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Influence of growth hormones on production of trichosanthin (TCN) from *in vitro* cultures of *Trichosanthes* species

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ABSTRACT

During the *in vitro* cultures of *Trichosanthes anguina* and *T. kirlowii*, effect of various concentrations of plant growth regulators (PGR's) were studies on the production of a secondary metabolite, *"trichosanthin"* (TCN) which is effective in inhibiting human immunodeficiency virus I replication in Lymphocytes and macrophase cells *in vitro.* It is a wonder drug used for curing AIDS[1] NAA Kinetin TDZ and BAP attached the yield of trichosanthin (TCN). Identification of trichosanthin in each of the tissues was carried out by thin layer chromatography (TLC).

KEYWORDS

Trichosanthin (TCN);
Plant growth regulators;
Auxins;
Cytokinins;
Thin layer chromatography;
In vitro.

INTRODUCTION

Plants represent a valuable resource for a great variety of secondary metabolities of pharmaceutical importance. Secondary metabolites have been reported from tissue cultures of *Datura species*[2], *Atropa belladonna*[3], *Hyoscyamus niger*[4] and others. Trichosanthin (TCN) is a single chain RIP[5]. Bioactive protein and functions as a defensive protein in plants[6].

The effect of plant growth regulators (PGR's) on the growth and secondary metabolite content was studies by Sharma in *Atropa belladonna*[7], *Datura metel*[8] and *Hyoscymus niger*[9]. So far, there is no report on the effect of PGR's on trichosanthin production in the *species of Trichosanthes*.

METHODS AND RESULTS

Callus was initiated from different explants of *Trichosanthes species viz T. anguina and T. kirlowii.* The explants were cultured on MS medium supplemented with auxins, NAA, IAA and 2,4-D either singly or in combination. The callus thus formed was established and maintained by frequent subculturing on MS medium supplemented with various concentrations of cytokinins, TDZ, Kinetin and BAP. These static cultures were maintained at 26°C , 55% relative humidity and diffused light (300Lux).

Eighteen month old calli maintained on MS medium was transferred to fresh MS medium supplemented singly with various concentrations (1.0mg/l, 2.0mg/l and 3.0mg/l) of 2,4-D, IAA NAA, TDZ, kinetin and BAP.

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TABLE 1 : Effect of various concentration of PGR's on the production of "Trichosanthin" (mg/g dry tissue) in eight week old tissue cultures of *Trichosanthes anguina* and *Trichosanthes kirlouri*

Medium		T. anguina Trichosanthin content mg/g dry tissue			T. kirlowii Trichosanthin content mg/g dry tissue		
MS		0.215			0.270		
MS+		in mg/l			in mg/l		
	PGR's	1.0	2.0	3.0	1.0	2.0	3.0
Auxins	2,4-D	0.225	0.245	0.211	0.280	0.292	0.220
	IAA	2.234	0.269	0.208	0.292	0.320	0.215
	NAA	0.260	0.279	0.202	0.310	0.375	0.210
Cytokinins	TDZ	0.365	0.369	0.325	0.325	0.342	0.320
	Kinetin	0.372	0.380	0.315	0.340	0.375	0.318
	BAP	0.370	0.390	0.302	0.362	0.392	0.309

The tissues were harvested separately after eight weeks and their growth indices calculated. These tissues were dried separately at 105°C for 20 min and then at 60°C until the tissue reached a constant weight. The dried samples were powdered. Identification of trichosanthin in each of the tissues was carried out by thin layer Chromatography. The test samples and the reference trichosanthin were maintained. The spots coinciding with those of the reference trichosanthin were marked and their R_f values calculated. Ten replicates of each sample were examined and mean R_f values taken. Each isolated compound was crystallized and further analysed for their IR spectral studies with their authentic sample and confirmation. The various extracts were subjected to quantitative estimation using spectrophotometer (Baunch and Lomb) by adopting the procedure of Feldman and Robb[10]. Concentration of the trichosanthin in each of the test sample was calculated and presented in the TABLE 1.

Trichosanthin content was low in callus cultures grown on MS medium without PGR's. PGR's had a marked effect on the production trichosanthin in callus cultures. The content increased with concentration of auxins and cytokinins upto 2.0mg/l. At 3.0mg/l of all the PGR's there was a marked decrease in trichosanthin. Cytokinins caused increased trichosanthin content; BAP proved most potent.

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