SUMMARY

The present studies were conducted in 10 species and hybrids of epiphytic orchids which are economically important and/or endangered genotypes. With an aim to develop an efficient micropropagation system and to achieve mass cloning of these different explants procured from aerial stem (Rhynchostylis retusa), pseudobulb (Coelogyne cristata, C. ovalis), foliar (Cattleya cv. 'Almakee', Eria bambusifolia, Luisia teretifolia, Rhynchostylis gigantea, R. retusa, Vanda cristata, Vanda cv. Kasem's Delight 'Tom Boykin', V. teres), root (Cattleya cv. 'Almakee', Luisia teretifolia, R. gigantea, R. retusa, Vanda cv. Kasem's Delight 'Tom Boykin', V. teres), floral parts [Ovary (C. cristata), perianth (C. ovalis and V. cristata), anther (R. retusa and V. cristata)] were successfully used for regeneration.

Regeneration in uninodal stemdiscs (with an internodal region), was markedly influenced by the position on the source explant and quality of growth stimulus in the nutrient pool. BAP (1 mg/l) favoured regeneration in the nodal regions, whereas, an additional use of peptone (0.5 mg/l) proved useful in activating adventive meristem in the adjoining internodal regions. The efficacy of peptone was better expressed when medium was either supplemented with more or an additional dose of calcium ions. Pseudobulb segments of Coelogyne cristata and C. ovalis were successfully used for regeneration. The percent regeneration frequency and the pathway, therein, varied with the species and chemical
stimulus in the nutrient pool. In *C. cristata* nearly half of the explants generated shoot buds in medium containing 10mg/l KN alone or in combination with NAA (5mg/l), whereas, replacement of KN with BAP besides, improving the regeneration frequency (to 62.5%), modified the regeneration pathway to Plbs. On the other hand, in *C. ovulis* BAP/KN alone favoured Plb mediated regeneration in 50% explants, however, their simultaneous treatment generated shoot buds in 87.5% explants.

Foliar explants from axenic *in vitro* cultures regenerated frequently, whereas, those obtained from the greenhouse grown plants responded infrequently, such a differential response appears to be related to the extent which the explants exuded phenolics (!) into the medium, the former exuded poorly or none, however, the latter ones exuded profusely. In the *in vitro* sourced explants, juvenility of the tissues emerged as a major factor controlling the activation of proliferative loci in the foliar explants. The ability of very young leaves to proliferate all along the adaxial surface and of somewhat older ones at the base conforms to the earlier suggestions that the cells in the proximal leaf segments retain their plasticity longer than in those with distal segments. The proliferative loci were invariably traced to the dermal (epidermis/hypodermis) cells. Their activation on both the abaxial and adaxial surface in most of the present taxon, indicates that both the surfaces of bifacial leaves in these taxa are meristematically equipotential. The role of growth adjuncts in initiation and
multiplication of meristemoids and regulation of their subsequent development was apparent in the present cultures. In this connection, a simultaneous treatment with 1mg/l of KN and NAA favoured callus mediated development (Cattleya cv. `Almakee', Eria bambusifolia, Rhynchostylis gigantea, R. retusa, Vanda cv. Kasem's Delight `Tom Boykin'), however, elimination of NAA in the latter medium, favoured direct Plb.,,development (E., bambusifolia, R. gigantea, Vanda `Kasem's Delight Tom Boykin) whereas for the similar results in V. teres, a higher dose (2 mg/l) of KN was used. Additional use of NAA and AC, modified the regeneration pathway (Callus mediated Plb development) in the same plant. Replacement of KN with BAP in the latter medium favoured direct development of shoots (Cattleya cv. `Almakee'). With a view to dermal cell proliferations, in the foliar explants the regenerative potential of foliar peels was assessed. Uniseriate peels failed to respond, whereas, multiseriate ones (2-3 layered) responded through direct or callus mediated Plb development. Individual/ or combined treatment with ) 0.25 mg/l each of KN/IAA/IBA favoured Plb mediated regeneration in Vanda cv. `Kasem's Delight `Tom Boykin', while the Cattleya cv. `Almakee' peels required an higher dose of the growth adjuncts.

