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# Effect of Different Phyto-Hormones on Callogenesis in *Gymnema sylvestre* R. Br. : An Important Anti-Diabetic Plant

[1]Bishnupriya Hansdah, [2]S. L. Sahoo
[1]Assistant Professor of Botany, North Orissa University, Takatpur, Baripada, Odisha, India
[2]Professor of Botany, Utkal University, Vani Vihar, Bhubaneswar, India
Corresponding Email- santi_bot_uu@yahoo.co.in

## ABSTRACT

*Gymnema sylvestre* R. Br., belongs to the family Asclepiadaceae and commonly called as "Gudmar" is one of the popular medicinal plants acknowledged as a potent drug plant in Ayurveda as well as in homeopathic systems. This plant is mostly used medicinally in India and Southeast Asia for treatment of "sweet urine" or what we refer it as diabetes and also employed in the treatment of many other health problems. This study developed a novel cell culture system for in vitro callus induction, proliferation and growth of this species with improving the active principles in the plant. Nodal explants were inoculated in MS basal media, with supplementation of different concentrations (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l) of Auxins (2, 4-D and NAA) and Cytokinins (Kinetin and BAP). The explants were allowed to grow and the callus was maintained on the same medium for weekly analysis. The maximum callusing was obtained in the medium supplemented with combinations of Kn (2.5 mg/l) along with 2, 4-D (2.5 mg/l). The maximum fresh weight and dry weight was found when MS medium fortified with 2, 4-D (2.5 mg/l) followed by NAA at 2.5 mg/l. Among cytokinins, the Kinetin supplemented medium yield more callus then that of BAP at 2.0 mg/l. From the above study, it was quite conclusive that, the phytohormones play a significant role in regulation of the growth of callus and proliferation.

**Keywords :** *Gymnema sylvestre* R. Br., Diabetes, Callogenesis, Proliferation, Phytohormones.

## I. INTRODUCTION

### General Introduction to Tissue Culture

Medicinal plants are nature's gift, which has curative properties due to the presence of various complex chemical substances of different composition (1). The use of plants as medicine due to the presence of various chemical substances is ever increasing day by day. But since most of these plants are taken from the wild, hundreds of species are now on the verge of extinction. In view of the growing world population, increasing anthropogenic activities, rapidly eroding natural ecosystem etc the natural habitat for a great number of herbs and trees are dwindling. Many of them are facing extinction. To cope up with alarming situation the recent exciting developments in plant tissue culture technique have come as a boon (2)(3). *In vitro* culture of plant cells and tissue has attracted considerable interest over recent years because it provides the means to study plant physiological and genetic processes in addition to offering the potential to assist in the breeding of improved cultivars by increasing genetic variability (4)(5). *Gymnema sylvestre R. Br.* (Asclepiadaceae), a vulnerable species is a slow growing, perennial medicinal woody climber found in South China, India and Vietnam. It is widely used in indigenous system of medicine for treatment of Diabetes (6)(7). Besides, the plant species is also used in the treatment of asthma, obesith, eye complaints, imflammations and snakebite

(8)(9)(10)(11). In addition, it possesses anti-microbial, anti-hypercholesterolemic and sweet suppressing activities (12)(13)(14)(15).

To fulfill the increasing demand of *G. sylvestre*, its large scale propagation is essential and plant tissue culture technique acts as alternative methods for the development and multiplication of this medicinal plant species. Callus induction is a powerful tool to regenerate plants. Callus is a disorganized mass of undifferentiated tissue comprised of actively dividing cells. The cells of callus dedifferentiate and thus regain their meristematic properties, including rapid proliferation. Our main objective of this study was to investigate the optimal cultivating conditions and effects of phytohormone for this *G. sylvestre* by means of callus induction and proliferation.

