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In-vitro propagation of anti-diabetic plant (*Gymnema sylvestre* R.Br.)

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ABSTRACT

Plants are useful source of various bioactive compounds which have direct and indirect use in the treatment of various human indispositions. Plant have always played prominent roles in traditional as well as modern medicinal system. The Apocynaceae family is the one of the most medicinally diverse family. This study focuses on the micropropagation of Gymnema sylvestre and investigation of efficiency of various plant growth regulators (PGRs) on its invitro propagation. Plant tissue culture as an important tool for continues production of active compounds including secondary metabolites and designed molecules. The result demonstrate the potential of in vitro techniques for rapid and efficient propagation of G.sylvestre, which has significant implications as anti-diabetic plant.

KEYWORDS - *Gymnema sylvestre,* In-vitro, Micropropagation, Anti-diabetic, Benzyl adenine.

INTRODUCTION

Gymnema sylvestre is one of the powerful medicinal plants used from prehistoric times. It is perennial woody climber spread up to height 600m and commonly known as "Gurmer". (Karale P A and Karale M A 2017). *G.sylvestre* is generally disseminated in India, Malaysia, Sri Lanka, Indonesis, Japan, Vietnam, Tropical Africa and South Western region of the people's republic of China. It is also found in Banda, Kokan, Western ghat and Deccan extending to the parts of western and northern India (Karale P.A and Karale M.A 2017). *Gymnema sylvestre* leaves contain triterpene saponins belonging to oleanane and dammarene classes. Oleanane saponins are gymnemic acid and gymnemasaponins while dammarene saponins are gymnemasides (Parijat K *et.al.* 2007). Other phytoconstituents include anthraquinones , flavones , hentriacontane , pentatriacontane , phytin , resin , tartaric acid , formic acid , butyric acid , lupeol β- amyrin related glycosides , stigmasterol and

calcium oxalate. (Tiwari P *et al.,* 2014)

G. Sylvestre is used in folk, Ayurvedic system to treat type 1 and 2 diabetes. It is also used in the treatment of urinary complaints, stomach problems, piles, chronic cough, breathing troubles, asthma, eye complaints, cardiopathy, constipation, jaundice, and bronchitis. (Vimala C *et al.,* 2023). The propagation of G. *sylvestre* has been done through stem cutting or seeds. However these methods are slow, season dependent and seeds germination is difficult because the seeds have a low viability. Micropropagation is potential and alternative source for the large scale production of desired and disease free plantlet with rapid growth.

MATERIAL AND METHOD

A. Experimental material:

Meristematic regions were taken from the healthy plant growing under the in-vivo conditions were taken as explant.The material of *Gymnema sylvestre* R.Br were collected from Botanical garden of Sir Parashurambhau College (Autonomous) Pune, India.

B. Culture medium:

The MS medium was supplemented with different concentrations (0.1, 0.2, 0.3, 0.4, 0.5 mg/l) of Benzyl adenine (BA) and in combination with 0.1 mg/l 2, 4-dichlorophenoxyacetic acid (2, 4-D) for callus induction. The MS medium were congealed with agar (0.8%) and sucrose (3%) was used as source of carbohydrate .pH of the medium was adjusted to 5.8-6.0 using 0.1N NaOH / 0.1N HCL. Volume of medium was adjusted with distilled water before being autoclaved at 15l bps and 121℃ for 20 minutes.

C. Cleaning:-

These explants were cut 2-3cm and washed under running tap water for 30 minutes, followed by washing with labolene and tween 20 (fungicide) for 5 minutes each and then wash with distilled water to remove dust particles and microbes.

D. Inoculation:

Surface sterilization is carried out in laminar with 70% alcohol and 0.1% $HgCl_2$ followed by washing with sterile distilled water to remove traces of $HgCl_2$. Fresh cuts were given to explants after sterilization to remove undesirable or dead portions. The explants were then inoculated on MS medium.

E. Incubation:

All the cultures were incubated at temperature of 25℃ ± 2℃. Light and dark condition were maintain 16 hrs light and 8 hrs darkness, illuminated by fluorescent light of about 1800-

2000 lux intensity

F. Data Analysis:

A minimum of 10 replica were used per treatment and the experiments were repeated thrice. Data were analysed statistically by using mean.

