Variability of *Corynespora cassiicola* Isolates from Non-Traditional Rubber Growing Areas

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Introduction

Corynespora leaf fall disease (CLFD) caused by *Corynespora cassiicola* (Berk and Curt) is the most distructive foliar disease reported in Sri Lanka and it has become a serious threat to the world natural rubber industry (Fernando *et al*., 2012). CLFD was first detected in Sri Lanka during the year 1985 and the first epidemic was experienced in 1985-1986, devastating the clone RRIC 103, one of the most prestigious genetic materials bred by Sri Lankan scientists. Very recently, a leaf spot disease with uncommon symptoms was reported from non- traditional rubber growing areas. The disease samples were collected from the field plants of RRIC 121 and the isolation of the samples consistently resulted *Corynespora cassiicola* isolates. The objective of this investigation was to report the newly recorded disease symptoms, isolate the pathogen and also to study the variability in cultural and reproductive characteristics.

Methodology

Diseased leaf samples were collected from the several traditional and non- traditional rubber growing areas and the different symptoms were recorded. Five isolates were collected, purified and single conidia cultures were raised on to potato dextrose agar (PDA).

Mycelial plug (5.0 mm) from the advancing margin of a seven-day old culture of the test isolates and were placed at the centre of Petri plates containing PDA. The plates were incubated at RT under normal light and dark regimes. Colony colour, texture and the growth were recorded after 8 days of incubation period. The growth was also recorded. The cultural characteristics in different culture media were also observed. Ten-day old cultures of the isolates grown on PDA were used to prepare conidia suspensions. Reproductive characteristics were evaluated by using slide cultures, conidia shapes and their sizes. The conidial concentration of the filtrates was measured using a haemocytometer. Pathogenicity of the isolates was tested using detached leaf method (Brown and Soepena, 1994). Conidial suspensions at a concentration of 2×10^{4} conidia/ ml) were used for artificial inoculations. Apple green leaves of the clone, RRIC121 clone were used for pathogenicity tests. Toxin production was evaluated by using leaf wilt bio assay and leaf puncture bio assay.

Results and Discussion

Symptoms of the two disease samples from non-traditional rubber growing areas (intermediate and dry zones) showed more or less similar lesion types and the other three disease samples showed different types of symptoms *viz* characterstic railway track lesions, irregular and polyhedral lesions with prominent yellow halos and symptoms similar to old Oidium patches. One of the unique features of this pathogen is the high variation in symptom production and this has been reported by the previous workers (Fernando *et al*., 2009). *C. cassiicola* isolates showed variations in colony colour and texture. The isolate A was olevaceous green on top / dark brown to black on lower surface, isolate B was grey coloured with yellow shade / dark grey, isolate C

was olevaceous green / dark grey to black, isolate D was ash/ dark brown and the isolate E was ash/ dark grey to black. The texture of the isolates too showed variability. The isolates A, B and C showed puffy and dense growth while D and E showed thin flat growth. All the isolates showed even margins. Significant variations were observed on the growth rates among the isolates under investigation. The isolates showed the maximum growth after 8 days of incubation. Isolate B showed the highest growth rate while the isolate D showed the lowest growth rate. Based on the statistical analysis using ANOVA procedure in SAS system, the growth rate of the 5 isolates were significantly different from each other. Significant variations were observed in the shapes of the conidia produced among the different isolates. Some isolates (A and E) produced abundant conidia and their shapes were obclavate, cylindrical, oval, curved or straight. The dimensions of the conidia were (2-25) µm × (0.5-1.25) µm showing a variability in the sizes among the different isolates of *C. cassiiclola* under investigation. The five isolates showed significant variation ($p<0.0001$) in conidia production. Isolates E and A were high conidia producers while the isolates B, C and D were poor producers. Within the isolates from non-traditional rubber growing areas, isolate A was a higher conidia producer than the isolate B. Within the isolates from traditional rubber growing areas isolate E showed a higher conidia production. All the isolates showed morphological variations on different media after 8 days of incubation. More profuse growth was observed on the media PDA, CDA and MEA. The growth of the isolates on LBA and BA was poor. Effect of different culture media on conidia production showed significant variations. Isolate A and E produced abundant conidia (>5000 cm^{-2}) on PDA while on the media MEA, LBA, CDA the same isolates produced less number of conidia. Comparatively the poor sporulators B, C and D also showed higher number of conidia on PDA and MEA. The pathogenicity of the five different isolates tested on detached tender leaves (apple green stage, RRIC121) showed significant variability. The grouping of the lesions was statistically converted in to mean lesion scores. According to the statistical analysis a high mean lesion score was shown for the isolate A while the isolate C showed the lowest mean lesion score. In leaf wilt bio assay the degree of wilting in different isolates varied markedly suggesting significant variations in the toxin production among the different isolates. Isolates A, E and D showed a clear wilting symptom compared to the culture filtrates of the other isolates. Isolate B showed the lower mean lesion score indicating lower aggressiveness.

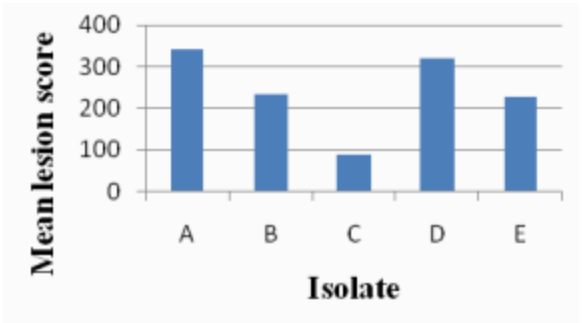

Figure 1. Mean lesion scores for the pathogenicity of C. cassiicola isolates on the clone RRIC 121.

Conclusions

The results of this investigation indicated a clear variation in symptoms, cultural characters, reproductive characters and pathogenicity amongst the population of *C. cassiicola* in non-traditional rubber growing areas. Positive correlations were revealed between conidia production and pathogenicity. The knowledge on this population of *C. cassiicola* should be considered in the clonal and fungicide screening programmes.

References

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