

## Research Article



## ***In vitro* Callus Induction and Plantlet Regeneration of *Swertia chirayita*: A Critically Endangered Medicinal Plant**

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### ABSTRACT

The present study has been carried out to investigate the indirect shoot organogenesis of *Swertia chirayita*, a high value medicinal herb of temperate Himalayas. Sterilized explants were prepared by using 0.1% HgCl<sub>2</sub> and 0.5% Bavistin and Callus was initiated from leaf explants and were placed on Murashige and Skoog (MS) medium with varying combinations and concentrations of naphthalene acetic acid (NAA), 6-benzylaminopurine (BAP), 2, 4 dichlorophenoxyacetic acid (2, 4-D) and thidiazuron (TDZ) either alone or in combinations. The best concentration for the callus induction was observed in 2, 4 D (1.0mg/l) + BAP (0.5mg/l) and TDZ (0.5mg/l). Calli were then transferred to different concentrations of growth regulators either alone or in combinations for shoot regeneration. The highest number of adventitious shoots was formed in Kinetin (1.5mg/l) + Indole 3 acetic acid (2.0mg/l) and Gibberlic acid (0.2mg/l). The results demonstrated that the leaf explants were a good source of callus induction and indirect shoot regeneration.

**Keywords:** *Swertia chirayita*, callus, plantlet regeneration, Murashige, Skoog (MS).

### INTRODUCTION

*Swertia chirayita* is one of the critically endangered medicinal herbs belonging to the Gentianaceae family. It grows in the temperate Himalayas from Kashmir to Bhutan and in Khasi hills of Meghalaya at an altitude of 1100 to 3000 meter<sup>3</sup>. The plant stems and leaves are reddish in color when mature. The leaves are lanceolate, and have five nerves varying in the length from 8-9 cm. The Flowers are greenish yellow and tinted with purple. It can attain height up to 1.5 meters. Seed dispersal takes place once the plant has matured in late November or December (depending on the area). It generally grows in acidic soil condition with pH range lies 4.7 to 5.5<sup>2</sup>. *Swertia chirayita* is much prized as a bitter tonic without aroma and astringency. It is an official drug of the Indian Pharmacopoeial list 1946.

It is a well known herb in Ayurvedic system of medicine and has been used for curing various diseases such as malarial fever, cough, scabies, leucoderma, asthma, ulcers, chronic fever and cold<sup>4</sup>. The main active principle of *Swertia chirayita* is Chiretin. The bitter principles are the main constituents of the plant which are involved in the Seco-irodoid glucoside group and are Amarogentin and Amaraswerin. Collection of *Swertia chirayita* has reduced its population to a very low level resulting in its categorization in an endangered species; It will therefore be desirable to apply novel technique of *in vitro* conservation and micro-propagation for its conservation and production of a large number of disease-free and true-to-type plants or to save natural populations by *in vitro* production so that plants from natural habitats are not uprooted.

### MATERIALS AND METHODS

#### Source and preparation of explants

The plants of *Swertia chirayita* were procured from Jaypee University Waknaghat Solan. Green young leaf segments of *Swertia chirayita* were collected and washed thoroughly in running tap water for 5-10 minutes. These were then treated with 0.2% Bavistin containing 3-5 drops of detergent for half an hour, on a rotary shaker followed by rinsing three times with sterile distilled water. They were surface sterilized in 70% ethanol for 10-15 seconds and immersed in 0.1% HgCl<sub>2</sub> for 3-5 minutes. Then the explants were finally rinsed with autoclaved distilled water for 3-4 times.

#### Culture medium and growth conditions

MS medium with 3% sucrose and varying concentration of phyto-hormones NAA, BAP, 2, 4-D and TDZ were used for the induction of callus. The pH of the medium was adjusted to 5.8 before gelling with agar (0.8% w/v) and autoclaved for 15 to 20 minutes at 15 p.s.i. at 121°C. After the preparation and solidification of media, the sterilized explants (leaf segments) were inoculated on the MS medium and incubated in culture room. Cultures were incubated at 24 ± 2°C under cool fluorescent light (1500 – 2000Lux) with a 16 h/8 h light dark cycle.

