Gladiolus

Plate-1: Callus formation and somatic embryos from Gladiolus.

A: Callus tissues obtained from the stem segment.
MS + 4 mg/l 2,4-D and 3 mg/l Kinetin.

B: Somatic embryos formation from callus.
MS + 5 mg/l 2,4-D and 2 mg/l Kinetin.
Gladiolus

Plate-2: Micropropagation of Gladiolus from isolated corms and cormels.

A: In vitro proliferation of cormels and corms
MS + 4 mg/l 2,4-D and 3 mg/l Kinetin.

B: In vitro proliferated well developed shoots and development of plantlets.
MS + 5 mg/l 2,4-D and 2 mg/l Kinetin.
Gladiolus

Plate-3: *In vitro development of plantlets of Gladiolus.*

A: Callus cultures showing developed and differentiated plantlets after subculture to regeneration and maintenance medium.

MS + 4 mg/l 2,4-D and 3 mg/l Kinetin.

B: In vitro developed plantlets from proliferated buds culture

MS + 4 mg/l 2,4-D and 3 mg/l Kinetin.
Gladiolus

**Plate-4:** *Shoot multiplication and plantlet production in Gladiolus.*

A: In vitro initiation and multiplication of shoots from callus cultures (45-days).
   MS + 4 mg/l 2,4-D and 3 mg/l Kinetin.

B: Initiation and development of regenerated plantlets from somatic embryos.
   MS + 5 mg/l 2,4-D and 2 mg/l Kinetin.

C: In vitro developed plantlets showing well developed roots.
   Hormone free full strength MS media.
Gladiolus

**Plate-5:** *Multiple shoot production through Leaf culture in Gladiolus.*

A: Swollen leaf explant exhibiting callus induction.

MS + 4 mg/l 2,4-D and 3 mg/l Kinetin.

B: Development of multiple shoots.

MS + 4 mg/l 2,4-D and 3 mg/l Kinetin.

C: Different stages in in vitro development of plantlets.

MS + 4 mg/l 2,4-D and 3 mg/l Kinetin.
Gladiolus

Plate-6: Development of corms and cormels of Gladiolus.

A: Different sizes of corms and cormels (In clusters and isolated)
B and C: In vitro developed plantlets and their transplantation.
1:3:1 (Farm yard manure:soil:sand).
**Gladiolus**

**Plate-7:** Large scale transplantation and flowering in Gladiolus.

A: “True to type” Flowering in in vitro developed plants.

B: Large scale field performances.

C: “True to type” blooming spikes of in vitro developed gladiolus.
Chrysanthemum

Plate-8: Initiation and maintenance of callus tissues from stem segments of chrysanthemum.

A: Swollen explants showing callus tissues.
MS + 5 mg/l BAP and 1 mg/l NAA.

B: Development of green, hard, nodular bodies on callus.
MS + 5 mg/l BAP and 1 mg/l NAA.

C: White, hard, notched 36 weeks old callus maintained on
MS + 5 mg/l BAP and 1 mg/l NAA.
*Chrysanthemum*

**Plate-9:** *Regeneration from callus cultures of chrysanthemum.*

A: Embryogenic calli.

5 mg / l 2,4-D and 2 mg / l NAA.

B: Subsequent growth and micro shoots production of embryogenic calli after regular subculture on maintenance media.

5 mg / l 2,4-D and 2 mg / l NAA.

C: Intense growth and development of in vitro root culture.

Hormone free ½ strength MS medium.
**Chrysanthemum**

*Plate 10:* *Regeneration and multiplication of in vitro raised shoots in chrysanthemum.*

A: Callus tissues showing initiation of regenerants.
MS + 5 mg/l BAP and 1 mg/l NAA.

B: Apical bud elongation and showing multiple shoot formation.
MS + 5 mg/l BAP and 1 mg/l NAA.

C: Nodal variants observed in in vitro cultures showing complete developed multiple bud cluster and plantlets.
MS + 5 mg/l BAP and 1 mg/l NAA.
Chrysanthemum

Plate-11: Initiation and proliferation in chrysanthemum leaf explants.

A: Differentiation at the base of leaf explant.
   5 mg/l 2,4-D and 2 mg/l NAA.

B: Proliferation of differentiated micro shoots and roots.
   5 mg/l 2,4-D and 2 mg/l NAA.
Chrysanthemum

Plate-12: Micropropagation of chrysanthemum.

