



## ***In vitro* direct plant regeneration from shoot tip explants of *Clitoria ternatea* - An important medicinal plant**

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### **Abstract**

A simple micropropagation method from shoot tip explants was reported here for *Clitoria ternatea* L., an economically important medicinal plant. High frequency of micro shoots were obtained from this explants on MS medium supplemented with various concentrations of Indole Acetic acid (IAA) (0.5 - 2.0 mg/l) and KN (0.5 - 2.0 mg/l). Maximum number of shoots was obtained from shoot tip explants in the medium containing 0.5 mg/l IAA and 1.0 mg/l Kinetin (KN). The regenerated shoots were further elongated on MS medium supplemented with IAA (0.1- 0.6 mg/l) and 6-Benzylaminopurine (BAP) (0.1 - 0.6 mg/l). Among these concentrations, high length of shoot elongation was achieved in the medium containing BAP (0.5 mg/l) and IAA (0.5 mg/l).

**Key words:** *Clitoria ternatea* L, Tissue culture, Explants, BAP, IAA

### **Introduction**

Mass propagation of plant species through *in vitro* culture is one of the best and most successful examples of commercial application of plant tissue culture technology (Sridhar and Naidu, 2011). *Clitoria ternatea* Linn. belongs to the family Fabaceae. This is a perennial twining herb, stems terete, more or less pubescent. Leaves imparipinnate, petioles 2-2.5 cm long; stipules 4mm long, linear, acute. Leaflets 5-7, subcoriaceous, 2.5-5 by 2-3.2 cm, elliptic-oblong, obtuse or caute; stipules filiform. Flowers -axillary, solitary, standard bright or blue or sometimes white, with an orange centre, seed-6-10, yellowish brown, smooth. Two types- white variety and blue flowered variety; widely distributed throughout Bangladesh, used as ornamental plant.

Various parts of *C. ternatea* have been reported to have nootropic activity, anxiolytic activity, tranquilizing property, anti-inflammatory and analgesic activity, antipyretic, antimicrobial activity and immunomodulatory activities (Mukherjee *et al.*, 2008). The plant is found to possess antibacterial activity (Malabadi *et al.*, 2005). The flavonol glycoside present in roots is reported to have antibacterial activity (Yadava and Verma, 2003). Considering the high economical and pharmacological importance of secondary metabolites, industries are deeply interested in utilizing plant tissue culture technology. *C. ternatea* has been reported to have

anti-inflammatory, hepatoprotective (Solanki and Jain, 2011), antihyperlipidemic (Solanki and Jain, 2010) and immunoinhibitory activities. The present investigation elucidates *in vitro* multiple shoot regeneration through nodal segments of *C. ternatea* and screening of their antibacterial properties.

### **Materials and Methods**

#### **Plant material**

*Clitoria ternatea* plants were brought from the bank of river of Kollidam in Tiruchirappalli, Tamilnadu and its identity was confirmed by Botanical Survey of India (Southern Circle), Coimbatore, Tamilnadu, India.

#### **Explants and surface sterilization**

Shoot tips were collected from the field grown five-day-old plants of *Clitoria ternatea* and washed repeatedly with distilled water and finally treated with  $HgCl_2$  (0.1%) for 4 min in a laminar flow cabinet and washed three times with autoclaved distilled water to remove any trace of  $HgCl_2$ . After surface sterilization, shoot tips were excised at the base and divided into pieces as explants of size 25 - 30 mm.

#### **Culture medium and conditions for plant regeneration**

Under a laminar flow, cabinet explants were inoculated aseptically on MS (Murashige and Skoog, 1962) medium supplemented with various concentrations of Indole Acetic Acid

(IAA) and Cytokinins [6-benzylaminopurine (BAP) and Kinetin]. All media were adjusted to pH 5.8, and 0.8% agar and 30 g/l sucrose were added. About 15 ml of the medium were dispensed in each culture bottle and sealed with plastic cover before autoclaving at 121 °C for 15 min under pressure of 15 Pis. The media were left to cool as slant in the culture room until use. All cultures were maintained at 16 hr light of 1000 lux using fluorescent lamps at 25 ± 2 °C. Results were observed at regular intervals and data were collected from three independent experiments and presented as average ± standard error (SE).

#### Transfer of the explants

Maximum precautions were taken during the time of transfer of the explants. The hands were thoroughly washed with detergents and wiped with spirit. The surface sterilized explants were cut into required size, and inoculated on pre-sterilized media in the presence of a spirit lamp. Non-absorbent cotton plug wrapped with white open-wove bandage cloth was used to plug the culture tubes.