#### Data analysis

The percentage of explants initiating calli and somatic embryo, number of days for initiation, texture and morphology, number of days for shoot regeneration and percentage of survival were determined after 6 weeks of subculture.



## RESULTS AND DISCUSSION

### Explant sterilization

Sterilization of explants was carried out in combination of bavistin 0.2 per cent and HgCl<sub>2</sub> 0.1 per cent at varying time intervals. The data obtained from the leaf explants is given in Table 1, and it shows that the maximum per cent of contaminated cultures was found in bavistin 0.2 per cent for 2-4 min followed by HgCl<sub>2</sub> 0.1 per cent for 4-5 min yielding 55-70 per cent uncontaminated cultures. The best effective treatment was Bavistin 0.2 per cent for 5-10 min followed by HgCl<sub>2</sub> 0.1 per cent for 3-4 min yielding 80-90 per cent uncontaminated cultures (Table. 1).

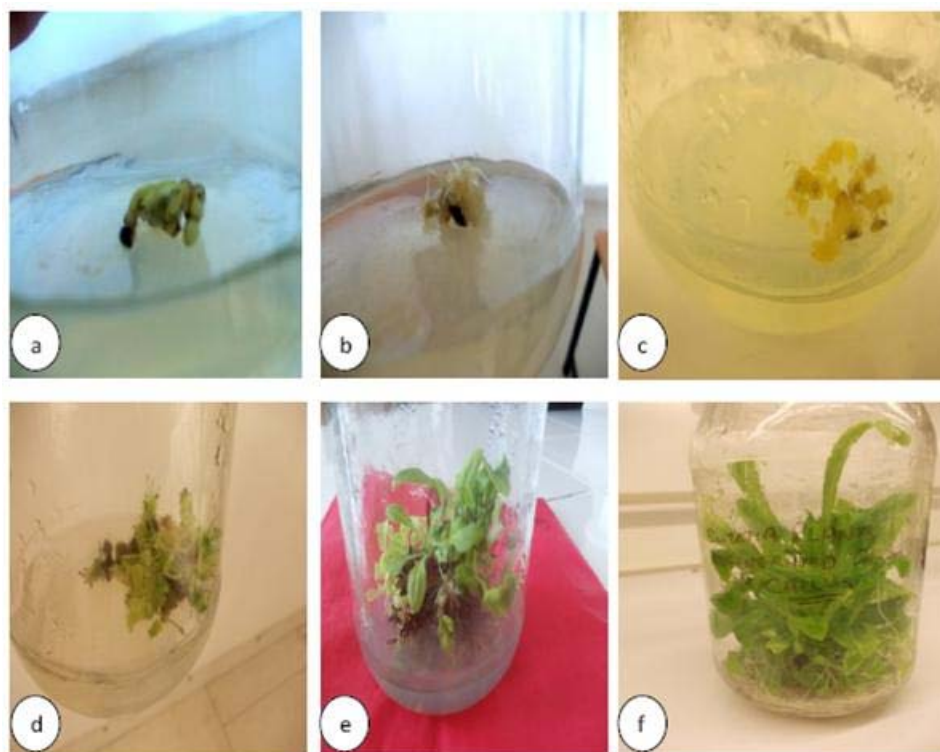
### Callus Induction

Aseptically sterilized leaf segments from field grown plants of *Swertia chirayita* were cultured on MS Medium

containing different concentrations of auxin and cytokinin either alone or in combination i.e. 2,4-D (1.0mg/l - 3.0 mg/l), NAA (0.5mg/l - 3.0 mg/l), BAP (0.5 - 3.0mg/l) and TDZ (0.1mg/l - 0.5mg/l). No signs of active growth were seen in basal medium and BAP alone in which browning of explants occurred. The highest frequency of callus induction was observed in, 2, 4-D (1.0mg/l) + BAP (0.5mg/l) and TDZ (0.5mg/l), after 6 weeks of culture. A similar study was described previously<sup>11</sup> from the root derived callus culture of *Swertia chirayita* where he found BAP (13.32µM) + 2, 4-D (0.90µM) to be the best concentration for the callus induction, multiplication and shoot induction. Different combinations of 2, 4-D with IAA, IBA and TDZ were tested at different concentrations, where 2, 4-D (1mg/l) + IAA (0.5mg/l) was found to be the best for the multiplication of callus culture of *Swertia chirayita* (Figure.1.c).

