# RT-PCR BASED CLONING AND SEQUENCING OF POTATO LEAF ROLL VIRUS-COAT PROTEIN (PLRV-CP) GENE FOR CHARACTERIZATION AS A BANGLADESHI PLRV ISOLATE AND ITS PHYLOGENETIC ANALYSIS

**Dr. Md. Belal Hossain**

Professor, Dept. of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh

## Abstract

An experiment was conducted in Molecular Biology and Plant Virology Laboratory under the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. Total RNA was extracted from Potato leaf roll virus (PLRV) positive leaves and complementary DNA (cDNA) were synthesized from total RNA. Reverse transcriptase polymerase chain reaction (RT-PCR) based detection conditions were optimized by using coat protein (CP) gene specific primers. In PCR amplification cDNA and in nucleotide sequencing PCR product was used as a template. A 346 bp amplicon of PLRVCP gene was amplified and amplified gene region was sequenced. The expected nucleotide sequence of amplified PLRV-CP gene showed 95 to 98% homology when compared with the isolates sequences reported in Gene Bank database. This explored novel PLRV-CP gene was characterized as a PLRV Bangladeshi isolate (Accession number, Bankit 2274496, MN605963). PLRV-CP gene protein modeling was carried out using Expert Protein Analysis System (ExPaSy), DNASTAR's protein tools server used for 3D protein modeling. Phylogenetic analysis was also carried out, the tree was made by using MEGA 4.0 software and maximum parsimony method was selected to construct phylogenetic tree. The RT-PCR based molecular technique optimized in this study, would be a useful for early detection, epidemiological studies of PLRV as well as in seed tubers certification program and the novel hyper variable sequenced region of PLRV-CP gene will be useful in pathogen derived resistance breeding program against the PLRV local strain.

**Keywords:** potato, PLRV-CP Gene, PCR-Based Cloning, PLRV-Bangladeshi isolate.