

APPENDIX

Reagents

I Reagents for genomic DNA preparation

1. Insect DNA extraction buffer

1M Tris pH 8.0:	100 µl
0.5 M EDTA pH:	25 µl
1M NaCl:	25 µl
10% SDS:	25 µl
Proteinase K (2 mg/ml):	25 µl

Add sterile distilled water to the final volume of 500 ml

2. Cassava DNA extraction buffer (2X CTAB buffer)

1 M Tris pH 8.0:	50 ml
0.5 EDTA pH 8.0:	20 ml
NaCl:	40.91 g
CTAB:	10 g

Add distilled water to reach a final volume of 490 ml, then autoclave. Put the buffer at room temperature until it reaches 60 °C, then add 2% CTAB and bring the final volume to 500 ml. Add 0.02% mercaptoethanol before use.

3. 1 M Tris pH 8.0

Tris (hydroxymethyl) aminomethane: 121.14 g

Dissolve 121.4 g in 800 mL of distilled water, then adjust the pH to 8.0 with the appropriate volume of concentrated HCl. Bring the final volume to 1 L with distilled water. Autoclave and then store at room temperature.

4. 0.5 EDTA pH 8.0

Ethylenediaminetetraacetic acid disodium salt $2H_2O$

Dissolve 186.1 g in 800 mL of distilled water and approximately 20 g of NaOH pellets while stirring to adjust the pH to 8.0. Bring the final volume to 1 L with distilled water. Autoclave and then store at room temperature.

5. 1M NaCl

Sodium chloride: 5.88 g

Dissolve 5.88 g in 80 mL of distilled water, then add distilled water to a final volume of 100 mL. Autoclave and store at room temperature.

6. 3M Sodium acetate pH 5.2

Sodium acetate trihydrate: 40.8 g

Dissolve 40.8 g in 80 mL of distilled water, adjust the pH to 5.2, and then add distilled water to a final volume of 100 mL. Autoclave and store at room temperature.

7. 10% SDS

Sodium dodecyl sulfate: 10 g

Dissolve 10 g in 80 mL of sterile, deionized water, and then add sterile, deionized water to a final volume of 100 mL. Store at room temperature.

8. 1X TE buffer

10 mM Tris pH 8.0

0.1 mM EDTA pH 8.0

Add distilled water to the final volume of 100 mL, autoclave, and then store at room temperature.

9. Proteinase K (20 mg/ml), Store -20 °C**10. RNase A (10 mg/ml), Store -20 °C****11. Chloroform/Isoamyl alcohol (24:1)**

Chloroform: 24 mL

Isoamyl alcohol: 1 mL

Store at room temperature

12. Phenol/Chloroform/Isoamyl alcohol (25:24:1)

Phenol: 25 mL

Chloroform: 24 mL

Isoamyl alcohol: 1 mL

Store at 4 °C

II Reagents for Fluorescent in situ hybridization (FISH)

1. 6% H₂O₂ (Hydrogen peroxide) in 100% Ethanol

Ethanol: 94 ml

H₂O₂: 6 ml

2. PBST (Phosphate buffer saline with Tween 20)

1X PBS 50 ml

0.02% Tween20 1 ml

Store at room temperature

3. Hybridization buffer 500 µl

Stock reagent	Volume (µl)	Final concentration
5M NaCl	90	900 mM
1M Tris	10	20 mM
Formamide	150	30%
5% SDS	0.5	0.01%
H ₂ O	249.5	

4. Hybridization buffer and probe 500 µl

Stock reagent	Volume (µl)	Final concentration
5M NaCl	90	900 mM
1M TRIS	10	20 mM
Formamide	150	30%
10 µM Probe	5	100 nM
5% SDS	0.5	0.01%
Distilled water	244.5	

III Reagents for PCR

1. Primers were used in PCR amplification

No.	Primer name	Sequence (5'-3')
1	AV1 (Forward)	GTTGAAGGTACTTATTCCC
2	AV1 (Reverse)	TATTAATACGGTTGTAAACGC

2. PCR reaction mixture

Components	Volume (μ l)	Final concentration
10X PCR buffer, Minus Magnesium Chloride	2	1X
10 mM dNTP mixture	0.4	0.2 mM each
50 mM Magnesium Chloride	0.6	1.5 mM
10 μ M forward primer	0.5	0.5 μ M
10 μ M reverse primer	0.5	0.5 μ M
Template DNA	2	
<i>Taq</i> DNA Polymerase	0.1	
Sterile Distilled water	13.9	

3. PCR condition

PCR program	Temperature ($^{\circ}$ C)	Time (min)
Initial denaturation	94	5
Denaturation	94	0.4
Annealing	55	0.4
Extension	72	0.4
Final extension	72	5

} 35 cycles

Gel electrophoresis of cassava and whitefly

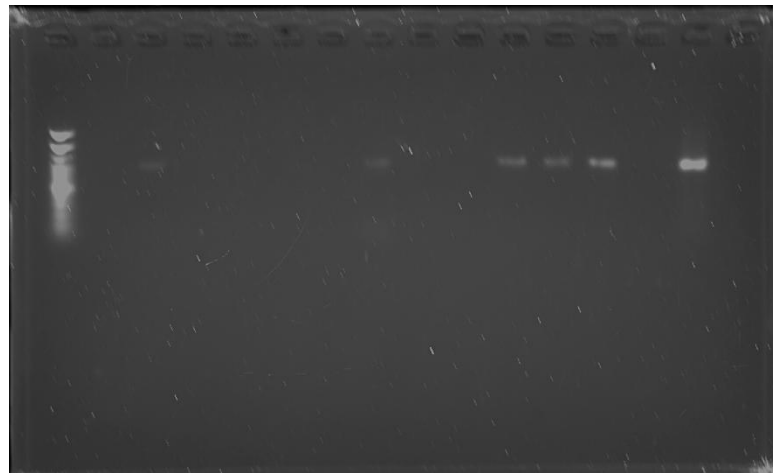


Figure 1. Positive and negative results of SLCMV virus testing in cassava.

