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Callus Induction and *In vitro* Plantlet Regeneration of *Gymnema sylvestre* R. Br. (Retz.)

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ABSTRACT

Higher plants are the source of a large number of chemicals with wide range of medicinal, pharmacological and insecticidal properties. In recent years there is a great demand for plant-based products because of the broad biological activities, safety without any toxic side effects and low impact on environment. *Gymnema sylvestre* R. Br. (Family- Asclepiadaceae) is one of the important medicinal plants of India. Through plant tissue culture technique, it is possible to produce disease free and high fielding plantlets of commercially important plants in large quantity of meet the huge demand from the cultivator's

KEYWORDS: *Gymnema*, antidiabetic, gymnemicacid, antioxidant,antiobesiy biotechnology.

INTRODUCTION

GymnemaSylvestreR. Br. is a valuable herb belonging to the family Asclepiadaceae, and widely distributed in India, Malaysia, Srilanka, Australia, Indonesia, Japan, Vietnam, tropical Africa and the southwestern region of the People's Republic of China. The plant is commonly known as Periploca of the woods (English); Gurmar (Hindi); The word "Gymnema" is derived from a Hindu word "Gurmar" meaning "destroyer of sugar" and it is beli;eved that it might neutralize the excess of sugar present in the body in Diabetes mellitus.The plant tissue culture is the in-vitro manipulation of plant cell and tissue, which is which allows one types of tissue or organ to be initiated from another type. In this keystone in the foundation of plant bio technology; it is useful in plant propagation. The principle of plant tissue culture is based upon the concept of'; totipotency''i.e. every plant cells have a capacity to develop whole plant. The history of plant tissue culture dates back on at least to 1902 when Haberlandt, a German botanist proposed

that all plant could be cultured invitro. He is said to the father of tissue culture. Gautheret (1934) got encouraging result with culturing cambial tissue of carrot. The first plant growth hormones Indole acetic acid (IAA) was discovered in mid-1930 by Kogl et al. In 1934, Prof. White successfully cultured tomato root. In 1939, Gautheret successfully cultured carrot tissue. Both Gautheret and White were able to maintain the culture for about 6 years by sub-culturing them on fresh medium. Their experiment demonstrated that culture could be not only initiated but also maintained over a long period of time.

Taxonomic position

It is an herb native to the tropical forests of southern and central India where it has been used as a naturopathic treatment for diabetes for nearly two millennia. It is commonly known as Periploca of the woods, Gudmar, Gurmari, Gurmarbooti the taxonomic position of this plant is as follows.

Kingdom : Planate

Division : Magnoliophyta

Class : Magnoliophyta

Species *: sylvestre*

Order : Gentianales

Family : Asclepiadaceae

Genus :*Gymnema*

The plant tissue culture is the in-vitro manipulation of plant cell and tissue, which is which allows one types of tissue or organ to be initiated from another type. In this keystone in the foundation of plant bio technology; it is useful in plant propagation. the principal of plant tissue culture is based upon the concept of totipotency of biology which tells that a single living cell of plants have the capacity to regenerate the whole plant which is phenotypically as well as genetically similar to mother plant. Through plant tissue culture technique, it is possible to produce disease free and high fielding plantlets of commercially important plants in large quantity of meet the huge demand from the cultivator's

MATERIAL AND METHODS-

The present study entitled "Development of callus and Micropropagation protocol of *gymnemasylvestre* R.Br. (Retz)" was conducted in plant Tissue Culture Laboratory, Department of Biotechnology.

Study site:

The study site is situated on the border of Chitrakoot district of Uttar Pradesh and Satna district of Madhya Pradesh. The average annual rainfall is about 781 mm. the climate is generally dry except during the south-west mansoon that brings 75% of the total normal rainfall (june-september).

The experimental healthy plant for the study had been choosed was *GymnemaSylvestre*(Gudmar). The plant selected in Deendayal Research Institute ChitrakootSatna (M.P.). These plants were sprayed with fungicide before use.

