CHAPTER III

MATERIALS & METHODS
3.1 PLANT MATERIAL

3.1.1 Ginger

The following three varieties of Ginger were used for in vitro studies.

3.1.1.1 Variety - Suprabha (PGS-35)

YEAR OF RELEASE : 1988

INSTITUTE : High Altitude Research Station, Orissa University of Agriculture & Technology, Pottangi - 764 039, Orissa.

PEDIGREE : A clonal selection (PGS-35) from Kundulli local.

AREAS OF ADOPTION : All the ginger growing tracts of Orissa.

MATURITY : 229 days.

AVERAGE YIELD : 18.6 tonnes fresh rhizomes / ha

POTENTIAL YIELD : 22.8 tonnes fresh rhizomes / ha

QUALITY ATTRIBUTES : Oleoresin : 8.9% Essential : 1.9% oil

MORPHOLOGICAL CHARACTERS

Colour of aerial shoot : Green

Plant height (cm) : 51.8

Leaf length/ breadth (cm) : 17.0 / 1.9

No. of tillers per clump : 11.1

No. of leaves per tiller : 14.4

Colour of rhizome core : Whitish Yellow

Weight of fresh rhizomes per clump (g) : 200

Dry recovery % : 20.5

Crude fibre (%) : 4.4
REACTION TO MAJOR PESTS AND DISEASES

Rhizome rots (Pythium aphanidermatum) : Susceptible
Bacterial wilt (Pseudomonas solanacearum) : Susceptible
Leaf spot (Phylllosticta zingiberi) : Susceptible
Shoot borer (Conogethes punctiferalis) : Susceptible
Rhizome scales (Aspidiella hartii) : Susceptible

SPECIAL CHARACTERS

Plumpy flat rhizomes with bright glazy skin and brown scales. Suitable for both green and dry ginger. Performs well in hilly areas, drought prone areas and in both early and late sowing conditions.

3.1.1.2 Variety - Suruchi (PGS-19)

YEAR OF RELEASE : 1990

INSTITUTE : High Altitude Research Station, Orissa University of Agriculture & Technology, Pottangi - 764 039, Orissa.

PEDIGREE : A clonal selection (PGS-19) from Kundulli local.

AREAS OF ADOPTION : All the ginger growing tracts of Orissa.

MATURITY : 218 days.

AVERAGE YIELD : 11.6 tonnes fresh rhizomes / ha

POTENTIAL YIELD : 21.8 tonnes fresh rhizomes / ha

QUALITY ATTRIBUTES : Oleoresin : 10% Essential : 2.0%

MORPHOLOGICAL CHARACTERS

- Colour of aerial shoot: Green
- Plant height (cm) : 51.3
- Leaf length/ breadth (cm) : 17.7 / 1.9
- No. of tillers per clump : 11.8
No. of leaves per tiller : 13.5
Colour of rhizome core : Greenish Yellow
Weight of fresh rhizomes per clump (g) : 205
Dry recovery % : 23.5
Crude fibre (%) : 3.8

REACTION TO MAJOR PESTS AND DISEASES

Rhizome rots (Pythium aphanidermatum) : Susceptible
Bacterial wilt (Pseudomonas solanacearum) : Susceptible
Leaf spot (Phyllosticta zingiberi) : Susceptible
Shoot borer (Conogethes punctiferalis) : Susceptible
Rhizome scaler (Ampidiella hartii) : Susceptible

SPECIAL CHARACTERS

Fingers slender, cylindrical with prominent nodes and reddish brown scales. Performs well in irrigated, rainfed as well as late sown conditions.

3.1.1.3 Variety - Suravi


INSTITUTE : High Altitude Research Station, Orissa University of Agriculture & Technology, Pottangi - 764 039, Orissa.

PEDIGREE : Induced mutant (VK-3) from Rudrapur local.

AREAS OF ADOPTION : Throughout ginger growing areas in Orissa.

MATURITY : 225 days.

AVERAGE YIELD : 17.5 tonnes fresh rhizomes / ha

POTENTIAL YIELD : 21.6 tonnes fresh rhizomes / ha
QUALITY ATTRIBUTES

Oleoresin : 10.2%  Essential : 2.1% oil

MORPHOLOGICAL CHARACTERS

Colour of aerial shoot : Deep Green
Plant height (cm) : 62.0
Leaf length/ breadth (cm) : 15.3 / 1.6
No. of tillers per clump : 14.5
No. of leaves per tiller : 14.3
Colour of rhizome core : Deep Yellow

Weight of fresh rhizomes per clump (g) : 220
Dry recovery % : 23.0
Crude fibre (%) : 4.0

REACTION TO MAJOR PESTS AND DISEASES

Rhizome rots (Pythium aphanidermatum) : Susceptible
Bacterial wilt (Pseudomonas solanacearum) : Susceptible
Leaf spot (Phylllosticta zingiberi) : Susceptible
Shoot borer (Conogethes punciferalis) : Susceptible
Rhizome scales (Aspidiella hartii) : Susceptible

SPECIAL CHARACTERS

Plumpy, cylindrical rhizomes with dark glazy skin and deep brown scales. Can be grown both under irrigated and rainfed conditions.
3.1.2 Turmeric

The following varieties of Turmeric were utilised in the present investigation.

