APPENDIX-I

List of Instruments

1. **Autoclave (Horizontal Rectangular Steam Sterilizer)**- Yorco, Ghaziabad, India
2. **Chlorophyll Meter**- CCM200, Opti Sciences, Hudson, NH, USA
3. **Confocal Microscope**- Zeiss LSM510 meta GmbH, Germany
4. **Confocal Microscope**- Zeiss LSM510 meta GmbH, Germany
5. **Deionized Water Purification System**- SA 67120, Millipore, Molsheim, France
6. **DNA Sequencer**- ABI PRISM™ 310 Genetic Analyzer or ABI 3130xl Genetic Analyzer (Applied Biosystems, USA)
7. **DNA/RNA Electrophoresis**- Bangalore GeNei, India; Bio-Rad, USA
8. **Drying Oven**- MSW-215, MAC, Delhi, India
10. **Gas Chromatography**- Agilent Technologies, CA, USA
11. **Gel Documentation System**- Alpha DigiDoc, Alpha Innotech, CA, USA; Bio Rad,
12. **Growth Chamber**- Kaleidoscope, Bangalore, India
13. **HH2 Moisture Meter**- Delta-T Devices Ltd. Cambridge, UK
14. **High Performance Liquid Chromatography**- LC-20AT pump, DGU-20A5 degasser, CBM-20A array detector, Shimadzu, Kyoto, Japan
15. **Hybridization Chamber**- 5895, Amersham, Life Sciences, UK
16. **Incubated Shaker**- Innova 4230, New Brunswick Scientific, USA
17. **Infra Red Gas Analyzer**- Li-COR, Lincoln, NE, USA
18. **Laminar Air Flow Cabinet**- 15000, Klenzaids, Bioclean Devices, India
19. **Light Microscope**- Labophot, Nicon Corp., Japan
20. **Lyophilizer**- TFD8503, Ilshin Lab Co. Ltd., Korea
21. **Magnetic Stirrer**- 1199/2, Jain Scientific, India
22. **Microfuge**- D-37520, Osterode am Harz, Germany
23. **Microscope** - ZIESS, Axio Imager, USA
24. **Multimode Microplate Reader**- Synergy™ HT, Winooski, VT, USA
25. **Nanodrop**- ND-1000, UV/VIS, USA
26. **PCR Machine**- S 1000™; Bio-Rad, Hercules, CA, USA; GeneAmp® PCR System 9700 (Applied Biosystems, USA).
27. **pH Meter** - Cyberscan 510<sup>PC</sup>, Eutech Instruments Ltd., Singapore
28. **Rapid Visco-Analyser (RVA)** - 3-D, Newport Scientific Co. Ltd, Narrabeen, Australia
29. **Real Time PCR** - MX 3000P, Stratagene
30. **Refrigerated Centrifuge** - 3K30, Sigma, Labozentrfuzen, Germany; S 1000<sup>TM</sup>, Bio-Rad, Hercules, CA, USA
31. **Rocker** - Rocker-100, GeNei, Bangalore, India
32. **Scanning Electron Microscope** - S-3400N, Hitachi, Japan
33. **Spectrophotometer** - SPECORD 200, Analytik Jena, Germany; System 9700, Applied Biosystems, USA
34. **Speed Vac Concentrator** - DNA120-230, Thermo Scientific, USA
35. **Thermal Cycler** - iCycler, Bio Rad, USA
36. **Ultra Low Temperature Freezer** - U 535, New Brunswick Scientific, USA
37. **UV Cross Linker** - Hoefer UVC 500, Amersham Biosciences Inc., USA
38. **UV Transilluminator** - TFX-35M
39. **Vacuum Manifold** - Millipore corp., USA
40. **Water Bath** - Ecoline RE 204, Lauda, Germany
APPENDIX-II

MS (Murashige and skoog 1962) Basal Media Preparation

To Make One liter of Media with dH₂O

- Major salt (MS) stock: 100 ml
- Vitamin stock (VS): 10 ml
- Minor elements (ME) stock: 1 ml
- NH₄NO₃ (Ammonium nitrate) stock: 25 ml
- KNO₃ (Potassium nitrate) stock: 25 ml

Stock Solutions Preparations:

**MS Stock: 3 litres (Added Stepwise)**

- Na₂EDTA₂H₂O: 1.116 g
- FeSO₄.7H₂O: 0.834 g
- MgSO₄.7H₂O: 11.1 g
- CaCl₂.2H₂O: 13.2 g
- KH₂PO₄: 5.1 g

**VS Stock: 200 ml**

- Glycine: 40 mg
- Meso-inositol: 2 g
- Nicotinic acid: 10 mg
- Pyridoxine: 10 mg
- Thiamine: 2.2 mg

