Identification of possible pathogenic RNA sequences in Malaysian oil palm

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Introduction

The present distribution of viroid diseases especially cadang-cadang is confined to southern island of Philipines. Whether the disease spread towards further down south is not known. Leaf spots locally known as genetic yellowing has been observed in locally grown oil palms. In the early stage non-necrotic, translucent, bright orange leaf spots appear. In the mid-stage leaf spots become larger and more frequent so that fronds begin to appear chlorotic from a distance. In the late stage, leaf spots are almost confluent and more numerous on the older fronds. Molecular diagnostic methods have been developed that rely on detection of the viroid in test samples. When cellular nucleic acids purified from coconut tissue are analyzed on polyacrylamide gels, the molecular forms were identified by their relative mobility. Currently, the RNA is still under investigation by polymerase chain reaction. The PCR products will be used as templates for synthesis of radioactively labeled complementary RNA or DNA probes. These will be used in hybridization assays to detect nucleotide sequences

Materials and Methods

Leaf samples with distinct symptoms from various locations and stored frozen until used. Several methods of extraction of total nucleic acids were evaluated in laboratory. The total RNAs of each virus were isolated from purified virus using the proteinase K and phenol-SDS procedure as described by Sambrook *et al.* (1989). In each eppendorf tube 200 µl (1 µg/µl) of virus was mixed with an equal volume of extraction buffer(0.03 M of KCl, 3 mM $MgCl_2$, 0.01 M of SDS, 0.02 M Tris HCl) and 50 µl of proteinase K (2 mg/ml) and incubated for 20 min at 50° C. Then 80 µl of 1 M NaCl and one volume of equilibrated phenol (50° C) were added. The preparation was mixed vigorously and incubated for 5 min at 50° C. After centrifugation at 12,000 g for 5 min, the aqueous phase was removed and subjected to another phenol extraction. The aqueous phase was removed and one volume of chloroform isoamylalcohol (24:1) was added and centrifuged at 12,000 g for 10 min at 4° C. The aqueous phase was removed and subjected to two further chloroform isoamylalcohol extractions. The final aqueous phase was removed and two volume of ethanol and one tenth volume of 3 molar sodium acetate were added and placed at -70^0 C. The precipitate was collected by centrifugation at 12,000 g for 15 min and washed once in 70 % ethanol dried and suspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.3) and stored at -20° C.

Results and Discussion

From electrophoresis, several RNA components were observed in the unfractionated nucleic acid extracts. For confirmation, the samples were sent to University of Adelaide, for hybridization studies and sequencing. For further detail studies a Malaysian PhD candidate is pursuing the studies on collaborative basis with the University of Adelaide, Australia

Conclusions

There are indication that the symptom genetic yellowing in oil palm could be due to some pathogenic nucleic acids

Benefits from the study

Able to detect diseases before they become widespread by using modern and sensitive diagnostic procedures

Patent(s), if applicable:

Nil

Stage of Commercialization, if applicable:

Nil

Project Publications in Refereed Journals:
Nil

Project Publications in Conference Proceedings:
Nil

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