3.1.2.1 Variety - Krishna

<table>
<thead>
<tr>
<th>YEAR OF RELEASE</th>
<th>Clonal selection from Tekurpeta collection from Andhra Pradesh.</th>
</tr>
</thead>
<tbody>
<tr>
<td>INSTITUTE</td>
<td>Maharashtra Agricultural University, Kasba Digraj, MAHARASHTRA.</td>
</tr>
<tr>
<td>PEDIGREE</td>
<td>Maharashtra</td>
</tr>
<tr>
<td>AREAS OF ADOPTION</td>
<td>Maharashtra</td>
</tr>
<tr>
<td>CROP DURATION</td>
<td>240 days.</td>
</tr>
<tr>
<td>AVERAGE YIELD</td>
<td>9.2 tonnes fresh rhizomes / ha</td>
</tr>
<tr>
<td>POTENTIAL YIELD</td>
<td>11.8 tonnes fresh rhizomes / ha</td>
</tr>
<tr>
<td>QUALITY ATTRIBUTES</td>
<td>Curcumin : 2.8% Oleoresin :2.8% Essential oil : 2.0%</td>
</tr>
</tbody>
</table>

MORPHOLOGICAL CHARACTERS

<table>
<thead>
<tr>
<th>Colour of aerial shoot</th>
<th>Yellowish Green</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant height (cm)</td>
<td>118</td>
</tr>
<tr>
<td>Leaf length/ breadth (cm)</td>
<td>50/16</td>
</tr>
<tr>
<td>No. of tillers per clump</td>
<td>1.8</td>
</tr>
<tr>
<td>No. of leaves per clump</td>
<td>14.4</td>
</tr>
<tr>
<td>Yield of rhizomes per clump (g)</td>
<td>450</td>
</tr>
<tr>
<td>No. of mother rhizomes</td>
<td>1</td>
</tr>
<tr>
<td>Weight of mother rhizomes (g)</td>
<td>70.0</td>
</tr>
<tr>
<td>No. of primaries</td>
<td>6</td>
</tr>
<tr>
<td>Weight of primaries (g)</td>
<td>300</td>
</tr>
</tbody>
</table>
No. of secondaries : 7
Weight of secondaries : 175
Colour of rhizomes : Light yellow
Dry recovery (%) : 16.4

REACTION TO MAJOR PESTS AND DISEASES

Rhizome rots (Pythium graminicolum) : Moderately resistant
Leaf blotch (Taphrina maculana) : Moderately resistant
Leaf spot (Colletotrichum capsici) : Moderately resistant
Rhizome scales (Aspidiella hartii) :
Shoot borer (Conogethes punctiferalis) : Moderately resistant

SPECIAL CHARACTERS

Long plumpy fingers with light yellow rhizomes.
Moderately resistant to rhizome-fly.

3.1.2.2 Variety - BSR-1

YEAR OF RELEASE : 1986

INSTITUTE : Department of Spices & Plantation Crops,
Faculty of Horticulture,
T.N.A.U.,
Coimbatore - 641 003,
Tamil Nadu.

PEDIGREE : Selection (5378-3-1) from Erode Local irradiated with X-ray

AREAS OF ADOPTION : Tamil Nadu
CROP DURATION : 285 days.
AVERAGE YIELD : 28.7 tonnes fresh rhizomes / ha
POTENTIAL YIELD : 39.6 tonnes fresh rhizomes / ha
QUALITY ATTRIBUTES : Curcumin : 4.2% Oleoresin : 4.0%
Essential oil : 3.7%
MORPHOLOGICAL CHARACTERS

Colour of aerial shoot : Green
Plant height (cm) : 73.8
Leaf length/ breadth (cm) : 38.8 / 11.7
No. of tillers per clump : 3.9
No. of leaves per clump : 15.6
Yield of rhizomes per clump (g) : 805
No. of mother rhizomes : 2.7
Weight of mother rhizomes (g) : 185
No. of primaries : 9.1
Weight of primaries (g) : 455
No. of secondaries : 21.2
Weight of secondaries (g) : 170
Colour of rhizomes : Bright Yellow
Dry recovery (%) : 20.5

REACTION TO MAJOR PESTS AND DISEASES

Rhizome rots (Pythium graminicolum) : Susceptible
Leaf blotch (Taphrina maculans) : Susceptible
Leaf spot (Colletotrichum capsici) : Susceptible
Rhizome scales (Aspidiella hartii) : Susceptible
Shoot borer (Conogethes punctiferalis) : Susceptible

SPECIAL CHARACTERS

Rhizomes bright yellow in colour with short internodes. Suitable to drought prone, water logged and hilly areas as well as saline and alkaline areas.
3.1.2.3 Variety - Sugandham

