

Appendix A

Instrument and Apparatus

All instruments and apparatus used during the course of research are as following:

Centrifuges:

- ❖ Avanti™ J-251 and J2-M1 Centrifuge (Beckman, USA)
 - Rotor JA 14 and JA 20
- ❖ Centrifuge 5417R and 5415D (Eppendorf®, Germany)
- ❖ L-80 Ultracentrifuge (Beckman, USA)
 - Rotor 80Ti
- ❖ Mini Spin Plus Centrifuge (Eppendorf®, Germany)

Electrophoresis apparatus :

- ❖ Power PAC 300 (Bio-Rad, USA)
- ❖ Mini-PROTEAN® Cell (Bio-Rad, USA)
- ❖ Mini Trans-Blot® Electrophoresis Transfer Cell (Bio-Rad, USA)

Other equipments and apparatus:

- ❖ AlphaImager 2200 Gel Documentation System (Alpha Innotech, USA)
- ❖ Arium® 611 Water Purification System (Sartorius, UK)
- ❖ Autoclave machine (TOMY, Japan)
- ❖ Balances or Weigher
- ❖ Beakers (50 ml, 100 ml, 250 ml, 500 ml, 1000 ml)
- ❖ 3D Belly Dancer (Rocky, USA)

- ❖ BioPhotometer (Eppendorf[®], Germany)
- ❖ Bunsen Burner
- ❖ Centrifuge tubes, round bottom (Nalgene, USA)
- ❖ Ultracentrifuge tubes, fit to 80Ti rotor (Beckman, USA)
- ❖ Miracloth (Calbiochem, USA)
- ❖ Dry bath
- ❖ Drying Oven (Memmert, Germany)
- ❖ Bottles (50 ml, 100 ml, 250 ml, 500 ml, 1000 ml) (Schott Duran, UK)
- ❖ Environmental Shaker
- ❖ Filter Papers
- ❖ -20 °C Freezer
- ❖ -80 °C Freezer
- ❖ Fume Hood
- ❖ GENios Multimode Research Reader (Tecan, Austria)
- ❖ Gloves
- ❖ Ice Maker
- ❖ 37 °C Incubator (Memmert, Germany)
- ❖ Innova[™] 2000 Platform Shaker (New Brunswick Scientific, UK)
- ❖ Innova[™] 3100 Water Bath Shaker (New Brunswick Scientific, UK)
- ❖ Liquid Nitrogen and Tank
- ❖ Magnetic Stirrer
- ❖ Microcentrifuge Tubes (0.5 ml and 1.5 ml)
- ❖ Microwave Oven (Panasonic, Malaysia)
- ❖ Paper Towels
- ❖ Petri Dishes (75mm)
- ❖ pH Metre (Hanna, Malaysia)

- ❖ Pipetman™ Pipettes (Gilson, France)
- ❖ Pipette Tips
- ❖ Planting Materials (including soils, fertilisers, planting pots and watering pots)
- ❖ 4 °C Refrigerator (Panasonic, Malaysia)
- ❖ Funnel (100 ml)
- ❖ Super Blender (National, Japan)
- ❖ Syringe Filters (0.22 µm)
- ❖ Syringes and Needles
- ❖ 15 ml and 50 ml Tubes (Falcon, Becton Dickinson, USA)
- ❖ 50 ml Tubes (Grenier Bio-One, Germany)
- ❖ Universal Bottles (30 ml)
- ❖ Vortex Mixer (Mika, USA)
- ❖ Wash Bottles
- ❖ Water Bath
- ❖ Water Distiller

Appendix B

General Media and Solutions

Chloramphenicol (34 µg/ml)

34 mg in 1ml ethanol

Filter sterilize through 0.22 µm filter

Store at -20 °C

70 % Ethanol (100 ml)

70 ml Ethanol

30 ml dH₂O

Store at room temperature

Kanamycin (sulfate) (30 µg/ml)

30 mg/ml in deionized water.

Filter sterilize and store at -20 °C.

100 mM IPTG (isopropyl-D-thiogalactopyranoside)

2.38 g IPTG in 100 ml deionized water.

Filter sterilize and store at -20 °C.

