Annals of Phytomedicine 4(2): 67-70, 2015

Journal homepage: www.ukaazpublications.com

ANNALS OF PHYTOMEDICINE
An International Journal

ISSN: 2393-9885

In vitro clonal propagation of *Salacia oblonga* WALL. An endangered medicinal plant

K.G.K. Deepak, G. Suneetha and Ch. Surekha

Department of Biochemistry and Bioinformatics, Institute of Science, GITAM University, Visakhapatnam-530 045, Andhra Pradesh, India

Received September 20, 2015: Revised October 20, 2015: Accepted October 27, 2015: Published online December 30, 2015

Abstract

An efficient protocol has been developed to micropropagate *Salacia oblonga* WALL, an highly endangered medicinal plant of the Indian subcontinent and Sri Lanka. Endangered nature of *S.oblonga* is due to over exploitation for its medicinal significance and poor seed germination rate. Therefore, there is an immediate necessity to micropropagate the plant in *in vitro* conditions in order to conserve the plant from the brink of extinction. For the first time, the plant has been propagated successfully through *in vitro* conditions using nodal explants. Various hormonal concentrations were experimentally checked to micropropagate *S. oblonga*. Maximum shooting response was observed from the axils of nodal explants on MS media, supplemented with BAP (3.5 mg/l) + IBA (1 mg/l), followed by BAP (4 mg/l)+ IAA(1 mg/l). Successful rooting was observed in MS media, supplemented with 0.5 mg/l IBA. The rooted plantlets were shifted to green house for acclimatization. The results will help to facilitate the conservation and propagation of endangered medicinal plant *S. oblonga*.

Key words : *Salacia oblonga* WALL., endangered, micropropagation, explants, medicinal plants

1. Introduction

Salacia oblonga WALL belonging to Celestaceae family, is an important endangered woody plant that is distributed throughout India and Sri Lanka. In India, it is found in the rain forest of Western Ghats from Konkan southwards to Kerala (Anshul *et al.*, 2013). It is a small climbing shrub, leaves are ovate and flowers are greenish yellow with fruits about 3 cms in diameter, light brown or orange when ripe, with 1-8 seeds embedded in the pulp. The roots, stems and leaves of *S. oblonga* have been extensively used in Ayurvedic medicine for treating various diseases. The active principles are known to possess many biological activities such as antidiabetic, antirheumatic, antigonnorhoeic, antioxidant, antimicrobial nephroprotective, antimutagenic and against skin diseases (Collene *et al.*, 2005; Matsuda *et al.*, 1999, 2002, 2005; Kirtikar and Basu, 1987). The methanolic extracts of root and stem contain two potent α-glucosidase inhibitors (salacinol and kotalanol) and an aldose reductase inhibitor kotalagenin-16-acetate (Matsuda *et al.*, 1999; Yoshikawa *et al.*, 1998).

Salacinol and kotalanol binds competitively to the α-glucosidases present in the small intestine and inhibit breakdown of carbohydrates into glucose thereby, resulting in low blood glucose levels. Kotalagenin-16-acetate, competitively binds to aldose reductase and prevents the accumulation of sorbitol in the eye, thus preventing formation of cataracts (Deepak *et al.*, 2014, 2015a). Due to its high medicinal value, the roots and stems are extensively used in Indian ayurvedic system and this resulted in over exploitation and uncontrolled harvest from the natural habitat. In addition, lack of organised cultivation practices, insufficient attempts for the replacement of harvested plants, poor seed germination and poor regeneration capacity, restricts the propagation of *S. oblonga* (Oomen *et al.*, 2000; Dhanasri *et al.*, 2014) and resulted the plant to endangered status.

Therefore, there is an immediate necessity to conserve and propagate *S.oblonga* through non-conventional methods, *i.e.*, *in vitro* and *ex situ* propagation. Reports on conservation of *Salacia* sps.are very rare. Dhanasri *et al.* (2014) reported successful micropropagation of *S. reticulata*, using axillary buds as explants on different concentrations and different combinations of 6-Benzylaminopurine (BAP), Indole-3-acetic acid (IAA), Kinetin (Kn) and Indole-3-butyric acid (IBA). Deepak *et al.* (2015b) have reported a simple and effective method for vegetative propagation of *S.oblonga*, using stem and root cuttings as explants by treating with different concentrations of IBA (0-500 ppm). Therefore, the aim of the present work is to establish micropropagation of *S.oblonga* in order to conserve and produce genetically stable plantlets for future generations.

