

THE CONSTRUCTION AND EXPRESSION OF PCAMBIA1304::16D10::S2F3 IN AGROBACTERIUM TUMEFACIENS FOR GLOBAL INTERACTOME ANALYSIS OF BANANA – MELOIDOGYNE INCOGNITA 16D10 GENE INTERACTION VIA AFFINITY TAGGING PURIFICATION-MASS SPECTROMETRY (AP-MS) APPROACH

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Meloidogyne incognita is one of the most damaging root-knot nematode (RKN) species. Its infestation contributed to 78 billion USD annual yield losses of crops including bananas. Bananas are consumed as a staple food in the tropics and subtropics. Economically, bananas are important in the trade market, documented to value up to 12 million USD in 2012. At present, an effective management procedure to control *M. incognita* spread in banana plantations is yet to be developed. As the name suggests, symptoms of RKN-infected plants include galled root systems that appear as knots due to the formation of giant, multi-nucleated feeding cells induced by the nematodes. *16D10*, a parasitism gene, is responsible to encode effector protein that manipulates the host's defense mechanism to form these nutrient sinks by directly interacting with the host's transcription factors. The current study aims to first construct *16D10* gene fused with 2xStrep::3xFlag (S2F3) tags into pCAMBIA1304 vector and express in *Agrobacterium tumefaciens* in order to later identify the plant protein(s) interacting with *16D10* gene. Both mature 16D10 peptide and the full peptide were used in our study. Three types of plasmids were constructed namely pCAMBIA1304::16D10::S2F3, pCAMBIA1304::mature16D10::S2F3 and pCAMBIA1304::mature16D10::GFP::GUS::6xHis including plasmids without *16D10* gene as a negative control reaction. *Agrobacterium tumefaciens* were then transformed with these constructs. Total protein was isolated from transformed *A. tumefaciens* and subjected to purification procedure. Western blot analysis revealed that 16D10 protein was present in *A. tumefaciens* colonies transformed with pCAMBIA1304::mature16D10::S2F3 and pCAMBIA1304::mature16D10::GFP::GUS::6xHis.

Keywords: banana, *Meloidogyne incognita*, *16D10*, parasitism gene, *Agrobacterium tumefaciens*