## II. MATERIALS AND METHODS

The healthy plants were collected from apex of 2 years old genotype of *Gymnema sylvestre* R. Br. for standardization of callugenesis protocol in Department of Botany, Utkal University, Bhubanesware, Odisha. Collected explants were first washed with running tap water (10 minutes) and then surface sterilized with Teepol (5%), 70% ethanol for 1-2 minutes and were rinsed in distilled water thrice. Then, they were taken to the laminar air flow chamber where treated with 0.1% $HgCl_2$ for 2-3 minutes and washed with sterile double distilled water. It was then inoculated in the appropriate Murashige and Skoog's (16) basal medium, which contained sucrose (3%), and pH (5.8). The cultures were incubated at 25±2ºC under 2000 lux light intensity provided by white fluorescent lamp for 8 hours photoperiod. The basal MS medium was used with derived supplementation of phytoregulators for callus induction from leaf and nodal segment of *G. sylvestre*. The MS basal media were supplemented with different auxins: 2,4D 0.5-3 mg/l, and Naphthalene Acetic Acid (NAA) 0.5-3 mg/l and cytokinin: Benzyl Amino Purine (BAP) and Kinetin 0.5-3 mg/l. after callus induction periodically sub culture was done better callus developments and proliferation. The fresh and dry biomass was also calculated for appropriate estimation of callus production.

## III. RESULT AND DISCUSSION

### Callogenesis/ callus induction:

In order to study the effect of plant growth regulators (PGRs), the nodal explants were inoculated in MS medium supplemented with various concentrations of auxins and cytokinins. The remarkable callus proliferation was observed when MS medium was fortified with cytokinins i.e. kinetin (0.5 mg/l), BAP (0.5 mg/l) and 2, 4-D, NAA (0.5 mg/l) among the auxins. Ahmed *et al.* (17) reported that MS medium with PGRs auxins and cytokinins combination were suitable for callus proliferation of *G. sylvestre*, but they did not shown the other concentration and combination for callus proliferation accept 24D and Kinetin (data not shown here). According to our experiment, among the various treatments, best response (95 ± 1.20) towards the callus induction was obtained in MS medium supplemented with 2,4-D, kinetin (2.5mg/l) each. The single supplementation of Kinetin (2mg/l) (Fig 1, b), NAA at 2.5 mg/l (Fig 1, d), and BAP at 2 mg/l (Fig 1, e), caused 82.3±0.83%, 79.6±0.92% and 75.2±1.16% callus induction, respectively showing that kinetin was marginally more effective in causing induction of callus than of the other two plant growth regulators. However, the differences in the efficiency in induction of callus was marginal but significant ($F= 8.63$; $p \geq 0.05$). In all the media tested, 2, 4-D single or Kinetin found to be more effective for the callus proliferation.

**Table 1 :** Effect of MS basal medium supplemented with different concentration of plant growth regulators (PGRs) on induction of callus and characteristic of callus.

