3-O19-5

Use of response surface methodology (RSM) for the optimization *Lasiodiplodia* sp. LAMP assay in Philippine 'Carabao' Mango (*Mangifera indica* Linn.)

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Purpose: *Lasiodiplodia sp.* is a plant pathogen associated with the Stem-end-rot (SER) of mangoes. The disease is one of the primary concerns among mango production including the Philippine Carabao mango cultivar. In this study, we demonstrate the utilization the response surface methodology (RSM) to determine the optimal conditions employed in the rapid detection of *Lasiodiplodia sp.* using the loop-mediated isothermal amplification PCR (LAMP) for the early detection of the SER pathogen.

Methods: The primers used in the LAMP assay were designed to target the RNA-directed DNA polymerase II (rbp2) gene of *Lasiodiplodia sp* isolated from SER stricken mango fruits. Optimization the started with a fractional factorial design using the Fedorov's (1972) algorithm. The factors considered in the LAMP assay were the following: 1.) Mg^{2+} concentration; 2.) temperature; and 3.) time. The presence of LAMP amplicons was measured from the GelRed ™ fluorescence of the LAMP reaction tubes using the photographs of reaction and blank tubes. The response variable assigned in the RSM optimization was the ratio of pixel intensities of the test reaction tube and blank tubes.

Results: The LAMP reaction described best fits the second order with interaction response model (R-squared=0.918). In this model, the pure-quadratic (PQ) form of the factors respond significantly (p=0.039) towards synthesis of LAMP amplicons. Canonical analysis of the of the surface model suggests the optimum LAMP reaction conditions: $[Mg^{2+}]$ = 7.59mM; Temperature = 63.9^{o}C; and Time = 42mins.

Conclusions: Response surface methodology (RSM) could be applied to the optimization of the LAMP assay. The estimated values for each factor from the RSM canonical analysis could be used as a benchmark for the further development of the *Lasiodiplodia sp*-LAMP assay in field applications.
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