2.Sample Preparation: Extract DNA from the collected samples using a simpleextraction protocol (CTAB method).

3.LAMP Reaction Setup:

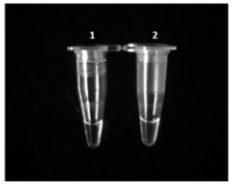
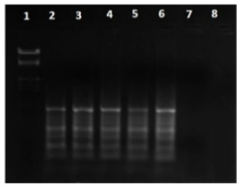
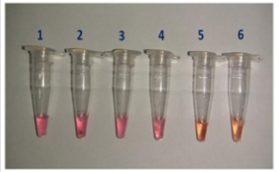
- Prepare the LAMP reaction mix according to the table 1 instructions.
- Add the extracted DNA to the reaction mix.
- Set up the LAMP reaction tubes.

4.Isothermal Amplification: Incubate the reaction tubes in a constant temperatureheating device (usually 60°C) and incubate for 30-60 minutes.

5.Result Interpretation: Observe the visual readout for a change in colour orfluorescence, indicating the presence of SLCMV.

Expected results:
When employing the Sri Lankan Cassava Mosaic Virus (SLCMV) LAMP technique in cassava, the following expected results can be anticipated:

Positive SLCMV Detection:
In cases where cassava plants are infected with SLCMV, the LAMP assay is expectedto produce a clear, positive result. This is indicated by a noticeable color change orfluorescence in the reaction tubes and ladder like bands in 2% agarose gel.

Presence of Turbidity	Visualization using nucleic acid dye (Ethidium bromide)	Visualization using agarose gel electrophoresis
		
➢ **Tube 1** - Healthy cassava lamp product (no turbidity) ➢ **Tube 2** - Infected cassava lamp product (turbidity)	➢ **Tube 1-3**-SLCMV infected sample ➢ **Tube 4** = Positive sample (SLCMV) ➢ **Tube 5 &6**= Healthy cassava leaf	➢ **Lane 1:** Ladder (1000 bp) ➢ **Lane 2-5:** SLCMV infected cassava leaf sample Lane 6: Positive sample (SLCMV) ➢ **Lane 7**: Healthy ➢ **Lane 8:** Without DNA

Conclusion
The Sri Lankan Cassava Mosaic Virus (SLCMV) LAMP technique in cassava is a powerful diagnostic tool that offers speed, sensitivity, and specificity in detecting the virus. This technology is instrumental in disease management, helping to reduce crop losses and ensure food security for communities relying on cassava as a staple crop. Its simplicity and rapid results make it an invaluable resourcefor farmers and researchers alike.

Authors:Dr. R. Arutselvan and Dr.T. Makeshkumar
Published by: Director,ICAR - CentralTuberCrops Research Institute, Sreekariyam, Thiruvananthapuram - 695017, Kerala, India
Email: director.ctcri@icar.gov.in; Telephone: (+91)(471) 2598551

Technical Leaflet

Sri Lankan Cassava Mosaic Virus (SLCMV) LAMP Technique in Cassava

ICAR - CentralTuberCrops Research Institute, Sreekariyam, Thiruvananthapuram - 695017, Kerala, India
Email: director.ctcri@icar.gov.in; Telephone: (+91)(471) 2598551

-Technical Leaflet

Sri Lankan Cassava Mosaic Virus (SLCMV) LAMP Technique in Cassava

Introduction

The *Sri Lankan Cassava Mosaic Virus* (SLCMV) poses a significant threat to cassava production in India and South East Asia, impacting food security and livelihoods in these regions where cassava is a staple as well as industrial crop (Figure 1). To combat this virus, a robust and efficient diagnostic technique is crucial. The Loop-Mediated Isothermal Amplification (LAMP) technique has emerged as a powerful tool for the detection of SLCMV in cassava.