2. Materials and Methods

2.1 Plant material

Defoliated young stems (10 cm) of *S. oblonga* WALL were collected from 7 year old plant, growing in the green house of GITAM University. The explants were washed with mild detergent and were then surface sterilized with 0.5% bavistin (a fungicide) for 5 min, followed by 0.1% mercuric chloride for 1 min and 5-6 times thorough washings with sterile distilled water. The explants were blotted dry with sterile filter papers. The surface sterilized defoliated young stems were cut into 3 cm long segments with one

Author for correspondence: Dr. Ch. Surekha
Department of Biochemistry and Bioinformatics, Institute of Science, GITAM University, Visakhapatnam-530 045, Andhra Pradesh, India
E-mail: challa_surekha@yahoo.co.in
Tel.: +91-8008116626

axil on either side and used as experimental sources. The nodal explants were cultured on MS media supplemented with different combinations of phytohormones, *viz*., Gibberellic acid (0-2.5 mg/l); Thidiazuron (1-5 mg/l) + Kn (0-1 mg/l); BAP (1-5 mg/l) + IBA (1 mg/l). Polyvinylpyrrolidine (0.1%), L- Proline (0.1%) and activated charcoal (0.1%) were added to MS media to inhibit formation and release of phenolics into media. The culture tubes were incubated at 25±2^{0}C, 50-60% relative humidity and 16/8 hr light / dark photoperiod, supplied by cool white fluorescent tubes of 3000 lux. The explants were subcultured into fresh MS media for every 21 days.

The shoots longer than 3 cm were excised from the axils and transferred into MS rooting medium, supplemented with or without 0-1 mg/l IBA. Well rooted explants with fully opened leaves were planted in plastic cups (5 cm diameter), containing autoclaved soil and sand (2:1) and regularly watered with sterile distilled water. The established plants were transferred to black covers containing soil and vermiculate in 1:1 ratio and covered with transparent polythene covers to prevent excess transpiration. The plants were exposed to sun light for an hour initially, followed by gradual increase in time of exposure and finally transferred into green house.

2.2 Statistical analysis

Each experiment was repeated 3 times with 20 explants per set. The data were subjected to statistical analysis for calculating the mean and the standard error.

3. Results and Discussion

The nodal explants were tested for regeneration, using 9 different combinations of phytohormones. Nodal explants supplemented with different combinations of phytohormones showed no significant changes in the first week of inoculation. Explants inoculated on GA_3(0.5-1.5 mg/l); TDZ (1-5 mg/l) + Kn (0.5 mg/l); TDZ (1-5 mg/l) + Kn (1 mg/l); BAP (2.5-3.5 mg/l) + IBA (1 mg/l) and BAP (2-4 mg/l) + IAA (1 mg/l) showed the same response, *i.e*., small protrusion or a bud emergence (indicated with arrows) from both the axils of nodal explants in a week as shown in the (Figure 1A). The percentage of explants showing this response at the axils is more or less similar in all the phytohormone combinations used. The buds in the axils increased in size to form a shoot primordia in 2 weeks (Figure 1B). The maximum percentage of shoot primordia development was observed on phytohormone combination of BAP (3.5 mg/l) + IBA (1 mg/l) with 50%, followed by 45% response on BAP (4 mg/l) + IAA (1 mg/l) when compared to others concentrations of phytohormones used. The shoot primordia increased in length during the 3rd week and the maximum shoot length of 1-2 cm was observed in BAP (3.5 mg/l) + IBA (1 mg/l). The number of explants inoculated per each treatment, percentage of explants that showed bud emergence at the axils of explants and length of shoot primordia were listed in Table 1. Opening of small leaves from the shoot primordia was observed during the 4th week (Figure 1C). In most of the explants, a single shoot elongated and opened its leaves from the axillary region of each explant. Leaf expanded and continued to increase in size during the 8-9 weeks (Figure 1D). In few explants, the explant started yellowing from the base and the yellowing reached to top, leading to death in 9-10 weeks.

