

IMPEDIMETRIC DNA BIOSENSOR AS EARLY WARNING SYSTEM TOOL IN MANAGEMENT OF PAPAYA DIEBACK DISEASE

Nur Azura Mohd Said¹, Lau Han Yih¹, and Norliza Abu Bakar²

¹Biodiagnostic-Biosensor Programme, Malaysian Agricultural Research & Development Institute (MARDI), Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia

²Agri-Omic & Bioinformatic Programme, Biotechnology & Nanotechnology Research Centre, Malaysian Agricultural Research & Development Institute (MARDI), Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia

Correspondence: nazurams@mardi.gov.my, hylau@mardi.gov.my, lizaab@mardi.gov.my

Agriculture losses due to crop infections are a major concern across the globe. In Malaysia, the persistent issue of papaya dieback disease is a threat to the industry. Advance disease detection in papaya is of paramount importance in order to minimize the damage, yield and economic losses. With this regard, early detection by electrochemical DNA biosensor offers the most cost-effective and efficient means in plant disease management. We report here the development of DNA impedance biosensor for the detection of *Erwinia mallotivora* bacteria, the causal agent for papaya dieback disease. Genome comparison via bioinformatic analysis on six *Erwinia* spp. has identified 45 gene clusters with 114 genes sequence found to be unique to *E. mallotivora*. One of them, a unique gene for a Hypothetical protein (Hyp); was selected for primer design, PCR-amplified reaction and purified to be used as DNA target for DNA sensor application. Concentrations ranging from 50 ng to 250 ng of DNA probe designed from the Hyp protein gene sequence were immobilized on thiol-modified gold screen-printed electrodes for electrochemical studies. Electrochemical impedance spectroscopy (EIS) technique was carried out in 5 mM ferri/ferrocyanide in 0.1 M phosphate buffer, pH 7.0 solution. Nyquist plots' semi-circles and charge transfer resistance (ΔR_{ct}) for 0 ng -125 ng target DNA increased linearly with $R^2=0.9962$. At 125 ng and 250 ng however, the calculated ΔR_{ct} were 7003 ± 682 and 6536 ± 444 , respectively. The decreased value indicates the saturation of the gold surface with target DNA as well as steric hindrance. This preliminary result indicates the ability of the impedimetric DNA sensor to detect the DNA sequence of Hyp protein gene at lower concentrations in early detection of papaya dieback disease which ultimately will circumvent the great loss in papaya industry.

Keywords: *Erwinia mallotivora*, dieback disease, electrochemical impedance spectroscopy, biosensor, plant disease management