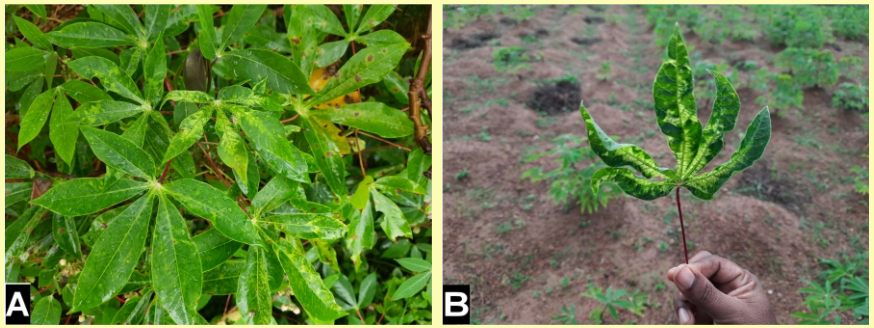


Figure 1: **(A)** Sri Lankan cassava mosaic virus (SLCMV)-incited chlorotic speck or mosaic symptoms on cassava leaves; **(B)** Leaf area is reduced and in extreme cases leaf distortion and shoe string were observed.

Key Features of LAMP technique:

1.Isothermal Amplification

The LAMP technique operates under isothermal conditions, eliminating the need for complex, temperature sensitive equipment. This ensures that the assay can be performed in-resource limited settings, making it highly accessible to farmers and researchers alike.

2.Specificity

The SLCMV LAMP assay is highly specific, targeting unique regions within the SLCMV genome. This specificity minimizes the risk of false positives and allows for precise identification of SLCMV in cassava plants.

3.Sensitivity

The LAMP technique is exceptionally sensitive, capable of detecting SLCMV even at very low concentrations ($0.02\ \mu g\ \mu l^{-1}$) . This early detection is critical in managing and controlling the spread of the virus within cassava crops.

4.Speed

Results can be obtained within 30-60 minutes, significantly reducing the time required for diagnosis compared to traditional methods which takes one or more days. This rapid turnaround time aids in making timely decisions regarding disease management.

5.Visual Readout

The LAMP assay can be designed to produce a visual readout, such as a colour change or fluorescence, making it easy for non-specialists to interpret results in the field.

Protocol

(i)Materials Required:

- LAMP primer sets designed for SLCMV.
- Sample collection materials (e.g., plant leaves).
- Reaction tubes.
- LAMP reaction mix.
- Heating equipment capable of maintaining a constant temperature (e.g., heat blocks or water
- baths or PCR).

(ii)LAMP reaction mixture:

Incubate the following reaction at 60°C for 30-60 minutes.

Table 1: Components of the LAMP Reaction Mixture

Component	Reaction volume	Final Concentration
10X ThermoPol Buffer	2.5 µl	1X (contains 2 mM MgSO4)
MgSO4 (100 mM)	1.5 µl	6 mM (8 mM total)
dNTP Mix (10 mM)	3.5 µl	1.4 mM each
FIP/BIPPrimers (25X)	1 µl	1.6 µM
F3/B3 Primers (25X)	1 µl	0.2 µM
LoopF/B Primers (25X)	1 µl	0.4 µM
Bsm DNA Polymerase, Large Fragme (8,000 U/ml)	1 µl	320 U/ml
Betaine	1 µl	0.8 M
DNA Sample	Variable	> 10 copies or more
Nuclease-free Water	to 25 µl	Variable
Total Reaction Volume	25 µl	-

General Guidelines:

1.A LAMP Primer Mix can be prepared with all 4 or 6 (with Loop) primers. A 25XPrimer Mix should contain: 40 µM FIP, 40 µM BIP, 5 µM F3, 5 µM B3, 10 µM LoopF,10 µM LoopB in TE or water.
2.Reactions should be setup on ice. If room temperature setup is desired, use *Bst* 2.0WarmStart® DNA Polymerase (NEB #M0538).

3.If analyzing via agarose gel electrophoresis or other method requiring opening LAMPreaction vessels, setup secondary analysis area and equipment to avoid contamination.

4.Running a non-template control is strongly recommended to ensure amplificationspecificity.

5.If optimization is desired, try titrating Mg2+ (4–10 mM final) or *Bst* DNA Polymerase LargeFragment (0.04–0.32 U/µl), or changing reaction temperature (50–68°C).

(iii)LAMP Procedure

1.Sample Collection: Collect leaf samples from cassava plants suspected of SLCMVinfection. Ensure samples are representative and properly labelled.