Appendix-A

Thesis related publication till March, 2013


**DATABASE DESIGNED:**

**Bambooinfoline**- a database for North Bengal Bamboo’s
(Availability:  [http://www.bamboodb.ind.in/](http://www.bamboodb.ind.in/))
Appendix-B

Buffers and chemicals used for DNA fingerprinting studies

*CTAB- buffer*

100mM Trizma Base (Sigma, Cat# T1503) (pH-8.0)
20mM EDTA (Merck India, Cat# 60841801001730) (pH-8.0)
1.4 M NaCl (Merck India, Cat#60640405001730)
2% (w/v) CTAB (Hexadecyl cetyl trimethyl ammonium bromide) (Sigma, Cat# H6269)
12.11g of molecular grade Trizma base was dissolved in 400 ml double distilled water, pH was adjusted to 8.0 and was divided into two parts of equal volume. To one part 7.44g EDTA was added and to the other part 81.8g NaCl and 20g CTAB. Both the parts were than mixed and the final volume was made up to 1000ml with double distilled water prior to autoclaving. The buffer was autoclaved at 121°C and 15 psi for 20 mins and stored at room temperature for further use.

*Note:* Add 1% PVP (Polyvinylpyrrolidone) (Sigma, Cat #P5288) and 0.3% β-mercaptoethanol (Sigma, Cat# M3148) just before use.

*5X TBE (Tris-borate-EDTA) buffer*

Trizma base (Sigma, Cat# T1503) = 27 gm
Boric acid (Sigma, Cat# 15663)= 13.75 gm
0.5M EDTA (pH 8.0)=1.86 gm
All the reagents were dissolved separately and finally mixed together and the final volume was made up to 1000ml with double distilled water prior to autoclaving. The buffer was autoclaved at 121°C and 15 psi for 20 mins and stored at room temperature for further use.

*1X TE:*

Tris-Cl (pH 8.0) (i.e. 10Mm) =0.6055gm
EDTA (pH 8.0) (i.e. 1mM) =0.186 gm
Both the reagents were dissolved separately and finally mixed together and the final volume was made up to 1000ml with double distilled water prior to autoclaving. The buffer was autoclaved at 121°C and 15 psi for 20 mins and stored at room temperature for further use.

*3M Sodium Acetate (Sigma, Cat# S9513):*

The required amount of sodium acetate i.e.12.31 g was dissolved in 50ml double distilled water prior to autoclaving. The solution was autoclaved at 121°C and 15 psi for 20 mins and stored at room temperature for further use.

*6X gel loading buffer:*

TYPE 3:

0.25% Bromophenol blue (Sigma, Cat# B0126)
0.25% Xylene cyanol FF (Sigma, Cat# X4126)
30% Glycerol (Merck India, Cat#61756005001730) in water
Store at 4°C.

*RNase A:*

The RNase A enzyme (Sigma, Cat# R4875) was dissolved at a concentration of 10mg/ml in 0.01M sodium acetate (Sigma, Cat# S9513) (pH 5.2). The solution was heated at 100°C for 15 minutes in a water bath and allowed to cool slowly to room temperature. The pH was adjusted by adding 1/10 volume of 1M Tris-Cl (pH 7.4) and stored at -20°C for further use.

*Note:* Both 0.01M sodium acetate and 1M Tris-Cl were prepared and autoclaved at 121°C and 15 psi for 20 mins prior to use.

A2
# Appendix-C

## Composition of Murashige and Skoog medium (Hi media Cat# PT018)

<table>
<thead>
<tr>
<th>Macroelements</th>
<th>Amount required (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH$_2$PO$_4$</td>
<td>170.00</td>
</tr>
<tr>
<td>KNO$_3$</td>
<td>1900.00</td>
</tr>
<tr>
<td>MgSO$_4$</td>
<td>180.54</td>
</tr>
<tr>
<td>NH$_4$NO$_3$</td>
<td>1650.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Microelements</th>
<th>Amount required (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoCl$_2$.6H$_2$O</td>
<td>0.025</td>
</tr>
<tr>
<td>CuSO$_4$.5H$_2$O</td>
<td>0.025</td>
</tr>
<tr>
<td>FeNaEDTA</td>
<td>36.70</td>
</tr>
<tr>
<td>H$_2$BO$_3$</td>
<td>6.20</td>
</tr>
<tr>
<td>KI</td>
<td>0.83</td>
</tr>
<tr>
<td>MnSO$_4$.H$_2$O</td>
<td>16.90</td>
</tr>
<tr>
<td>Na$_2$MoO$_4$.2H$_2$O</td>
<td>0.25</td>
</tr>
<tr>
<td>ZnSO$_4$.7H$_2$O</td>
<td>8.60</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vitamins</th>
<th>Amount required (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>2.00</td>
</tr>
<tr>
<td>Myoinositol</td>
<td>100.00</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>0.50</td>
</tr>
<tr>
<td>Pyridoxine HCl</td>
<td>0.50</td>
</tr>
<tr>
<td>Thyamine HCl</td>
<td>0.10</td>
</tr>
</tbody>
</table>

