DETECTION OF FUSARIUM WILT IN OIL PALM BY POLYMERASE CHAIN REACTION METHODS

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Vascular wilt of oil palm is caused by a soil-borne fungus, *Fusarium oxysporum* f.sp. *elaeidis* (*Foe*). This disease is seriously affecting the oil palm industry in Africa, where yield in some areas have drastically been reduced. Currently, *F. oxysporum* can be diagnosed up to the species level through a morphological and molecular technique namely, the Polymerase Chain Reaction (PCR). Detection of its *forma specialis* (f.sp.) pathogenic to the genus *Elaeis, i.e.* f.sp. *elaeidis*, is critically required for the purpose of quarantine, field testing of 'resistant' varieties and the determination of its presence in palms and plantation soils.

OBJECTIVE

The objective of this study is to develop molecular diagnostic tools for *Foe* detection.

METHODOLOGY

In the molecular technique of detection, it is challenging to develop specific primers for *Foe* as some have evolved multiple times (polyphyletic) for host specificity. Nevertheless, a reliable, robust and accurate method has been developed to detect the presence of *Foe*, both as spores and resting thick-walled chlamydospores in seeds (*Figure 1*), pollen (*Figure 2*), soils and infected palms.

Figure 1. Fusarium oxysporum *f. sp.* elaeidis (Foe) *penetrates seeds through the germination pores.*

Figure 2. Fusarium oxysporum *f.sp.* elaeidis (Foe) *on male inflorescences sampled from the Democratic Republic of Congo.*

PCR PATHOTYPES PRIMERS

For the amplification of homologue gene fragment from *Foe* isolates only, specific primers were designed by comparing the sequences from two different *Foe* isolates and several different *F. oxysporum* pathotypes. The specific primers were designed to amplify PCR products of 550 base pairs (bp) of *Foe*.

The primer pair of ORF-F1 and ORF-R1 developed based on secreted effector proteins was able to amplify a unique DNA fragment of approximately 550 bp (*Figure 3*) for all *Foe* isolates from different geographic backgrounds. This primer pair on the other hand, did not amplify *Fusarium* spp., *Trichoderma* sp., *Aspergillus* sp. and *Sclerotinia sclerotiorum* tested (*Figure 4*). This shows that the primers were specific to *Foe*.

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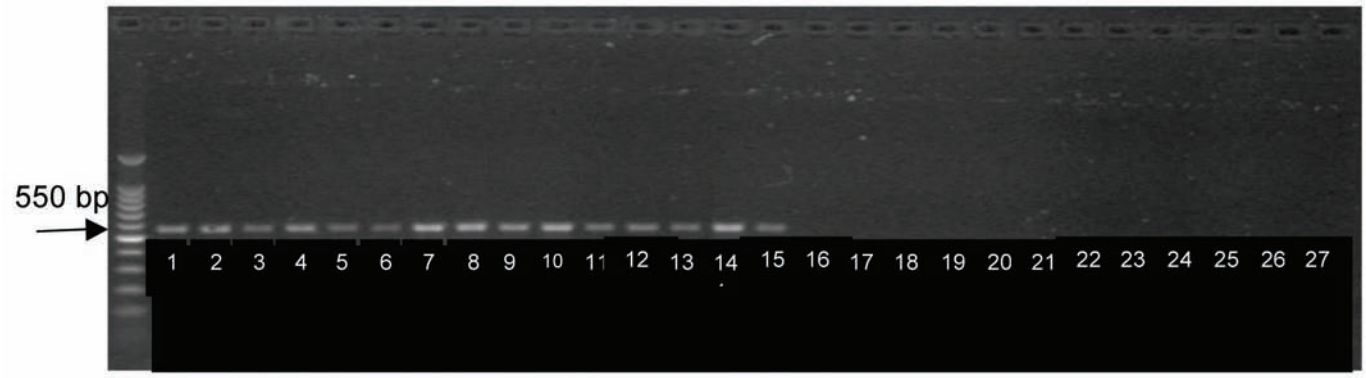

Figure 3. Agarose gel electrophoresis of PCR-amplified results using Foe *specific primers ORF-F1 and ORF-R1. The 100 bp DNA ladder marker was used. Lanes: 1-15:* F. oxysporum *f.sp.* elaeidis *(oil palm); 16:* F. oxysporum *f.sp.* phaseoli *(bean); 17-20:* F. oxsyporum *f.sp.* lycopersici *(tomato); 21:* F. oxysporum *f.sp.* radicis lycopersici *(tomato); 22:* F. oxysporum *f.sp.* vasinfectum *(cotton); 23:* F. oxysporum *f.sp.* tulipae *(tulips); 24:* F. oxysporum *f.sp.* narcissi *(narcissus); 25-26:* F. oxysporum *f.sp.* albedinis *(date palm) and 27:* F. oxysporum *f.sp.* canariensis *(canary palm).*

Figure 4. DNA fragments of two Foe *isolates using the primer pairs ORF-F1 and ORF-R1 separated on 1% agarose gel. DNA fragment corresponding to the electrophoresis peak of 550 bp. Lanes: 1-2:* F. oxysporum *f.sp.* elaeidis *(oil palm); 3:* F. oxysporum *f.sp* dianthi*; 4:* F. oxsyporum *f.sp.* basilici*; 5:* F. oxysporum *f.sp.* perniciousum*; 6-8:* F. oxysporum *f.sp.* cubense*; 9:* F. culmorum*; 10:* F. graminearum*; 11:* F. phaseoli*; 12:* F. redolens*; 13:* F. fujikuroi*; 14:* F. foetens*; 15:* F. lateritium*; 16:* F. verticilium*; 17:* F. mischanti*; 18:* F. hostae*; 19:* F. commune*; 20:* F. avenaceum*; 21:* F. nygamai*; 22:* F. chlamydosporum*; 23:* Sclerotinia sclerotiorum*; 24:* Aspergillus *sp.; 25:* Trichoderma *sp. and 26-27:* F. oxysporum.

CONCLUSION

Plant pathogenic microbes use small secreted proteins, called effectors, to suppress or evade basal immune responses that would otherwise inhibit host colonisation. Many are recognised by co-evolved host resistance genes and this often dictates specificity of plant-pathogen interactions. On the basis of plant-pathogen interactions, the *Foe* specific primers have been successfully developed based on the virulence effector genes products. This molecular diagnostic tool developed can potentially help the oil palm industry to detect and avoid *Foe* epidemics in Malaysia.

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