

## Somatic Embryogenesis in *Clerodendrum phlomidis* L.

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**Abstract:** *Clerodendrum phlomidis* is an important medicinal plant of Lamiaceae. Leaves, internode and petiole explants were cultured on Murashige and Skoog (MS) medium supplemented with combinations of 2, 4-dichlorophenoxy acetic acid (2, 4-D), kinetin (kn) and indole-3 butyric acid (IBA). Leaf explants formed clusters of globular somatic embryos directly from epidermal cells within 12-15d when cultured on Murashige and Skoog (MS) medium supplemented with 2.00 mg/l 2,4-D, 0.50 mg/l Kn and 0.025 mg/l IBA. Somatic embryo germination successfully achieved on plant growth regulator (PGR) free ½ MS medium.

**Key words:** 2, 4-Dichlorophenoxy Acetic Acid • *Clerodendrum* • Somatic Embryo • Indole-3 Butyric Acid • Kinetin • Lamiaceae

### INTRODUCTION

*Clerodendrum* is a medicinally important genus of Lamiaceae [1-5]. Roots of this plant are important ingredient of Ayurvedic preparations like Dashmoolakwatha, Chyanprashavleh, Haritakiavleh, Ayushyavardhaak tel, Narayan tel etc., valued for the treatment of variety of ailments [6]. It is used to treat several inflammatory diseases and arthritis in Indian traditional system and folk medicine [7]. *C. phlomidis* has so many medicinal properties which have been used since very ancient times of Ayurveda. Roots are mainly exploited for medicinal purposes. Therefore there is an urgent attention require for conservation of *C. phlomidis* to meet the increasing demand for medicinal purposes. Micropropagation studies on *C. phlomidis* using nodal explant is reported [8-10]. Somatic embryogenesis is an important method for large scale propagation of many plants [11-13]. There is only one report available on somatic embryogenesis on *C. indicum* [14]. In the present investigation we have studied potentials of various explants of *C. phlomidis* for Somatic embryogenesis.

### MATERIALS AND METHODS

Actively growing twigs 15-20 cm was collected from 10 year old plant from Medicinal Plant Garden of Anand Agriculture University in morning time between 8 AM to 9 AM in the month of December to February. Explants

were washed under running tap water for 10 min and then treated with 0.1% (w/v) mercuric chloride solution (1, 3, 5 and 10 min) followed by rinsing them for five times with sterile distilled water. Internode, petiole and leaves were inoculated on Murashige and Skoogs (MS) medium [15] supplemented with 2,4- dichlorophenoxy acetic acid (2,4-D) and indole-3- butyric acid (IBA) and kinetin (Kn) with 3% sucrose. Medium was adjusted to pH 5.8 using 1N NaOH or HCL and gelled with 0.8% agar. Somatic embryos and embryogenic callus obtained from leaf, internode and petiole were transferred to ½ MS (mineral nutrient reduced to half strength) medium devoid of plant growth regulators for germination. Cultures were kept in plant tissue culture lab at 16h photoperiod at a photosynthetic photon flux density of 35  $\mu\text{mol m}^{-2} \text{s}^{-1}$  provided by cool-white fluorescent tube at 25 $\pm$  2°C temperature. Embryogenic callus (At different developmental stages) were fixed in FAA [70% ethanol/acetic acid/formaldehyde, 18: 1: 1 (v/v)] for 24 h for histological studies. Later, fixed callus samples were dehydrated with a graded ethanol-xylene series followed by paraffin embedding [16]. Ten  $\mu\text{m}$  thick sections were taken using a rotatory microtome. The resulting paraffin ribbons were passed through a series of deparaffinising solutions containing alcohol: xylene series [16], followed by Periodic acid–Schiff (PAS) reagents [17]. Sections were mounted on slides using DPX mounting. Sections were examined under and photographed with a Zeiss Axioplan light microscope.

## RESULTS AND DISCUSSION

In present study, work was carried out to standardise procedure for somatic embryogenesis of *C. phlomidis* using leaf, internode and petiole explants. An experiment was performed to optimize the time of HgCl<sub>2</sub> treatment require for leaf, petiole and internode explants of *C. phlomidis* [Table 1]. It was observed that there is variation in HgCl<sub>2</sub> treatment for leaf, petiole and internode explants. Leaf and petiole explants turn brown under long duration of HgCl<sub>2</sub> treatment. Due to variation in duration of HgCl<sub>2</sub> treatment consistent aseptic and healthy cultures was not found. Therefore, only healthy cultures n=24 were counted plant growth regulators (PGRs) studies on various explants.

