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In Vitro Organogenesis of *Lycianthes bigeminata* Bitter.

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Abstract

Lycianthes bigeminata Bitter (Solanaceae) is an important medicinal herb distributed in the sholas of Nilgiris and chiefly used for curing ulcer. It is reported that the species is present in the sholas with poor population size in comparison to other constituent species. Owing to the demand and subsequent exploitation, it is predicted that it may occupy still poor association in the sholas of Nilgiris in course of time. Hence *in vitro* regeneration through employing tissue culture technique is needed. The preliminary attempt in the present study reports that the MS medium supplemented with Benzyl Amino Purine (BAP) and Naphthalene Amino Acid (NAA) at 0.5 mg/l each, induced effective callus formation. However further studies on hardening is suggested to know the survivability of this species.

Key Words : *Lycianthes bigeminata*, callus, *in vitro* organogenesis.

Introduction

Nilgiri Biosphere Reserve (NBR) is located in the Western Ghats between the co- ordinates of 76 c- 76 45 E and 11 15 – 12 15 N lying at the trijunction of the three states of Kerela, Karnataka and Tamilnadu and covering an area of about 5520 sq. km. Many indigenous, exotic and endemic plants of greater economical importance are harbouring

this region. Due to the poor ecological and physiological behaviours, some of them are restricted in their distribution. The species, *Lycianthes bigeminita* (Solanaceae) (Fig. 1a) a medicinal herb, chiefly used for the purpose of curing ulcer (Jain, 1996) is perpetuated very poorly in the shola understories due to lower survival rate of seedlings despite the higher seed out put. In the present study, an attempt has been made to know the response of the plant with respect to callus formation to basal medium supplemented with various concentrations of hormones.

Materials and Methods

Leaf explants were collected from mature selected superior phenotypes of the species, *Lycianthes bigeminata* in Thiashola, the Nilgiris. The explants were cut into small discs (0.5cm diameter) and washed thoroughly with running tap water for 2-3 minutes. Surface sterilization of the explants was carried out with 2-3% teepol (10mins) followed with 70% ethanol (10 mins) and 0.1% $HgCl_2$ (10 mins). The explants were then rinsed with sterile water 4-5 times and inoculated horizontally on the MS medium (Murashige and Skoog, 1962) in aseptic conditions.

The medium containing 30g/l of sucrose gelled with 8g/l ditco-bacto agar supplemented with and without 6-benzyl amino purine (BAP) and ∝-napthalene acetic acid (NAA) in various combinations was employed in the present study. The medium was adjusted to pH 5.8 prior to autoclaving and maintained at 24 ± 1^0 C under total darkness for 24 hrs and then 16 hrs cool white, fluorescent light (ca.4000 lux). Subculturing for shoot proliferation was carried out from the callus developed on the basal medium containing BAP and NAA at optimum levels. Similarly, the green adventitious shoots were excised carefully and subcultured on the medium with optimum level of NAA for root initiation. Five replications each with 20 leaf discs for callogenesis and each with 10 culture bottles for subculturing experiments were maintained.

Results and Discussion

Ten days after the inoculation of explants onto the medium, the callus was proliferated differentially according to the concentration of the hormones, BAP and NAA (Table1). The callus initiation began, at the cut ends which gradually extended to the entire leaf surfaces of the explants within a week of culture. The callus was compact, pale white in colour and generally translucent and uneven at the surface. The percentage of leaf discs, producing callus was ranging between 70 and 100 across the different combinations of BAP and NAA. The study revealed that all combinations of BAP and NAA induced higher degree of callus formation over the pure basal medium without hormones. However, the combination of BAP and NAA at the concentration of 0.5 mg/l each, induced the callus in all the inoculated explants (Table1 and Fig. 1b). Hence, the hormones, BAP and NAA at the concentration of 0.5mg/l each in the basal medium are found to be optimum for callus induction in the leaf discs of *L. bigeminata*.

