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Full Length Research Paper

In vitro propagation of *Gymnema sylvestre* Retz. R.Br through apical bud culture

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***Gymnema sylvestre Retz. R.Br*. is a very important medicinal plant used in many medical formulations mainly for the treatment of diabetes. The extensive use of *G. sylvestre* has resulted in its over-exploitation and the plant is rare in many states of India. In the present study multiple shoot were regenerated using apical buds as an explant. Several concentrations of cytokinins viz. kinetin and BAP were attempted with MS medium for efficient shoot induction. Highest shoot frequency was obtained in MS medium fortified with BAP (4.44 µM) and KN (4.64 µM) with 3% sucrose. Shoots obtained were rooted using half strength MS media with IAA. Eighty five percent of rooted shoots survived in the field.**

Key words: *Gymnema sylvestre* Retz. R.Br., *in vitro* propagation, apical bud.

INTRODUCTION

Gymnema sylvestre Retz. R.Br. (family Asclepiadaceae) commonly called as “Gudmar” (gud -jaggery, mar -kills) a medicinally important branched woody climber, distributed throughout India in dry forest up to 600 m. *Gymnema* is believed to stimulate release of *in vitro* insulin by increased membrane permeability (Parsaud, 1999)**.** Out of the constituent of the *Gymnema* leaves viz. triterpene gymnemic acid inhibits glucose absorption in the small intestine (Chattopadhyay, 1998).

G. sylvestre Retz. RBr. is one of the popular medicinal plants recognized as a potent drug plant in ayurveda and as mother tincture in extracts in homeopathic system. Besides these *G. sylvestre* also possess antimicrobial, antiatherosclerotic (Bishayee and Chatterjee, 1994) and hepatoprotective activities (Rana and Avadhoot, 1992). The plant is an antidote for snake bite used by tribals. It has been recognized by natural products industry in North America and Europe and a number of commercial, over - the - counter herbal products are now available that contain varying amounts of *Gymnema* leaf extract (Reddy et al., 1998).

G. sylvestre has been reported rare in Madhya Pradesh, Maharashtra and Andhra Pradesh due to excessive collection of entire plant for medicine and trade (Jain and Patole, 2001; Udayan et al., 2009). Besides its conventional propagation is hampered due to its poor seed viability, low rate of germination and poor rooting ability (Komalavalli and Rao, 2000).

MATERIALS AND METHODS

Plant material

Apical buds of (7 - 10 mm) were collected from mature (2 - 3 years old) plants of *G. sylvestre*. A sequential sterilization of the explants was done 5 min in 0.01% labolene followed by a rinse with sterile distilled water. The explants were then treated with 70% ethanol (0 - 5 min) and washed with distilled water. Explants were then immersed in 0.1% mercuric chloride for 2 - 5 min and finally rinsed with sterilized water 2 - 3 times. The explants were then kept in presterilized filter paper and then inoculated into test tubes.

Culture medium and incubation conditions

Explants were inoculated under aseptic conditions on to the sterile culture medium in test tubes on MS medium (Murashige and Skoog, 1962) containing 3% sucrose and plant growth regulators (PGRs) particularly cytokinins viz. BAP (0.44, 2.22, 4.44, 22.22, 44.44 µM)

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Abbreviations: MS, Murashige and Skoog’s; **BAP,** benzylamino purine; **KN,** kinetin; **IAA,** indole 3 acetic acid; **2,4-D,** 2,4-dicholorophenoxy acetic acid; **IBA,** indole butyric acid.

Table 1. Effect of various concentrations of BAP and KN on mean shoot number and mean shoot length on *G. sylvestre.*

PGR (µm)	Mean shoot number	Mean shoot length
BAP 0.00	1 ± 0.00	2.10 ± 0.04
0.44	1 ± 0.00	3.21 ± 0.02
2.22	1.24 ± 0.33	3.55 ± 0.34
4.44	2.24 ± 0.12	4.51 ± 0.03
22.22	1.31 ± 0.03	3.16 ± 0.02
44.4	1 ± 0.00	2.79 ± 0.08
KN 0.00	1 ± 0.00	2.10 ± 0.04
0.46	1 ± 0.00	3.18 ± 0.01
2.32	1.24 ± 0.55	3.41 ± 0.03
4.65	1.35 ± 0.04	4.68 ± 0.1
23.2	1.11 ± 0.11	3.89 ± 0.02
46.5	1 ± 0.00	2.80 ± 0.08

Table 2. Effect of BAP and KN combination on mean shoot number and mean shoot length regenerated from apical bud explant of *G. sylvestre*.