| Phytohormone concentration (mg/l) | | | | Callus response (%) | Texture and Color |
|---|---|---|---|---|---|
| **2,4-D** | **Kinetin** | **NAA** | **BAP** | **(Mean ± SEM)*** | |
| 0.5 | | | | 40±1.46 | White and friable |
| 1.0 | | | | 49.9±1.50 | White and friable |
| 1.5 | | | | 60 ± 1.58 | White and friable |
| 2.0 | | | | 73 ± 1.05 | White and friable |
| **2.5** | | | | **90 ± 1.20** | **Light brown and friable** |
| 3.0 | | | | 75 ± 1.02 | Light brown and friable |
| | 0.5 | | | 36 ± 0.99 | White and friable |
| | 1.0 | | | 60 ± 0.89 | White and friable |
| | 1.5 | | | 81 ± 0.91 | White and friable |
| | **2.0** | | | **82 ± 0.83** | **White and less hard** |
| | 2.5 | | | 79 ± 1.50 | Light yellow and less hard |
| | 3.0 | | | 72 ± 1.20 | Light yellow and less hard |
| 0.5 | 0.5 | | | 42 ± 1.03 | White and friable |
| 1.0 | 1.0 | | | 64 ± 0.95 | White and friable |
| 2.0 | 2.0 | | | 78 ± 1.05 | White and friable |
| **2.5** | **2.5** | | | **95 ± 1.11** | **White and friable** |
| 3.0 | 3.0 | | | 67 ± 0.98 | Light brown and friable |
| | | 0.5 | | 43 ± 1.04 | White and friable |
| | | 1.0 | | 62 ± 1.08 | White and friable |
| | | 1.5 | | 70 ± 1.40 | White and friable |
| | | 2.0 | | 75 ± 0.89 | Fluorescent green and friable |
| | | **2.5** | | **79 ± 0.92** | **Fluorescent green and granular** |
| | | 3.0 | | 76 ± 1.30 | Fluorescent green and granular |
| | | | 0.5 | 50.9 ± 1.20 | White and friable |
| | | | 1.0 | 65 ± 1.41 | White and friable |
| | | | 1.5 | 72 ± 0.87 | White and friable |
| | | | **2.0** | **75 ± 1.16** | **Light green and compact** |
| | | | 3.0 | 70 ± 1.92 | Light green and compact |
| | | 0.5 | 0.5 | 47 ± 1.54 | Light green and friable |
| | | 1.0 | 1.0 | 56 ± 0.78 | Light green and friable |
| | | 2.0 | 2.0 | 70 ± 1.22 | Light green and compact |
| | | **2.5** | **2.0** | **82 ± 1.01** | **Green and compact** |
| | | 3.0 | 3.0 | 66 ± 0.89 | Light green and compact |

Similar results were found in many plant including *G. entiana* spp. (18) and other dicot plants (19). Komalavalli and Rao (20), reported that there could be good positive effect of coconut and malt extract with 2, 4-D on callusing of *G. sylvestre,* when these are supplemented to MS medium. The callus response was increased with increasing concentration of auxins and cytokinins but at higher concentration the percentage of callus decreased. The friable and white/brown callus were obtained in MS medium fortified with 2, 4-D and Kinetins but the callus became green and compact when MS medium supplemented with BAP and Kinetin, singly or in combination.