G. Calculation of response percentage:

The response percentage rate on each media formulation was calculated using the following formula (Ramasubramaniyan. M.R et al., 2015).

$$\%\,\text{Response} = \frac{\text{Number for explants showing response}}{\text{Total number of explant inoculated}} \times 100$$

RESULT AND DISCUSSION

The callus was successfully initiated on MS media on day 16 after inoculation. Callus initiation of the various BA with 2 4 –D tested, MS medium augmented with (0.4, mg/l) BA was found to be optimum for inducing maximum callus from the apical bud explants of *G. sylvestre* (Table-1)

Table 1. Effect of BA and 2 4-D on in vitro callus initiation from apical bud explants of *Gymnema sylvestre* after 4 weeks of culture.

BA (mg/l)	(2,4-D) (mg/l)	% Responding Cultures
0.1 mg/l	0.1mg/l	56.66
0.2 mg/l	0.1mg/l	63.33
0.3 mg/l	0.1mg/l	73.33
0.4 mg/l	0.1mg/l	93.33
0.5 mg/l	0.1mg/l	66.66

Fig.1 A - Inoculation of apical meristem of *Gymnema sylvestre* R.Br (MS +0.4 Mg/l BA and 0.1mg/l 2-4 D)

Fig.1 B - Callus initiation (16 days)

Fig.1 C - Maximum callus (24 days)

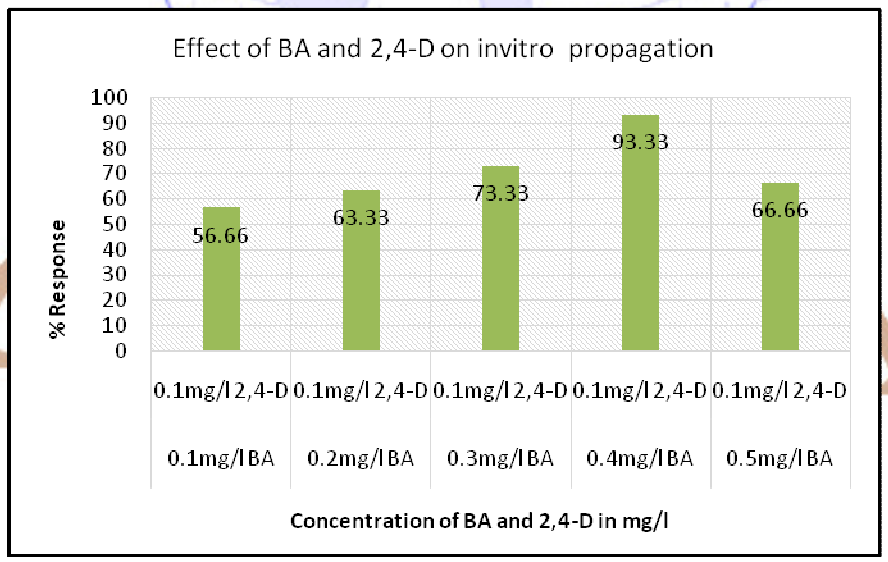

Fig. 2 - Effect of BA and 2, 4-D on invitro propagation of *Gymnema sylvestre*

CONCLUSION

An efficient micropropagation protocol was developed for *Gymnema sylvestre* using axillary bud as explant . MS medium augmented with 0.4 mg/l BA and 0.1 mg/l 2 4-D induced maximum callus at day 24 after inoculation.

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REFEREANCE

- Karale P.A, Karale M.A (2017). "A review on phytochemistry and pharmacological properties of milkweed family (Asclepiadaceae)". *Asian journal of pharmaceutical and clinical research.*30.
- M.R. Ramasubramaniyan , Kalpana Rajesh , M. Priya Dharishini , M. Krishna moorthy, A. Radha5, K. Balasubramanian , B. Sai Shruti and S. Raja Nandhini (2015). "Studies on Optimization of Medium in Induction and Regeneration of Callus and Shoot from *Costus igneus* and its Phytochemical Profile". *Journal of Academia and Industrial Research (JAIR).* 76.
- Parijat K, Rekha S and Mdhusudan K (2007). "*Gymnema sylvestre:* A mimoir". *Recent advances in Indian herbal drug research.*77.
- Tiwari P, Mishra B.N and Sangwan N.S (2014). "Phytochemical and pharmacological properties of Gymnema sylvestre". *Hindawi publishing corporation.*1.
- Vimala C, Ancy S, Mala G, Appavoo M and Irene Wilsy J (2023). "Gymnema sylvestre (Retz.) R.Br. Ex Sm.: A Botanical, Ethnopharmacological, Phytochemical, And Pharmacological Overview". *Journal of pharmaceutical negative result.*1153. Volume 14.