Subculturing experiments were attempted for shoot initiation from the callus developed on the basal medium supplemented with BAP and NAA (0.5 mg/l each). The percentage of shoot forming callus was varied greatly (20 – 95) according to the variation in concentration of BAP in basal medium (Table 1). The medium containing no BAP (control) had very poor response of 20% shoot forming callus. On the other hand, the MS medium containing BAP at 0.5 mg/l induced a maximum of 95 percentage of callus for shoot formation (Fig. 1c), and so the basal medium with BAP in this concentration is most suitable for effective shoot formation. The importance of BAP for shoot proliferation was already discussed and documented well (Das *et al.,* 1990; Subbaiah and Minocha, 1990).

It was further observed that root initiation was started after 10 days of subculturing on the basal medium containing various concentrations of NAA and no NAA (Table 1). It was standardized that the basal medium with NAA at 1.0mg/l induces 90% of shoots for root formations in the *in vitro* conditions (Fig. 1d). In addition, it is noted that this standardized basal medium produced higher number of roots (5/ shoot). Ray *et al.,* (2001) already reported that the NAA concentration at the level of 1mg/l initiates roots effectively for many endangered medicinal plants. The preliminary studies by Molinar *et al.,* (1996) also confirmed the requirement of NAA at the rate of 1ml/l in the basal medium for the high degree of root initiation in the shola element of Nilgiris, *Berberis trifoliata.*

The hormones BAP and NAA in the basal medium played greater role in the callus formation and subsequent shoot and root initiations in the species, *L. bigeminata*. Chalupa (1974) and Campbell and Durzan (1975) stated that the hormone, benzylaminopurine (BAP) plays effective role in the morphogenesis in dicots. However, Bonga (1977) pointed out that the response of plants with respect to organogenesis is species specific. Steinitz *et al.,* (2003) reported that Solanaceae members generally responded appreciably to the basal medium containing BAP. Austin *et al., (*1988), Bu *et al.,* (1993) and Wali and Siddiqui (2003) already reported the success of *in vitro* propagation of many Solanaceae members by using the basal medium containing auxin type of growth hormones. Paulsamy *et al.,* (2004) reported the success of callogenesis for another shola species of Nilgiris, *Berberis tinctoria.*

Conclusion

It is concluded that for the effective *in vitro* regeneration of the shola medicinal herb, *L. bigeminata,* the basal medium is standardised with the hormone concentration as follows: 0.5mg/l of BAP and NAA each for effective callus formation; 0.5 mg/l of BAP for effective shoot formation; 1.0mg/l of NAA for effective root initiation. The massive production of plantlets through this method of *in vitro* propagation and transfer of them to the shola habitats of Nilgiris will ensure the species conservation effectively. However, this fact will be confirmed only after knowing the survivability rate of seedlings through proper hardening experiments.

Table 1. Response of hormones BAP and NAA on callus formation, shoot proliferation and root initiation for the species, *Lycianthes bigeminata.*

Experiment for callus formation		Experiment for shoot proliferation		Experiment for root initiation		
Content of BAP and NAA (mg/l) in MS medium	* Percent leaf discs producing callus	Content of BAP (mg/l) in MS medium	** Shoot forming callus (%)	Content of NAA (mg/l) in MS medium	** Percent shoots producing roots	No. of roots per shoot
0.5	80 ± 2.5	0.5	95 ± 4.5	0.5	70 ± 6.1	3 ± 0.2
1.0	75 ± 3.3	1.0	80 ± 3.5	1.0	90 ± 5.9	5 ± 0.4
2.0	85 ± 4.5	2.0	90 ± 3.9	2.0	80 ± 6.8	3 ± 0.2
3.0	80 ± 2.8	3.0	85 ± 4.2	3.0	70 ± 3.5	4 ± 0.4
4.0	90 ± 3.2	4.0	70 ± 5.1	4.0	60 ± 3.8	3 ± 0.2
0.5	100 ± 6.5	MS medium without BAP	20 ± 1.8	MS medium without NAA	40 ± 2.5	1 ± 0.2
1.0	95 ± 6.1					
2.0	85 ± 5.7					
3.0	80 ± 5.8					
4.0	85 ± 6.1					
0.5	90 ± 5.9					
1.0	85 ± 5.8					
2.0	85 ± 6.5					
3.0	85 ± 6.0					
4.0	80 ± 6.2					
0.5	85 ± 4.8					
1.0	85 ± 3.9					
2.0	80 ± 5.3					
3.0	75 ± 5.5					
4.0	75 ± 4.8					
MS medium without BAP and NAA	70 ± 6.3					

* Mean value of 5 replication each cultured with 20 leaf discs.
** Values are arrived at by the observations made on10 test culture bottles in each replication.