**YEAR OF RELEASE**: 1982

**INSTITUTE**: Spices Research Station, Gujarat Agricultural University, Jagundhan - 382 701, GUJARAT.

**PEDIGREE**: A clonal selection from germplasm

**AREAS OF ADOPTION**: Gujarat.

**CROP DURATION**: 210 days.

**AVERAGE YIELD**: 15.0 tonnes fresh rhizomes / ha

**POTENTIAL YIELD**: 20.0 tonnes fresh rhizomes / ha

**QUALITY ATTRIBUTES**: Curcumin: 3.1% Oleoresin: 11% Essential oil: 2.7%

**MORPHOLOGICAL CHARACTERS**

- Colour of aerial shoot: Light Green
- Plant height (cm): 85
- Leaf length/breadth (cm): 47/15
- No. of tillers per clump: 2
- No. of leaves per clump: 7
- Yield of rhizomes per clump (g): 220
- No. of mother rhizomes: 2
- Weight of mother rhizomes (g): 22
- No. of primaries: 12
- Weight of primaries (g): 130
- No. of secondaries: 16
- Weight of secondaries (g): 88
Colour of rhizomes : Reddish Yellow

Dry recovery (%) : 23.3

**REACTION TO MAJOR PESTS AND DISEASES**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Tolerance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizome rots (Pythium graminicolum)</td>
<td>Moderately tolerant</td>
</tr>
<tr>
<td>Leaf blotch (Taphrina maculans)</td>
<td>Moderately tolerant</td>
</tr>
<tr>
<td>Leaf spot (Colletotrichum capsici)</td>
<td>Moderately tolerant</td>
</tr>
<tr>
<td>Rhizome scales (Aspidiella hartii)</td>
<td>Moderately tolerant</td>
</tr>
<tr>
<td>Shoot borer (Conogethes punctiferalis)</td>
<td>-</td>
</tr>
</tbody>
</table>

**SPECIAL CHARACTERS**

Thick and stout rhizomes with long internodes.

**3.1.2.4 Variety - Co-1**

**YEAR OF RELEASE** : 1982

**INSTITUTE** : Department of Spices & Plantation Crops, Faculty of Horticulture, T.N.A.U., Coimbatore - 641 003, Tamil Nadu.

**PEDIGREE** : Vegetative mutant (5307-1-1) by X-ray irradiation of Erode local.

**AREAS OF ADOPTION** : Tamil Nadu.

**CROP DURATION** : 285 days.

**AVERAGE YIELD** : 30.0 tonnes fresh rhizomes / ha

**POTENTIAL YIELD** : 35.0 tonnes fresh rhizomes / ha

**QUALITY ATTRIBUTES** : Curcumin : 3.2% Oleoresin : 6.7% Essential oil : 3.2%
### MORPHOLOGICAL CHARACTERS

<table>
<thead>
<tr>
<th>Character</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour of aerial shoot</td>
<td>Green</td>
</tr>
<tr>
<td>Plant height (cm)</td>
<td>50.8</td>
</tr>
<tr>
<td>Leaf length/ breadth (cm)</td>
<td>36.7 / 10.3</td>
</tr>
<tr>
<td>No. of tillers per clump</td>
<td>4.4</td>
</tr>
<tr>
<td>No. of leaves per clump</td>
<td>28.0</td>
</tr>
<tr>
<td>Yield of rhizomes per clump (g)</td>
<td>537</td>
</tr>
<tr>
<td>No. of mother rhizomes</td>
<td>2</td>
</tr>
<tr>
<td>Weight of mother rhizomes</td>
<td>56.7</td>
</tr>
<tr>
<td>No. of primaries</td>
<td>8.8</td>
</tr>
<tr>
<td>Weight of primaries</td>
<td>180.3</td>
</tr>
<tr>
<td>No. of secondaries</td>
<td>19.4</td>
</tr>
<tr>
<td>Weight of secondaries</td>
<td>138</td>
</tr>
<tr>
<td>Colour of rhizomes</td>
<td>Orange yellow</td>
</tr>
<tr>
<td>Dry recovery (%)</td>
<td>19.5</td>
</tr>
</tbody>
</table>

### REACTION TO MAJOR PESTS AND DISEASES

- **Rhizome rots (Pythium graminicolum)**: Susceptible
- **Leaf blotch (Taphrina maculans)**: Susceptible
- **Leaf spot (Colletotrichum capsici)**: Susceptible
- **Rhizome scales (Aspidiella hartii)**: Susceptible
- **Shoot borer (Conogethes punctiferalis)**: Susceptible

### SPECIAL CHARACTERS

Rhizomes big and orange yellow in colour. Suitable to drought prone, water logged and hilly areas as well as saline and alkaline areas.
3.1.2.5 Variety - Suguna (PCT-13)

YEAR OF RELEASE : 1991

INSTITUTE : National Research Centre for Spices (ICAR), Calicut - 673 012, Kerala.

PEDIGREE : A selection from the germplasm (PCT-13) collected from Andhra Pradesh.

AREAS OF ADOPTION : Kerala and Andhra Pradesh

CROP DURATION : 190 days.

