



In vitro Callus Induction from Stem Explants of *Cissus quadrangularis* L. (Hadjod)

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Stem explant of Cissus quadrangularis L., belongs to vitaceae family were cultured on MS media supplemented with 2,4D, NAA, IBA. Callus formation on the surface of stems on high and low concentrations of NAA alone and combined with BAP, stimulated the callus induction. However, concentrations of IBA used here, inhibited Shoot induction. Induced Callus is used for the study of secondary metabolite. Within 90 to 120 days of the culture it showed growth up to 90 %. Aim of this study is to enhance the phytochemical after applying tissue culture technique.

Key words: NAA, 2,4D, callus, phytochemical. Explant

Introduction

Cissus quadrangularis L. is a climber plant species endemic to the India, shrlanka, Pakistan and other tropical countries belongs to the vitaceae family. This valuable medicinal plant is widely used in India. It is popular among the traditional healers having expertise in treatment bone related troubles. It is one of the very frequently used herbs by traditional bone setters of India. (In Hindi Had=bone; Jod=to fix). It is also used for piles, asthma, digestive troubles, cough, and loss of appetite. Drug occurs as pieces of stem of varying lengths. Stem is slender dichotomously branched, sub-angular, glabrous, brown, fleshy, fibrous, smooth with 4-winged. Climbing herb, tendrils simple, opposite to the leaves, leaves simple or lobbed, sometimes 3-foliate, dentate Toothed trilobe leaves 2 to 5 cm wide appear at the nodes. Each has a tendril emerging from the opposite side of the node. Racemes of small white, yellowish, or greenish flowers; globular berries are red when ripe. Powder is Brown in colour; it shows fragments of vessels, fibers, parenchymatous cells and a few rosette crystals of calcium oxalate, starch grains and idioblast containing raphides and isolated acicular crystals of calcium oxalate. (Unknown) Active constituents are oxo and keto steroids are reported in *Cissus qdrangularis L.* studied by **Sen S.P. 1964**. Stem isolates include 3- keto steroids, onocer-7-en-3 α , 21 β -diol (I) and onecer-7-en-33 α , 21 β : **Pankaj Oudhia, 2007**. *Cissus quadrangularis* contains high amount of vitamin C, carotene A, anabolic steroidal substances and calcium. The international organizations like IUCN in 1986. reported this as vulnerable stag e in Chhattisgarh. So this herb requires conservation. When these organisations will report the extinction of Hadjod from Chhattisgarh, only after this report, the local authorities will start project in order to conserve it. Thus the potential of this species may be economically exploited as medicinal climber. There are so many formulas are available in market. So the demand of this is so high but cultivation is less. The aim of the present study was to determine for the first time, suitable culture media and growth regulators on callus induction and direct organogenesis.

Materials and Methods:

Callus tissue derived form the stem explant of *Cissus quadrangularis L.* was collected from MFP park (Minor forest production) park, Barkhera Pathani, Bhopal. The stem of fresh plant were surface sterilized

with 70 % (v/v) ethanol for 5 min followed by immersing them in a 0.01 % (w/v) HgCl₂ solution for 1.5 and 3.5 min, respectively. After this treatment, the short branches were rinsed five times with sterile distilled water. The plant material of *Cissus quadrangularis* L. Petioles were separated from the stems and were cut into 10 and 13 mm long pieces. They were placed in MS or supplemented with 10gm/lit agar (Difeo Bacto), 4% sucrose and various concentrations of plant growth regulators, the pH were adjusted to 6.0. This experiment was conducted in every month of the year, and observed the result. Explants were inoculated on different basal medium supplemented with various level of 2,4D (CM1, CM2, CM3, CM4, CM5, CM6, CM7, CM8) and NAA (CM9, CM10, CM11, CM12, CM13, CM14, CM15, CM16). These cultures were kept at 28^oC under dark. The number of explant showing callusing was observed after 45 days of callus initiation. Now these callus subculture in to same medium, it showed profuse callusing. Callusing quality is depends upon the combination of PGR's is proven by; **Mustafa, Rateb, Seham, Ei-Hawary, Ali M. Ei-Shamy, Essam MA , Yousef 2007 ; Khan , Alam And Nath ,2004; Yung-I-Lee And Nean Lee , 2003; Aniruddha Dattamn Alka , Bansal And Shrivastava 2003.**

Result and discussion:

Approximately 90% of the stem segments were callused on the respective callusing medium in about 90 to 120 days after the culture. In starting callus was slimy brown friable callus. In subculture the proliferation rate of the callus increased and tissue became friable. 2, 4 D is usually used for the induction of callus. Callus indication was observed from piece of the stem grown on MS medium supplemented with various concentration and combination of 2,4 D and NAA in different month of the year, for the same duration (TABLE 1,2) **Gita Rani, Virk And Avinawh Nagpal, 2003**

The callus induction was shown best in concentration of CM6, CM7& CM 8 in this medium it showed light brown slimy friable callus with 90% of growth. This callus is useful for the phytochemical analysis. Best callus responding are observed in the month of May –June and July -August.

TABLE: 1- Responses of callusing of *Cissus quadrangularis* L.(HADJOD) in different month of year.

S.NO.	Months	Callus induction
1	Jan – Feb	++
2	Mar-April	+
3	May-June	++++
4	July- Aug	++++++
5	Sep_Oct.	+
6	Nov- Dec.	+

TABLE: 2 Effect of various level and combinations of PGR's for callusing of *Cissus quadrangularis* L.(HADJOD)

S.No.	MS-Medium	2,4,D	NAA	Type of the callus	Responses in %
1	CM1	0.5	-	No callus induction	0%
2	CM2	1.0	-	Dark brown friable callus	25%
3	CM3	1.5.	-	Brown friable callus	35%
4	CM4	2.0	-	Light brown friable callus	50%
5	CM5	2.5	-	Light brown friable callus	65%
6	CM6	3.0	-	Slimy light brown friable callus	85%
7	CM7	3.5.	-	Brown non friable callus	90%

8	CM8	4.0	-	Brown non friable callus	92%
9	CM9	-	0.5	Light brown non friable callus	35%
10	CM10	-	1.0	Light green non friable callus	40%
11	CM11		1.5	Light brown whitish callus	50%
12	CM12		2.0	Light green whitish callus	50%
13	CM13		2.5	Light brown whitish callus	50%
14	CM14		3.0	Light brown white non friable callus	65%
15	CM15		3.5	Light brown white callus	70%
16	CM16		4.0	Light brown non friable callus	75%

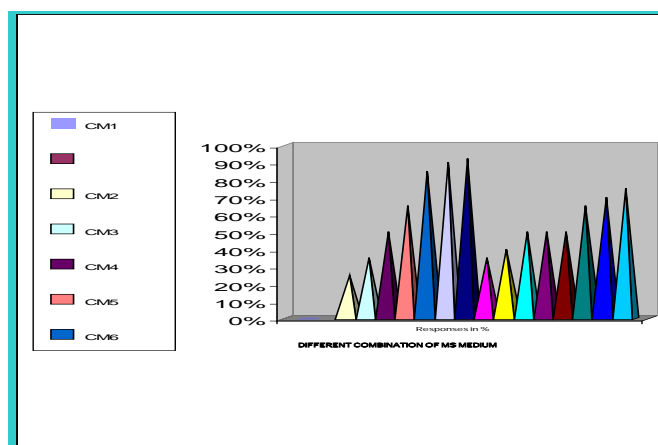


Fig: 18 Effect of Various Level and Combinations of PGR's For Callusing of *Cissus Quadrangularis* L. (Hadjod)

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