Root explants from greenhouse grown plants exuded (Phenolics!) profusely and perished without showing morphogenetic changes. Those from the axenic culture in vitro behaved differentially depending upon the level of maturity of their tissues. The mature explants with intact root cap showed an extended
growth, whereas, those from juvenile roots with ill developed root caps regenerated. This differential response is attributed to the higher endogenous level of auxin (IAA) in mature roots, as the root cap is an active site of IAA accumulation, was well developed in mature roots. It supports the earlier contention that its (IAA) supra-optimal level maintains root meristem, whereas, its sub-optimal level changes the developmental pathway to shoot meristem. Proliferations in the juvenile root explants was auxin and/ or cytokinin quality dependent. Callus was generated in KN and NAA supplemented medium, in most of the present taxon, however, replacement of KN with higher dose (3 mg/l) BAP, favoured Plb mediated development (Cattleya cv. 'Almakee', R. gigantea, R. retusa).

Cell proliferation in the exised ovaries, were pollination and chemical stimulus dependent. The ovaries callused to BAP (10 mg/l) and peptone (2g/l) treatment in the nutrient pool, however, initiation in response in the petals was obligatory to the use of labellum as donor petal and an auxin and cytokinin combined treatment. Cytokinin (BAP/KN) were more effective when used in a dose double than that of NAA, their efficacy varied with the species; KN was effective in C. ovalis cultures and BAP in those of V. cristata. For differentiation in the callus, raised from ovary and petals, a low osmoticum was required in the BM.

The embryogenic competence of the anthers was dependent upon the proper staging of the microspores presence of operculum and the chemical stimulus in
the nutrient pool. The recalcitrant behaviour of uninucleate tetrads was overcome at their late-uninucleate stage of development. A treatment with BAP and/or P proved beneficial in initiating cultures. The embryogenic potential earlier in *R. retusa* was obligatory to simultaneous treatment of KN (10 mg/l) and NAA (1 mg/l).

For the multiplication of culture in taxa (*C. cristata* and *E. bambusifolia*), which regenerated poorly, the proliferative potential of Plb explant was successfully exploited. The regeneration was invariably callus mediated, however, their subsequent development was chemical stimulus dependent.

Synthetic 'seeds' were best formed using 4% Sodium alginate and 100 mM CaCl$_2$·2H$_2$O and were complexed for 40 mins in CaCl$_2$·2H$_2$O solution. Among the different nutrient media used as substrates, Agar-gelled nutrient medium gave the highest percent germination. Room temperature (25°C) was optimum while a lower temperature (4°C) reduced the percent germination.

The mortality rate during lab to land transfer was significantly decreased when plantlets were hardened *in vitro* prior to their transfer to the greenhouse.

AC, besides checking the release of brownish (Phenolics !) exudates, proved beneficial in pseudobulb (*C. cristata*, *C. ovalis*), foliar and root (*Rhynchostylis gigantea* and *R. retusa*) explants.
Based on present observations, the following explants and chemical stimuli are suggested for micropropagating plants under present investigation:

### Micropropagation Blue Print for Genus *Cattleya*

<table>
<thead>
<tr>
<th>Status</th>
<th>Source of Explant</th>
<th>Time of Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial</td>
<td>Foliar (0.5 - 1 cm long)</td>
<td>August</td>
</tr>
</tbody>
</table>

#### Three Step Sterilization

<table>
<thead>
<tr>
<th>Sterilant</th>
<th>Name</th>
<th>Conc.</th>
<th>Manufacturer</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterilant</td>
<td>Streptomycin</td>
<td>0.1%</td>
<td>Sarabhai Chemicals</td>
<td>20 mins.</td>
</tr>
<tr>
<td></td>
<td>Mercure Chloride</td>
<td>0.1%</td>
<td>BDH, E. Merck (India)</td>
<td>12 mins.</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>70%</td>
<td>BDH, E. Merck (India)</td>
<td>3-4 sec.</td>
</tr>
</tbody>
</table>

#### Medium for

(i) Initiation: BM + KN (4 mg/l)*
(ii) Multiplication: BM + KN + NAA
(iii) Maintenance: BM + KN + NAA*