AVERAGE YIELD : 29.3 tonnes fresh rhizomes / ha

POTENTIAL YIELD : 60.3 tonnes fresh rhizomes / ha

QUALITY ATTRIBUTES : Curcumin : 4.9% Oleoresin : 13.5% Essential oil : 6.0%

MORPHOLOGICAL CHARACTERS

Colour of aerial shoot : Green
Plant height (cm) : 107
Leaf length/ breadth (cm) : 46/12.3
No. of tillers per clump : 1.9
No. of leaves per clump : 12.8
Yield of rhizomes per clump (g) : 529
No. of mother rhizomes : 1.8
Weight of mother rhizomes (g) : 15.0
No. of primaries : 9.3
Weight of primaries (g) : 210.0
No. of secondaries : 26.4
Weight of secondaries (g) : 337.4
Colour of rhizomes : Orange
Dry recovery (%) : 20.4*

REACTION TO MAJOR PESTS AND DISEASES

Rhizome rots (Pythium graminicolum) : Moderately Tolerant
Leaf blotch (Taphrina maculans) : Moderately Tolerant
Leaf spot (Colletotrichum capsici) : Susceptible
Rhizome scales (Aspidiella hartii) : Susceptible
Shoot borer (Conogethes punctiferalis) : Susceptible

SPECIAL CHARACTERS

A short duration variety with thick and plumpy rhizomes and high yield potential. Field tolerant to rhizome rot.

3.1.2.6 Variety - Roma (PTS-10)

YEAR OF RELEASE : 1988
INSTITUTE : High Altitude Research Station, Orissa University of Agriculture & Technology, Pottangi - 764 039, Orissa.
PEDIGREE : A clonal selection from T. Sunder (PTS-10)
AREAS OF ADOPTION : In the states of Orissa, Tamil Nadu, Himachal Pradesh, Andhra Pradesh & Kerala.
CROP DURATION : 250 days.
AVERAGE YIELD : 20.7 tonnes fresh rhizomes / ha
POTENTIAL YIELD : 40.0 tonnes fresh rhizomes / ha
QUALITY ATTRIBUTES : Curcumin : 9.3% Oleoresin : 13.2% Essential oil : 4.2%
MORPHOLOGICAL CHARACTERS

Colour of aerial shoot : Green
Plant height (cm) : 74.2
Leaf length/ breadth (cm) : 38.5 / 12.8
No. of tillers per clump : 3.4
No. of leaves per clump : 25.5
Yield of rhizomes per clump (g) : 260
No. of mother rhizomes : 2.8
Weight of mother rhizomes (g) : 26.3
No. of primaries : 15.0
Weight of primaries (g) : 147.3
No. of secondaries : 22.0
Weight of secondaries (g) : 86.4
Colour of rhizomes : Orange Yellow
Dry recovery (%) : 31.0

REACTION TO MAJOR PESTS AND DISEASES

Rhizome rots (Pythium graminicolum) : -
Leaf blotch (Taphrina maculans) : Tolerant
Leaf spot (Colletotrichum capsici) : Tolerant
Rhizome scales (Aspidiella hartii) : Tolerant
Shoot borer (Conogethes punctiferalis) : Susceptible

SPECIAL CHARACTERS

Performs well under late sown conditions. Suitable for both irradiated and rainfed conditions. Ideally suited for hilly areas.
3.1.2.7 Variety - Sudarshana (PCT-14)

YEAR OF RELEASE : 1991

INSTITUTE : National Research Centre for Spices (ICAR), Calicut - 673 012, KERALA.

PEDIGREE : A selection from the germplasm (PCT-13) collected from Singhat, Manipur.

AREAS OF ADOPTION : Kerala and Andhra Pradesh

CROP DURATION : 190 days.

AVERAGE YIELD : 28.8 tonnes fresh rhizomes / ha

POTENTIAL YIELD : 54.9 tonnes fresh rhizomes / ha

QUALITY ATTRIBUTES : Curcumin : 7.9% Oleoresin :15.0% Essential oil : 7.0%

MORPHOLOGICAL CHARACTERS

Colour of aerial shoot : Green
Plant height (cm) : 136
Leaf length/ breadth (cm) : 37.4/12.1
No. of tillers per clump : 1.9
No. of leaves per clump : 14.3
Yield of rhizomes per clump (g) : 565
No. of mother rhizomes : 1.8
Weight of mother rhizomes (g) : 17.0

No. of primaries : 10.1
Weight of primaries (g) : 236.0
No. of secondaries : 20.1
Weight of secondaries (g) : 310.0
Colour of rhizomes : Orange
Dry recovery (%) : 20.6*

REACTION TO MAJOR PESTS AND DISEASES

- Rhizome rots (Pythium graminicolum) : Moderately Tolerant
- Leaf blotch (Taphrina maculans) : Moderately Tolerant
- Leaf spot (Colletotrichum capsici) : Susceptible
- Rhizome scales (Ampidiella hartii) : Susceptible
- Shoot borer (Conogethes punctiferalis) : Susceptible

SPECIAL CHARACTERS

A high yielding, high quality short duration turmeric with thick plumpy rhizomes. Field tolerant to rhizome rot.