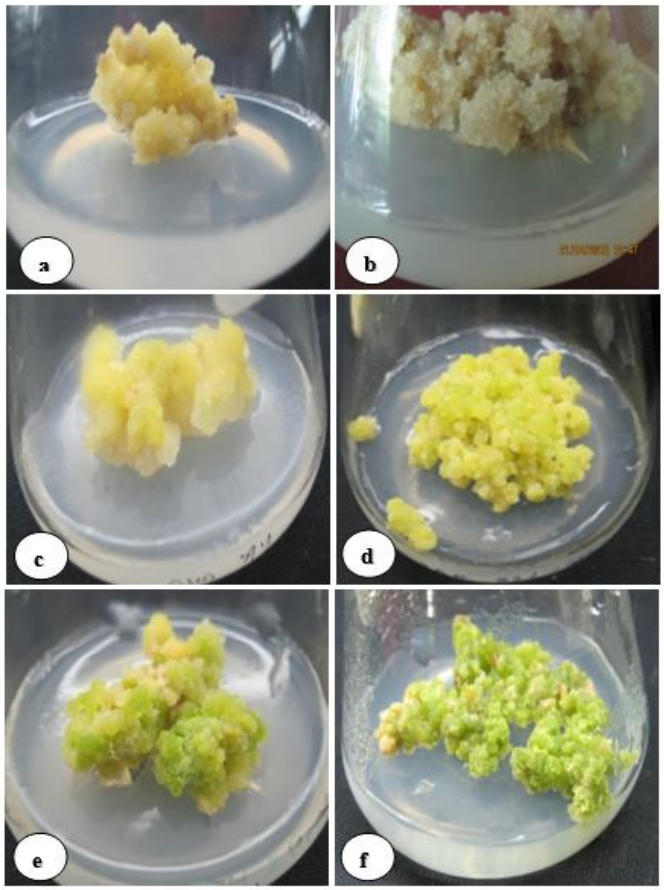


**Fig. 1.** Callogenesis in G.sylvestre: (a) friable callus in 2,4-D (2.5 mg/l), (b) White and less hard callus in Kinetin (2.0 mg/l), (c) White and friable callus at 2,4-D & Kinetin (2.5 mg/l) each, (d) Fluorescent green and granular at NAA (2.5 mg/l), (e) Light green and compact at BAP (2.0 mg/l), (f) Green and compact at NAA (2.5 mg/l) & BAP (2.0 mg/l).

**Growth index of callus tissue:**

**A. Fresh weight of the callus tissue:**

The growth of *Gymnema sylvestre* callus was determined by taking fresh weight measurement. The fresh weight of the callus was measured after 6 weeks time interval. The callus growth was found in MS medium fortified with auxins such as (2, 4-D and NAA) and cytokinins (BAP and Kinetin) at different concentration and combination. The yield of fresh biomass was high on the MS medium fortified with 2.5 mg/l concentration of 2, 4-D (11.66 ± 0.33 g/culture) followed by NAA (7.17 ± 0.28 g/culture) at 2.5 mg/l. Among the cytokinins, the Kinetin supplemented medium yield more callus i.e. 8.33 ± 0.23 g/culture at 2 mg/l than that of BAP (4.12 ± 0.08 g/culture) with 2mg/l concentration. The maximum callus growth was achieved when MS medium supplemented with 2, 4-D along with Kinetin (14.84 ± 0.23g/culture) at 2.5 mg/l each. The development of callus was increase with increasing concentration of auxins and cytokinins but at higher concentration i.e more than 2.5 mg/l, the callus growth declined.

**B. Dry weight of the callus tissue**

The result of dry biomass yield was achieved by taking dry weight of the callus tissue. At the end of the culture period, the cell mass was placed between the folds of blotting paper to remove excess water content and dry weight was measured after drying the fresh cell mass at room temperature. The dry callus was found when callus developed in the medium supplemented with auxins i.e 2, 4-D (0.84 ± 0.10 g/culture) at 2.5mg/l concentration followed by NAA (0.56 ± 0.10 g/culture) at 2.5mg/l concentration. Among the cytokinins, good amount of dry biomass yield was obtained when MS medium fortified with kinetin at 2mg/l i.e (0.61± 0.02 g/culture) where as BAP shows (0.36 ± 0.04 g/culture) dry biomass at

2mg/l concentration. The maximum dry biomass was obtained when MS medium fortified with 2, 4-D combination with Kinetin (0.92 ± 0.02 g/culture) at 2.5 mg/l each. The dry biomass was ten times less than that of fresh biomass due to high water content.

**Table 2 :** Effect of MS basal medium supplemented with different concentration of plant growth regulators (PGRs) on growth index of callus tissue.