STERILIZATION OF EXPLANT

Single nodal segment were collected form *Gymnemesylvestre* plants. Prior to use the explants washed with teepol (5 drops in 100 ml water) for 10 min. followed by washing under running tap water for 15 min. The nodal segment were then surface sterilized by treating with 0.1 % solution of Mercuric Chloride or 0.5 % Sodium Hypochlorite as According to the following Table . After three times washing in sterile distilled water the cut ends of the segment were trimmed and cultured on MS media.

Inoculation:

The surface sterilized explants were inoculated in screw cap jam bottles in 200 ml. The bottles contained 30-40 ml MS medium supplemented with various growth regulators according to the nature of experiment. The inoculation process was carried out under aseptic condition in laminar air flow cabinet (MAC India, Pvt. Ltd., New Delhi). It has a small motor to blow air which is first passed through a coarse filter, where it is freed from large particles and subsequently through a fine filter (HEPA filter), which removes particles larger than 0.3 µm and ultra clean air flow through the working area. The velocity of the air carrying out of the filter is about 27± 3 m per minute. It is also filter over head with white fluorescent tubes and UV tubes. Glass bead sterilizer was used for sterilization of forceps, scalpels and scissors.

Culture conditions**:** Explant inoculated jam bottles were kept in incubation chamber under the controlled environment of temperature (25±2 ºC) and light (13 hd^{-1} illumination of 30 to 40 µ molls M^{-2} S^{-1} spectral of Flux photons) and 60-70% relative humidity (RH). The light sources were white florescent tubes and incandescent bulbs (Philips India Ltd., Mumbai) and (ECE Industries Ltd., New Delhi) units fitted with the air conditioner; split air conditioning unit is used. The chamber was fumigated with potassium dichromate and formaldehyde after 20-25 days interval.

Multiplication-

The initiated plantlets were removed from the explants with the help of forceps and scalpels and cut from the nodal section. The segment was transferred into the multiplication medium with different concentration of hormones. After inoculation tubes/conical were placed upon the culture rack under fluorescent light of 14-16 hours at 24+_2ċ temperature.

Table 1-Media Composition for Shoot Multiplication

S.No.	Media Name	Media Composition
1	M1	MS basal medium + 2 mg BAP
2	M2	MS basal medium +3 mg BAP+1.5 mg NAA+50 mg AS
3	M3	MS basal medium +0.5 mg BAP

Rooting-After multiplication plantlets are inoculated in the rooting MS medium with different concentration of hormones the root initiation. After inoculation, cultured tubes were placed upon the culture rack under fluorescent light of 14-16 hours and 24 +_20C temperature.

Table 2– Media Composition for rooting

S.No.	Media Name	Media Composition
1	R1	MS ½ basal medium + 0.5 mg IBA
2	R2	MS ½ basal medium + 50 mg AC
3	R3	MS ½ basal medium

CALLUS CULTURE

The inoculation of leaf explants was carried under aseptic and sterile environment. The sterilized explants are put into sterile petri plate and inoculated in the culture tube the sterilized explants were put into sterile petri plates and inoculated in the culture tubes containing MS media supplemented with hormones. After inoculation culture tubes were placed upon culture rack in dark at 24+_2c.

Table-3 Media composition for callus initiation

S. No.	Media Name	Media composition
1	C1	MS basal medium +2 mg 2,4-D+1 mg kinetin
2	C2	MS basal medium +3 mg 2,4-D+ 1 mg kinetin
3	C3	MS basal medium + 4 mg 2,4-D +1mg kinetin

4	C4	MS basal medium + 5 mg 2,4-D + 1 mg kinetin

Result and Discussion- The present investigation has been planned to develop the Callus Induction and *In vitro* Plantlet Regeneration of *Gymnemasylvestre*R. Br. (Retz.) Therefore, the result of micro-propagation discussed under following heads.