Shoots longer than 3 cm, with opened leaves were transferred to MS rooting media, supplemented with IBA (0-1.5 mg/l) and 0.6% agar. Small roots were initiated in explants that were grown on BAP (3.5) + IBA (1 mg/l) when subcultured onto MS media, supplemented with 0.5 mg/l IBA (Figure 1E). The explants from GA_3, TDZ + Kn, BAP + IAA failed to root and they showed yellowing of leaf and browning of tissue. The plants that successfully rooted were transferred to cups, filled with sterilized soil and sand (2:1) as shown in Figure 1E. The cups are covered with polythene covers to prevent excess water loss from the plantlets. The explants are watered with sterile distilled water and exposed to sunlight every day for an hour. Plantlets with thick root formation (Figure 1G) were shifted to green house for acclimatization.

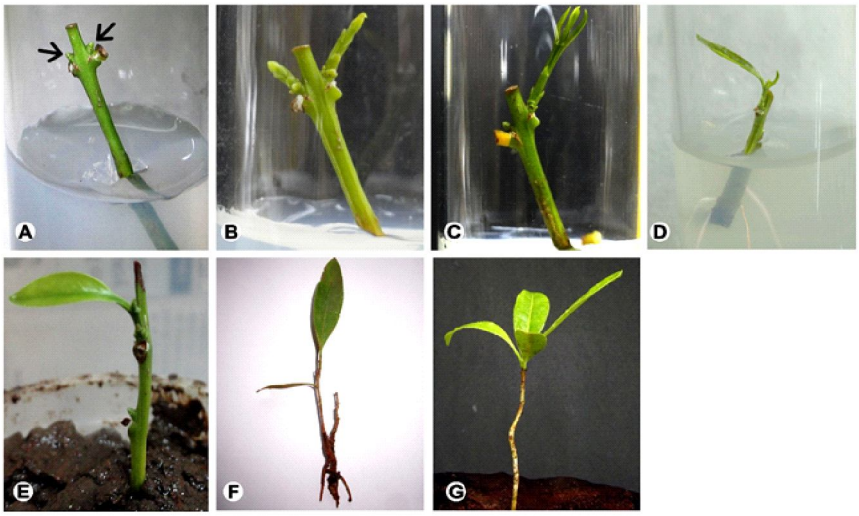

Figure 1: Different stages of micropropagation of S. oblonga WALL

A) Bud emergence from axils (indicated with arrows) of nodal explants in a week; B) Initiation of shoot primordia from emerged axillary buds in 2 weeks; C) Opening of small leaves from shoot primordia in 4 weeks; D) Rooting observed on MS medium supplemented with 0.5 mg/l IBA (indicated with arrows) from nodal explant in 12 weeks; E) Hardening of explant in sterile soil; F) Hardened plant with thick roots after 15 weeks; G) Acclimatized plant