| Phytohormone concentration (mg/l) | | | | Fresh weight of callus (g/culture) | Dry weight of callus (g/culture) |
|---|---|---|---|---|---|
| 2,4-D | Kinetin | NAA | BAP | (Mean ± SEM)* | (Mean ± SEM)* |
| 0.5 | - | - | - | 3.00 ± 0.25 | 0.28 ± 0.03 |
| 1.0 | - | - | - | 4.18 ± 0.10 | 0.32 ± 0.04 |
| 1.5 | - | - | - | 5.19 ± 0.18 | 0.40 ± 0.01 |
| 2.0 | - | - | - | 8.25±0.23 | 0.67 ± 0.02 |
| **2.5** | **-** | **-** | **-** | **11.66 ± 0.33** | **0.84 ± 0.03** |
| 3.0 | - | - | - | 9.69 ± 0.33 | 0.53 ± 0.03 |
| - | 0.5 | - | - | 2.10 ± 0.13 | 0.14 ± 0.03 |
| - | 1.0 | - | - | 3.28 ± 0.13 | 0.19 ± 0.03 |
| - | 1.5 | - | - | 5.26 ± 0.12 | 0.43 ± 0.04 |
| **-** | **2.0** | **-** | **-** | **8.33 ± 0.23** | **0.61 ± 0.02** |
| - | 2.5 | - | - | 6.12 ± 0.41 | 0.47 ± 0.03 |
| - | 3.0 | - | - | 4.20 ± 0.25 | 0.32 ± 0.04 |
| 0.5 | 0.5 | - | - | 2.06 ± 0.33 | 0.21 ± 0.01 |
| 1.0 | 1.0 | - | - | 6.03 ± 0.48 | 0.44 ± 0.02 |
| 2.0 | 2.0 | - | - | 8.19 ± 0.17 | 0.63 ± 0.02 |
| **2.5** | **2.5** | **-** | **-** | **14.84 ± 0.23** | **0.92 ± 0.02** |
| 3.0 | 3.0 | - | - | 9.48 ± 0.16 | **0.61** ± 0.02 |
| - | - | 0.5 | - | 1.24 ± 0.14 | 0.08 ± 0.01 |
| - | - | 1.0 | - | 1.81 ± 0.07 | 0.11 ± 0.02 |
| - | - | 1.5 | - | 3.12 ± 0.16 | 0.29 ± 0.01 |
| - | - | 2.0 | - | 4.85 ± 0.14 | 0.32 ± 0.02 |
| **-** | **-** | **2.5** | - | **7.17 ± 0.28** | **0.56 ± 0.02** |
| - | - | 3.0 | - | 4.21 ± 0.21 | 0.27 ± 0.03 |
| - | - | - | 0.5 | 0.93±0.025 | 0.07 ± 0.01 |
| - | - | - | 1.0 | 1.36±0.24 | 0.09 ± 0.006 |
| - | - | - | 1.5 | 3.04±0.30 | 0.22 ± 0.03 |
| - | - | - | **2.0** | **4.13±0.08** | **0.36 ± 0.04** |
| - | - | - | 2.5 | 2.46± 0.27 | 0.22 ± 0.02 |
| - | - | - | 3.0 | 3.00±0.078 | 0.21 ± 0.02 |
| - | - | 0.5 | 0.5 | 1.06±0.27 | 0.07 ± 0.008 |
| - | - | 1.0 | 1.0 | 1.81±0.074 | 0.17 ± 0.04 |
| - | - | 2.0 | 2.0 | 2.90±0.070 | 0.25 ± 0.01 |
| - | - | **2.5** | **2.0** | **4.08±0.045** | **0.32 ± 0.01** |
| - | - | 3.0 | 3.0 | 3.11±0.072 | 0.22 ± 0.02 |

*: Data represents the mean of 10 replicates for each treatment.

This part of study present the percentage of callus response increases with increasing concentration of phytohormone i.e. auxins and cytokinins but auxins are most favored for callus growth and development (21). In all the media tested, 2, 4-D single or with Kinetins found to be more effective for the callus proliferation. The maximum callus biomass was obtained with auxins and cytokinins combination at 40 days of culture.

## IV. CONCLUSION

The present investigation revealed that the induction of regenerative callus from cultured explants is mostly induced on the exogenous application of auxins and cytokinins. But auxins are the most preferred phytohormones for growth and development of callus. The maximum percentage of callus was achieved when MS medium was fortified with 2, 4-D+Kinetin, which gave whitish and friable callus as compared to other hormones. In NAA supplemented medium the calli were found to be green fluorescent in color, highly compact and granular in nature whereas with kinetin in the medium calli became yellowish white and friable. Fresh biomass yield was found to be maximum in the medium containing 2, 4-D along with Kinetin followed by Kinetin and then BAP independently. The same pattern was also observed in case of fresh weight and dry weight biomass of the callus tissue.

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