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In vitro Callus Induction from Different Explants of *Gymnema sylvestre* R. Br.

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Abstract: ***The different parts of in vitro raised plant of Gymnema sylvestre were tested on the two different type medium MS and WAP in different concentrations supplemented with 2,4-D, IAA and NAA for callus induction. Various growth regulators greatly influenced the growth of callus on both medium but highest efficient callus were established from in vitro leaves part on WAP full strength medium containing 2 mg/l 2, 4-D. Higher concentrations of auxins represent decreased callus production.***

Abbreviations: IAA Indole-3-acetic acid, NAA Naphthalene acetic acid 2, 4-D 2 4 Dichlorophenoxy acetic acid, MS Murashige and Skoog, WAP Woody Plant Medium.

Keywords: Gymnema sylvestre, Auxins, in vitro, Callus.

1. Introduction

Ayurveda or the 'science of life' is an ancient holistic system for diagnosis and treatment perhaps the oldest system of medicine. *Gymnema sylvestre* a member of family Asclepiadaceae, commonly known as madhunashini. It is a valuable antidiabetic medicinal plant found in India, Asia, Tropical Africa, Malaysia and Srilanka (**Keshavamurthy, 1990**). It is a potent antidiabetic plant and used in Ayurvedic and Homeopathic system of medicine as a destroyer of 'Madhumeha' (glycosuria) and other urinary disorders. **(Kapoor, 1977; Ravi and Wahi, 1995; Mitra et al, 1995)** The main property of this plant leaves are appreciating the taste of sugar.(**Mr. D. Hooper in, 1887)** It is a very effective medicinal plant use in against of asthma, eye complaints, inflammations, family planning, weight loss and snake bite **(Anonymous, 1956; Uniyal, 1993 and Kurihara Y., 1992).** Vegetative propagation is only the method for cultivation, but it is very slow growing process in different climate conditions. In nature less seed germination with poor viability is responsible for its diminishing population size. The poor propagation coupled with indiscriminate collection from natural resources for its diverse medicinal applications ***Gymnema sylvestre*** has been fast disappearing and threatened; therefore, there is urgent need to conserve the plant by biotechnological approach like tissue culture (**Choudhury, 1988***)*. The use of *in vitro* techniques in germplasm conservation is increasing and has been successfully applied to the conservation of several rare species, both for propagation and for long-term storage and large scale production. Tissue culture studies on *Gymnema sylvestre* have largely been restricted to monitor the gymnemic acid synthesis in callus culture. (**C. Subathra Devi et.al. 2006)** Little tissue culture work has been reported on ***Gymnema sylvestre*** callus culture by **C. Subathra Devi et.al. 2006; Gopi & Vatsala, 2006;Kanetkar *et al*., 2006; Lee *et al*., 2006; Abdul Bakrudeen Ali Ahmed *et al*., 2009.** In the present investigation, we report the successful callus production of ***Gymnema sylvestre*** by using *in vitro* raised plant leaf.

2. Materials and Methods

The seeds were collected from Kasturi Herbal farm Misrod, Bhopal an elite vine (2-3 years old). Plant Identified by Laghu Vanupaj Prasannskarn & Anusandhan Kendra Barkheda Pathani, Bhopal M.P. Explants were immersed in water with a little amount of fungicide-Bavistin for 1 to 3 hours and then rinse with tap water five to six time. Further surface sterilization treatment was conducted in a laminar air flow chamber. The explants were sterilized with 0.1% (w/v) fresh prepared mercuric chloride solution for 1min, and then washed with double DW two times. There after explants were carefully transferred to sterile blotting paper placed over sterile Petri plate to remove excess water & then inoculated into MS medium.

The plant material was collected in *invitro* raised seedling of *Gymnema sylvestre* R.Br. The *in vitro* raised plantlets were aseptically cut into various parts like cotyledonary leaf, leaf, nodal section, Inter nodal section and root. They were carefully inoculated onto the MS (**Murashige & Skoog, 1962)** and WPM **(Lloyd and Mc Cown, 1981)** culture media using sterile forceps under aseptic conditions for callus formation. The culture medium augmented with 3% sucrose and gelled with 0.8% agar and supplemented with various concentrations of auxins (0.5-2mg/l) 2,4-D, IAA and NAA (Table. 1, Fig .1). All cultures were incubated in culture room at 27$\pm$2 ^{0}C in darkness or cool white light of 3,500 Lx. at plant level provided by fluorescent tubes with 55-60% relative humidity. The response of callus induction was observed after 10 days of culture. The callus induction frequency was determined by the number of explants producing callus as the percentage of the total number of explants. The explants with induced cultures were transferred to culture bottles containing fresh medium after 15-20 days intervals. Callus growth was measured after 45-60 days of culture in fresh weight and dry weight. The dry weight was used for gymnemic acid analysis.

3. Result and Discussion

The callus initiation from different explants, explants were grown on different medium and different concentrations of Auxins, respectively observed within 10 days.