LB (Luria-Bertani) Agar (1 L)

20 g LB Agar powder

Add dH₂O to final volume

Autoclave at 121 °C and 15 psi for 15 minutes

Store at room temperature

LB (Luria-Bertani) Broth (1 L)

20g LB Broth powder

Add dH₂O to final volume

Autoclave at 121 °C and 15 psi for 15 minutes

Store at room temperature

1.0 M Sodium Phosphate Buffer (pH 7.4)

61.0 ml 1M Na₂HPO₄

39.0 ml 1M NaH₂PO₄

Phosphate-Buffered Saline (PBS) (pH 7.5)

8.0 g NaCl

0.2 g KH₂PO₄

2.9 g Na₂HPO₄·12H₂O

0.2 g KCl

Add dH₂O

Adjust to pH 7.4

Top up with dH₂O to final volume

Store at 4 °C

50 mM Tris-HCl (pH 7.5)

50 mM Tris base

Adjust to pH 7.5 using HCL

Appendix C

CMV purification protocol

Buffer A

0.5 M Sodium Citrate, pH 7.0

5 mM EDTA

0.5 % Thioglycolic acid (added just before use)

Store at 4 °C

Buffer B

5 mM Sodium Borate, pH 9.0

5 mM EDTA

2 % Triton X-100

Store at 4 °C

Cushion II

5 mM Sodium Borate, pH 9.0

5 mM EDTA

10 % Sucrose

Store at 4 °C

Buffer C

5 mM Sodium Borate, pH 9.0

5 mM EDTA

Autoclave at 121 °C and 15 psi for 20 minutes

Store at 4 °C

Appendix D

Plant extraction protocol

Extraction Buffer, pH 7.4

0.5 M Tris-HCl

30% w/v Sucrose

0.2% v/v SDS

2% β -mercaptoethanol

Tris-Phenol, pH 7.5

Commercially available

Ammonium Acetate in Absolute Methanol

100 mM Ammonium Acetate in 500 ml methanol

Acetone

0.2% DTT

Protein Resuspension Buffer (pH 7.5)

20 mM Sodium Phosphate Buffer

2% w/v SDS

20% v/v Glycerol

Appendix E

Solutions for IMAC

Binding Buffer, pH 7.4

20 mM Sodium Phosphate Buffer (pH 7.4)

500 mM NaCl

20 mM Imidazole

Adjust pH to 7.4

Elution Buffer, pH 7.4

20 mM Sodium Phosphate Buffer (pH 7.4)

500 mM NaCl

500 mM Imidazole

Adjust pH to 7.4

Appendix F

Solutions for Polyacrylamide Gel Electrophoresis (PAGE)

30 % Acrylamide Solution

30.0 g Acrylamide:biacrylamide (37.5:1)

Store in dark at 4 °C

10 % Ammonium Persulphate (APS)

0.02 g Ammonium persulphate

200 µl dH₂O

Always freshly prepare

3X Sample Protein Buffer

187.5 mM Tris-HCl, pH 6.8

125.0 mM DTT

6 % SDS

30 % Glycerol

0.03 % Bromophenol Blue

Store at room temperature

1X Running Buffer

25 mM Tris Base

192 mM Glycine

0.1% SDS

Store at room temperature

12% Separating Gel

1.32 ml dH₂O

1.6 ml 30% Acrylamide Solution

1.0 ml 1.5M Tris-HCl pH 8.8

0.04 ml 10% SDS

0.04 ml 10% APS

4 µl TEMED

4% Stacking Gel Solution

0.36 ml dH₂O

0.095 ml 30% Acrylamide Solution

0.16 ml 0.5M Tris-HCl pH 6.8

10 µl 10% SDS

10 µl 10% APS

1 µl TEMED

Fixing Solution

50% Ethanol

10% Glacial Acetic Acid

40% dH₂O

Commasie Blue Staining Solution

40 % Methanol

7 % Acetic Acid

0.025 % Commasie brilliant blue R250

Store at room temperature

Destain Solution I

40 % Methanol

7 % Acetic Acid

Store at room temperature

Destain Solution II

5 % Methanol

7 % Acetic Acid

Store at room temperature

Silver Staining Solutions :

