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DEVELOPMENT OF A DNA PROBE TO DETECT LEAF CURL VIRUS IN CHILLI PLANT TISSUES

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Chilli (*Capsicum annuum* L.) is a major cash crop grown in Sri Lanka. Viral diseases are considered as a major limiting factor in chilli production. As preventive measures are more effective than curative measures, early detection by reliable diagnostic tools would be more effective in controlling plant virus diseases. Chilli leaf curl virus belonging to the genus *Begomovirus* is one of the causal viruses of chilli leaf curl complex (CLCC), a major threat to chilli cultivation in Sri Lanka. Due to the complexity of symptoms of CLCC, infections due to chilli leaf curl virus cannot be identified by field symptoms. Hence, use of a molecular detection method has become essential. The present study was conducted to detect *Begomoviruses* in CLCC-infected plants using a specific DNA probe. A primer pair was designed targeting a common region in the coat protein gene sequence of chilli leaf curl virus (CLCV), using MFFT software and Primer 3 software, based on sequence information obtained from the early phase of this study. The forward and reverse primer sequences designed were 5' gggcaagatatggatggatg 3' and 5' acctggtaacgatccctgta 3' respectively. Genomic DNA extracted from the plants showing CLCC symptoms were subjected to PCR amplification with the newly-designed primers. Findings revealed that the designed primer pair was successful in amplifying a PCR product to detect CLCV-infections at a 87.5 % success rate. The PCR product amplified by the newly-designed primer pair was purified by *Suprex* column (*TAKARA*, Japan) and labeled with digoxygenin. Dot blot hybridization was done using the DNA extracted from CLCC-infected, deformed and healthy chilli leaves. The prepared probe developed positive signals only with DNA dots obtained from PCR products of CLCC-infected plants. The probe prepared in the present study successfully detected CLCV-infections at a plant genomic DNA concentration higher than 200 ng/µl and when the PCR product concentration was more than 60 ng/µl. Success of the applicability of the designed probe for routine use in plant virus diagnostics will be further validated through plant tissue imprint hybridization.

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