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RESEARCH ARTICLE

An Investigation on the *In Vitro* Propagation of *Trichosanthes cucumerina* L. - An Important Medicinal Plant

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ABSTRACT

Trichosanthes cucumerina L. is an important medicinal plant belonging to the family cucurbitaceae. It is an annual climber. It contains wide range of medicinal properties like anti-diabetic, anthelmintic, anti-cardiac failure, hypoglycemic, anti-fertility, anti-inflammatory and against to HIV. Present investigation was carried out with a view to standardize an *in vitro* culture technique for propagation of *Trichosanthes cucumerina* L. Shoot tips and nodal explants from healthy grown plants were used as explants and cultured on MS media alone and MS media supplemented with different concentrations of cytokinins-kinetin(KIN) and benzyl amino purine (BAP) for shoot proliferation. High frequency of shoot proliferation was observed in MS media supplemented with KIN 2mg/l + IBA 1.0 mg/l and best rooting was observed in MS media along with IBA 2.0 mg/l.

KEYWORDS

Trichosanthes Cucumerina L. *Nodal Segment, Cytokinin, Benzyl Amino Purine*

INTRODUCTION

Medicinal plants are among our oldest medicines and their increasing use in recent years in evidence of public interest in alternatives to conventional medicine. Plant-based medicines are currently in demand and their popularity is increasing day by day. About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine. Nowadays, people are becoming aware of the potency and side effect of synthetic drugs, there is an increasing interest in the natural product remedies with a basic approach towards the nature. This led to sudden increase in the number of herbal drug manufactures[2].

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India is a vast repository of medicinal plants that are used in traditional medical treatments[4]. India was the largest supplier by far, with 10,055 tons of plants and 14 tons of vegetables, alkaloids and their derivatives[10]. However, it is only during the last decade that the real significance of the medicinal plants sector has begun to be realized by mid 1980s, there was a renewed interest in natural materials and approaches to health care, coupled with the recognition that technology alone could not solve the pressing health care needs of the world's population[15].

Recent estimates suggest that, over 9,000 plants have known medicinal applications in various cultures and countries, and this is without having conducted comprehensive research amongst several indigenous and other communities. According to the World Health Organization (WHO), over 80% of the world's

population or 4.3 billion people rely upon such traditional plant based systems of medicine to provide them with primary health care. Interest in medicinal plants as a re-emerging health aid has been also fulled by the rising costs of prescription drugs in the maintenance of personal health and well-being.

The World Health Organization (WHO) has estimated the present demand for medicinal plants is approximately US$ 14 billion per year. The demand for medicinal plant-based raw materials is growing at the rate of 15-25% annually and according to an estimate of WHO the demand for medicinal plants is likely to increase more than US$ 5 trillion in 2050. In India, the medicinal plant-related trade is estimated to be approximately US$ 1 billion per year[7].

Among the various medicinal plants, *Trichosanthes cucumerina* L. plant was chosen for the present study. Since, the plant is a rich source of nutrition. It is constituted with proteins, fat, fibre, carbohydrates, vitamin A and E. The total phenolics and flavonoids contents are 46.8% and 78.0% respectively[1]. The triterpenoids found are 23, 24-dihydrocucurbitacin D, 23,24-dihydrocucurbitacin B and cucurbitacin B. Recently, cucurbitacins are also known to possess a number of potent pharmacological effects, deriving largely from their cytotoxic, anti-cancer and anti-inflammatory properties. Hence, an attempt has been made to standardize a reproducible protocol for *in vitro* propagation of *Trichosanthes cucumerina* L.

MATERIALS AND METHOD

Trichosanthes cucumerina L. plant and its seeds were collected from kolli hills. The seeds were grown in the green house, Department of Plant Science, Bharathidasan University, Tiruchirappalli. Its identity was confirmed in the Rapinat herbarium (Voucher number: BHMP001), St. Joseph's College, Tiruchirappalli. Shoot tips and nodal segments were excised from the healthy grown plants and washed thoroughly in running tap water for 10 min. then washed with a drop of teepol solution for 5 min. under laboratory condition. The explants were surface sterilized under laminar air flow chamber with 70% alcohol for 30 seconds. The explants were then disinfected with 0.1% $HgCl_2$ solution for 5 min. after which the explants were rinsed four to five times in sterilized distilled water to remove the traces of the mercuric chloride ($HgCl_2$). The explants were cultured on MS basal medium[11] fortified with different concentrations of plant growth hormones along with 3% sucrose and 0.8% agar. The pH of the medium was adjusted and maintained to 5.7 with the help of 0.1N NaOH or 0.1N HCl. The media was sterilized by autoclaving at 121^{o}C for 30 min. All cultures were maintained at 16 hr photo period with 2000 lux light intensity at 25 ± 2^{0}C. Results were observed at regular intervals and tabulated.