To it was added 3% sucrose (Hi media Cat# RM134), 0.332 mg/l CaCl$_2$ (Merck India Cat# 61764405001730). pH was adjusted to 5.6±0.1 and the volume was made up to 1000ml with double distilled water. It was then autoclaved for 20 minutes at 121ºC and 15psi and cooled and plant growth regulators are added (if any) as per requirement.

**Note:** In case of solid media agar (Hi media Cat#RM026) is added at the rate of 0.8%.
Appendix-D

Chemicals and buffers used for antioxidant profiling

Solvents:
1. Methanol (Merck India, Cat# 60600905001730)
2. Acetone (Merck India, Cat# 60001405001730)
3. Double distilled water

Standards:
1. Gallic acid (Himedia, Cat# RM233)
2. Quercetin (Himedia, Cat# RM6191)
3. Ascorbic acid (Himedia, Cat# CMS1014)
4. Cathechin (Sigma, Cat#C0567)

Total Phenol:
1. Folin Ciocalteu’s reagents (SRL, Cat# 062015)
2. Sodium Carbonate (Merck India, Cat# 61778705001730) (20%)

Total Flavonoid:
1. Sodium Nitrite (Himedia, Cat# RM417) (5%)
2. Aluminium Chloride (Sd Fine, Cat#) (10%)
3. Sodium hydroxide (Merck India, Cat# 6184305001730) (1mM)

Total Flavonol:
1. Aluminium Chloride (Sd Fine, Cat#) (2%)
2. Sodium acetate (Sigma, Cat# S9513) (5%)

Total Proanthocyanidin:
1. Vanillin (Himedia, Cat# RM616) (4%)
2. Hydrochloric acid (Merck India, Cat# 61762505001730)

DPPH scavenging activity:
1. DPPH (Himedia, Cat# RM2798)
2. Methanol (Merck India, Cat# 60600905001730)

Ferrous reducing power assay:
1. Phosphate buffer (0.2M) pH 6.6
   Potassium dihydrogen phosphate (Merck India, Cat# 60487305001730)
   Dipotassium hydrogen phosphate (Merck India, Cat#61788005001730)
2. Potassium ferrocyanide (Merck India, Cat# 61843605001730) (1%)
3. Trichloro acetic acid (Himedia, Cat# RM7570) (10%)
4. Ferric chloride (Himedia, Cat# RM1379) (0.1%)

Hydrogen peroxide scavenging activity:
1. Hydrogen peroxide (Merck India, Cat# 61765305001730) (2mM)
2. Phosphate buffer (pH 7.4)
   Potassium dihydrogen phosphate (Merck India, Cat#60487305001730)
Continues from previous page

Potassium hydroxide (Merck India, Cat#60503305001730)

HPLC analysis:
1. Methanol (HPLC grade) (Sigma, Cat# 65548)
2. Water (HPLC grade) (Sigma, Cat# 95304)
3. Rutin (Sigma, Cat# R5143)
4. Gallic acid (Sigma, Cat# 27645)
5. β- sitosterol (Sigma, Cat# 172286)
6. Quercetin (Sigma, Cat# Q4951)

In vivo testing:
1. Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6- pyrimidinetetrone) (Sigma, Cat#A7413)
2. Phosphate buffer (50mM) (pH 7.0)
3. EDTA (0.1 mM)
4. NADPH (1.5 mM) (Sigma, Cat# N5130)
5. Glutathione (GSH) (0.01 mM) (Sigma, Cat#G4251)
6. Glutathione Reductase (0.24 U) (Sigma, Cat#G3664)
7. t-butyl hydro-peroxide (12mM)
8. Carbonate buffer (0.05M)
9. 30mM epinephrine (Sigma, Cat#E4250) in 0.05% acetic acid (Merck India, Cat#60006305001730)
10. 1, 1, 3, 3- tetramethoxy propane (Sigma, Cat#108383)
11. SDS (sodium dodecyl sulfate) (Sigma, Cat# 436143) (8.1%)
12. 20% acetic acid (Merck India, Cat#60006305001730)
13. 0.6% thiobarbituric acid (Sigma, Cat#T5500)
14. Butanol
15. Pyridine (Sigma, Cat# 270970)
16. Glibenclamide