**Table 1:** Effect of sterilants on surface sterilization of leaf explants of *Swertia chirayita*

Sr. no.	Sterilizing agent	Time (min.)	% established cultures
1	Bavistin 0.2 %	2-4	57.40
	Hgcl <sub>2</sub> 0.1 %	2-4	
2	Bavistin 0.2 %	5-10	81.60
	Hgcl <sub>2</sub> 0.1 %	3-4	
3	Bavistin 0.2 %	7-8	64.60
	Hgcl <sub>2</sub> 0.1 %	5-10	



**Figure.1 (a ) and (b)** Callus initiation from leaf explants cultured on MS + 2, 4-D (1.5mg/l) + Bap (0.5mg/l) + TDZ (0.5mg/l) after 4 weeks of culture **(c)** Multiplication of callus on 2, 4-D (1mg/l) + IAA (0.5mg/l) **(d)** Regeneration of shoot buds from leaf-derived callus of *S. chirayita* cultured on MS + KN (1.5mg/l) + IAA (2.0mg/l) + GA<sub>3</sub> (0.2mg/l) after 4 weeks **(e)** and 6 weeks of culture **(f)** Plantlet formation after 8 weeks.

**Table 2:** Effect of different concentrations of growth regulators used for callus induction of *Swertia chirayita*

S. No.	Media composition (mg/l)							Response	Texture
	2,4-D	NAA	KN	6-BA	IBA	IAA	TDZ		
1	-	-	-	-	-	-	-	-	-
2	3.0	-	1.0	-	-	-	-	-	-
3	-	1.5	-	3	-	-	-	callus	Soft friable, light yellowish
4	2.0	-	-	-	-	-	-	callus	Hard compact and yellow
5	-	2.5	-	1.5	-	-	-	-	-
6	2.0	-	-	-	1.5	-	-	-	-
7	2.5	-	-	0.7	-	-	-	callus	Compact and yellowish
8	-	0.5	0.5	-	-	-	-	-	-
9	1.0	-	-	-	-	-	0.5	-	-
10	1.0	-	-	0.5	-	-	0.5	callus	Friable, yellowish & embryogenic

### Plantlet regeneration

After 6 weeks of culture in the callus multiplication medium (MS + IAA (0.5mg/l) + 2, 4-D (1.0mg/l)) the calli were sub cultured in the media containing different concentrations of BAP, KN and TDZ and IAA, NAA or 2, 4-D for shoot bud regeneration. The calli differentiated into dark green shoot buds (Figure.1.d&e) in the media supplemented with IAA (2.0mg/l) + Kinetin (1.5m/l) + GA<sub>3</sub> (0.2mg/l). BAP or KN alone did not induce any morphogenetic response and also in combination with 2, 4-D did not help in regeneration of shoot buds from callus. Cytokinins along with auxins play an important role in the plant growth and shoot regeneration. Morphogenesis is controlled by the interplay of auxins and cytokinins<sup>12</sup>. Synergistic effect of cytokinins with auxins has been reported to produce superior results for the shoot proliferation in many medicinal plants<sup>5, 8, 13</sup>. On the other hand<sup>1</sup> reported the use of 1 mg/l IAA in their patented media composition for faster propagation of *Swertia chirayita*. This variation in *Swertia chirayita* response might be due to the difference in the genotypes used in the two studies and difference in the physiological state of the explants.

### CONCLUSION

The study describes an efficient protocol for plant regeneration system in *Swertia chirayita* through somatic embryogenesis which may help in the conservation of the species and possibly lead to the production of secondary metabolites and extraction of active compounds from callus sources.

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