**ME Stock: 100 ml**

- KI: 83 mg
- H₃BO₃: 620 mg
- MnSO₄.4H₂O: 2230 mg
- (If MnSO₄.5H₂O): 1690 mg
- ZnSO₄.7H₂O: 860 mg
- Na₂MoO₄.2H₂O: 25 mg
- CuSO₄.5H₂O: 2.5 mg
CoCl$_2$.6H$_2$O  2.5 mg

Ammonium Nitrate (NH$_4$NO$_3$) 500 ml:  33 g
Potassium Nitrate (KNO$_3$) 500 ml:  38 g

Sucrose:  2%

pH  5.7 (Adjusted by 0.1 N HCl and 0.1M NaOH)

Agar  0.8% (Agar was added after the pH was taken)

Media was autoclaved at 121°C, 15Psi (1.06 Kg m$^{-2}$s$^{-1}$) for 20 min and left overnight to solidify.

**DNA Isolation Solution**

**Composition of CTAB Extraction Buffer:**

a)  2% CTAB (w/v)

b)  100 mM Tris-Cl (pH = 8.0)

c)  20 mM EDTA (pH = 8.0)

d)  1.4 M NaCl

e)  1% PVP

**Stock solutions for CTAB extraction buffer:**

a)  2% CTAB (w/v):
    
    2 g CTAB in 100 ml d H$_2$O.

b)  100 mM Tris-Cl:
    
    Stock 1M (500 ml): 48.45 g Trizma base was dissolved in 400 ml dH$_2$O and pH was adjusted to 8 by 1 N HCl. The final volume was made upto 500 ml. Use 10 ml of 1M in 100 ml CTAB extraction buffer to get final concentration of 100 mM.

c)  20 mM EDTA:
    
    Stock 0.5 M (500 ml): 93.05 g of sodium EDTA (Na$_2$EDTA) was added to 300 ml of dH$_2$O, added few pellets of KOH, kept on stirrer then added 100 mM KOH drop wise till the pH reached upto 8 (EDTA dissolves completely at exactly at pH = 8). The final volume was made to 500 ml with dH$_2$O. Use
4 ml of this stock in 100 ml CTAB extraction buffer to get final concentration of 20 mM.

d) 1.4 M NaCl:  
8.19 g of NaCl was dissolved in 100 ml CTAB extraction buffer.

e) 1% PVP:  
1 g PVP was dissolved in 100 ml CTAB extraction buffer.

All stocks were autoclaved prior to use

**TE buffer:** 10 mM Tris-Cl (pH = 8) and 1 mm EDTA (pH = 8).

**RNase A (10 mg ml⁻¹):** Dissolved RNase A 10 mg in one ml of 10 mM Tris-Cl (pH = 7.5) and 15 mM NaCl. Heated at 100°C for 15 min. Cooled to RT and stored at -20°C for further use.

**3 M Sodium acetate (pH = 4.8):** Dissolved 408.1 g of CH₃COONa.3H₂O in 800 ml dH₂O. Adjusted the pH to 4.8 with CH₃COOH (glacial acetic acid) and final volume was made to 1 l with dH₂O.

**Media for Bacterial Growth**

**Luria Bertani (LB) media 1 litre:**

- Tryptone 10 g
- NaCl 10 g
- Yeast extracts 5 g

The above components were dissolved in dH₂O and final volume was made to one liter. pH was set to 7.0 and 1.5% agar was added if the media was to solidify.

**Yeast Extract Mannitol (YEM) Media 1 litre:**

- Stock A 10 ml
- Mannitol 10 g
- Yeast Extract 1 g
- Stock B 0.8 ml
The above components were dissolved in dH₂O and final volume was made to one liter. pH was set to 7.0 and 1.5 % agar was added if the media was to solidify.

Stock A: 5 mg K₂PO₄ and 1 g NaCl dissolved in 100 ml dH₂O
Stock B: 246.4 mg MgSO₄.7H₂O dissolved in 100 ml dH₂O

Stock Solution for Chemicals used

Silver Thiosulfate (STS)
- Equal volume of sodium thiosulphate (Na₂S₂O₃.5H₂O) (2.382 g 100 ml⁻¹) and silver nitrate (AgNO₃) (0.204 g 100 ml⁻¹) were mixed to get a stock of 6 mM.
- The STS stock was kept at 4 °C for subsequent use.

Tri-iodo Benzoic acid (TIBA)
- 50 mg of TIBA was dissolved in 10 ml of ethanol and stored at 4°C for further use.