Sterilization- The explant was collected from the field and then sterilized with $HgCl_2$ and alcohol. A total number of 36 explants were taken and the surface sterilized with 2 different type combinations of 70 % alcohol and 0.1% $HgCl_2$. After observation, it was found that both combinations were suitable to sterilize the nodal explants of *gymnemasylvestre.*

Table 4. Table 15- Survival number of nodal explant of *Gymnemasylvestre* in various treatments

Treatment	contamination	Dead	survive	Survival %
T1	3	0	20	86%
T2	4	0	15	78%

T1=30 Seconds in 70% alcohol and 90 Second in 0.1% $Hgcl_2$

T2=60 Seconds in 70% in alcohol and 120 Seconds in 0.1% $HgCl_2$

INOCULATION

The sterilized nodal explant of *Gymnemasylvestre* was inoculated in MS medium supplemented with different combination of growth hormones. After 21 days, it was found that MS medium supplemented with 4.4 mg BAP was best to initiate the nodal explant of most suitable for shoot initiation from nodal segment and have 2.5 shoot with 4.2 cm height. MS medium supplemented with 4.4 mg BAP gave the best response. Reddy et al (1998) reported incorporation of NAA (1.6 µM) with high concentration of BAP (22µ M) in the medium. But in the present study only BAP gave good response. Explant inoculated at higher concentration of BAP alone or in combination with NAA produced clumps of highly-reduced shoots with smaller leaves (Reddy et al, 1998).

Table-5 Effect of various concentrations of BAP and KN on number of nodes shoots length and number of leaves on *Gymnemasylvestre*

NO	MEDIA	Number. Of Nodes	MEAN SHOOT LENGTH	Number of leaves
1	MS+0.4mg BAP	1	3.2cm	1

2	MS+2.2 mg BAP	1.24	3.5cm	2
3	MS+ 4.4mg BAP	3.5	4.5cm	4.25
4	MS+5mg BAP	1.3	3.1 CM	3.1
5	MS+5.6mg BAP	1	2.7cm	3
6	MS+ KN 0.46	1	3.1cm	2
7	MS+ KN 2.32	1.2	3.4cm	1
8	MS+ KN 4.6	1.3	4.6cm	3.5
9	MS+ KN 5mg	1.1	3.8cm	2
10	MS+ KN 5.5 mg	1	2.8cm	2.5

MS medium with 4.4 mg BAP showed the maximum number of bud sprouting. (Fig-3)Kinetin also showed bud initiation but not as induced by BAP.

The addition of cytokinin promotes precocious axillary shoot development (Hussey, 1976; Bhojwanf4nd Razdah, 1983). Although BAP and KN are equally effective in axillary bud proliferation, BAP has been considered to be more active (Rahman and Blake, 1988, Misra,1996).In *Hemidesmusindicus*. Patnaik and Debata (1996) reported such abnormal shoots in low concentration of BAP.

.**Multiplication**- The initiated shoots were transferred in MS medium supplemented with different combination of growth hormones. Based on 20 days data, data were recorded for number of shoots, shoot height (cm), number of nodes and number of leaves.

Table-6 Multiplication of Initiated Shoot

Media	No of Shoot	Shoot height (cm)	No of leaves	No of Nodes	No of Roots	Remark
M1	2.5*	2.9	2.5	1	0	
M2	4.5	2.8	1.55	1	0	
M3	2.5	2.45	3.5	4.56	1	Callusing observed

*Average

The average number of shoots per explant was 2.5, 4.5 and 2.5 with height of 2.9,2.8 and 2.45 cm in M1,M2 and M3 medium respectively.2.5,1.55 and 3.5 leaves (average) were found in M1,M2 and M3 medium respectively. The average number of nodes was 1, 1 and 4.56 in M1, M2 and M3 medium respectively.(fig-1).

In addition to these data, rooting and callusing at the base of micro shoots were also observed in different mediums. The rooting was observed in M3 medium. While some explants have basal callusing, which were inoculated in M3 medium from these data, it is clear that M2 and M3 medium has better response in

comparison to M1 medium. In M2 medium number was shoot was 4.5, but it only 1 node, while in case of M3 medium, number of shoot was 2.5 and have 4.56 nodes per explant and also have root. Therefore , M3 medium i.e. MS supplemented with MS basal medium +BAP 4.4 mg+ KN 4.6 mg was best for shoot proliferation, and it also yielded rooting.