There are some predetermined embryogenic cells present on different parts of plants e.g. leaf, internode, petiole, immature zygotic embryos, etc [18]. Usually, somatic embryogenesis is induced in the presence of auxin alone [19] or in combination with cytokinin [20,21]. In plant growth regulator free medium, no responses were obtained in all explants and explants remained green for 15 days and later all were

turned to brown within 30 days. 2,4-D has been proven to be most potent and effective for somatic embryogenesis and because it increase endogenous level of indole-3 acetic acid [22]. Embryogenic callus formation was observed in all concentrations of 2,4-D in combinations with kinetin (kn) and indole-3 butyric acid (IBA) irrespective of PGRs type concentrations and explant type [Figure 1 E,D,F]. Histological studies confirmed about somatic embryogenesis [Figure 1A]. It was observed that 2 mg/L 2, 4- D + 0.5 mg/l IBA + and .025 mg/L Kn tend to be most effective medium for embryogenic callus induction from internode, petiole and leaf explants and when this callus was transferred on to ½ MS (mineral nutrient) medium resulted in germination of somatic embryos [Table 2, Figure 1 B]. Conversion of somatic embryo into plantlets with root is used for acclimatization and filed transfer into filed conditions [Figure 1 C]. There is further study is require for standardization of somatic embryogenesis for large scale multiplication of *C. phlomidis*. This protocol will be useful for further development and standardisation of *C. phlomidis* and other *Clerodendrum* species.

Table 1: Surface sterilization with mercuric chloride (HgCl<sub>2</sub>) on node, internode and leaf explants of *C. phlomidis*

| Treatment       | Petiole |       |       |        | Internode |       |       |        | Leaf  |       |       |        |
|-----------------|---------|-------|-------|--------|-----------|-------|-------|--------|-------|-------|-------|--------|
|                 | 1 min   | 3 min | 5 min | 10 min | 1 min     | 3 min | 5 min | 10 min | 1 min | 3 min | 5 min | 10 min |
| Contamination % | 27.78   | 19.44 | 16.67 | 0      | 22.22     | 19.44 | 5.56  | 5.56   | 16.17 | 8.33  | 0     | 0      |
| Browning %      | 0       | 0     | 20.83 | 41.67  | 0         | 0     | 0     | 0      | 0     | 0     | 27.78 | 44.44  |

Data about contamination and browning was collected after 30 days of inoculation. Percentage (%) data was calculated. N=24

Table 2: Callus derived from leaf, petiole and internode explant MS medium supplemented 2,4-D+ Kn or 2, 4-D + Kn+ IBA and transferred to ½ MS *C. phlomidis*

| PGRs (mg/l)* |      |       | Leaf  |          | Petiole |           | Internode |           |
|--------------|------|-------|-------|----------|---------|-----------|-----------|-----------|
| 2,4-D        | Kn   | IBA   | %     | Number   | %       | Number    | %         | Number    |
| 0.00         | 0.00 | 0.00  | 0.00  | 0.00     | 0.00    | 0.00      | 0.00      | 0.00      |
| 1.00         | 0.50 | 0.00  | 41.67 | 12.9±2.8 | 50.00   | 11.60±2.9 | 33.33     | 9.25±2.0  |
| 2.00         | 0.50 | 0.00  | 75.00 | 20.7±5.4 | 41.67   | 20.33±5.8 | 58.33     | 19.86±5.8 |
| 3.00         | 0.50 | 0.00  | 79.17 | 21.5±5.1 | 54.17   | 20.62±5.0 | 62.50     | 20.60±5.0 |
| 4.00         | 0.50 | 0.00  | 83.33 | 26.2±5.3 | 58.33   | 25.50±5.6 | 66.67     | 26.06±5.4 |
| 1.00         | 0.50 | 0.025 | 87.50 | 24.4±5.5 | 66.76   | 24.00±6.1 | 70.83     | 24.00±5.9 |
| 2.00         | 0.50 | 0.025 | 91.67 | 27.4±7.7 | 70.83   | 27.41±8.1 | 79.17     | 27.68±8.3 |
| 3.00         | 0.50 | 0.025 | 87.50 | 24.1±7.7 | 58.33   | 23.43±8.7 | 75.00     | 23.17±7.6 |
| 4.00         | 0.50 | 0.025 | 83.33 | 23.2±4.7 | 45.83   | 21.36±2.4 | 58.33     | 23.14±4.4 |

Here number indicates number of well developed somatic embryo. Various stages of embryo were not counted.