A: In vitro raised well developed shoots with roots.
  MS + 5 mg/l BAP and 1 mg/l NAA.

B and C: Successful transplantation of in vitro produced plantlets.
  1:3:1 (Farm yard manure:soil:sand).
Chrysanthemum


A and B: Large scale transplantation to pot (Green house)

C: In vitro developed “True to type” plantlets under field condition showing vigorous growth and flowering.
Lily

Plate-14: Initiation and maintenance of callus in lily.

A: Initiation and growth of callus tissues from bulb scales explants.
   MS + 5 mg/l 2,4-D and 2 mg/l Kinetin.

B: Initiation of protuberances on callus from callus maintenance medium.
   MS + 5 mg/l 2,4-D and 2 mg/l Kinetin.

C: Embryogenic calli from petal segments.
   MS + 4 mg/l 2,4-D and 2 mg/l Kinetin.
**Lily**

*Plate-15:* Micropropagation of lily from leaf explants and their transplantation.

A: Initiation and development of swollen tissue culture, showing differentiation, after subculture to maintenance medium.

\[ \text{MS} + 4 \text{ mg/l 2,4-D and 2 mg/l Kinetin.} \]

B: Proliferation and differentiation at later stage of growth and development of plantlets on maintenance medium.

\[ \text{MS} + 4 \text{ mg/l 2,4-D and 2 mg/l Kinetin.} \]

C: Regeneration of complete plantlets with well developed roots showing vigorous growth.

\[ \text{MS} + 4 \text{ mg/l 2,4-D and 2 mg/l Kinetin.} \]
Lily

Plate-16: *In vitro regeneration and development of roots in lily.*

A: *In vitro regenerated roots from roots explants.*
   Hormone free full strength MS media.

B: *Intense growth of in vitro produced roots.*
   Hormone free full strength MS media.
*Lily*

**Plate-17:** *Callus masses showing proliferation of protuberances in lily.*

A: Callus masses showing proliferation of protuberances.  
MS + 5 mg / l 2,4-D and 2 mg / l Kinetin.

B: Regeneration of shoots from callus tissues.  
MS + 5 mg / l 2,4-D and 2 mg / l Kinetin.

C: Well-developed multiple shoots with developed roots.  
Hormone free full strength MS medium.

D: Acclimatization and transplantation of in vitro obtained plantlets followed by hardening.
**Lily**

*Plate-18:* Acclimatization, transplantation and hardening resulted in flowering in lily.

- **A:** Lily bulb with developed roots and leaves.
- **B:** In vitro raised complete plantlet.
- **C and D:** “True to type” blooming lily flowering.
- **E:** Development of in vitro raised complete plantlet after hardening.
**Plate-19:** Secondary metabolites (Thin Layer Chromatography) (TLC).

Characterization and Colour Identification (according to age of callus in weeks).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Colour</th>
</tr>
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<tbody>
<tr>
<td>beta-sistostirol</td>
<td>Purple</td>
</tr>
<tr>
<td>Tigogenin</td>
<td>Brown grey</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Pink</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>Dark grey.</td>
</tr>
<tr>
<td>n-butenol</td>
<td>Deep yellow</td>
</tr>
<tr>
<td>Iso-propanol</td>
<td>Light yellow</td>
</tr>
</tbody>
</table>
Plate-20: Electrophoretic study.

A: Activity of peroxidase
   Glad: Gladiolus
   Chry: Chrysanthemum
   Lily: Lily

B: Activity of Catalase
   Glad: Gladiolus
   Chry: Chrysanthemum
   Lily: Lily

C: Activity of Protease
   Glad: Gladiolus
   Chry: Chrysanthemum
   Lily: Lily

D: Activity of RNase
   Glad: Gladiolus
   Chry: Chrysanthemum
   Lily: Lily
Dehydration

Plate-21: Long term preservations chrysanthemums.

A: Sterilized flowers of chrysanthemum prepared for dehydration in iron plates.

B: Flowers covered with washed fine sand.

C: Hot air oven (Temperature 40°C for 37 hours for flowers and 31 hours for leaves specimens)

D: Dehydrated flowers carefully separated from sand and oven.

E: Arrangement of dehydrated flowers and leaves.

F: Dehydrated leaves of different size.
Dehydration

Plate-22: Care and maintenance of dehydrated parts of chrysanthemums.

A: Dehydrated flowers and leaves arranged for decorations.
B and C: Vacuumed acrylic jars and glass containers were used for interior decoration and personal usage (Paper weights and Photo plates).