Annual Academic Sessions 2011
ISSN 2012-9912; ©2011 Open University of Sri Lanka

IN VITRO PROPAGATION OF GYMNEMA SYLVESTRE

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INTRODUCTION

Gymnema sylvestre **(Retz.) R.Br. ex SCHULT** is a vulnerable medicinal plant belonging to the family Asclepiadaceae or milk weed family. The plant is commonly known as Masbedda in Sri Lanka. This plant is indigenous to India and grows wild in tropical forests of western and southern parts of India and in the tropical regions of Africa, Australia and South East Asia. It is occasionally cultivated. In Sri Lanka it is rather common in the low country especially in the dry and intermediate regions (Jayaweera and Senaratne, 2006).

The leaves and leaf extracts of *Gymnema* are extensively used mainly in India and also in many other parts of the world in many forms such as in tea bags, tablets, beverages and confectionaries in controlling mainly diabetes and obesity (Kanetkar, Singhal, Kamat, 2007; Nakamura, 1988; Ueno,1997). In Sri Lanka also, in ayurvedic medicine the leaves are used as a remedy for diabetes (Ayurveda Pharmacopoeia,1961).

Although *Gymnema* is in high demand in the world now due to its hypoglycemic properties, it is a slow growing plant in which the pod set is also rare. The seeds lose viability in a short period of storage (Reddy, Gopal, Sita,1998). Poor seed germination also restricts its multiplication (Komavali and Rao,2000). Conventional propagation of *Gymnema* by shoot cuttings is also difficult due to poor rooting (Komavali and Rao, 2000; personal communication, Ayurvedic Research Institute).

Therefore propagation of this plant by alternate methods is essential. In this context, *in vitro* propagation of *Gymnema* by direct organogenesis through axillary nodes was investigated as the main objective of this research project. At the same time a study of indirect organogenesis of calli induced either on internodal or on leaf segments was also carried out.

METHODOLOGY

- The Standardization of the sterilization procedure was carried out by using different concentrations of $HgCl_2$ alone and in combinations with NaOCl for different exposure times.
- The nodal segments were cultured in Murashige and Skoog medium (MS,1962) with the auxin 0.1mg/L NAA along with cytokinins BAP and/or Kinetin in seven different combinations i.e.,a) 0.1mg/L Kin b) 0.5 mg/L Kin c) 1.0 mg/L Kin d)0.5 mg/L BAP e)1.0 mg/L BAP f) 1.5 mg/ L BAP g) 1.0mg/L BAP and 0.5mg/L Kin, for shoot proliferation.
- The effect of explant orientation of internodal segments was observed by culturing them in vertical, inverted, horizontal and angular positioning in the MS medium supplemented with 1.0 mg/L BAP, 0.5mg/L Kin and 0.1 mg/L NAA.
- Inter nodal and leaf segments were cultured in the MS medium supplemented with the same hormonal combinations (a-g) for callus induction.

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- Leaf segments alone were cultured on MS media with 2.5mg/L and 4.0mg/L 2,4-D also for callusing. In addition, to study the effect of 2, 4-D pulse on callus induction from leaf segments, they were exposed to 1.7μM 2,4-D in liquid MS medium for 90 minutes and cultured them on MS Basal medium and in the MS medium containing 1.0 mg/L BAP, 0.5mg/L Kin and 0.1 mg/L NAA.
- All the above media were fortified with 100mg/L each of citric acid and malt extract and 3% sucrose. And the cultures were maintained in the dark for 7 days and then transferred to light.
- Regeneration from callus was carried out on a medium with MS salts, B5 vitamins, 0.5 μM BAP and 2% sucrose and maintained in light.
- There were 7 experiments and these were set up according to CRD model and ANOVA and mean separation (LSD) of data were carried out using SAS ver. 6.12 software package.

RESULTS AND DISCUSSION

Standardization of the Sterilization Procedure

Standardization of the sterilization procedure was carried out with 14 different treatments by using $HgCl_2$ and NaOCl or with both of the sterilants. Among the 14 different treatments tested on both the types of explants (leaf and stem), the best effect was exhibited by 0.5% $HgCl_2$ for 10 minutes exposure time. This was the only treatment that allowed over 70% survival of each explant type. In all the other treatments, the explants were either contaminated (mainly by fungi or otherwise by bacteria) or they appeared to be dead due the harmful effects of the sterilant/s at high concentrations.

Effect of Growth Regulators on Shoot/ Callus Induction from Cultured Explants

Axillary shoot sprouting was initiated at all levels of BAP and Kin along with 0.1 mg/L NAA (Table 1). From the results, it could be concluded that BAP supplemented media was more effective in shoot proliferation than Kin supplemented media. BAP at 1.0mg/L was found to be the best among the concentrations tested and produced the highest shoot induction frequency (80%) Shoot proliferation was also observed on this medium. Two shoots were regenerated from one explant on this medium whereas only a single shoot was produced from explants in all other growth regulator combinations.

Growth of the induced shoots, as measured by the mean shoot length, increased with the increase in the Kin concentration in the medium. However Reddy *et al.* (1998) reported that there was no significant effect of Kin, on shoot length. And the study proved statistically that medium incorporated with 1.0 mg/L BAP and 0.1 mg/L NAA has a significant effect on shoot proliferation.