Explants	Mean shoot number		Mean shoot length	
	Subculture 1	Subculture 2	Subculture 1	Subculture 2
BAP 4.44 + KN 0.46	2.67 ± 0.08	4.42 ± 0.12	5.81 ± 0.10	8.75 ± 0.21
BAP 4.44 + KN 2.32	3.40 ± 0.08	7.38 ± 0.15	6.42 ± 0.12	10.46 ± 0.27
BAP 4.44 + KN 4.65	5.47 ± 0.08	12.27 ± 0.15	7.29 ± 0.09	12.02 ± 0.18

and KN (0.46, 2.32, 4.64, 23.2, 46.4 µM) alone and in combination were used for shoot induction and multiplication. Root induction was attempted from shoots using auxins IBA (0.49, 2.46, 4.92, 24.6 and 49.2 µM), IAA (0.57, 2.85, 5.71, 28.55, 57.1 µM), NAA (0.53, 2.68, 5.37, 26.85, 53.70 µM), and 2,4-d (0.45, 2.25, 4.54, 22.7 and 45.4 µM) with MSM at full and half strength.

The cultures were maintained in culture tubes and conical flasks and were kept in the culture room at a temperature of 25 ± 2°C, relative humidity (RH) of 60-70% and a light intensity of approximately 1500 Lux provided by cool, white, fluorescent tubes under a photoperiod of 16/8 hr (light/dark). Cultures were maintained through regular subcultures. The cultured tissues were aseptically transferred to fresh media without being subjected to chemical sterilization. The morphogenetic responses of the explants were assessed and recorded in the form of mean number of shoot and mean shoot length. For each treatment, 15 replicates were maintained and each experiment was repeated at least three times.

RESULTS AND DISCUSSION

Initiation of cultures and shooting

Bud break of the inoculated explants occurred within 7 - 10 days of inoculation. New shoots emerged by the third week of inoculation (Figure 1a). A precocious outgrowth of buds, shoot number and shoot length occurred with an increase in the concentration of BAP (0.44 - 4.44 µm) and KN (0.46 4.64 µm) in MS media. (Table 1) Highest bud break was achieved in BAP (4.44 µm), while Kn (4.46 µm) resulted in a high shoot length (Table 1). Shoots produced using Kn were thicker and leaves were darker green in color to those in BAP.

Effect of combinations of BAP and KN on shoot number and shoot length

High shoot number and shoot length was achieved in BAP and KN combination as well. Highest shoot number and shoot length were achieved in BAP (4.44 µm) + Kn (4.64 µm). Shoot number and shoot length increased dramatically in the second subculture, with some explants producing 10 - 12 shoots and growing up to 13.5 cm (Figure 1b (Table 2). Efficient shoot multiplication using a combination of cytokinins from axillary bud explant has been reported earlier (Reddy et al., 1998; Saraswathy et al., 2002; Baskaran et al., 2007).

Rooting

Regenerated shoots were rooted in media containing auxins. High frequency of rooting was achieved by

