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SHORT COMMUNICATION

EFFICIENT *IN VITRO* SHOOT MULTIPLICATION OF *GYMNEMA SYLVESTRE* R.Br. - AN ANTIDIABETIC MEDICINAL PLANT

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An efficient *in vitro* shoot multiplication protocol was developed for *Gymnema sylvestre* R.Br. which is a potent anti-diabetic plant. Nodal explants were inoculated in half strength MS media containing different auxins (IAA and IBA) and cytokinins (BAP and Kinetin) at various concentrations for rapid multiple shoot induction. Multiple shooting (3.25±0.62) was obtained in presence of BAP and Kn at 3.0 mg/l each. Best response in terms of shoot length was recorded (1.97±0.06 cm) in the media supplemented with Kn and IAA at 1.5 mg/l each. Microshoots obtained *in vitro* were inoculated in ¼ MS media which produced maximum of 5 roots at 1.0 mg/l IBA.

Key words: **Anti-diabetic plant, *In vitro* multiplication, *Gymnema sylvestre*, Gymnemic acid**

Gymnema sylvestre R.Br. (Asclepiadaceae) has potent anti-diabetic properties. It is being used in ayurvedic and homeopathic system of medicine from time immemorial (Dixit and Pandey 1984, Kapoor 1977, Mitra *et al*. 1995). The word gymnema has been derived from a Hindu word "Gurmar", a destroyer of sugar. This species is woody climber of tropical and subtropical regions (Anonymous 1997). The bioactive compound found in the leaves of *G. sylvestre* is commonly known as 'Gymnemic acid'. This substance inhibits glucose absorption in small intestine and decreases high glucose levels in blood (Shimizu *et al.* 1997). This plant is also helpful in the treatment of asthma, eye complaints, inflammation, family planning and snake poison (Uniyal 1993, Selvanayagam *et al.* 1995). Gymnemic acid IV isolated from leaves of *G. sylvestre* has antisweet, antihyperglycemic, glucose uptake inhibitory, glycosidase inhibitory and lipid lowering effects (Gurav *et al*. 2007, Ali Ahmed *et al.* 2008). The hyperglycemic effect of diabetogenic hormones (Somatotropin, Corticotropin) are inhibited by extract of *G. sylvestre* leaves. Purified Gymnemic acid also inhibits glucose stimulated secretion of gastric inhibitory peptide hormone (Fushiki *et al.* 1992). Indiscriminate collection and over-utilization of this plant for commercial purposes by pharmaceutical companies caused *G. sylvestre* as threatened with extinction and fast disappearing plant (Chaudhury 1988). Conventional propagation faces problem due to its poor seed viability, low rate of germination and poor rooting ability of vegetative cuttings. Tissue culture offers an alternative propagation method which accelerates large scale multiplication, improvement and conservation of plant. Some work has been reported on *G. Sylvestre* in context of direct regeneration (Reddy *et al.* 1998, Komalivalli and Rao 2000), indirect organogenesis (Komalivalli *et al.* 2005) and somatic embryogenesis (Kumar *et al*. 2002). This paper describes cost effective *in vitro* multiplication of *G. sylvestre* by using half strength MS media.

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Disease and pest free plantlets of *G. sylvestre* were collected from the vicinity of Varanasi i.e., Ramnagar, Rajiv Gandhi South Campus of BHU at Barkacchha, and from BHU campus. They were planted and established in Horticulture garden of the Institute of Agricultural Sciences BHU. Nodal explants (1.5-2.0 cm long and 0.3-0.4 cm thick) were obtained from two months old parent plant for use of *in vitro* propagation studies. The explants were washed in running tap water. These were subjected to different surface sterilization treatment using, firstly with 2-3 drops of Tween-80 for 10 min, and then mixture of $HgCl_2$, (0.1%) and sodium hypochlorite (1%) in equal amount for 8 min. Finally the explants were rinsed 5-6 times with double distilled sterile water.

Half strength MS media (Murashiga and Skoog 1962) were fortified with different auxins (IAA and IBA) and cytokinins (BAP and Kn) at various concentration (mg/l, w/v) for multiple shoot induction, both alone as well as in combination: (i) BAP and Kn singly (ii) BAP+Kn (iii) BAP+IBA (iv) Kn+IBA (v) Kn+IAA. The pH of media was adjusted at 5.8. Sucrose (3%) and agar 0.8% (w/v) were added to the media. The *in vitro* shoots were transferred to ¼ MS medium having IBA.

