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Short Communication

Effect of different plant hormones on callus induction in *Gymnema sylvestris* R.Br. (Asclepiadaceae)

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The use of *Gymnema sylvestris* as an alternative remedy for diabetes was well known from ancient times in India and the plant is valuable for its pharmaceutical properties. The present study describes callus induction of the plant *G. sylvestris* using internodal explants and the influence of different plant hormones like 2,4-D, kinetin, IAA, BAP on the growth of calli. Internodes were proved to be the best explant for culture, which were grown on MS basal medium (Murashige and Skoog, 1962) with different concentration of various growth regulators. The standard plant tissue culture protocol for callus culture and/or micropropagation was adopted. The highest efficiency of callus formation was observed in the medium containing different concentration of 2,4-D and kinetin.

Key words: *Gymnema sylvestris*, tissue culture, micropropagation, plant growth regulators, callus.

INTRODUCTION

The use of *Gymnema sylvestris* R.Br. (Asclepiadaceae) commonly known as “Gurmar” (Destroyer of sugar) or “Periploca of the woods” was well-known to the Indian people since ancient days (“Meshashring”) as a source of antidiabetic drugs. In recent years, it became one of the better known names in the world of herbal medicine. It is rich source of many bioactive compounds such as gymnemic acid (GA-I-X) quercitol, lupeol, β-amyrin, stigmasterol, gymnemin, gymnemagenin, gurmarin, etc. which are mainly effective in lowering of blood sugar.

The normal propagation method of *G. sylvestris* requires a lot of time (about 6 - 8 months) to grow a developed plantlet from seed. In addition it requires sufficient field, constant manuring and a constant observation which in turn requires a lot of manpower. Apart from this, getting seeds from the plant is difficult and moreover the chance of getting a disease/contamination free plant is less.

On the contrary a standardized method of micropropagation of *G. sylvestris* can provide a greater percentage of yields of pathogen free plant in a shorter time and in a smaller place. Also, in order to obtain those active compounds one need not to regenerate cseveral omplete plants (Jha and Ghosh, 2005). Extracts from pathogen free calli if generated *in vitro* will prove beneficial. The present study describes the procedure for the callus induction and culture of *G. sylvestris* following standard plant tissue culture protocol using different meristimatically active plant parts, growth regulators and studying their effect on callus induction.

MATERIALS AND METHODS

Materials

The mother plant was sprayed with 0.5% Bavistin, 0.5% Rugor, organic nutrients and organic fertilizer with a gap of 7 - 10 days in between. Then the twigs were collected and washed thoroughly under water. Surface sterilization was done with different concentrations of $HgCl_2$. These surface sterilized twigs nodal region, internodal region (0.5 inch.) and apical buds were cut and chosen as explant. The material preparation was performed following the method of Smith (2000) with minor alteration.

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Abbreviations: IAA, Indole acetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; KIN, kinetin; BAP, benzylaminopurine; GA, gymnemic acid.

Table 1. Effect of MS basal media supplemented with different concentration plant growth regulators on induction of callus and characteristic of callus.

Treatment (mg/l)	Days to Callus Initiation	Average rate of increase per day	Texture	Colour
2,4-D 2	20-25	0.20%	Friable	Fluorescent green
2,4-D 3	20-25	0.05%	Friable	Fluorescent green
2,4-D 4	20-25	0.02%	Friable	Pale green
2,4-D 5	20-25	0.02%	Friable	Pale green
2,4-D 2+KIN 1	20-25	0.05%	Friable	Pale green
2,4-D 3+KIN 1	20-25	0.05%	Friable	Fluorescent green
2,4-D 4+KIN 1	15-20	0.20%	Friable	Fluorescent green
2,4-D 5+KIN 1	15-17	0.80%	Friable	Fluorescent green
2,4-D 6+KIN 1	20-25	0.20%	Friable	Fluorescent green

Table 2. Effect of different concentration plant hormones on callus growth after subculture from initiating media.

Initial media (g/l)	Transferred to (mg/l)	Average rate of increase per day
2,4-D 2	2,4-D 2	0.20%
	MS without GR	0.50%
2,4-D 4+KIN1	2,4-D 2	0.15%
	MS without GR	0.10%
	2,4-D 4+KIN1	0.50%
2,4-D 5+KIN1	2,4-D 2	0.50%
	MS without GR	0.10%
	2,4-D 5+KIN1	0.20%

Medium preparation and culture

The explants were inoculated into Murashige and Skoog`s (1962) culture medium (MS) (supplemented with 0.8% agar with pH 5.8). MS culture medium was supplemented with different combinations of growth regulators for callus induction. The media were autoclaved at 15 lb/inches^2 pressure, at 121°C for 20 min. Cultures were maintained in 25 ± 1°C, under 16 h photoperiod provided for callus initiation according to standard protocols (Rajdhan, 1995). The effect on callus induction was studied with different concentrations of IAA and BAP and their combination(s). Further 2,4-D and kinetin and their combination(s) were also evaluated (De, 2000). The calli so developed in different medium at different rates were subcultured every two weeks into the same or other medium to determine if there is any altered effect of growth regulators on callus growth.