### Micropropagation Blue Print for Genus *Coelogyne cristata*

<table>
<thead>
<tr>
<th>Status</th>
<th>Source of Explant</th>
<th>Time of Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial, Threatened</td>
<td>Pseudobulb (2-3 cm long), Ovary (3 cm long)</td>
<td>April</td>
</tr>
</tbody>
</table>

#### Three Step Sterilization

<table>
<thead>
<tr>
<th>Sterilant</th>
<th>Name</th>
<th>Conc.</th>
<th>Manufacturer</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudobulb</td>
<td>Streptomycin</td>
<td>0.1%</td>
<td>Sarabhai Chemicals</td>
<td>20 mins.</td>
</tr>
<tr>
<td></td>
<td>Mercure Chloride</td>
<td>0.1%</td>
<td>BDH, E. Merck (India)</td>
<td>12 mins.</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>70%</td>
<td>BDH, E. Merck (India)</td>
<td>3-4 sec.</td>
</tr>
</tbody>
</table>

#### Medium for

(i) Initiation: BM + P (2 g/l) + BAP
(ii) Multiplication: BM** + BAP + NAA *
(iii) Maintenance: BM + BAP + NAA
### MICROPROPAGATION BLUE PRINT FOR

**Coelogyne ovata I**

**Status**
Threatened

**Source of Explant**
Petal (Labellum), 0.25-0.50 cm long

**Time of Collection**
September

#### Three Step Sterilization

<table>
<thead>
<tr>
<th>Sterilant</th>
<th>Name</th>
<th>Concentration</th>
<th>Manufacturer</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three Step Sterilization</td>
<td>Streptomycin</td>
<td>0.1%</td>
<td>Sarabhai Chemicals, Baroda</td>
<td>12 mins.</td>
</tr>
<tr>
<td></td>
<td>Sodium hypochlorite</td>
<td>4%</td>
<td>BDH, E. Merck (India) Ltd., Bombay</td>
<td>15 mins.</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>70%</td>
<td>Bengal Chemicals and Pharm. Ltd., Calcutta</td>
<td>2-3 sec</td>
</tr>
</tbody>
</table>

#### Medium for

(i) **Initiation**
BM + KN (5 mg/l) + NAA (5 mg/l)

(ii) **Multiplication**
BM **+ KN + NAA**

(iii) **Maintenance**
BM + KN + NAA

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### MICROPROPAGATION BLUE PRINT FOR

**Eria bambusifolia**

**Status**
Commercial, Rare

**Source of Explant**
Pseudobulb (2.5 - 3 cm long)

**Time of Collection**
March - April

#### Three Step Sterilization

<table>
<thead>
<tr>
<th>Sterilant</th>
<th>Name</th>
<th>Concentration</th>
<th>Manufacturer</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three Step Sterilization</td>
<td>Streptomycin</td>
<td>0.1%</td>
<td>Sarabhai Chemicals, Baroda</td>
<td>20 mins.</td>
</tr>
<tr>
<td></td>
<td>Mercurochrome</td>
<td>0.1%</td>
<td>BDH, E. Merck (India) Ltd., Bombay</td>
<td>12 mins.</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>70%</td>
<td>Bengal Chemicals and Pharm. Ltd., Calcutta</td>
<td>3-4 sec</td>
</tr>
</tbody>
</table>

#### Medium for

(i) **Initiation**
BM + P (2 g/l) + KN + NAA

(ii) **Multiplication**
BM + KN + NAA

(iii) **Maintenance**
BM + KN

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### MICROPROPAGATION BLUE PRINT FOR

**Luisia teireti folia**

**Status**
Commercial, Rare

**Time of Collection**
Foliar

**Time of Collection**
March - April

#### Three Step Sterilization

<table>
<thead>
<tr>
<th>Sterilant</th>
<th>Name</th>
<th>Concentration</th>
<th>Manufacturer</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three Step Sterilization</td>
<td>Streptomycin</td>
<td>0.1%</td>
<td>Sarabhai Chemicals, Baroda</td>
<td>20 mins.</td>
</tr>
<tr>
<td></td>
<td>Mercurochrome</td>
<td>0.1%</td>
<td>BDH, E. Merck (India) Ltd., Bombay</td>
<td>15 mins.</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>70%</td>
<td>Bengal Chemicals and Pharm. Ltd., Calcutta</td>
<td>3-4 sec</td>
</tr>
</tbody>
</table>