All the cultures were kept at 25±1^{0}C and 16:8 hour cycle of light (2000 lux of light from cool fluorescent tubes). Observations were recorded after an interval of two weeks and data are presented 30 days after inoculation. Different parameters were examined i.e. number of shoots, length of shoots and number of leaves. Total of 4-8 replicates were used. Data were presented in the form of mean ± SE, and means followed by the same letter within the columns are not significantly different (P = 0.05) using Duncan's multiple range test.

Nodal explants were inoculated in half strength MS media with different auxins (IAA and IBA) and cytokinins (BAP and Kn) at various concentrations (0.2, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0 mg/l). Almost at every concentration shoot primodia were seen within 4-8 days of inoculation. The results are presented in Table 1 and Fig. 1. The parameters recorded (Number of shoots, shoot length, number of leaves) differed at different concentrations. With BAP alone, explants showed more efficient multiple shooting in comparison to kinetin.

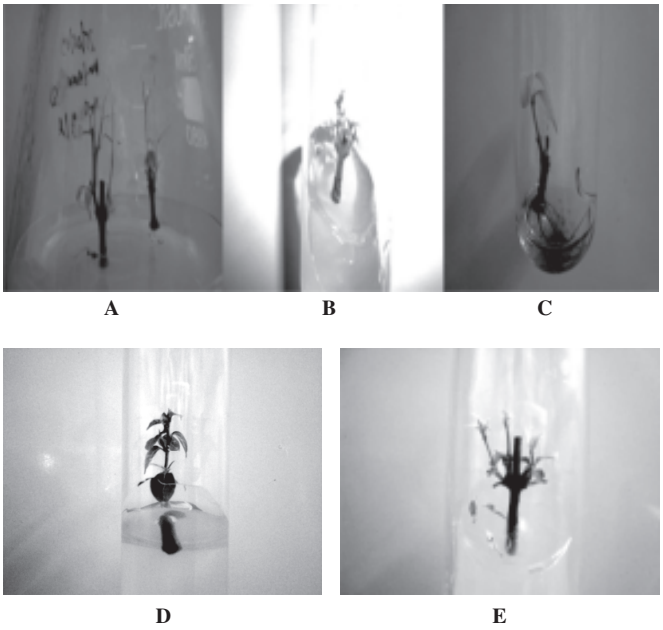

Fig.1 (A-E). ***In vitro*** **shoot induction and rooting of** ***Gymnema sylvestre*** **microshoots.**
(A) Shoot formation in ½ MS supplemented with Kn+IAA (1.5 mg/l each), (B) shoot formation in ½ MS+Kn (0.2 mg/l), (C) Multiple shoot formation in BAP and Kn (3.0 mg/l each), (D) Multiple shooting in ½ MS+BAP (0.5 mg/l), (E) ***In vitro*** **rooting in ¼ MS supplemented with IBA (1.0 mg/l).**

Maximum number of shoots (2.75±0.47) was observed at 0.5 mg/l of BAP alone, besides maximum shoot length (0.75±0.09 cm) and number of leaves (9.25±0.47). Similar responses were observed in previous studies but with MS full medium in *Gymnema sylvestre* (Reddy *et al.* 1998, Komalivalli and Rao 2000). BAP was effective in axillary shoot regeneration, in present study, similar to those reported in other medicinal plants like *Holarrhene antidysenterica* (Agarwal *et al.* 2005), *Eclipta alba* (Ray and Bhattacharya 2008), *Ocimum basilicum* (Siddique and Anis 2008), *Spilanthes acmella* (Singh *et al*. 2009). BAP was superior in shoot induction probably due mainly to the ability of plant tissues to metabolize BAP more readily than other synthetic growth regulators, or ability of BAP to induce production of natural hormones such as zeatin within the tissue (Malik *et al.* 2005). Kinetin when used singly at 0.2 mg/l produced best response in terms of all the parameters recorded i.e. shoot number (2.00±0.40), shoot length (1.70±0.17 cm) and number of leaves (6.75±0.62) (Table 1). Overall observation indicates that shoot length decreases with increasing concentration of BAP and Kinetin, when used