Table 1 : Response of nodal explants to various concentrations of phytohormones

Sl.No.	Phytohormone used (mg/l)	No. of explants inoculated	No. of explants showing bud emergence	No. of explants showing shoot elongation	No. of explants rooted	No. of explants hardened
1.	GA_3 (0.5)	20	2.6 ± 0.8	1.6 ± 1.2	0	0
	GA_3 (1)	20	6 ± 0.5	5.3 ± 0.3	0	0
	GA_3 (1.5)	20	0.6 ± 0.3	0.3 ± 0.3	0	0
	GA_3 (2)	20	0	0	0	0
	GA_3 (2.5)	20	0	0	0	0
2.	TDZ (1) + Kn (0.5)	20	3 ± 0.5	2.8 ± 0.6	0	0
	TDZ (2) + Kn (0.5)	20	1.2 ± 0.6	0.9 ± 0.2	0	0
	TDZ (3) + Kn (0.5)	20	2.6 ± 0.4	1.5 ± 0.4	0	0
	TDZ (4) + Kn (0.5)	20	5.3 ± 0.9	4.3 ± 0.6	0	0
	TDZ (5) + Kn (0.5)	20	3.1 ± 0.5	1.8 ± 0.2	0	0
3.	TDZ (1) + Kn (1)	20	1.6 ± 0.5	1.3 ± 0.8	0	0
	TDZ (2) + Kn (1)	20	1.4 ± 0.3	1 ± 0	0	0
	TDZ (3) + Kn (1)	20	2.3 ± 0.6	2 ± 0.5	0	0
	TDZ (4) + Kn (1)	20	3.6 ± 1.2	3 ± 1.1	0	0
	TDZ (5) + Kn (1)	20	2 ± 0.5	1.3 ± 0.3	0	0
4.	BAP (2) + IBA (1)	20	0	0	0	0
	BAP (2.5) + IBA (1)	20	2.6 ± 1.2	2 ± 2.5	1.2 ± 0	0.6 ± 0
	BAP (3) + IBA (1)	20	6 ± 0.5	5 ± 0.5	2.1 ± 0	1 ± 0
	BAP (3.5) + IBA (1)	20	10.6 ± 0.8*	9.6 ± 0.8*	5.2 ± 0	3.2 ± 0
	BAP (4) + IBA (1)	20	0	0	0	0
5.	BAP (1) + IAA (1)	20	0	0	0	0
	BAP (2) + IAA (1)	20	2.6 ± 0.3	2.1 ± 0.3	0	0
	BAP (3) + IAA (1)	20	5 ± 1.1	4.1 ± 0.4	0	0
	BAP (4) + IAA (1)	20	9.3 ± 0.8*	8.5 ± 0.7*	0	0
	BAP (5) + IAA (1)	20	0	0	0	0

*significant at $p \leq 0.05$ level of probability

The study reports a simple, reproducible method for micropropagating *S. oblonga*. The effects of cytokinins and auxins on morphogenesis of nodal explants, are observed in this study. Cytokinins are reported to overcome apical dominance, induce high number of shoot buds and also release lateral buds from dormancy (Rout *et al.*, 2000; Rout, 2005). But in the present study, cytokinins did not promote intensive shoot multiplication but developed only one shoot per axil.

The highest number of bud emergence, short elongation, rooting and hardening was obtained in MS medium supplemented with 3.5 mg/l BAP and 1 mg/l IBA. Concentration of BAP higher than 4 mg/l exhibited negative effect on shoot regeneration and continued exposure of explants to high concentration of BAP during shoot induction inhibited the growth of shoots. The synergistic effect of auxin and cytokinin has been demonstrated in medicinal plants like *Rauvolfia teraphylla, Santolina canescens, Bupteurum fruticosum* and *Rotula aquatic.* The results of the present study are in accordance with Pandey *et al.* (2015) with the use of BAP for shoot multiplication.

The presence of auxins at lower concentration in medium facilitated better root formation. MS medium supplemented with 0.5 mg/l IBA was found superior to IAA and NAA in inducing roots.The results are in harmony with Fracro and Echeverrigancy (2001) and Shahzad *et al.* (2007) in showing the superiority of IBA over other auxins in root formation.

4. Conclusion

S. oblonga WALL. is an endangered medicinal plant, widely used in Ayurvedic system of medicine to treat diabetes and various other ailments. Increased demand for roots and stems had resulted in extensive clearing of *S. oblonga* from the natural habitats due to which the plant is endangered. Therefore, the present study is

focused on micropropagating *S. oblonga*, using nodal segments as explants. Successful shooting and rooting was achieved on MS media, supplemented with BAP (3.5 mg/l) + IBA (1 mg/l) and IBA (0.5 mg/l), respectively. These efforts could help to propagate plants with high medicinal value and thereby, meeting the demands of the growing human population.

Acknowledgement

KGKD, GS and CS are thankful to the GITAM University, Visakhapatnam, India for providing the facility and support. CS is thankful to Department of Science and Technology (DST) and University Grants Commission (UGC), New Delhi for the project funding SR/FT/LS-32/2010 and 42-180/2013(SR), respectively KGKD is thankful to DST, New Delhi for supporting with Junior research fellow and Senior research fellow in the project SR/FT/LS-32/2010. GS is thankful to UGC, New Delhi for supporting with Project fellow fellowship in the project 42-180/2013(SR). We profusely thank Dr.N.Nageswara Rao Reddy, GITAM University, Visakhapatnam, India for critical comments and reviewing of the manuscript.

