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Mass Multiplication of *Gymnema sylvestre* (Retz.) R. Br. ex Schult. Through *In vitro* Shoot Organogenesis from Callus

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Abstract

For large scale production through shoot organogenesis from callus, seeds of *Gymnema sylvestre* were cultured on MS containing various concentrations of 2,4-D. An optimum callusing (62.40%) was observed on MS with 1.5 mg/l 2,4-D. Upon transfer to multiplication medium, the calli multiplied 2.5 times in 45 days. The calli were then tested for shoot induction and an optimum result in terms of both per cent response (52.04%) and an average number of shoots (47.2) were observed on MS supplemented with 1.0 mg/l BAP. Maximum shoot elongation was obtained on 0.5 mg/l Kn and best rooting took place on half strength MS with 0.5 mg/l IBA. This protocol can be followed for the large scale *in vitro* multiplication of *G. sylvestre*.

Introduction

Gymnema sylvestre (Retz.) R. Br. ex Schult. of Asclepiadaceae is a slow growing woody climber distributed in the moist and dry deciduous forests and also in the plains of all districts of Kerala, India. The plant is also known by its synonym *Periploca sylvestris* Retz. The local names of the plant in Malayalam are Chakkarakolli and Madhunaasini (Sasidharan 2004). The word Gymnema is derived from the Hindi word Gurmar meaning destroyer of sugar, because the leaves of this plant possess potential chemicals to neutralize excess of sugar present in the body of diabetes mellitus patients (Keshavamurthy and Yoganarasimhan 1990). The principal antidiabetic constituent of the plant is gymnemic acid, which is used to cure diabetes and obesity (Kanetkar et al. 2007).

The plant possesses many active chemical components. The major secondary metabolites in *G. sylvestre* include a group of nine closely related acidic glycosides, mainly gymnemic acids A-D which are found in all parts of the plant (Yoshikawa et al. 1989). Gymnemic acids and gymnema saponins are members of oleane type of saponins

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whereas gymnemasides are dammarane saponins (Khramov et al. 2008). The other important phytoconstituents reported from the plant included gymnemagenin, gymnestrogenin (Rao and Sinsheimer 1971), anthroquinones, flavones, hentriacontane, pentatriacontane, phytin, resins, tartaric acid, formic acid, butyric acid, lupeol, β- amyrin related glycosides, stigmasterol and calcium oxalate (Sinsheimer et al. 1970). The plant has also been reported to possess several important bioactive properties like antimicrobial (Pasha et al. 2009), antiobesity (Kumar et al. 2013), antiinflammatory (Malik et al. 2008), antihypercholesterolemic (Bishayee and Chatterjee 1994) and hepatoprotective (Rana and Avadhoot 1992) activities.

Because of the immense medicinal importance and low multiplication rate of the plant, there is an urgent need to develop and standardize an effective *in vitro* propagation technique for the mass production of *G. sylvestre*. There are some reports on micropropagation of this plant (Reddy et al. 1998, Komalavalli and Rao 2000, Akshitha et al. 2014).

Materials and Methods

Mature green fruits were collected from plants growing in Nambiakulam, Kottayam District, Kerala, India. The fruits were surface sterilized immediately before culture by using 0.1% $HgCl_2$ (w/v) for five min and then washed thrice with sterilized double distilled water. The fruits were then dissected with the help of a scalpel and a pair of forceps. The seeds were isolated and inoculated on MS supplemented with different concentrations of 2,4-D (0.5 - 2.0 mg/l) for callus induction. The calli were transferred to MS supplemented with BAP (1.0 mg/l) for multiplication. The calli were subsequently cultured on MS with various concentrations of BAP (0.5 - 1.5 mg/l) and Kn (0.4 - 0.6 mg/l) alone or in combination with IAA (0.4 - 0.6 mg/l), NAA (0.4 - 0.6 mg/l) or IBA (0.4 - 0.6 mg/l) for obtaining maximum shoot organogenesis. The shoots were transferred to MS with different concentrations of Kn (0.1 - 1.0 mg/l) for shoot elongation. Elongated shoots were then cultured on half strength MS with IBA (0.4 - 0.6 mg/l) for root induction.