Rooting-Further micro shoots were subjected to rooting for the development of full plantlet. Although rooting was observed in the multiplication stage in M3medium, but in this callusing was also observed in some of the micro shoots. Callusing at the junction of root and shoot can end up to death of plantlet after hardening. This might because the root was not attached with shoot and it can be originated from the cell of callus, therefore, it cannot supply nutrient to the shoot, which will followed to the death of the plant.

The micro shoots were transferred into half strength MS basal medium as well as MS medium with various auxins like NAA, IAA and IBA (Table-4).

Table-7**Effect of auxins on *in vitro* root formation from regenerated shoots of *Gymnemasylvestre***

Concentration (µM)	**Root induction (%)**	**No of roots (%)**
NAA 5.37	46 %	4.0%
IAA 5.71	13.3%	1.33%
IBA 4.92	6.60%	0.33%
Ms Basal ('/2 Strength)	66.6%	6.66%

One of the major obstacles to micro propagation of plants is rooting and acclimatization of plantlets (Torres, 1989). The cultures which are inoculated on auxin free half strengths MS basal medium, showed root initiations. However, rooting was slow and the percentage was very less. With the addition of auxin callus formation was there which did not favour the root formation. Thus *ex vitro* rooting is preferred in commercial laboratories (Alderson et al, 1988).

Hardening- Well-developed plantlets (shoot and root) was transferred into sterile sand, soil and vermicomposting mixture (1:1:1) for their acclimatization to soil. After 21 days, it was observed that 80% plants were survived.

Callus induction- Young leaves of *Gymnemasylvestre*was inoculated in to C medium i.e. MS medium supplemented with 11 µm BA and 2.26 µm 2, 4-D. when 7 to 8 days a full mass of cell i.e. callus was

observed which was white in colour. Higher concentration of Auxin with lower concentration of cytokinin is supposed to be inducing the callusing. Sairker*et al.* (2009) also used this media for the callus induction of *Stevia rebudianna*.

The callus induction was noted within 4-5 days on explants inoculated on media

Supplemented with 11 µM BA and 2.26 µM 2, 4-D.

BA / 2,4 D	0	2.2	6.6	8.8	11
0	+	+	+	-	++
2.26	+	+	+	+++	++++
6.78	+	+	++	+	++
9.04	-	++	+	-	-
11.3	+	+	+	+	+

When different levels of BA were used in combination with 2.26 µM 2, 4-D maximum of 70% of callus induction was observed in 11 µM BA. The callus induced on this combination was friable and fast growing .fig(6).Initiation of callus was observed in *Calotropisgigantea*Lin on MS media supplemented with KN (0.5 mg/1) and 2, 4-D (1 mg/1). In our studies simple MS medium supplemented with 11.1 µM of BA and 2.26 µM of 2, 4-D gave fast growing and profuse callusing than the medium supplemented with NAA. Even though the callus growth was very fast

