Fusarium* and *F. oxysporum* species PCR diagnostic probe that is more robust than previous ones as it has been shown to exclude fungi most closely related to *F. oxysporum*, such as *F. foetens* (Schroers *et al.*, 2004). These technologies indicate a more reliable, accurate, and sensitive method for detection of the fungal pathogen in seed, soil, sand, and pollen.

REFERENCES

Beckman, C H and Roberts E M (1995). On the nature and genetic basis for resistance and tolerance to wilt diseases of plants. *Advance Botanical Research 21*: 35-77.

Cheng, Z S; Tang, W C; Su, Z J; Cai, Y; Sun, S F; Chen, Q J; Wang, F H; Lin, Y C; She, Z G and Vrijmoed, L L P (2008). Identification of mangrove endophytic fungus 1403 (*Fusarium proliferatum*)

based on morphological and molecular evidence. *J. Forestry Research, 19*: 219-224.

Davis, R M; Colyer, P D; Rothrock, C S and Kochman, J K (2006). *Fusarium* wilt of cotton: Population diversity and implications for management. *Plant Disease, 90:* 692- 703.

Hibbett, D S (1992). Ribosomal RNA and fungal systematics. *Transaction of the British Mycological Society, 33*: 533-556.

Ignjatov, M; Milošević, D; Nikolić, Z; Gvozdanović-Varga, J; Jovičić, D and Zdjelar, D (2012). *Fusarium oxysporum* as causal agent of tomato wilt and fruit rot. *J. Pesticides and Phytomedicine, 27:* 25-31.

O'donnell, K and Cigelnik, E (1997). Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are non-orthologous. *Molecular Phylogenetic and Evolution, 7:* 103-116.

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

Rusli, M H; Wheals, A E; Sharma, S; Idris, A S and Cooper, R M (2017). Disease epidemiology and genetic diversity of *Fusarium oxysporum* f.sp. *elaeidis*, cause of *Fusarium* wilt of oil palm (*Elaeis guineensis* Jacq.). *J. Oil Palm Res., Vol. 29:* 548-561.

Schroers, H J; Bayeen, R P; Meffert, J P and De Gruyter, J (2004). *Fusarium foetens*, a new species pathogenic to begonia elatior hybrids (*Begonia* x *hiemalis*) and the sister taxon of *Fusarium oxysporum* species complex. *Mycologia, 96:* 393-406.

White T J; Bruns, T; Lee, S and Taylor J W (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols. A Guide to Methods and Applications*. (Innis, M A; Gelfand, D H; Sninsky, J J and White, T J eds.). Academic Press, San Diego, CA, USA. p. 315-322.

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