GENETIC CHARACTERISATION AND BIOCONTROL OF TOBACCO ETCH VIRUS INFECTING PEPPERS (*Capsicum chinense* var. JACQ) IN JAMAICA.

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ABSTRACT

Tobacco etch virus (TEV) is a member of the genus *Potyvirus*, family *Potyviridae*, with a global distribution, is an economically important pathogen of solanaceous crops, including Scotch Bonnet pepper (*Capsicum chinense* var. Jacq.), especially in Jamaica. One approach that forms a prelude toward controlling TEV is by understanding its genetic diversity through molecular characterization, using PCR, RT-PCR, RFLP, molecular cloning, sequencing, nucleotide and amino acid sequence analysis along with phylogenetic analysis. Ten symptomatic samples were collected from each of the following locations: Bodles Agricultural Research Station, Old Harbour, St. Catherine; Ebony Park HEART Academy, Ebony Park, Clarendon; CARDI and UWI Botany Gardens, UWI, Mona. Primers complementary to the coat protein (CP), helper component proteinase (HC-Pro) and P1 proteinase regions of the viral genome were designed, and used to amplify the specified regions. Purified PCR and RT-PCR products were cloned in a cloning vector, and the putative clones analyzed and sequenced.

RFLP analysis of PCR and RT-PCR amplified fragments showed similar restriction profiles among the TEV amplicons. Amino acid analysis of the CP gene indicated two highly conserved DAG motifs of the N-terminal domain that are typical of highly aphid transmissible (HAT) TEV. Multiple alignments of

amino acid and nucleotide sequence revealed similarity of 66.8%-100%, and 84.6% and 100%, respectively. Amino acid analysis of the HC-Pro gene revealed several conserved and highly conserved motifs typical of this potyviral gene. These include; the KITC motif of the N-terminal domain, two IGN motifs and a CCC motif of the conserved central domain, and the PTK motif of the C-Terminal domain. Two highly conserved residues C_{345} and H_{418} represent the active sites of the proteolytic C-terminal domain. Amino acid and nucleotide sequence analysis revealed similarity indices of 88.9%-99.6%, and 92%-99.9%, respectively. The P1 gene also revealed several highly conserved amino acid residues (H_{213}, D_{222}, and S_{255}) representing the proteolytic triad of the C-terminal domain.

Although varied, the high nucleotide and amino acid sequence similarity indices of the TEV isolates confirmed that they were closely related. The magnitude of the relatedness among TEV isolates was confirmed unequivocally by phylogenetic reconstructions from nucleotide and amino acid sequence data matrices and RFLP data profile. Phylogenetic analysis inferred a single clade of TEV isolates, indicating a very strong relationship among isolates.

Single and dual inoculations of pepper seedlings with plant growth-promoting rhizobacteria (PGPR); *Pseudomonas putida* NCIMB-9571, and *Bacillus* sp. UWI-3 were used as biocontrol agents against TEV disease under greenhouse conditions. There were significant increases in stem length, shoot dry weight, root dry weight, shoot phosphorus and shoot potassium levels of all PGPR treated

plants when compared with TEV inoculated control. The titre of TEV in PGPR treated plants and TEV inoculated control were assessed at four and eight weeks post viral inoculation, using tissue immunoblotting augmented by DAS ELISA. PGPR treatments reduced viral titre by up to 90%, when compared with TEV inoculated control. PGPR treatments also significantly reduced the titre and the spread of TEV in inoculated leaves by 72% and 32%, respectively when compared with TEV inoculated control. *Pseudomonas putida* NCIMB-9571 appeared to be chiefly responsible for promoting plant growth, while *Bacillus* sp. strain UWI-3 appeared to be responsible for viral suppression.