RESULTS AND DISCUSSION

The concentration of $HgCl_2$ to be used for sterilization was first standardized. Different concentration namely 0.1, 0.15, 0.2, 0.25, 0.3, 0.35 and 0.4% were used. Among them 0.25% concentration proved best for sterilization as explants remain healthy and contamination free after 10 days also. After this, the best probable explant for callus induction was standardized. For that reason different parts of explants were inoculated in different composition of medium and among them the internodal explants were found to be most effective as swelling was observed and green colour of the explant persisted which indicated the retention of photosynthesis.

After standardizing the explant and sterilization, for the growth of *G. sylvestris* callus culture different composition and combination of growth regulators were tested. indole acetic acid (IAA) and benzyl aminopurin (BAP), 2,4-dichlorophenoxyacetic acid (2,4-D), and lastly 2,4-D with kinetin(KIN) were tested. 2,4-D alone (1-2 mg l^{-1}) shows proliferation and deep greening which turned into a small friable callus, but the growth rate was very slow. The friable microcallus originated from cut surface of the internode (Figure 1a). The best callus initiation was observed with 2,4-D in combination with KIN (1 – 5 mg l^{-1}:1 mg l^{-1}) (Table 1). It shows fluorescent green coloured friable callus which was initiated in a very short period of time, 20 - 25 days.

The effect of different growth regulator on callusing and on callus growth was the objective of this study. It was observed that to maintain the callus 2,4-D medium was not effective enough whereas the callus remain healthy and increase in different concentration of 2,4-D:KIN combination or in simple MS basal medium (1962) without any growth regulator(s) (Table 2). After 20 - 30 days of initiation of the friable callus, some embryo-like structures resembling embryo (globular/heart shaped) seem appeared from the surface of the callus. Komalavali and Rao (2000) reported that there could be a good positive effect of coconut and malt extract with 2,4-D on callusing of *G. sylvestris* if supplemented in the MS basal medium.

The tissue cultures of medicinal plants have a wide range of industrial application (Ghosh, 2005). Explant sterilization is a major step in culture establishment of perennial trees or shrub. Proper concentration of sterilizing agent is a key factor (Roy and Saha, 1997). This was determined by applying different concentration of $HgCl_2$ in the present investigation. Further, internodal region is highly responsive in several plant species (Bhojwani and Razdan, 2004) and similar results were found in *Gymnema* too. The best result found in case of

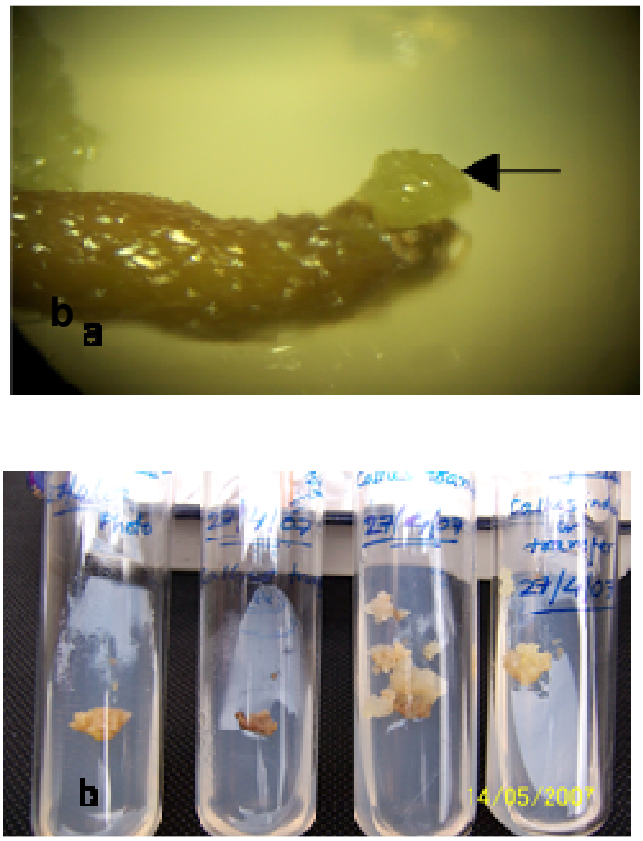

Figure 1. a. Initiation of microcallus (10X) from axillary bud viewed through stereomicroscope [Olympus Magnus MS24]. **b.** Comparative callus initiation and proliferation in different mediums.

maintenance was observed in MS medium supplemented with 2,4-D and KIN in combination. The effect was more or less similar with other dicot plants (De Kalvan, 2004). The effect of 2,4-D, KIN, IAA, BAP on callus initiation and maintenance so observed will be helpful and attempts can be made to assay the concentrations of bioactive molecules as this plant is rich in bio-active constituents. Desired secondary metabolite can be manipulated as conditions required from the callus (Kumar, 2003). It can be precisely con-trolled and cell cultures or organ cultures can be done. Creating genetically modified variety will also be possible from this callus (Gamborg and Phillips, 1995). This *in vitro* study will help future workers on developing related manipulations.

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