GENETIC VARIATION OF *Rigidoporus microporus* ISOLATES FROM DIFFERENT HOST PLANTS IN THE VICINITY OF RUBBER PLANTATIONS

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Introduction

Rigidoporus microporus **(Polyporales, Basidiomycota) syn.** ***Rigidoporus lignosus*** **is the most destructive root pathogen of rubber plantations distributed in tropical and sub-tropical regions.** ***R. microporus*** **causes white root disease (WRD) in several tropical species such as forest crops, plantation crops, fruit crops, medicinal and ornamental plants. This deadly disease annually causes huge economic loss for rubber industry. Recent studies have shown that the WRD is spreading in Sri Lankan rubber cultivations at an alarming rate. Expansion of the host range and the genetic diversity of the pathogen are some of the reasons behind this phenomenon.** ***R. microporus*** **produces rhizomorphs associated with wood in the soil and capable of infecting adjacent tree roots. The control and management of the WRD of rubber in most tropical countries is hindered due to limited knowledge of the population genetics of the different isolates. There is no detailed in Sri Lankan study on the population genetic variability of this economically important pathogen. Therefore, this study was carried out to investigate genetic variability of** ***R. microporus*** **isolates from different host plants in vicinity of rubber plantations.**

Methodology

Collection of pathogen isolates: **Diseased root samples were collected from different hosts;** ***Hevea brasiliensis, Mucuna bractiata, Cinnamomum zeylanicum, Camellia sinensis, Artocarpus nobilis, Murraya koenigii, Salix babylonica, Alstonia macrophylla, Alstonia heterophyllus*** **in the vicinity of rubber plantations (Table 1). The samples were collected from wet (WZ) and intermediate (IZ) zones of Sri Lanka. All the isolates obtained were purified and fungal tip cultures were raised on Malt Extract Agar (MEA) (Oxoid) at the room temperature (28±2 ^{o}C).**

Table 1. ***Rigidoporus microporus*** **isolates obtained from different host plants**

Isolate	Host plant	Place of collection	Agro-ecological zone
R1	*H. brasiliensis*	Kaluthara	WZ
R2	*M. bractiata*	Daraniyagala	WZ
R3	*C. zeylanicum*	Kuruwita	WZ
R4	*H.brasiliensis*	Monaragala	IZ
R5	*C. sinensis*	Kuruwita	WZ
R6	*A. nobilis*	Kaluthara	WZ
R7	*M. koenigii*	Kaluthara	WZ
R8	*S. babylonica*	Kaluthara	WZ
R9	*A.macrophylla*	Padukka	WZ
R10	*A.heterophyllus*	Kegalle	IZ

R11	*H. brasiliensis*	Kegalle	IZ	
R12	*H. brasiliensis*	Galle	WZ	

RAP

D-PCR Analysis

Preparation of cultures: **A tiny needle piece of mycelia of different isolates was inoculated into eppendorf tubes (1.5 mL) with 500 µL of 2% Malt extract liquid medium and incubated for 4 days at room temperature (28±2 ^{o}C).**

DNA extraction: **The DNA was extracted using the method described by Madushani *et al*., (2014). The mycelium disc was ground quickly with 400 µL of extraction buffer (200 mM TrisHCl -pH 8.5), 250 mM NaCl, 25 mM EDTA containing 10%SDS in 9:1 ratio) using a motor and pestle. The liquid phase was transferred into a 1.5 mL eppendorf tube. Then 150 µL of 3 M sodium acetate (pH 5.2) was added and centrifuged at 10,000 rpm for 4 min. The supernatant was pipetted in to a new epppendorf tube. Equal volume of cool iso-propanol was added and incubated for 2 min at room temperature. The resulting solution was mixed well by slowly inverting the tube for 1 min; initially, cloudy appearances of DNA threads were visible and centrifuged at 10,000 rpm for 4 min to pellet out the DNA. Supernatant was decanted and pellet was washed twice with 300 µL of 70% ethanol and centrifuged at 10,000 rpm for 1 min. Pellet was air dried and re-suspended in 50 µL of autoclaved ultra-purified water and stored at -20 ^{o}C till use.**