3.1.2.8 Variety - Suroma (PTS-24)

YEAR OF RELEASE : 1989

INSTITUTE : High Altitude Research Station, Orissa University of Agriculture & Technology, Pottangi - 764 039, Orissa.

PEDIGREE : A clonal selection from T. Sunder (PTS-24)

AREAS OF ADOPTION : In the states of Orissa, Tamil Nadu and Himachal Pradesh.

CROP DURATION : 253 days.

AVERAGE YIELD : 20 tonnes fresh rhizomes / ha

POTENTIAL YIELD : 44.9 tonnes fresh rhizomes / ha

QUALITY ATTRIBUTES :
- Curcumin : 9.3% Oleoresin : 13.1%
- Essential oil : 4.4%
MORPHOLOGICAL CHARACTERS

Colour of aerial shoot : Green
Plant height (cm) : 76.5
Leaf length/ breadth : 40.4 / 13.7 (cm)
No. of tillers per clump : 2.6
No. of leaves per clump : 18.2
Yield of rhizomes per clump (g) : 262
No. of mother rhizomes : 2.7
Weight of mother rhizomes (g) : 23.0
No. of primaries : 16.0
Weight of primaries (g) : 143.6
No. of secondaries : 30.0
Weight of secondaries (g) : 95.4
Colour of rhizomes : Light orange yellow
Dry recovery (%) : 26.0

REACTION TO MAJOR PESTS AND DISEASES
Rhizome rots (Pythium graminicolum) : -
Leaf blotch (Taphrina maculans) : Tolerant
Leaf spot (Colletotrichum capsici) : Tolerant
Rhizome scales (Aspidiella hartii) : Tolerant
Shoot borer (Conogethe punctiferalis) : -

SPECIAL CHARACTERS

A variety with high curcumin round and plumpy mother rhizomes and slender fingers with dark brown scales, light orange yellow flesh and reddish brown skin.
3.1.2.9 Variety - Suvarna (PCT-8)

YEAR OF RELEASE : 1987

INSTITUTE : National Research Centre for Spices (ICAR), Calicut - 673 012, KERALA.

PEDIGREE : A selection from germplasm (PCT-8) collected from Assam.

AREAS OF ADOPTION : Kerala, Karnataka and Andhra Pradesh

CROP DURATION : 200 days.

AVERAGE YIELD : 17.4 tonnes fresh rhizomes / ha

POTENTIAL YIELD : 43.5 tonnes fresh rhizomes / ha

QUALITY ATTRIBUTES : Curcumin : 4.0% Oleoresin : 13.5% Essential oil : 7.0%

MORPHOLOGICAL CHARACTERS

- Colour of aerial shoot : Green
- Plant height (cm) : 69.4
- Leaf length/breadth (cm) : 66.4 / 17.4
- No. of tillers per clump : 2.6
- No. of leaves per clump : 16.4
- Yield of rhizomes per clump (g) : 460
- No. of mother rhizomes : 3.0
- Weight of mother rhizomes (g) : 34
- No. of primaries : 21
- Weight of primaries (g) : 232
- No. of secondaries : 28.2
- Weight of secondaries : 201
Colour of rhizomes: Deep Orange

Dry recovery (%): 26.0

REACTION TO MAJOR PESTS AND DISEASES

Rhizome rots (Pythium graminicolum): Field tolerant
Leaf blotch (Taphrina maculans): Field tolerant
Leaf spot (Colletotrichum capsici): Field tolerant
Rhizome scales (Aspidiella hartii): Field tolerant
Shoot borer (Conogethes punctiferalis): Field tolerant

SPECIAL CHARACTERS

A high yielding, short duration turmeric with deep orange coloured rhizome.

3.2 PREPARATION OF MEDIA

3.2.1 Stock Preparation

1. All chemicals to be used for the preparation of stocks were fresh and stored as directed by the manufacturers.

2. Stock solutions were kept away from strong sunlight and not kept in temperatures above room temperature for longer than a working day.

3. The containers used for storing the stock solutions were of a type that can be easily poured from and have a tight fitted lid.

4. Before actual use the solutions were visually screened to check for chemical precipitation (where the chemical has separated out of solution).
Colour of rhizomes : Deep Orange
Dry recovery (%) : 26.0%

REACTION TO MAJOR PESTS AND DISEASES

Rhizome rots (Pythium graminicolum) : Field tolerant
Leaf blotch (Taphrina maculans) : Field tolerant
Leaf spot (Colletotrichum capsici) : Field tolerant
Rhizome scales (Aspidiella hartii) : Field tolerant
Shoot borer (Conogethes punctiferalis) : Field tolerant

SPECIAL CHARACTERS

A high yielding, short duration turmeric with deep orange coloured rhizome.

3.2 PREPARATION OF MEDIA

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3. The containers used for storing the stock solutions were of a type that can be easily poured from and have a tight fitted lid.

4. Before actual use the solutions were visually screened to check for chemical precipitation (where the chemical has separated out of solution).
5. The preparation of the individual stock is important. Care was taken in the weighing of the individual chemicals, the final volume adjustment and the complete dissolving of the chemical.