Table 1. *In vitro* propagation of *Gymnema sylvestre.*

Plant growth regulator (mgl^{-1})				Number of Shoot	Shoot length (cm)	Number of leaves
BAP	**Kinetin**	**IAA**	**IBA**			
0.0	0.0	0.0	0.0	1.00 ± 0.00^{a}	0.27 ± 0.025^{a}	1.25 ± 0.25^{a}
0.2	0.0	0.0	0.0	2.00 ± 0.00^{abcd}	0.75 ± 0.06^{ghi}	7.50 ± 0.86^{jklmnop}
0.5	0.0	0.0	0.0	2.75 ± 0.47^{de}	0.75 ± 0.09^{ghi}	9.25 ± 0.47^{op}
1.0	0.0	0.0	0.0	1.75 ± 0.25^{abcd}	0.47 ± 0.02^{abcdef}	4.75 ± 0.47^{cdefgh}
1.5	0.0	0.0	0.0	2.25 ± 0.25^{bcd}	0.65 ± 0.05^{fgh}	6.75 ± 0.47^{hijklmn}
2.0	0.0	0.0	0.0	2.00 ± 0.00^{abcd}	0.65 ± 0.05^{efgh}	6.00 ± 0.40^{efghijkl}
3.0	0.0	0.0	0.0	2.25 ± 0.25^{bcd}	0.62 ± 0.02^{efgh}	9.50 ± 0.05^{p}
4.0	0.0	0.0	0.0	2.50 ± 0.86^{cde}	0.62 ± 0.02^{fgh}	6.50 ± 0.64^{ghijklmn}
5.0	0.0	0.0	0.0	2.75 ± 0.47^{de}	0.60 ± 0.08^{defgh}	7.00 ± 0.70^{hijklmno}
0.0	0.2	0.0	0.0	2.00 ± 0.40^{abcd}	1.70 ± 0.17^{lm}	6.75 ± 0.62^{hijklmn}
0.0	0.5	0.0	0.0	1.75 ± 0.25^{abcd}	0.75 ± 0.02^{ghi}	8.00 ± 1.08^{klmnop}
0.0	1.0	0.0	0.0	1.00 ± 0.00^{a}	0.52 ± 0.11^{bcdef}	3.75 ± 0.85^{bcde}
0.0	1.5	0.0	0.0	1.50 ± 0.28^{abc}	0.45 ± 0.05^{abcdef}	6.00 ± 0.81^{efghijkl}
0.0	2.0	0.0	0.0	2.00 ± 0.00^{abcd}	0.57 ± 0.10^{cdefg}	6.50 ± 0.50^{ghijklmn}
0.0	3.0	0.0	0.0	1.75 ± 0.25^{abcd}	0.52 ± 0.04^{bcdef}	6.00 ± 0.91^{efghijkl}
0.0	4.0	0.0	0.0	2.25 ± 0.25^{bcd}	0.37 ± 0.02^{abc}	3.75 ± 0.62^{bcde}
0.2	0.2	0.0	0.0	1.50 ± 0.28^{abc}	0.47 ± 0.04^{abcdef}	4.25 ± 0.47^{bcdefg}
0.5	0.5	0.0	0.0	1.25 ± 0.25^{ab}	0.40 ± 0.04^{abcd}	3.75 ± 0.47^{bcde}
1.0	1.0	0.0	0.0	1.50 ± 0.28^{abc}	0.42 ± 0.02^{abcde}	3.50 ± 0.28^{bcd}
1.5	1.5	0.0	0.0	2.00 ± 0.00^{abcd}	0.35 ± 0.02^{ab}	3.25 ± 0.25^{abc}
2.0	2.0	0.0	0.0	1.75 ± 0.25^{abcd}	0.60 ± 0.04^{defgh}	7.25 ± 0.47^{ijklmnop}
3.0	3.0	0.0	0.0	3.25 ± 0.62^{e}	0.42 ± 0.06^{abcde}	9.25 ± 0.75^{op}
4.0	4.0	0.0	0.0	2.50 ± 0.50^{cde}	0.37 ± 0.02^{abc}	5.00 ± 0.40^{cdefghi}
5.0	5.0	0.0	0.0	1.75 ± 0.25^{abcd}	0.32 ± 0.04^{ab}	6.50 ± 1.04^{ghijklmn}
0.2	0.0	0.0	0.2	1.50 ± 0.28^{abc}	0.87 ± 0.08^{i}	8.75 ± 1.65^{nop}
0.5	0.0	0.0	0.5	1.25 ± 0.25^{ab}	0.65 ± 0.05^{fgh}	8.50 ± 1.19^{mnop}
1.0	0.0	0.0	1.0	1.75 ± 0.25^{abcd}	0.80 ± 0.08^{hi}	12.50 ± 0.70^{q}
1.5	0.0	0.0	1.5	1.00 ± 0.00^{a}	0.80 ± 0.07^{hi}	6.25 ± 0.75^{fghijklm}
2.0	0.0	0.0	2.0	1.75 ± 0.25^{abcd}	0.47 ± 0.04^{abcdef}	7.00 ± 0.40^{hijklmno}
3.0	0.0	0.0	3.0	1.25 ± 0.25^{ab}	0.52 ± 0.06^{bcdef}	5.75 ± 0.25^{defghijk}
4.0	0.0	0.0	4.0	1.50 ± 0.28^{abc}	0.37 ± 0.02^{abc}	5.25 ± 0.62^{cdefghij}
5.0	0.0	0.0	5.0	2.00 ± 0.00^{abcd}	0.37 ± 0.06^{abc}	8.25 ± 0.62^{lmnop}
0.0	0.2	0.0	0.2	1.00 ± 0.00^{a}	0.77 ± 0.08^{ghi}	2.00 ± 0.00^{ab}
0.0	0.5	0.0	0.5	1.50 ± 0.28^{abc}	0.50 ± 0.07^{bcdef}	3.00 ± 0.70^{abc}
0.0	1.0	0.0	1.0	1.75 ± 0.25^{abcd}	0.80 ± 0.07^{hi}	4.00 ± 0.40^{bcdef}
0.0	1.5	0.0	1.5	2.00 ± 0.40^{abcd}	0.65 ± 0.06^{fgh}	3.75 ± 0.75^{bcde}
0.0	0.2	0.2	0.0	1.00 ± 0.00^{a}	1.12 ± 0.04^{j}	3.25 ± 0.25^{abc}
0.0	0.5	0.5	0.0	1.00 ± 0.00^{a}	1.42 ± 0.02^{k}	4.25 ± 0.25^{bcdefg}
0.0	1.0	1.0	0.0	2.00 ± 0.00^{abcd}	1.80 ± 0.09^{mn}	4.25 ± 0.25^{bcdefg}
0.0	1.5	1.5	0.0	1.00 ± 0.00^{a}	1.97 ± 0.06^{n}	5.25 ± 0.25^{cdefghij}
0.0	2.0	2.0	0.0	2.00 ± 0.00^{abcd}	1.47 ± 0.02^{k}	8.25 ± 0.25^{lmnop}
0.0	3.0	3.0	0.0	2.25 ± 0.25^{bcd}	1.57 ± 0.02^{kl}	8.25 ± 0.62^{lmnop}
0.0	4.0	4.0	0.0	2.50 ± 0.28^{cde}	1.92 ± 0.04^{n}	9.50 ± 0.86^{p}
0.0	5.0	5.0	0.0	1.75 ± 0.25^{abcd}	1.70 ± 0.04^{lm}	9.25 ± 0.47^{op}