RESULTS AND DISCUSSION

The present study established a protocol for *in vitro* propagation of *Trichosanthes cucumerina* L. through nodal and shoot tip culture. The highest rate of micropropagation often depended not only on the selection of the most suitable explants but on a medium containing the correct combination of growth regulators for tissue. An exogenous supply of growth regulators has been essential for *in vitro* shoot induction, elongation and rooting in many plant species[6].

The effect of different concentrations of BAP (0.5-2.5 mg/l) and Kinetin (KIN) (0.5-2.5mg/l) were assessed. Among the two cytokinins, KIN was superior over BAP on nodal explants (Table-1). Likewise, nodal explants showed better shoot proliferation than the shoot tip explants. Again, the kinetin was tested along with different concentrations of auxins-NAA, IAA and IBA (0.5-2.5 mg/l). A lower concentration of IBA and higher concentration of KIN (KIN 2mg/l + IBA 1.0 mg/l) was better for active shoot formation. The addition of low concentrations of auxin along with cytokinin has increased the rate of shoot formation in many species[14].

Previous reports states that, addition of IAA, IBA or NAA to the MS medium produced rooting [5, 8, 9, 12, 13].

Table 1: Shoot formation from nodal explants of *Trichosanthes cucumerina* L. using different concentrations of auxin and cytokinins

Hormone	Concentration (mg/l)	Number of shoots/ explant	Average shoot length/ explant(cm)	Frequency (%)
Basal	Control	1 ± 0^{d}	1.8 ± 0.19^{h}	77 ± 2.23^{ab}
BAP	0.5	1 ± 0^{d}	0 ± 0.09^{h}	0 ± 1.12^{ab}
	1.0	1 ± 0^{d}	2.18 ± 0.05^{h}	73.6 ± 2.33^{ab}
	1.5	1.6 ± 0.24^{bcd}	3.58 ± 0.05^{f}	69.8 ± 1.42^{ab}
	2.0	1.6 ± 0.24^{bcd}	4.36 ± 0.06^{e}	80.4 ± 0.74^{ab}
	2.5	1.8 ± 0.2^{bc}	4.52 ± 0.03^{de}	72 ± 1.78^{ab}
KIN	0.5	1 ± 0^{d}	2.18 ± 0.05^{h}	48 ± 16.5^{c}
	1.0	1.6 ± 0.24^{bcd}	3.66 ± 0.07^{f}	77.8 ± 1.49^{ab}
	1.5	1.8 ± 0.2^{bc}	4.64 ± 0.04^{de}	66 ± 1.51^{b}
	2.0	2.6 ± 0.24^{a}	8.1 ± 0.04^{b}	80.8 ± 1.01^{ab}
	2.5	2.2 ± 0.2^{ab}	6.04 ± 0.40^{c}	74.2 ± 1.62^{ab}
KIN + IBA	2.0 + 0.5	2.6 ± 0.24^{a}	9.6 ± 0.04^{a}	74.4 ± 1.69^{ab}
	2.0 + 1.0	1.6 ± 0.24^{bcd}	6.22 ± 0.31^{c}	81.2 ± 1.24^{a}
	2.0 + 1.5	1.2 ± 0.2^{cd}	4.96 ± 0.11^{d}	68.4 ± 1.20^{ab}
	2.0 + 2.0	1.4 ± 0.24^{cd}	2.92 ± 0.05^{g}	68.4 ± 1.20^{ab}
	2.0 + 2.5	1.4 ± 0.24^{cd}	2.2 ± 0.05^{h}	80.8 ± 1.01^{ab}

Based on the reports, in the present study, different concentrations of auxins –IAA, IBA and NAA (0.5-2.5mg/l) were used for root induction. The well-developed shoots were inoculated on the MS medium supplemented with IAA, IBA and NAA (0.5-2.5 mg/l). Among, the various auxins tested IBA 2.0 mg/l showed better root induction than IAA and NAA (Table-2).

Similar reports on the effectiveness of IBA have been observed in other species[3]. In this species, higher concentration of IBA inhibits root formation. It was supported by Hossain *et al.*, 1993 with the increased in the concentration of IBA, root formation was inhibited.

Table 2: Effect of auxins on root induction on *Trichosanthes cucumerina* L.