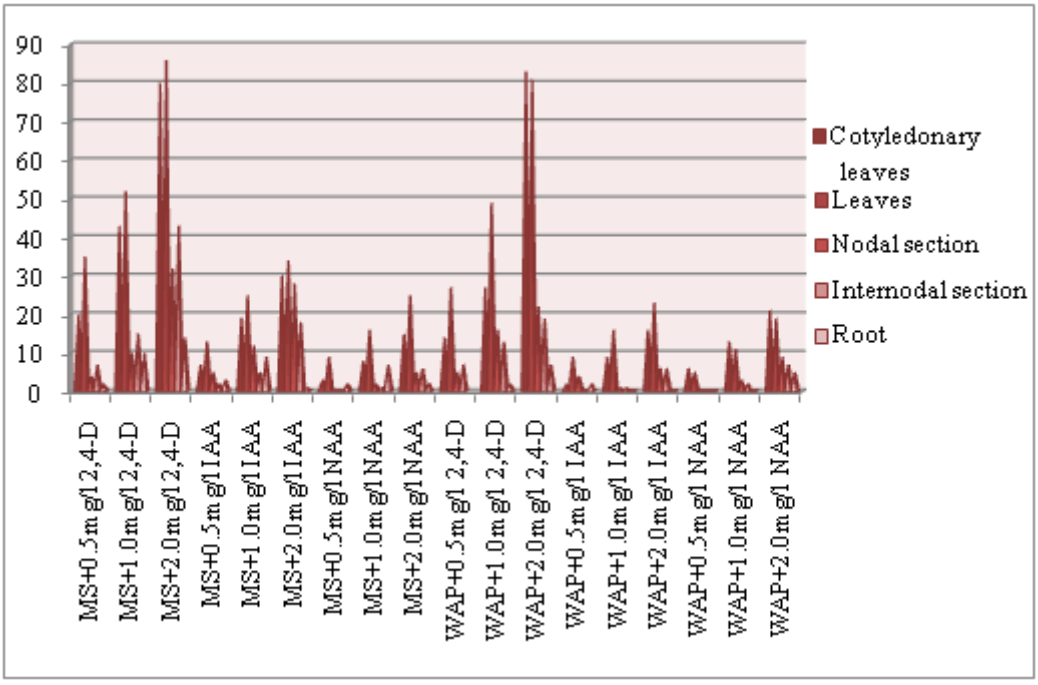
(Table 1) The maximum frequency of callus initiation was **86±0.11**% in MS medium containing (MS+2.0mg/l 2,4-D) from leaf explants in compare to WAP medium **81±0.26%.** On the other hand cotyledonary leaf explants were shows maximum callus induction **(83±0.33%)** on WAP with 2.0mg/l 2,4-D, in compare with MS+2.0mg/l 2, 4-D **(80±0.20%). C. Subathra Davi et.al., 2006 and Komalavalli *et al.*, 2007,** was also studied to develop same result on MS medium**.** Root explants were showed minimum response for callus induction on both medium, but WAP with IAA represent negative result. On the other side nodal and intermodal explants were present miner response for callus induction on both medium with all growth regulators. (Table. 1, Fig. 1) Maximum fresh weights of callus were 20.64 g and the dry weight were 4.15 g on MS media. The *in vitro* callus is having good quality of secondary metabolites in quantity and method is very useful to obtain high amount of phytochemicals in less time.

Table 1: Effect of callus Initiation on Different explants on Different medium supplemented with various concentration of Auxins

Callus induction frequency (% in mean± SE) from different parts of *invitro* plantlets

Medium combination	*Cotyledonary leaves*	*Leaves*	*Nodal section*	*Internodal section*	*Root*
MS+0.5mg/l 2,4-D	20±0.29	35±0.21	04±0.23	07±0.19	02±0.17
MS+1.0mg/l 2,4-D	43±0.32	52±0.25	10±0.27	15±0.22	10±0.02
MS+2.0mg/l 2,4-D	**80±0.20**	**86±0.11**	32±0.23	43±0.16	14±0.18
MS+0.5mg/l IAA	07±0.18	13±0.32	05±0.18	02±0.15	03±0.19
MS+1.0mg/l IAA	19±0.33	25±0.17	12±0.30	05±0.20	09±0.28
MS+2.0mg/l IAA	30±0.22	34±0.22	28±0.18	18±0.26	01±0.16
MS+0.5mg/l NAA	03±0.31	09±0.27	00±0.00	00±0.00	02±0.20
MS+1.0mg/l NAA	08±0.12	16±0.14	02±0.15	01±0.20	07±0.34
MS+2.0mg/l NAA	15±0.18	25±0.30	05±0.20	06±0.18	02±0.30
WAP+0.5mg/l 2,4-D	14±0.16	27±0.22	05±0.26	07±0.15	02±0.20
WAP+1.0mg/l 2,4-D	27±0.26	49±0.18	16±0.33	13±0.27	07±0.34
WAP+2.0mg/l 2,4-D	**83±0.33**	**81±0.26**	22±0.30	19±0.21	02±0.30
WAP+0.5mg/l IAA	02±0.33	09±0.16	04±0.27	00±0.00	00±0.00
WAP+1.0mg/l IAA	09±0.22	16±0.28	01±0.20	01±0.20	00±0.00
WAP+2.0mg/l IAA	16±0.31	23±0.14	06±0.14	06±0.18	00±0.00
WAP+0.5mg/l NAA	06±0.14	05±0.16	00±0.00	00±0.00	00±0.00
WAP+1.0mg/l NAA	13±0.20	11±0.20	03±0.19	02±0.20	00±0.00
WAP+2.0mg/l NAA	21±0.26	19±0.07	09±0.28	07±0.34	05±0.34

Figure 1: Effect of callus Initiation on Different explants on Different medium supplemented with various concentration of Auxins



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