6. If heat was required to dissolve certain chemicals then it was by gradual and of low heat intensity. If an acid or an alkaline was used for dissolving chemicals, then again as weak a solution as possible was to be used.

To prepare stock solutions of Auxins, Cytokinins, Gibberellins and Vitamins, 100mg of the compound was dissolved in 2-5ml of the solvent. Once the growth regulator powder got completely dissolved, the volume was made up to 100ml with double distilled water.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Indole-3-Butyric Acid</td>
<td>Ethyl Alcohol / 1.0N NaOH</td>
</tr>
<tr>
<td>2. Indole-3-Acetic Acid</td>
<td>- do -</td>
</tr>
<tr>
<td>3. 2,4 Dichloro Phenoxy Acetic Acid</td>
<td>- do -</td>
</tr>
<tr>
<td>4. Alpha Naphthalene Acetic Acid</td>
<td>1.0N NaOH</td>
</tr>
<tr>
<td>5. 2IP</td>
<td>1.0N NaOH</td>
</tr>
<tr>
<td>6. Kinetin</td>
<td>- do -</td>
</tr>
<tr>
<td>7. Zeatin</td>
<td>- do -</td>
</tr>
<tr>
<td>8. BAP</td>
<td>- do -</td>
</tr>
<tr>
<td>9. Nicotinic Acid</td>
<td>Water</td>
</tr>
<tr>
<td>10. Pyridoxin HCl</td>
<td>-do-</td>
</tr>
</tbody>
</table>
11. Thiamine HCl
12. Glycine
13. Citric Acid
14. Ascorbic Acid
15. GA3
16. Succinic Acid
17. Phloroglucinol

7. All stocks were clearly labelled with codes; and date, etc.

8. All stocks were checked daily. Any that precipitated were re-made.

9. Deionised water was the only water for making up stock solutions.

**Apparatus required 1**

1. 1 litre volumetric flask.
2. 1 litre graduated measuring cylinder.
3. 1 litre deionised water.
4. 500ml glass beaker.
5. Analytical balance.
6. Squeezy bottle or similar filled with deionised water.
7. Funnel.

**3.2.2 Procedure 1**

1. Stock container (s) were coded with appropriate label (s).

2. Chemicals were weighed out.

3. Added to 500ml beaker.

4. Added 2 x 300mls of deionised water.

5. Stirred/ mixed to dissolve.

6. When totally dissolved added to volumetric using the funnel.
7. Topped up volumetric using deionised water.
8. Stoppered and shaken thoroughly to mix.
9. Poured into stock container(s) and sealed tightly.
10. Stored in Refrigerator.

3.2.3 To make 1 litre Lindemaier & Skoog Medium (1965)

<table>
<thead>
<tr>
<th>Basic Stocks</th>
<th>Stock A</th>
<th>100mls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock B</td>
<td>10mls</td>
<td></td>
</tr>
<tr>
<td>Stock C</td>
<td>10mls</td>
<td></td>
</tr>
<tr>
<td>Stock E</td>
<td>10mls</td>
<td></td>
</tr>
<tr>
<td>Stock D</td>
<td>1mls</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>30grms</td>
<td></td>
</tr>
<tr>
<td>Deionised water to</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>adjust the final volume</td>
<td>to 1 litre pH.</td>
<td></td>
</tr>
<tr>
<td>Agar (added after pH)</td>
<td>8gms. adjustment</td>
<td></td>
</tr>
</tbody>
</table>

3.2.4 Preparation

1. Added the stock solutions as listed to a large enough container than can be stirred rapidly by hand without spillage.

2. Once all the stocks were mixed / dissolved the additions were added (when using charcoal it is preferable to make sure the sucrose has dissolved before adding the charcoal).

3. Adjusted with deionised water to 1 litre.
4. pH was adjusted to 5.7
5. Added Agar 0.8%.
3.2.5 Dispensing - Heating & Stirring

Heatng
Using a hot plate, etc., placed the heat proof vessel of media on the hot plate stirring often.

Media was never boiled – instead brought towards boiling point to dissolve all the agar and dispensed to final containers.

It was important to stir often when charcoal is added to the medium to give an even distribution.

Stirring
It is extremely important to make sure that the medium was thoroughly mixed throughout the dispensing process in order to give an even distribution of agar and charcoal per container. Dispensing was done with an agitator, keeping agar in suspension at all times.

Autoclaving:
Autoclaving at the correct time, pressure and temperature is important to ensure sterility. The following temperature and pressure was used:

Temperature 121 degrees Centigrade / 248 degrees Fahrenheit.
P.S.I. 18 Pounds (15 lbs is acceptable).
3.2.6 Storing Media

The containers of autoclaved media were carefully removed from the autoclave and transported to where they are to be stored.

Once the media was beginning to cool and the agar was setting the containers were not handled.

Preferably the media was stored for at least one week before it was used to check that the autoclaving has successfully sterilised all the containers / batch and that nothing has entered the container while cooling.

