

Genetic diversity of *Ralstonia solanacearum* phylotype II sequevar 4 strains associated with Moko disease of banana (*Musa* spp.) in Peninsular Malaysia

ABSTRACT

Moko disease caused by *Ralstonia solanacearum* (*R. solanacearum*) is a major disease affecting banana (*Musa* spp.) production. Although local reports suggested that this disease is widespread in Malaysia, molecular characterization of *R. solanacearum* strains associated with Moko disease in this country has not yet been done. During March 2011 to June 2012, 170 banana plants associated with Moko disease and the adjacent soil samples were collected in 12 different locations of five outbreak states in Peninsular Malaysia comprising Kedah, Selangor, Pahang, Negeri Sembilan and Johor, with disease incidence exceeding 80 % in some severely affected plantations. A total of 197 strains were identified as *R. solanacearum*-like colonies since they produced fluidal colonies that were white to pink coloration after incubation at 24 to 48 h at 29 °C on Kelman's TZC agar medium, appeared as Gram-negative rods, and positive for potassium hydroxide (KOH), Kovacs oxidase, catalase and lipase activity on Tween 80 solution tests. Biovar tests disclosed that only 30 strains displayed characteristics of biovar 1 *R. solanacearum* associated with Moko disease, which was negative for utilization of disaccharides and hexose alcohols. Pathogenicity assay showed that these 30 strains were virulence towards *Musa paradisiaca* cv. Nipah explants with diverse degrees of virulence. Phylotype-specific multiplex PCR (Pmx-PCR) revealed all strains belonged to phylotype II displaying a 372 bp amplicon. Phylogenetic analyses of endoglucanase (*egl*) sequences clustered all 30 strains into phylotype II/4, together with the reference sequences strains from Peru (UW129, UW162 and UW163) and Colombia (UW070). Pooled rep-PCR fingerprinting method defined two major groups; cluster 1 (sub-group A and B) and cluster 2 (sub-group C), with 35 % average similarity coefficient within these two clusters. The sub-groups in cluster 1 were represented by strains from Kedah, Selangor, Negeri Sembilan and Johor; while cluster 2 sub-group was represented exclusively by strains of Pahang. To our knowledge, this is the first description of *R. solanacearum* phylotype II/4 in Malaysia and the Asian region. Our findings may expand constructive documentation and describe a better understanding on diversity of Malaysian *R. solanacearum* Moko-causing strains populations, thus will be useful for designing disease control strategies.

Keyword: Banana; Genetic diversity; Moko disease; Phylotyping; *Ralstonia solanacearum* strains; Rep-PCR; Sequevar