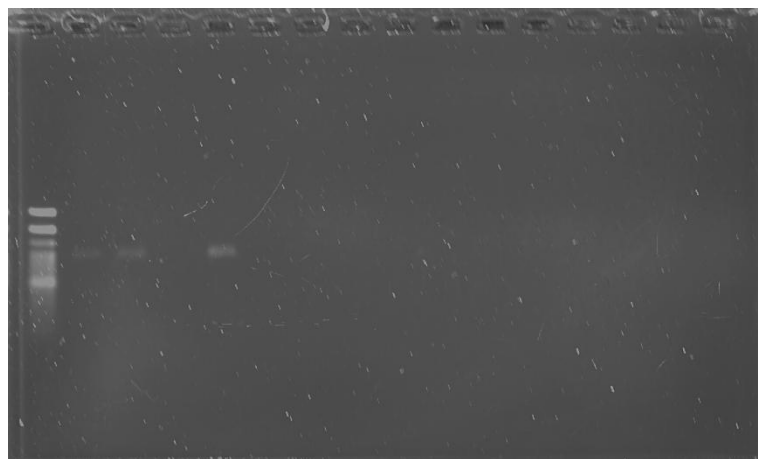


Figure 2. Positive and negative results of SLCMV virus testing in whiteflies.

Trichomes of six cassava cultivars

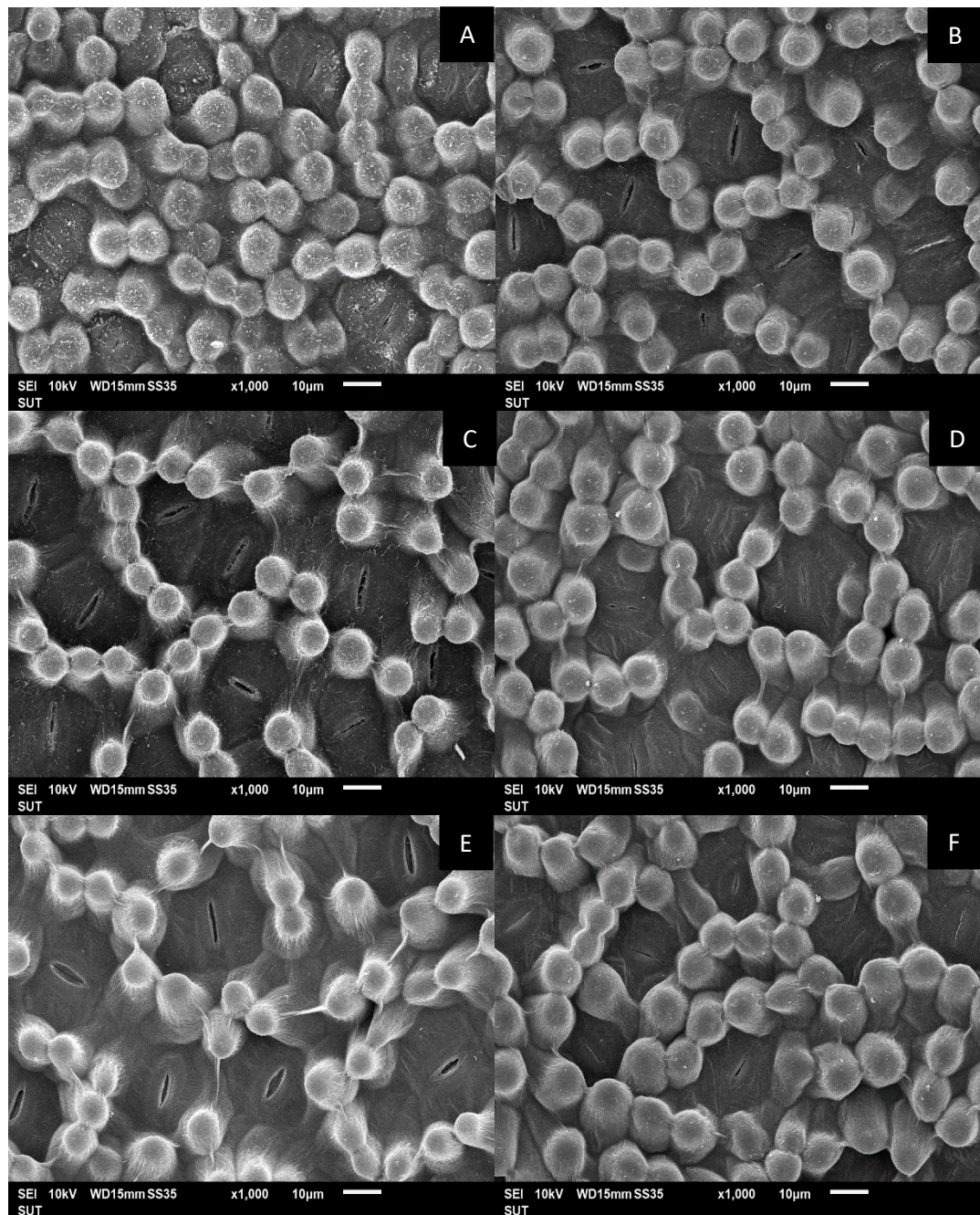


Figure 3. Trichomes of six cassava cultivars were observed through scanning electron microscopy (SEM) at 1000x magnification. Images show the leaf surface of (A) Huaybong 60, (B) CMR 89, (C) Kasetart 50, (D) Rayong 72, (E) Rayong 4, and (F) Pirun 2