Do's and Don'ts in Media Preparation

1. Do not store the media for more than 24 hours at room temperature, especially when the medium contains high levels of sucrose. Refrigerate.

2. Never use dirty stock or medium containers. Residue may carry over from batch to batch.

3. Do not boil media when heating to dissolve agar.

4. Always use deionised water.

5. Any discrepancies in pH readings should be noted and reported.

6. Do not leave batches in the autoclave once cycle has finished.
7. Handle all chemicals whether dry or in solution with care.

8. Follow autoclave procedures including safety procedures fully.
<table>
<thead>
<tr>
<th>Chemical</th>
<th>Weight per Litre of Stock Solution</th>
<th>Stock Solution / Volume of Stock / Litre of Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium Nitrate:</td>
<td>16.50 Grams</td>
<td></td>
</tr>
<tr>
<td>Potassium Nitrate:</td>
<td>19.00 Grams</td>
<td></td>
</tr>
<tr>
<td>Calcium Chloride:</td>
<td>04.40 Grams</td>
<td>A -----&gt; 100mls of Stock A</td>
</tr>
<tr>
<td>Magnesium Sulphate:</td>
<td>03.70 Grams</td>
<td></td>
</tr>
<tr>
<td>Potassium Di Hydrogen Phosphate.</td>
<td>01.70 Grams</td>
<td></td>
</tr>
<tr>
<td>Sodium EDTA</td>
<td>03.73 Grams</td>
<td>B -----&gt; 10mls of Stock B</td>
</tr>
<tr>
<td>Ferrous Sulphate:</td>
<td>02.78 Grams</td>
<td></td>
</tr>
<tr>
<td>Boric Acid</td>
<td>0.062 Grams</td>
<td></td>
</tr>
<tr>
<td>Manganese Sulphate:</td>
<td>02.23 Grams</td>
<td></td>
</tr>
<tr>
<td>Zinc Sulphate</td>
<td>01.06 Grams</td>
<td></td>
</tr>
<tr>
<td>Potassium Iodide:</td>
<td>00.83 Grams</td>
<td>C -----&gt; 10mls of Stock C</td>
</tr>
<tr>
<td>Sodium Molybdate Dihydrate:</td>
<td>00.0025 Grams</td>
<td></td>
</tr>
<tr>
<td>Cupric Sulphate:</td>
<td>00.0025 Grams</td>
<td></td>
</tr>
<tr>
<td>Cobalt Chloride:</td>
<td>00.0025 Grams</td>
<td></td>
</tr>
<tr>
<td>Thiamine</td>
<td>00.04 Grams</td>
<td>D -----&gt; 1mls of Stock D</td>
</tr>
<tr>
<td>Myo-Inositol</td>
<td>01.00 Grams</td>
<td>E -----&gt; 10mls of Stock E</td>
</tr>
</tbody>
</table>
3.3 Sterile Technique

The principle of the laminar flow cabinet is to take in air through the prefilter, removing dust particles, etc., then through the main filter which effectively directs purified air through the cabinet.

Please note the following:

1. Each object placed within the laminar flow cabinet will create air turbulence to a greater or lesser degree.
2. Keep exposed cultures away from objects such as the sterilipot which creates much air turbulence.
3. Cultures should be worked as close to the main filter as possible.
4. Never place objects directly behind the cultures.
5. Hands should work directly in front of the cultures to avoid excess air turbulence.
6. Providing hands are worked from the front any turbulence created by the hands will not affect the cultures.

The maintenance of a sterile environment during the culture of plant tissues is very important.

The culture media in which the plants grow contains high levels of nutrients and sugar which is ideal for fungus and bacteria to grow. If this happens the cultures are considered to be contaminated and have to be thrown away.
A fungal growth is usually found on the surface of the medium. It is usually caused by spores entering from outside. Bacteria are generally found as milky half circled veiling around the base of each culture.

1. Always keep all windows and doors closed to avoid draughts.
2. A minimum number of persons should be present in the vicinity of the transfer
3. Wash hands and wipe out the laminar flow cabinet / transfer chamber by a cloth which has been soaked with sterilant. Wipe down the sides, and floor of the cabinet. Wipe around the steripot and any other instruments in the cabinet. Place the cloth on a trolley.
4. Always wear a laboratory coat / sterile clothing and ensure that bangles, watches etc., are tucked inside the cuffs.
5. Avoid touching the filter with any kind of pointed instrument.
6. Avoid breathing directly into the cabinet. A surgical face mask can be worn.
7. Never pass the hand or arm over a sterile exposed surface.
8. Keep all sterile open surfaces as far back in the transfer chambers as conveniently as possible.
9. When pouring sterile liquids, grasp the flask at the base, and keep the hands as far as possible from the open tube or petridish receiving the liquid.

10. When pouring sterile petridish, hold the lid with the thumb and middle finger on opposite sides, and gently pull the lid back. That is never permit the fingertips to pass over the sterile bottom half of the dish.

11. At the conclusion of each step of the procedure, remove all unnecessary glassware, instruments, aluminiumfoil, and other materials that have been used.

The following additional notes could be useful for the operators of a micropropagation production unit.