Means followed by the same letter within the columns are not significantly different (P= 0.05) using Duncan's multiple range test.

singly. The similar trend was noticed in case of *Oroxylum indicum* (Boro *et al*. 2005). In the present study, at all concentrations of plant growth regulators, shoot length diminished with multiple shooting.

BAP and kinetin in combination shows synergistic effect in terms of maximum number of shoot (3.25±0.62) and number of leaves (9.25±0.75) at 3.0 mg/l each (Table 1). Maximum shoot length (1.97±0.06 cm) was obtained in combination of kinetin and IAA at 1.5 mg/l each. Media with 1.0 mg/l BAP and IBA each showed better growth in all the parameters in term of number of shoots (1.75±0.25), length of shoot (0.80±0.08 cm) and number of leaves (12.50±0.70).

There were 5 roots in number produced with mean length of 2.14 cm, when microshoots where transferred in ¼ MS media with IBA 1.0 mg/l (Table 2). Earlier reports also suggest that IBA is more effective auxin for root initiation in *Gymnema* species (Komalivalli and Rao 1997, 2000).

Table 2. *In vitro* rooting of microshoots using 1.0 mg/l IBA.

Parameters recorded	15 d	30 d
Number of roots	2	5
Length of root (in cm)	0.8 (mean)	2.14 (mean)

The protocol describes the best *in vitro* shoot multiplication of *G. sylvestre* in half strength MS media having BAP and Kn (3.0 mg/l), whereas the rooting of microshoots was best observed in one fourth MS media in presence of 1.0 mg/l IBA.

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