MS with appropriate growth regulators and 3% sucrose was employed throughout the experiment. The pH of the medium was 5.8. The medium was solidified using 0.8% agar. The medium was then sterilized in an autoclave at 121°C and 15 lbs pressure for 20 min. Cultures were incubated at 22 ± 2°C for 16 hrs in light (illuminated by 40 watts fluorescent tubes). Twenty-four cultures were raised for each treatment and all experiments were repeated three times. ANOVA and DMRT were used for comparison among treatment means.

Results and Discussion

Gymnema sylvestre is an important woody climber with very high medicinal value. Multiple shoots was produced from calli from seed explants of *G. sylvestre*. For callus induction from seeds MS with different concentrations of 2,4-D (0.5, 1.0, 1.5 and 2.0 mg/l) were tried. Induction of calli was observed after two weeks. The seedlings emerged from

seed coat after 10 days and callus was induced from all through the seedlings (Fig. 1A, B). A massive callus production was observed by about one month after culture (Fig. 1C). All the tested concentrations of 2,4-D showed callus induction at different intensities with an optimum of 62.40% callus production on MS with 1.5 mg/l 2,4-D. Concentrations below and above 1.5 mg/l 2,4-D reduced the production of callus. Similar results of 2,4-D assisted callus induction in *Gymnema sylvestre* was reported from nodal explants (Roy et al. 2008, Vats and Kamal 2013).

The calli obtained were then isolated and cultured on MS with 1.0 mg/l BAP for multiplication. For shoot induction from callus, the multiplied calli were subcultured on MS supplemented with BAP (0.5 - 1.5 mg/l) and Kn (0.4 - 0.6 mg/l) alone or in combination with IAA (0.4 - 0.6 mg/l), NAA (0.4 - 0.6 mg/l) or IBA (0.4 - 0.6 mg/l) (Table 1). Of the various combinations and concentrations of BAP, Kn, IAA, NAA or IBA used for shoot organogenesis, the optimum (52.04%) cultures responded with an average number of 47.2 shoots per culture on MS with 1.0 mg/l BAP. Similar observations were obtained by Nikam et al. 2009 from leaf explant in *Momordica cymbalaria* and by Vishwakarma et al. 2013 from petiole explant in *Viola serpens* Wall.

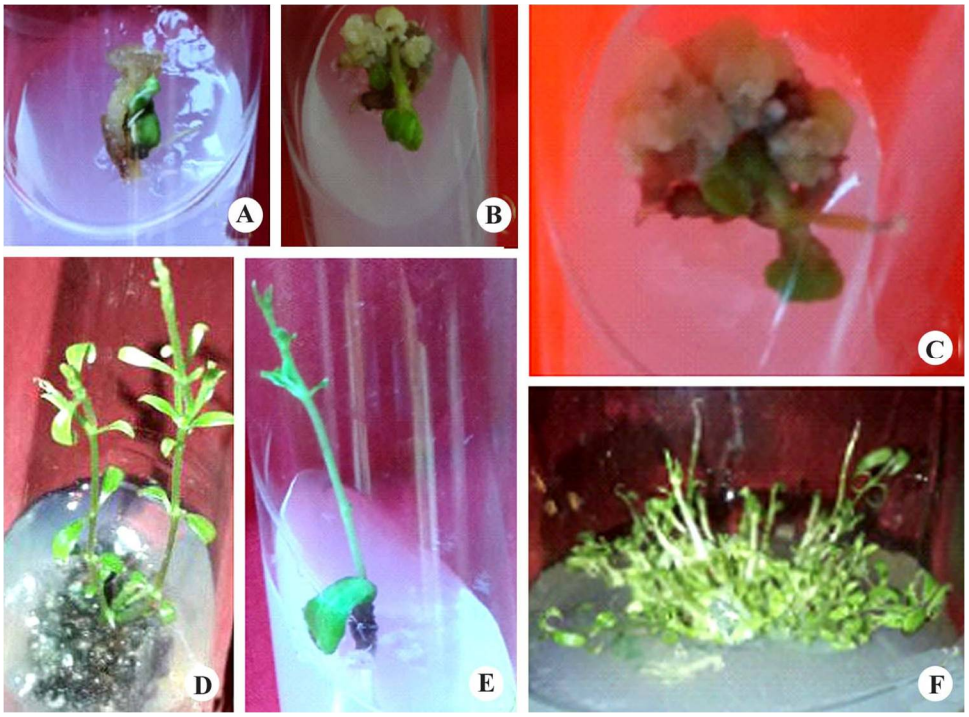

Fig. 1. Multiple shoot induction from green seedling of *G. sylvestre*. A. Green seedling and the calli induced from seed explant of *G. sylvestre* on MS. B. Same as in A after 21 days. C. Callus development after 28 days on seedlings. D. Shoot organogenesis from calli. E. Rooting of shoots on half MS after 45 days. F. Several healthy shoots were emerged from the calli after 45 days.