Fig-1-Graph showing sterilization of explant

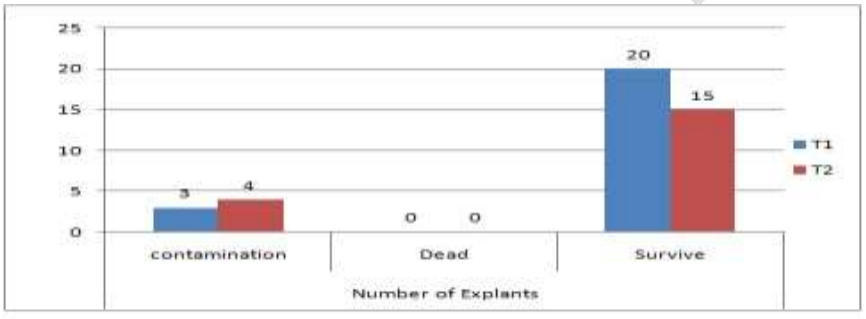


Fig-2 Graph showing multiplication of initiated shoot

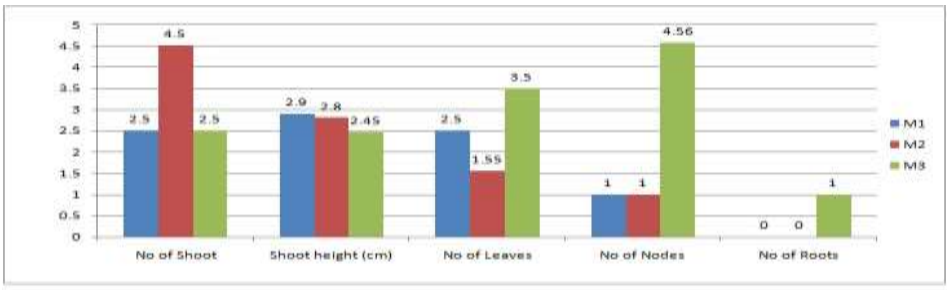


Fig-4 Induction of multiple shoots in *Gymnemasylvestre*

Fig-5a) Rooting .b) Roots of invitro derived plantlets

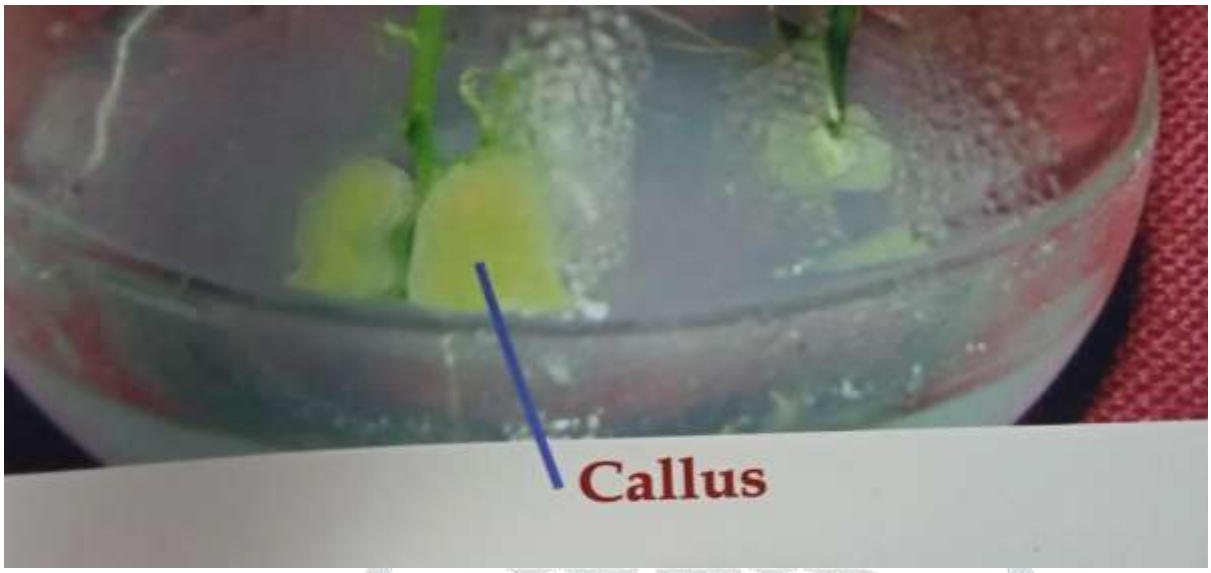
a) b)

Fig-6 Induction of callus from leaf explants of *Gymnemasylvestre*

(a) Callus formation on MS basal medium +BAP 4.4 mg+ KN 4.6 mg

(b) Further proliferation of callus on same medium

(a)



(b)

Conclusions

*Gymnemasylvestre*R.Br(Family: Asclepiadaceae) – commonly known as gurmar or sugar destroyer, is seen in various parts of India. The woody climber is used for various diseases and disorders in traditional medicines such as glycosuria, urinary complaints, chronic cough, piles, stomach problems, breathing troubles, asthma, eye complaints, cardiopathy, jaundice, constipation and bronchitis. The current updated review onthe Plant tissue culture technique, it is possible to produce disease free and high fielding plantlets of commercially important plants in large quantity of meet the huge demand from the cultivator's. The regeneration of whole plants from plant cell that have been genetically modified.

This updated review on the plant will be much more helpful for all those researchers who are all carrying out their investigations and research on this climber.

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