Growth hormone and concentration mg/l	**No. of roots/explants**	**Average root length/explants (cm)**	**Frequency (%)**
Control	0	0	0
IBA			
0.5	3.6 ± 0.24^{b}	2.64 ± 0.15^{d}	36 ± 2.44^{b}
1.0	3.2 ± 0.20^{bc}	2.72 ± 0.04^{d}	32 ± 2^{bcd}
1.5	3.6 ± 0.24^{b}	3.56 ± 0.07^{c}	34 ± 2.44^{bc}
2.0	$\mathbf{5.6\pm0.24^{a}}$	$\mathbf{4.76\pm0.07^{a}}$	$\mathbf{56\pm2.44^{a}}$
2.5	3.2 ± 0.20^{bc}	3.92 ± 0.05^{b}	32 ± 2^{bcd}
IAA			
0.5	1.6 ± 0.24^{f}	1.30 ± 0.05^{gh}	16 ± 2.44^{g}
1.0	1.6 ± 0.24^{f}	1.44 ± 0.04^{g}	16 ± 2.44^{g}
1.5	1.8 ± 0.20^{ef}	1.46 ± 0.05^{g}	18 ± 2^{fg}
2.0	2.6 ± 0.24^{cd}	1.72 ± 0.03^{f}	26 ± 2.44^{de}
2.5	2.6 ± 0.24^{cd}	2.08 ± 0.03^{e}	26 ± 2.44^{de}
NAA			
0.5	2.2 ± 0.20^{def}	1.14 ± 0.04^{h}	22 ± 2^{efg}
1.0	2.4 ± 0.24^{de}	1.14 ± 0.04^{h}	24 ± 2.44^{ef}
1.5	2.8 ± 0.20^{cd}	1.64 ± 0.04^{f}	28 ± 2^{cde}
2.0	2.8 ± 0.20^{cd}	1.76 ± 0.02^{f}	28 ± 2^{cde}
2.5	2.6 ± 0.24^{cd}	2.12 ± 0.03^{e}	26 ± 2.44^{de}

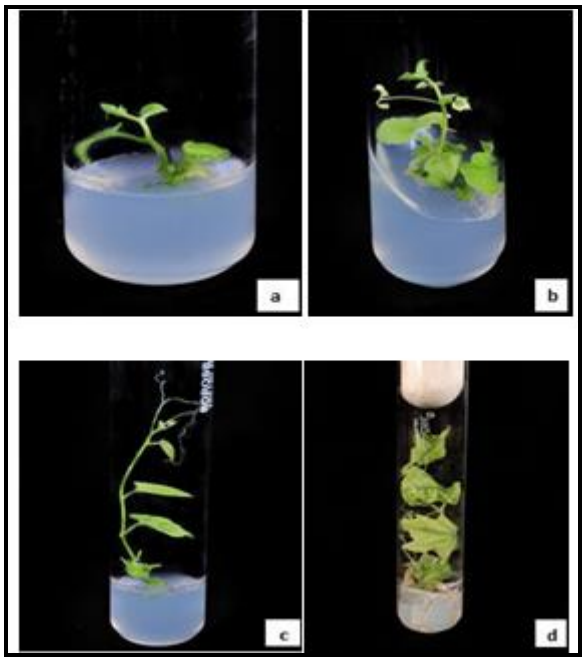

Figure 1: *In vitro* propagation from nodal explants of *Trichosanthes cucumerina* L.

a) Shoot formation on MS media with KIN 2.0mg/l from nodal explant after 15 days

b) Shoot proliferation on MS media with KIN 2.0mg/l from nodal explant after 25 days

c) Shoot elongation on MS media with KIN 2.0mg/l + IBA 1.0 mg/l from nodal explant after 40 days

d) Well-developed shoot and root on MS media with IBA 2.0mg/l

CONCLUSION

Most of the medicinal plants, even today, are collected from wild. The continued commercial exploitation of these plants has resulted in receding the population of many species in their natural habitat. To overcome this problem, *in vitro* propagation is an efficient means of ex situ conservation of medicinal plants. In the present investigation, an effective *in vitro* regeneration protocol through direct organogenesis for the mass propagation of *Trichosanthes cucumerina* L. was developed. Our results shows enhanced shoot formation by proliferation of nodal segments on a medium fortified with KIN 2.0mg/l + IBA 1.0 mg/l and rooting in IBA 2.0mg/l. The present attempt of regeneration using nodal segments developed would be useful in the large scale production of the important medicinal plant.

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