**Abbreviations:** 2, 4-D: 2, 4- dichlorophenoxy acetic acid; Kn: kinetin; IBA: indole-3-butyric acid. Data was collected after when embryogenic callus from leaf, internode and petiole was transferred to ½ MS (mineral nutrient diluted to ½) medium without plant growth regulators. (N=24).

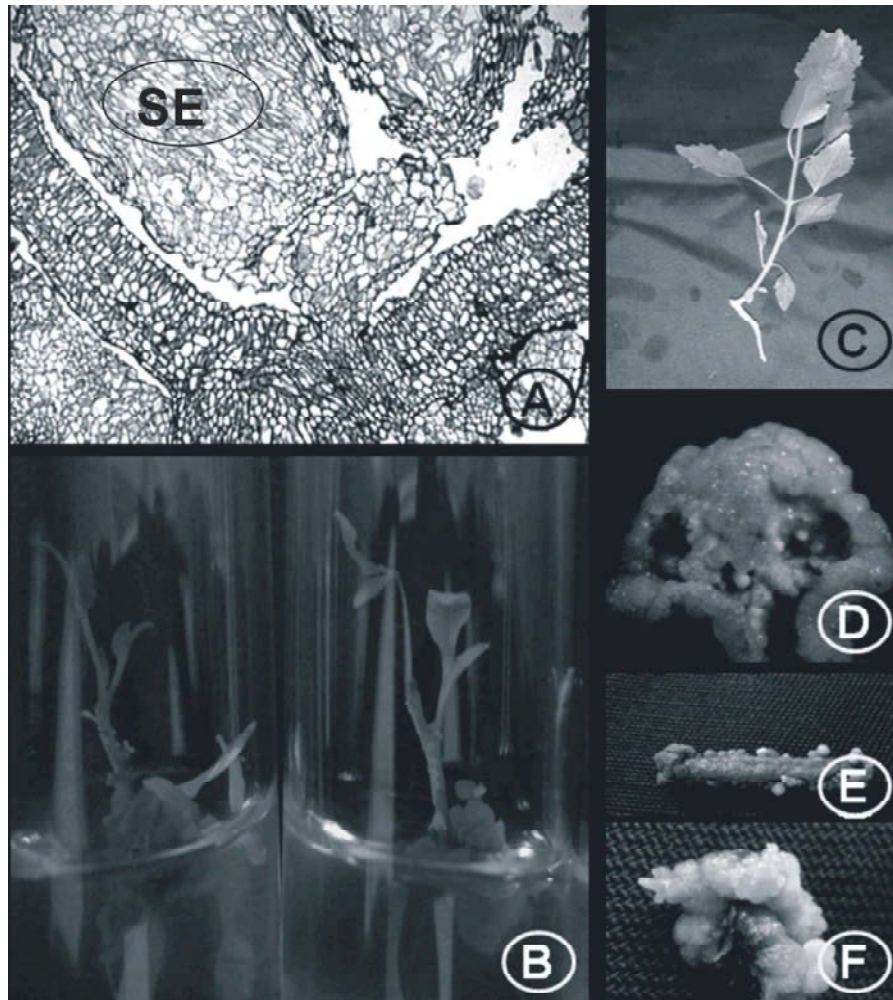


Fig. 1: Somatic embryogenesis in various explants of *Clerodendrum phlomidis*. A. Section showing globular embryo from leaf explant SE; somatic embryo. B. Germinated somatic embryo. C. Plant developed from somatic embryo. D. Embryogenic callus from leaf. E. Embryogenic callus formation from internode. F. Embryogenic callus formation from petiole explant.

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