1. Collect instruments and fit fresh blades onto scalpel handles.

2. Wipe the instruments and place them into sterilpot.

3. Wipe down boxes of media and stock boxes. Stack a maximum of 3 boxes in far left hand corner of cabinet. If stock and plants are being worked a maximum of 4 boxes should be allowed.

Check that it is the correct media code

4. Wipe packet of cardboard outside cabinet. Wash over cardboard with sterilant to check for any holes. Split cardboard open along widest edge, 2"down either side. Remove flap and place cardboard against side of cabinet.
5. Unwrap forceps take out 2 pieces of cardboard and lay down side by side (staggered) towards back of the cabinet.

**Check that correct clone number is being worked and for any bacterial / fungal contamination.**

6. Using forceps take out 2 pieces of cardboard and lay down side by side (staggered) towards back of cabinet.

7. Remove lid from stock box and place on top of stack of media. Slightly angle box to remove stock. Remove no more than 1/3 of box contents. Replace lid.

8. Work stock by recommended procedure.


10. Plant up onto fresh media.

11. Throw away rubbish. Wipe out floor of cabinet.

12. Label boxes with - clone number day number initials number of cultures per box.

13. Wrap boxes inside the cabinet and stack outside.

14. Wipe out floor or cabinet.

15. Blades should be changed as soon as they become blunt.

16. Change instruments if clone is changed.

17. Change forceps when planting a fresh box.
Fig. 5: Rhizomes of ginger cultivars: A - Suprabha (PGS-35), B - Suruchi (PGS-19) and C - Suravi
Fig. 5: Rhizomes of ginger cultivars: A - Suprabha (PGS-35), B - Suruchi (PGS-19) and C - Suravi
Fig. 6: Rhizomes of turmeric cultivars: A- Krishna, B- Sugandham, C- CO-1 and D- BSR-1
Fig. 7: Rhizomes of turmeric varieties: A- Suguna (PCT-13), B- Roma (PCT-10), C- Suroma (PCT-14), D- Suroma (PCT-8), and E- Suvarna (PCT-8)
3.4 Preparation and sterilization of explants

Rhizomes of 3 cultivars of ginger (Fig. 5) and 9 cultivars of turmeric (Figs. 6 & 7) were used as a stock / mother material.

3.4.1 Presterilization procedure

a. Rhizomes were washed under running water to remove soil particles.

b. Teepol (0.1%) wash was given for 5 minutes.

c. Any discoloured or damaged tissue were cut away.

d. Teepol (0.08%) was given again for 3 minutes.

3.4.2 Sterilization Procedure

a. Primary dissection was done and growing buds of rhizome were immersed in 70% ethanol for 30 seconds.

b. Sodium hypochlorite (NaOCl) treatment was given for 20 minutes. Other sterilising agents such as Mercuric hypochlorite and Calcium hypochlorite were also used.

c. The buds were washed in sterilised distilled water for 5 times.
3.5 Initiation of Explants

After sterilisation and final rinsing, the buds were put on a sterilised filter paper under a laminar airflow cabinet. Using a sterilised scalpel the buds were dissected again to remove the cut surface that has been in contact with the chemical sterilant. The buds were immediately inoculated onto L & S media in a way that the exposed tissue of the bud was in contact with the media. Different concentrations of cytokinin 6-benzylaminopurine (BAP) and (6-t-t-dimethyl allylamine purine) (2iP) were tested.

3.6 Multiplication, Shootin g and Rooting of Cultures

Since BAP was a better Cytokinin as compared to 2-iP it was decided to use only BAP in further experiments.

Before rooting the cultures, it is important that cultures were subjected to absorption of excess cytokinin by the shooting media. The shooting media also allows cultures to grow to an appropriate shoot size. This is extremely important to achieve the proper shoot / root ratio without which tissue culture plants donot establish in vivo.

Single shoots were inoculated on Lindemaier and Skoog basal medium with a range of Indole-3 acetic acid (IAA) and alpha-Naphthalene acetic acid (NAA).
All the cultures were maintained at a temperature 25 ± 2 C, a photoperiod of 16 hours per day and a light intensity of 4,000 lux.

3.7 Hardening of plants

Rooted cultures were taken out of the agar, washed gently in tap water and planted in planting trays with Soilrite potting medium. These were maintained in hardening tunnels for 4 weeks with 95% of humidity and 24 C of temperatures. After 4 weeks, planting trays were taken out and kept in shade for 2 weeks, before exposing to outside environment.

3.8 Field Trials

500 in vitro plants of ginger variety - Suprabha (PGB-35) and turmeric variety (CO-1) each were hardened and used for field trials.

The field trials loosened and the beds were prepared. Farm yard manure at the rate of 30 tonnes per hectare was applied at the time of preparing the beds. Rhizome bits of ginger and turmeric were used as controls alongwith the tissue culture plants. In case of ginger, NPK at the rate of 75:50:50kg/hectare in three split doses was applied. In case of turmeric, NPK at the rate of 30:30:60kg per hectare in three split doses was applied. The crop was harvested in 7 months.