The highest concentration of BAP (1.5 mg/L) used in the medium appeared to suppress shoot development. When BAP and Kin were combined and used at their optimal concentrations with NAA in the medium, healthy shoots were produced that were the longest. These shoot buds had many leaves that grew normally resembling the leaves of the mother plant. This is the medium that was proved to be the optimum for shoot proliferation by Komavali and Rao (2000).

Concentration of Growth Regulators (mg/L) NAA	BAP	Kin	Shoot Sprouting Frequency %	No of Shoots per Explant	Mean Length of Shoots (cm.)
0.1		0.1	20	01	0.7
0.1		0.5	40	01	0.95
0.1		1.0	10	01	0.84
0.1	0.5		10	01	0.8
0.1	1.0		80	02	0.9
0.1	1.5		40	01	0.74
0.1	1.0	0.5	40	01	1.36

Table 1: Effect of Growth Regulators on Shoot Induction from Nodal Explants

Callus induction from cultured internodes was very low on the media tested. Thirty percent of (30%) explants produced callus on media containing the highest level of BAP (1.5mg/L) and 10% produced callus on the highest Kin (1.0mg/L) incorporated media (Table 2). This agrees with the results of Gopi and Vatsala, (2006) indicating that BAP is more effective in producing callus than Kin.

Concentration of Growth regulators mg/L NAA	BAP	Kin	No. of Explants Producing callus
0.1		0.1	00
0.1		0.5	00
0.1		1.0	01
0.1	0.5		00
0.1	1.0		00
0.1	1.5		03
0.1	1.0	0.5	00

Table 2 : Effect of Growth Regulators on Callus Induction from Cultured Internodes

Internodal segments in horizontal orientation produced callus, in 30% of explants and in 10% of explants in the vertical down orientation (Table 3).

Explant Orientation	Callus Induction Frequency %
Vertical Up Position	00
Vertical Down Position	10
Angular Position	00
Horizontal Position	30

Table 3: Effect of Explant Orientation on the *In Vitro* Response from the Inter Nodal Segments

From this study, it is revealed that internodal explants are more responsive than leaf explants to the different media tested, wherein there is no significant observations produced. This also agrees with the previous reports of Kumar *et al* (2002), Gopi and Vatsala (2006) and Roy *et al* (2008). Calli induced did not show a positive response to the regeneration medium and culture conditions tested

CONCLUSION/ RECOMMENDATION

A suitable procedure for surface sterilization of explants obtained from *G. sylvestre* mother plants was developed. 0.5% w/v solution of $HgCl_2$ was proved to be the best sterilant with an exposure time of 10 minutes for both the leaf and stem explants.

Among the different growth regulator combinations tested, BAP 1.0 mg/L + Kin 0.5 mg/L + NAA 0.1 mg/L resulted in the healthiest shoot formation. This combination proves to be the best for shoot induction from axillary nodes of *G. sylvestre.* However it was statistically proved by the present study that the medium incorporated with 1.0mg/L BAP and 0.1 mg/L NAA was also equally good for shoot proliferation.

The internodal explants achieved a higher rate of callus induction when compared to leaf explants and a high concentration of BAP and Kin are required for this process. The results suggest that the horizontal orientation of the internodal explants is the best for callus induction. This study could further be used in developing suitable and economical techniques in obtaining true to type *G. sylvestre.*

REFERENCES

Ayurveda Pharmacopeia, (1961), Vol 1, Part 2, Ayurveda Department, 127

Dateo, G. P., Long, L. (1973) Gymnemic acid, The Antisaccharine Principle of *Gymnema sylvestre*. Studies on Isolation and Heterogenesity of Gymnemic Acid A. J. Agric. Food Chem., 21:899–903

Gopi, C., Vatsala, T. M., (2006) *In Vitro* Studies on Effects of Plant Growth Regulators on Callus and Suspension Culture Biomass Yield from *Gymnema sylvestre* R.Br. African Journal of Biotechnology, Vol 5 ,No.12 1215-1219

Jayaweera, D. M. A., Senaratne, L. K., (2006), Medicinal Plants Used in Ceylon Part1, National Science Foundation, 160-161

Kanetkar, P,. Singhal, R., and Kamat, M., (2007) *Gymnema sylvestre*: A Memoir Journal of Clinical Biochemistry and Nutrition; 41(2): 77–81.

Komalavali, N., Rao, M. V. (2000) *In Vitro* Micropropagation of *Gymnema sylvestre*: A Multipurpose Medicinal Plant. Plant Cell, Tissue and Organ Culture; 61:97–105.

Reddy, P. S., Gopal, G. R., Lakshmi Sita, G., (1998) *In Vitro* Multiplication of *Gymnema sylvestre* R.Br. An Important Medicinal Plant Curr. Sci .75 843-845

Roy, A., Ghosh, S.,Chaudri, M.,Saha, P. K., (2008), Effect of Different Plant Hormones on Callus Induction in *Gymnema sylvestris* R.Br.(Asclepiadaceae). African Journal of Biotechnology, Vol 7(13) 2209-2211