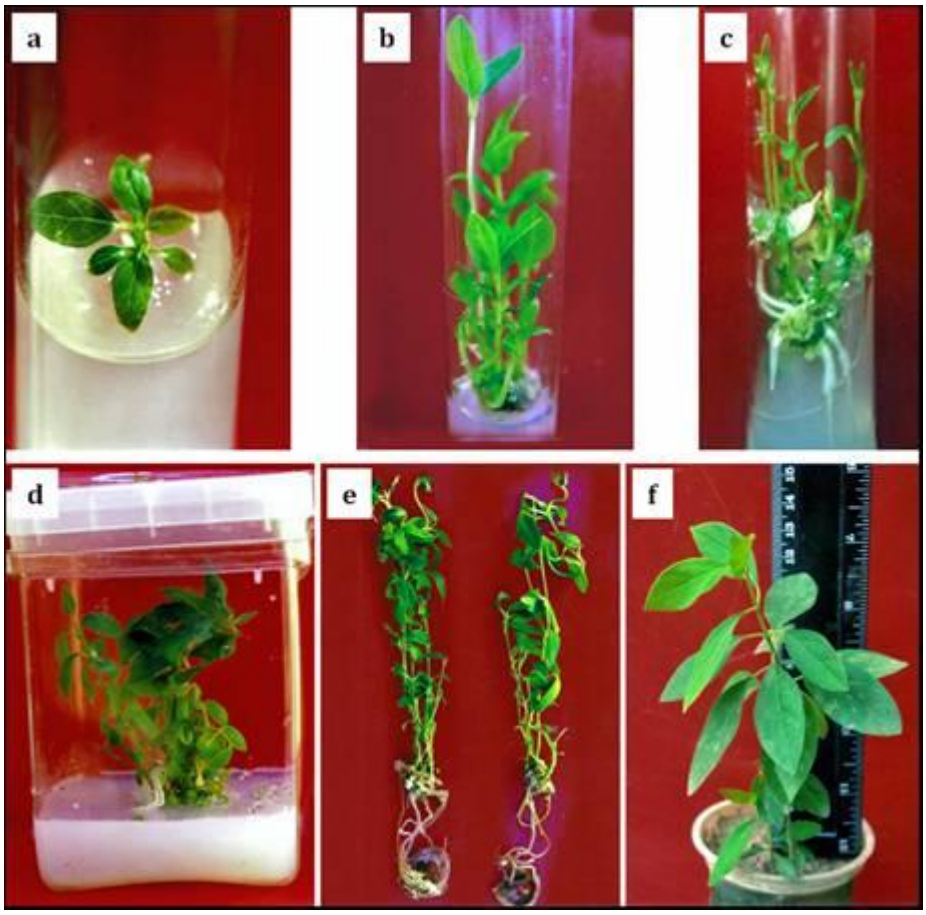

Figure 1. *In vitro* propagation of *G. sylvestre* (a) Apical bud break in BAP (4.44 µm). (b) Multiple shoots in BAP (4.44 µm) + KN (4.65 µm). (c-d) Rooting of multiple shoots in ½ MS with IAA (2.85 µm). (e) Elongated and rooted multiple shoots. (f) Acclimatized plant.

reducing the strength of media to half fortified with 2.85 µm of IAA within 20 days of subculture (Figure 1c - e).

Hardening and acclimatization

When the shoots of the in vitro regenerated plantlets attained a height of 8 - 10 cm, bearing healthy leaves and a good root system they were transferred into small plastic cups containing presterilized sand: soil (1:1) holed at bottom with a thin layer of soil and upper layer of sand and were covered with bell jars to protect excessive water loss from leaves in natural conditions. After a week the mouth of the bell jar was opened for an hour each day and after 15 - 20 days the bell jar was gradually removed. As the plantlets started growing in height they were transferred into bigger pots by carefully removing the plastic cup (Figure 1f).

In Conclusion, a simple and efficient protocol was developed for direct plant regeneration from apical bud explants of *G. sylvestre.* The protocol described here is rapid and reproducible and extremely useful in mass propagation of this extremely important medicinal plant.

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REFERENCES

Baskaran M, Rathinavel S, Sellathurai T, Prema S, Natarajan KK (2007). In vitro propagation and conservation of *Gymnema sylvestre (Retz) R. Br.* Plant archives. 7:283-286.

Bishayee A, Chatterjee M (1994). Hypolipemic and antiatherosclerotic effects of oral *Gymnema sylvestre* R.Br. leaf extract in albino rats fed on a high fat diet. Phytotherapy Res 8:118-120.

Chattopadhyay RR (1998). Possible mechanism of antihyperglycemic effects of *Gymnema sylvestre* leaf extract. Gen. Pharm. 31:495-496.

Jain AK, Patole SN (2001). Some threatened plants of Pachmari Biosphere reserve of Madhya Pradesh. J. Ind. Bot. Soc. 80: 151-155.

Komalavalli N, Rao MV (2000). In vitro micropropagation of *Gymnema sylvestre* – A multipurpose medicinal plant. Plant Cell Tiss. Org. Cult. 61:97-105.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497.
Persuad SJ, Al-Majed H, Raman A, Jones PM (1999). *Gymnema sylvestre* stimulates insulin release *in vitro* by increased membrane permeability. J. Endocrinol. 163 (2):207-212.
Rana AC, Avadhoot Y (1992). Experimental evaluation of hepatoprotective activity of *Gymnema sylvestre* and *Curcuma zedoaria.* Fitoterapia 63: 60-62.
Reddy PS, Ramagopal G, Lakshmisita G (1998). *In vitro* multiplication of *Gymnema sylvestre* R. Br. – An important medicinal plant. Curr. Sci. 75 (8):843-845.
Saraswathy S, Manavalan RSA, Vadivel E, Chezhiyan N, Vijaykumar M (2002). In vitro propagation studies in gymnema (Gymnema sylvestre R.BR.). South Indian Horticulture. 50: 664-666.
Udayan PS, Tushar KV, George S, Balachandran I (2009). Notes on a few rare, endemic and red listed plants from Trumula forest of Tirupati Chittor district, Andhra Pradesh. J. Econ. Tax. Bot. 33:277-284.