

RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) AND RANDOM AMPLIFIED MICROSATELLITES (RAMS) ANALYSES OF *GANODERMA* FROM OIL PALM AND COCONUT

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Introduction

The basal stem rot of oil palm caused by *Ganoderma* is the most devastating disease of oil palm in Malaysia. The highest disease incidence is in the coastal areas, especially in stands that were previously planted with coconut. It has been reported that trunks and stumps of coconut which are colonised by *Ganoderma* may become sources of inoculum (Turner, 1981). However, it is not known whether the *Ganoderma* species that infect oil palm are the same as those that colonise coconut stumps as it is not possible to distinguish them by their morphological and physiological characteristics and isozyme profiles (Latiffah, Z. unpublished data). The present investigation was carried out to study the random amplified polymorphic DNA (RAPD) and the random amplified microsatellites (RAMS) of the two groups of *Ganoderma* with a view to determine their similarities or differences.

Materials and Methods

A total of 68 *Ganoderma* isolates obtained from infected oil palms and coconut stumps were studied. The isolates were cultured in liquid medium for 14 days, after which the mycelia were harvested, lyophilised and used for DNA extraction following the phenol-chloroform method of Raeder and Broda (1985). For RAPD analysis, amplification conditions for polymerase chain reaction (PCR) were performed using the method of Williams et al. (1990). Four random oligonucleotide primers, OPI 01 (5' ACCTGGACAC 3'), OPI 07 (5' CAGCGACAAG 3'), OPI 12 (5' AGAGGGCACA 3') and OPI 14 (5' TGACGGCGGT 3'), were used for amplification. The amplified fragments were analysed by electrophoresis in 2% agarose gel. For RAMS analysis, amplification conditions for PCR were performed using the protocols of Hantula et al. (1996) in which 35 cycles were carried out and three microsatellite primers, (CCA)₅, (CGA)₅ and (GT)₅, were used. The amplified products were separated by electrophoresis in 1.7% SeaKem agarose. The standard marker for comparison of the banding patterns was a 100 bp molecular marker.

Results and Discussion

The four oligonucleotide primers produced amplified DNA fragments of between 1 - 10 bands and the size of the fragments ranged from 300 bp - 2072 bp. Most of the isolates showed polymorphic banding patterns. A high level of variation was observed within and between the isolates of the two groups of *Ganoderma*. Variations in the banding patterns were also shown by isolates from the same location. Although some common bands were shown by some of the isolates, these bands could not be used as diagnostic bands for the two groups of *Ganoderma* because they were not present in all the isolates. These results showed that RAPD could not be used to differentiate between *Ganoderma* isolates from infected oil palms and from coconut stumps. The microsatellite primers used in the RAMS analysis produced polymorphic banding patterns for both *Ganoderma* isolates from infected oil palms and coconut stumps. The size of the amplified DNA fragments ranged from 200 bp - 2072 bp. Variations of banding patterns were observed within and between the two groups of *Ganoderma* and among isolates from the same location. Although there were variations among the isolates, four common bands produced by primers (CGA)₅ and (CCA)₅ were observed in all the *Ganoderma* isolates. The two common bands produced by primer (CGA)₅ were 900 bp and 1200 bp and the other two common bands produced by primer (CCA)₅ were 350 bp and 380 bp. These common bands have the potential to be used as diagnostic bands for *Ganoderma* from infected oil palms and coconut stumps. However, more studies have to be conducted to determine whether these bands are unique to the *Ganoderma* isolates from oil palm and coconut.

Conclusions

Analysis using RAMS showed that two bands produced by primer (CCA)₅ and two by primer (CGA)₅ were common in all the isolates of the two groups of *Ganoderma*. These bands could be used as diagnostic bands for *Ganoderma* from oil palm and coconut, and they could also be used to develop specific probes to enable assessment of *Ganoderma* infection in oil palm tissues. This would be useful to the oil palm industry in allowing rapid assessment and early detection of the disease in infected palms.

References

- Hantula, J., Dusabenyagasani, M. and Hamelin, R. C. 1996. Random amplified microsatellites (RAMS). A novel method for characterising variation within fungi. *European Journal of Forest Pathology*. 26: 159-166.
- Raeder, U. and Broda, P. 1985. Rapid preparation of DNA from filamentous fungi. *Letters in Applied Microbiology*. 1: 17-20.
- Turner, P. D. 1981. *Oil Palms Diseases and Disorders*. Oxford University Press.
- Williams, J. D., Kubelik, A. R., Kenneth, J. L., Rafalski, J. A. and Scott, V. T. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*. 18: 6531-6535.