#### Results and Discussion

Tissue culture techniques are being increasingly exploited for clonal multiplication and *in vitro* conservation of valuable indigenous germplasm threatened with extinction. Greater demand for these plants especially for the purpose of food and medicine is one of the causes of their rapid depletion from primary habitats. Micropropagation offers a great potential for large scale multiplication of such useful species and subsequent exploitation (Boro *et al.*, 1998). Shoot tip of *Clitoria ternatea* were isolated aseptically and cultured on MS medium supplemented with Kinetin and Auxins for initiating vegetative growth and inducing maximum number of plantlets. MS medium was found to be most suitable compared to other media tried *viz.* B5, WB, Schenk and Hildebrandt, etc. Auxins, especially IAA, were reported to overcome apical dominance, release lateral buds from dormancy and promote shoot formation. The shoot tip explants were inoculated on MS medium containing different concentrations of Indole Acetic acid (IAA) (0.5 – 2.5 mg/l<sup>-1</sup>) and Kinetin (KN) (0.5 – 5.0 mg/l<sup>-1</sup>) for the production of multiple shoots (Table 1 & Fig. A & B). As a supplement of 0.5 mg/l IAA and 1.0 mg/l<sup>-1</sup> KN resulted in maximum proliferation was observed in shoot tip explants. The shoot tip explants produced the maximum number of

shoots per culture with a mean length of 7.6 ± 0.4 cm.

Table- 1: Response of Shoot tip explants in the production of shoots using different concentration of IAA and KN

Hormone Concentration		Shoot elongation after 3 week (cm) (Mean ± SD)
IAA	KN	
0.5	0.5	+++
0.5	1.0	++++
1.0	2.0	+++
1.5	3.0	++
2.0	4.0	++
2.5	5.0	++

++++ - High range, +++ - Moderate, ++ - poor

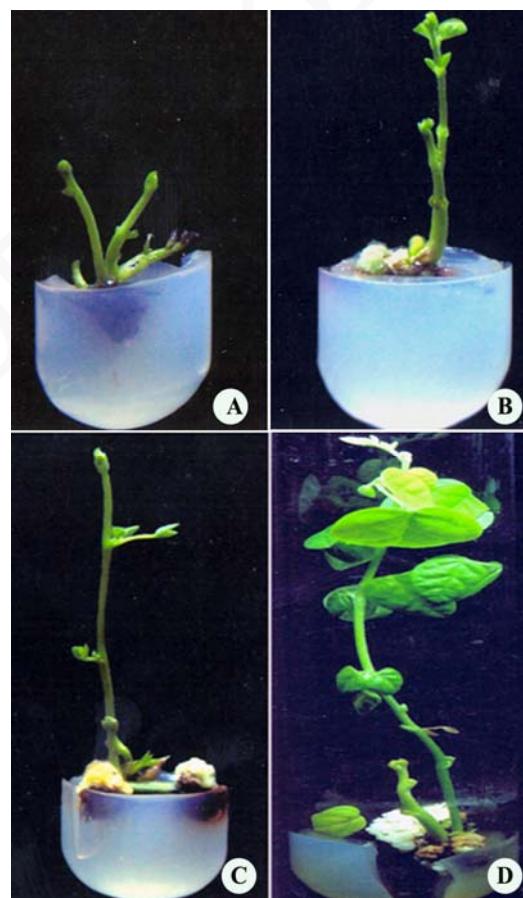


Fig.A. Shoot initiation (IAA + KN)

Fig.B Shoot elongation (2 week) (IAA + BAP)

Fig.C. Shoot elongation (3 week) (IAA + BAP)

Fig.D. Shoot elongation (4 week) (IAA + BAP)



Table- 2: Response of Shoot elongation from shoot tip explant of *Clitoria ternatea* using different concentration of IAA and BAP

Hormone Concentration		Shoot elongation after 3 week (cm) (Mean $\pm$ SD)
IAA	BAP	
0.1	0.1	-
0.2	0.2	3.6 $\pm$ 0.4
0.3	0.3	5.6 $\pm$ 0.9
0.4	0.4	5.6 $\pm$ 0.4
0.5	0.5	7.6 $\pm$ 0.4
0.6	0.6	7.2 $\pm$ 0.7

These explants were capable of directly developing multiple shoots on MS medium containing different concentrations of BAP and IAA. From the results (Table- 2), it is clear that a combination of BAP (0.5mg/l<sup>1</sup>) and IAA (0.5 mg/l<sup>1</sup>) at lower concentration was suitable for shoot elongation (Table-2 &Fig. 3 & 4). The effective role of NAA in combination with BAP for the induction of multiple shoots has been reported in *Rauwolfia serpentine* (Baksha *et al.*, 2007), *C. colocynthis* (Meena and Patni, 2007) and *Bupleurum distichophyllum* (Karuppusamy and Pullaiah, 2007). In contrast to the above mentioned results, some researchers observed that the combination of BAP and IAA on MS medium favoured multiple shoot buds in *Capsicum annuum* (Sobhakumari and Lalithakumari, 2003) and *Acalypha wilkesiana* (Sharma *et al.*, 2007). Combination of cytokinins also favoured multiple shoot proliferation in *Ocimum sanctum* (Girija *et al.*, 2006) and *Amygdalus communis* (Akbas *et al.*, 2009). These explants were capable of directly developing multiple shoots on MS medium containing different concentrations of BAP and IAA. From the results (Table 2), it is clear that a combination of BAP (0.5 mg/l) and IAA (0.5 mg/l<sup>1</sup>) at lower concentration was suitable for shoot elongation. The method developed for rapid micropropagation of *C. ternatea* is reliable and definitely a promising one for this valuable folklore medicine.

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