Fixative Solution

Ethanol 25 ml

Acetic Acid 5 ml

distilled water top up to 50 ml

Infiltrating Solution

Ethanol 15 ml

Sodium Acetate dihydrate 3.4 g

Glutaraldehyde 125 ul

Sodium Thiosulfate (penta) 0.1 g

Distilled water top up to 50 ml

Silver Solution

Silver Nitrate 0.05 g

Formaldehyde 10 ul

Distilled water top up to 50 ml

Staining Solution

Sodium Carbonate	1.25 g
Formaldehyde	5 ul
Distilled water	top up to 50 ml

Stopping Solution

EDTA (EDTA.Na.2H ₂ O)	0.73 g
Distilled water	top up to 50 ml

Conserving Solution

Glycerol	25 ml
Distilled water	top up to 50 ml

Appendix G

Solutions for Western and Dot blot Analysis

Protein Transfer Buffer

25 mM Tris base

192 mM Glycine

20 % Methanol

Store at 4 °C

Blocking Solution

5 % Skim milk in TBS buffer

Penta-Anti His IgG Mouse Antibodies

1/2000 Dilution in TBS Buffer

Secondary Antibody Solution

1:30,000 Dilution of Alkaline phosphatase-conjugated anti-mouse IgG in Blocking Solution

1X Tris-Buffered Saline (TBS) Buffer

8.0 g NaCl

0.2 g KCl

3.0 g Tris base

Adjust pH to 7.4

Autoclave at 121 °C and 15 psi for 20 minutes

Store at room temperature

1X TBST Buffer

0.05 % Tween 20 in 1X TBS

Adjust pH to 7.4

Autoclave at 121 °C and 15 psi for 20 minutes

Store at room temperature

BCIP Solution

1 tablet in 5 ml of BCIP solution

Appendix H

Solutions for Enzyme-Linked Immunosorbent Assay (ELISA)

Blocking Solution

5 % Skim milk in 1X PBS

Coating Buffer

1.59 g Na_2CO_3

2.93 g NaHCO_3

0.2 g NaN_3

Adjust pH to 9.6

Add dH₂O to 1 L

Store at 4 °C

General Extraction Buffer (GEB)

1.3 g Na_2SO_3 (anhydrous)

20.0 g PVP 40

0.2 g NaN_3

2.0 g Powdered egg (chicken) albumin, grade 2

Dissolve with 1X PBST to 1 L

0.05 % Tween 20

Adjust pH to 7.4

Store at 4 °C

ECI Buffer (1 L)

1.0 g BSA

20.2 g PVP 40

0.2 g NaN_3

Dissolve in 1X PBST to 1 L

Adjust pH to 7.4

Store at 4 °C

1X Phosphate-Buffered Saline (PBS)

8.0 g NaCl

0.2 g KH_2PO_4

2.9 g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$

0.2 g KCl

Add dH₂O

Adjust to pH 7.4

Top up with dH₂O to final volume

Store at 4 °C

1X PBST

0.05 % Tween 20 in 1X PBS

Store at 4 °C

1X PNP Buffer

0.01 g MgCl₂

0.02 g NaN₃

0.03 97.0 ml dietholamine

0.04 Adjust pH to 9.8

Add dH₂O to final volume

Store at 4 °C

PNP Solution

1 PNP tablet

5 ml 1X PNP buffer

Always freshly prepare

Appendix I

Protocol 1

Buffer I, Chloroform and 2% β -mercaptoethanol added to 20g fresh leaves



Blend till completely homogenize



Centrifuge at 15,000g (4 °C) for 10 minutes



Filter aqueous phase with damp miracloth and collect into a beaker



Add 10 PEG : 90 ml of collected aqueous phase



Stir for 45 minutes at 4 °C



Centrifuge again at 15,000g (4 °C) for 10 minutes



Decant supernatant completely



Pellet left to dry



Resuspend in Protein Resuspension Buffer overnight at 4 °C

Appendix J

Protocol 2 (small scale)

Homogenize 50 g plant tissues in 500 ml Extraction buffer

↓
Transfer homogenate to 250 ml centrifuge tube

↓
Add 2 M PMSF

↓
Incubate 1 hour at 4 °C with mixing

↓
Centrifuge 5 minutes , 50,000 rpm at 4 °C

↓
Transfer supernatant to new centrifuge tube and place on ice

↓
Resuspend the pellet in 500ml extraction buffer

↓
Add 2 mM PMSF

↓
Incubate as above

↓
Spin as above, combine with the supernatant with the first

↓
Resuspend the pellet in 500ml extraction buffer

↓
Add 500 ml acetone

↓
Incubate as above

↓
Spin to collect protein

↓
Resuspend pellet with Protein Resuspension Buffer

Extraction Buffer

0.1 M Tris HCl pH 8.3

0.5 M NaCl

5 mM DTT

5 mM EDTA

Store at 4 °C

40 mM PMSF

6.97 mg PMSF in 1 ml absolute Ethanol

Store at -20 °C