GUS Stock Preparation

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>For 10 ml</td>
<td></td>
</tr>
<tr>
<td>0.1 M Sod phosphate buffer (NaHPO₄), pH 7.0</td>
<td>9.49 ml</td>
</tr>
<tr>
<td>0.5 M EDTA, pH 8.0 with KOH</td>
<td>200 μl</td>
</tr>
<tr>
<td>0.1 M Ferrocyanide (in H₂O)</td>
<td>100 μl</td>
</tr>
<tr>
<td>0.1 M Ferricyanide (in H₂O)</td>
<td>100 μl</td>
</tr>
<tr>
<td>1 mM X-gluc (dissolved in Formamide)</td>
<td>100 μl</td>
</tr>
</tbody>
</table>

Antibiotic Stocks Solution

1. Cefotaxime: Dissolved 250 mg in 10 ml dH₂O
2. Carbenicillin: Dissolved 250 mg in 10 ml dH₂O
3. Hygromycin: Dissolved 250 mg in 10 ml dH₂O
4. Kanamycin: Dissolved 250 mg in 10 ml dH₂O
5. Streptomycin: Dissolved 250 mg in 10 ml dH₂O
6. Rifampicin: Dissolved 125 mg in 10 ml methanol (80%) 
7. Ampicillin: Dissolve 100 mg in 10 ml dH₂O
APPENDIX-III

Gene Sequences

*Macrophomina phaseolina* strain FIHB 1579 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

GenBank Accession Number: JQ257501.1

>gi|380749328|gb|JQ257501.1| Macrophomina phaseolina strain FIHB 1579 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

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Camellia sinensis thaumatin-like protein mRNA, complete cds

GenBank Accession Number: DQ444296.1

>gi|90995394|gb|DQ444296.1| Camellia sinensis thaumatin-like protein mRNA, complete cds

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CAGGCTGGAAGGCTACGTAAGGGCCAAAATACTGCTGATTCGATGAAACC
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TACCAGGCTACATAACCAATAATATTGTTGCACTGATGCGGCCCTGGGACCTGGG
CCTACAACTTTGTCCAAAGTCTTCAAGGATAGGTGCCCAGATGCTTATAGCTATCC
TCAGGATGATCACCAGTTGTCTTCACCTGCCCTGGTACCATTATGCTATTA
CCTTCTGCCCTTGA
Camellia sinensis Polyphenol oxidase (PPO) mRNA, complete cds

GenBank Accession Number: FJ656220.1

>gi|224037813|gb|FJ656220.1| Camellia sinensis polyphenol oxidase (PPO) mRNA, complete cds

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# Appendix-IV

<table>
<thead>
<tr>
<th>PGR combinations (mg l⁻¹)</th>
<th>√% Regeneration Response (%)*</th>
<th>No of shoots regenerated per explant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zeatin TIBA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.000</td>
<td>0.00</td>
<td>0.0 NSb</td>
</tr>
<tr>
<td>0.000</td>
<td>0.50</td>
<td>0.0 NSb</td>
</tr>
<tr>
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<td>1.25</td>
<td>0.2h</td>
</tr>
<tr>
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<td>2.50</td>
<td>0.0 NSb</td>
</tr>
<tr>
<td>0.050</td>
<td>0.00</td>
<td>0.0 NSb</td>
</tr>
<tr>
<td>0.050</td>
<td>0.50</td>
<td>0.0 NSb</td>
</tr>
<tr>
<td>0.050</td>
<td>1.25</td>
<td>0.1h</td>
</tr>
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<td>0.100</td>
<td>0.00</td>
<td>0.2h</td>
</tr>
<tr>
<td>0.100</td>
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<td>2.9c</td>
</tr>
<tr>
<td>0.250</td>
<td>0.00</td>
<td>2.4cd</td>
</tr>
<tr>
<td>0.250</td>
<td>0.50</td>
<td>2.2d</td>
</tr>
<tr>
<td>0.250</td>
<td>1.25</td>
<td>2.1de</td>
</tr>
<tr>
<td>0.250</td>
<td>2.50</td>
<td>4.1a</td>
</tr>
<tr>
<td>0.500</td>
<td>0.00</td>
<td>1.6ef</td>
</tr>
<tr>
<td>0.500</td>
<td>0.50</td>
<td>1.5ef</td>
</tr>
<tr>
<td>0.500</td>
<td>1.25</td>
<td>1.6ef</td>
</tr>
<tr>
<td>0.500</td>
<td>2.50</td>
<td>2.1de</td>
</tr>
<tr>
<td>3.000</td>
<td>0.00</td>
<td>0.8g</td>
</tr>
<tr>
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<td>0.50</td>
<td>0.8g</td>
</tr>
<tr>
<td>3.000</td>
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<td>2.5de</td>
</tr>
<tr>
<td>3.000</td>
<td>2.50</td>
<td>1.4f</td>
</tr>
</tbody>
</table>

Each value of % regeneration response and number of shoots regenerated per explant represents means of three replicates. Within a column, means followed by different letters indicated treatment differences are significant at $P \leq 0.05$. * Values in the column are the square root transformed values followed by real values within parentheses. NS: non-significant.