Individual shoots were then transferred to MS with different concentrations of Kn (0.1 - 1.0 mg/l) for shoot elongation and a maximum response was observed on MS with 0.5 mg/l Kn. On this medium, shoot reached an average height of about 5.22 cm in one month (Fig. 1D). A similar result was observed in *Tinospora cordifolia,* where Kn was found superior to BAP for shoot elongation (Raghu et al. 2006). However, in contrast to our observation, a study in *Gymnema sylvestre* showed optimum result on MS with 0.5 mg/l BAP (Akshitha et al. 2014).

Table 1. The effect of various phytohormones on shoot regeneration from callus of *G. sylvestre* 45 days after culture.

Plant growth regulators (mg/l)					Percentage of response [a]	Average no. of shoots per explants [a]
BAP	Kn	IAA	NAA	IBA		
0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5					47.4 ± 1.42d	44.0 ± 1d
1.0					52.04 ± 0.67e	47.2 ± 0.836d
1.5					49.96 ± 0.56d	41.6 ± 0.89d
	0.4				40.54 ± 1.08d	34.0 ± 1c
	0.5				42.72 ± 0.89d	38.6±1.14c
	0.6				38.76 ± 0.63c	36.4±0.89c
1.0	0.4				30.68 ± 0.54c	29.2±0.83b
1.0	0.5				33.28 ± 0.33c	31.4±1.51c
1.0	0.6				23.63 ± 0.43b	26.6±1.14b
0.5	0.5				32.2 ± 0.66c	21.6±0.54b
1.0	0.5				34.12 ± 0.66c	22.8±1.30b
1.5	0.5				29.96 ± 0.46b	19.0 ± 1a
1.0	0.5	0.4			18.82 ± 0.63a	39 ± 1.58c
1.0	0.5	0.5			21.28 ± 0.35b	43 ± 0.70d
1.0	0.5	0.6			17.22 ± 0.41a	38.8 ± 0.44c
1.0	0.5		0.4		11.24 ± 0.48a	17.2 ± 0.81a
1.0	0.5		0.5		14.18 ± 0.50a	20.0 ± 1a
1.0	0.5		0.6		10.06 ± 0.43a	15.2 ± 1.30a
1.0	0.5			0.4	26.16 ± 0.35b	26.0 ± 1b
1.0	0.5			0.5	28.26 ± 0.30b	29.4 ± 0.89b
1.0	0.5			0.6	24.4 ± 0.38b	25.2 ± 1.92b

Control without growth regulators. Culture period 45 days. [a] Values are means (± SE) obtained from three independent experiments. At least 24 cultures were maintained for each experiment. Means within a column followed by the same letter are not significantly different by DMRT ($p > 0.05$).

The elongated shoots were subsequently transferred to half strength MS with different concentrations of IBA (0.4 mg/l - 0.6 mg/l) for root induction. Optimum rooting was observed on half strength MS with 0.5 mg/l IBA. Here, 55.2% cultures responded with an average number of 4.33 roots per shoot after 45 days of culture (Fig. 1E). Roots were of an average 2.54 cm in length and originated from the basal end of the shoots. The

roots were healthy and white in colour. Previous reports revealed that most members of Asclepiadaceae exhibited maximum root induction and elongation on half strength MS. In another study, it was reported that *G. sylvestre* produced maximum root length in half strength MS with 3 mg/l IBA (Komalavalli and Rao 2000). Reddy et al. (1998) also reported that *G. sylvestre* produced roots on half strength MS without any growth regulators. Several shoots developed from calli on MS with BAP (1.0 mg/l) after 45 days of culture (Fig. 1 F). Plantlets with four leaves and well developed roots were successfully transferred to the soil after acclimatization.

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