PCR amplification: **Thirty random primers were screened and six primers yielded polymorphic banding patterns. Amplifications were performed in 20 µL reaction volume, with 50-100 ng genomic DNA, 1X PCR buffer,1.87 mM $MgCl_2$, 0.01 µM random 10-mer primers, 0.2 mM of dNTPs mix and 5u/µL Taq DNA polymerase (Promega Corporations, USA). Amplification was performed in Apallo DNA thermal cycler (ATC 201) programmed for an initial template denaturation at 94 ^{o}C for 1 min followed by 38 cycles of denaturation at 94 ^{o}C for 1 min, annealing at 36 ^{o}C for 1min and primer extension at 72 ^{o}C for 2 min with a final extension of 72 ^{o}C for 5 min. Each amplification reaction was performed three times to ensure that the results were reproducible. The amplified PCR products were resolved on 1.3% agarose gels containing ethidium bromide and visualized in a Quantum ST4 gel documentation system.**

Data analysis: **The amplified products of primers were scored as "1" for the presence of band sand ''0" for their absence. The discrete data set of reproducible polymorphic bands thus generated was analyzed. RAPDistance software program, version 1.04 was used to develop the genetic distance matrix and the dendrogram.**

Results and Discussion

Random Amplified Polymorphic DNA markers were used to investigate the genetic diversity among the twelve *Rigidoporus* isolates from different host plants. Among the 25 random primers, six primers (OPB17, OPL 08, OPL 10, OPM 05, OPN 13 and OPN 16) showed multi band patterns for each isolate. The primers amplified a total number of 74 bands from twelve isolates tested. The average number of bands per primer was 12.3. Band size ranged from 0.4 to 1 Kb.

A dendrogram resulted from cluster analysis showed two distinct groups designated as A and B (Fig. 1). Cluster A was sub clustered in to two groups and one of the sub clusters along with *C. sinensis* (R5). Cluster B was grouped with all *H. brasiliensis* isolates with *M.*

bractiata **(R2) and** ***C. zeylanicum*** **(R3) which are grown within the rubber plantations as a cover crop and an intercrop recommended by the Rubber Research Institute, Sri Lanka. The dendrogram showed a number of sub clusters, which indicated the high genetic variation among the investigated isolates. This variability may have arisen through point mutations, gene flow and/or recombination (Goodwin** ***et al.*****, 1994). However, any clear relationships between the geographical origin or host plants were not evident in the present study.**

In genetic distance matrix, genetic distance was ranged between 19% to 76% showed a higher genetic variation among them. Less genetic distance was observed between R4 (*H. brasiliensis*) and R5 (*C. sinensis*) revealed the least genetic variation. R 12 (*H. brasiliensis*) and R 7 (*M. koenigii*) shared 76% distance levels and most of the isolates laid their genetic distance between 50%-60%. Lower genetic distances were showed among the isolates collected from Kalutara district and were ranged between 31% to 52%, showing close relatedness among them. There is no correlation exists between host plants and their geographical origin. Therefore, the pathogen has great chance to spread disease over the plantations in Sri Lanka. On the other hand, due to high degree of genetic variability among isolates effectiveness of the fungicide recommendation can be low.

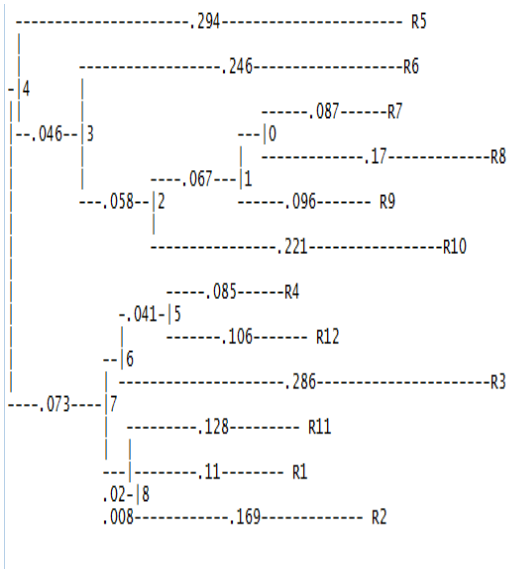

Figure 1. Dendrogram of eleven isolates constructed using RAP Distance program version 1.04 method – Neighbour joining tree

Conclusions and Recommendations

RAPD PCR analysis indicated a high genetic variation among the investigated isolates. The pathogen phylogeny will be the most important prerequisite in the future to reveal co-phylogeny of hosts, pathogen and biogeography in developing a sustainable strategy in management of WRD.

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