#### Medium for

(i) **Initiation**
BM + KN + IAA

(ii) **Multiplication**
BM + KN + IAA (2 mg/l)*

(iii) **Maintenance**
BM + KN + IAA

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### Micropropagation Blue Print for Genus Rhynchostylos
#### Species retusa
- **Status:** Commercial
- **Source of Explant:** Stem (0.75 - 2 cm long)
- **Time of Collection:** May - June

#### Three Step Sterilization
<table>
<thead>
<tr>
<th>Sterilant Name</th>
<th>Streptomycin</th>
<th>Mercuric Chloride</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>0.1%</td>
<td>0.1%</td>
<td>70%</td>
</tr>
<tr>
<td>Manufacturer</td>
<td>Sarabhai Chemicals</td>
<td>BDH, E. Merck (India)</td>
<td>Bengal Chemicals and Pharm. Ltd., Calcutta</td>
</tr>
<tr>
<td>Duration</td>
<td>20 mins.</td>
<td>12 mins.</td>
<td>3-4 sec</td>
</tr>
</tbody>
</table>

#### Medium for
- (i) Initiation: BM + P (0.5 mg/l) + BAP*
- (ii) Multiplication: BM + P (0.5 mg/l) + BAP*
- (iii) Maintenance: BM + KN + NAA*

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### Micropropagation Blue Print for Genus Vanda
#### Species cristata
- **Status:** Commercial, Rare
- **Source of Explant:** Foliar (2-2.5 cm long)
- **Time of Collection:** February - March

#### Three Step Sterilization
<table>
<thead>
<tr>
<th>Sterilant Name</th>
<th>Streptomycin</th>
<th>Mercuric Chloride</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>0.1%</td>
<td>0.1%</td>
<td>70%</td>
</tr>
<tr>
<td>Manufacturer</td>
<td>Sarabhai Chemicals</td>
<td>BDH, E. Merck (India)</td>
<td>Bengal Chemicals and Pharm. Ltd., Calcutta</td>
</tr>
<tr>
<td>Duration</td>
<td>20 mins.</td>
<td>15 mins.</td>
<td>3-4 sec</td>
</tr>
</tbody>
</table>

#### Medium for
- (i) Initiation: BM + BAP (10 mg/l) + IAA (5 mg/l)
- (ii) Multiplication: BM + BAP (10 mg/l) + IAA (5 mg/l)
- (iii) Maintenance: BM + BAP*

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### Micropropagation Blue Print for Genus Vanda
#### Hybrid cv. Kasem’s Delight ‘Tom Boykin’
- **Status:** Commercial
- **Source of Explant:** Foliar peel (0.75 - 1 cm long)
- **Time of Collection:** November

#### Three Step Sterilization
<table>
<thead>
<tr>
<th>Sterilant Name</th>
<th>Streptomycin</th>
<th>Mercuric Chloride</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>0.1%</td>
<td>0.1%</td>
<td>70%</td>
</tr>
<tr>
<td>Manufacturer</td>
<td>Sarabhai Chemicals</td>
<td>BDH, E. Merck (India)</td>
<td>Bengal Chemicals and Pharm. Ltd., Calcutta</td>
</tr>
<tr>
<td>Duration</td>
<td>20 mins.</td>
<td>12 mins.</td>
<td>3-4 sec</td>
</tr>
</tbody>
</table>

#### Medium for
- (i) Initiation: BM + KN (0.25 mg/l)
- (ii) Multiplication: BM + KN (0.25 mg/l)
- (iii) Maintenance: BM + KN (0.5 mg/l)

*BAP, KN, IAA, NAA used at 1mg/l unless indicated otherwise within parenthesis*

BM** Modified Mitra et al., (1976) medium (see text)