Conflict of interest

We declare that we have no conflict of interest

References

Anshul, C.; Singh, S. and Sharma, A.K. (2013). *Salacia oblonga* Wall: A review on its pharmacognostic, phytochemical and pharmacological aspects. Intl. J. Res. Pharm. Biomed. Sci., **4**:1215-1218.

Collene, A.L.; Hertzler, S.R.; Williams, J.A. and Wolf, B.W. (2005). Effects of a nutritional supplement containing *Salacia oblonga* extract on postprandial glycemia, insulinemia, and breath hydrogen responses in healthy adults. Nutrition, **21**:848-854.

Deepak, K.G.K.; Nageswara Rao Reddy, N. and Surekha, C. (2014). Role of antidiabetic compounds on glucose metabolism-A special focus on medicinal plant: Salacia sps. Med. Chem., **4**:373-381.

Deepak, K.G.K.; Rathna Giri, P.; Kavi Kishor, P.B. and Ch. Surekha. (2015a). Salacia as an ayurvedic medicine with multiple targets in diabetes and obesity. Ann. Phytomed., **4(1)**:46-53.

Deepak, K.G.K.; Suneetha, G. and Surekha, Ch. (2015b). A simple and effective method for vegetative propagation of an endangered medicinal plant *Salacia oblonga* Wall. J. Nat. Med., 70:115-119; DOI 10.1007/ s11418-015-0932-6.

Dhanasri, G.; Reddy, M.S.; Naresh, B. and Cherku, D. (2014). Micropropagation of *Salacia reticulata*-an endangered medicinal plant. Plant Tissue Cult Biotechnol., **23(2)**:221-229.

Fracaro, F. and Echeverrigaray, S. (2001). Micropropagation of cunilagalioides, a popular medicinal plant of south Brazil. Plant Cell Tissue Organ Cult., **64(1)**:1-4.

Kirtikar, K.R. and Basu, B.D. (1987). Indian medicinal plants. CAB MDirect.

Matsuda, H.; Murakami, T.; Yashiro, K.; Yamahara, J. and Yoshikawa, M. (1999). Antidiabetic principles of natural medicines. IV. Aldose reductase and alpha-glucosidase inhibitors from the roots of *Salacia oblonga* Wall. (Celastraceae): Structure of a new friedelane-type triterpene, kotalagenin 16-acetate. Chem. Pharm. Bull. (Tokyo), **47**:1725-1729.

Matsuda, H.; Morikawa, T. and Yoshikawa, M. (2002). Antidiabetogenic constituents from several natural medicines. Pure Appl. Chem., **74(7)**:1301-1308

Matsuda, H.; Yoshikawa, M.; Morikawa, T.; Tanabe, G. and Muraoka, O. (2005). Antidiabetogenic constituents from *Salacia* species. J. Trad. Med., **22**:145-53.

Oommen,S.; Vend, D.K. and Krishnan.R. (2000). Tropical Indian medicinal plants: Propagation methods. FRLHT, Bangalore, pp: 351.

Pandey, A.; Dosad, S.; Chawla, H.S. and Arora, S. (2015). *In vitro* organogenesis and plant regeneration from seed-derived callus cultures of finger millet (*Eleusinecoracana*). Braz. J. Bot., **38(1)**:19-23.

Rout, G.R.; Samantaray, S. and Das P. (2000). *In vitro* manipulation and propagation of medicinal plants. Biotechnol. Adv., **18**:91-120.

Rout. G.R. (2005). Micropropagation of *Clitoria ternatea* Linn. (Fabaceae) - An important medicinal plant. *In vitro* Cell. Dev. Bio. Plant, **41**:516-519.

Shahzad, A.; Faisal, M. and Anis, M. (2007). Micropropagation through excised root culture of *Clitoria ternatea* and comparison between *in vitro* regenerated plants and seedlings. Ann App.Bio., **150(3):**341-349.

Yoshikawa, M.; Murakami, T.; Yashiro, K. and Matsuda, H. (1998). Kotalanol-A potent glucosidase inhibitor with thiosugarsul foniumsulfate structure, from antidiabetic Ayurvedic medicine *Salacia reticulata*. Chem. Pharm. Bull., **46**:1339-1340.