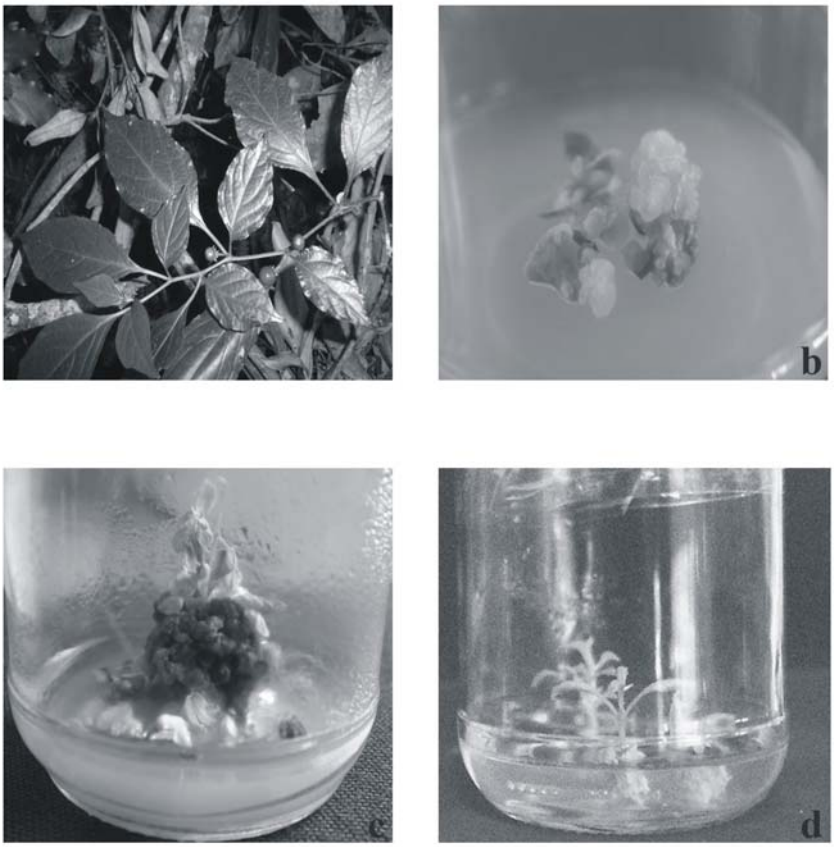

Fig. 1. Habit and formation of callus and roots from leaf explants of *L. bigeminata* in standardized basal medium during *in vitro* regeneration.

a. The herb, *L. bigeminata.*

b. High degree of callus formation in the basal medium contains BAP and NAA at 0.5 mg/l each.

c. Effective shoot formation in basal medium with BAP at 0.5 mg/l.

d. High frequency of rooting in the basal medium supplemented with NAA at 1.0mg/l·

References

1. Austin, S.E., Lojkowska, M.K., Ehlenfeldt, Kelman and Helgenson, J.P. Fertile interspecific somatic hybrids of Solanum: A noval source of resistance to Erwinia soft rot. **Phytopathology** 78: 1216-1220, (1988).

2. Bonga, J.M. Application of tissue culture in Forestry. In : J. Reinert and Y.P.S. Bajaj (eds.). **Applied and Fundamental aspects of Plant Cell, Tissue and Organ Culture.** Springer – Verlag, Berlin. pp. 93-107, (1977).

3. Bu, G.Z., Gong, M.L., Ding, C.M. and Yao, S.M. A new breeding line of tobacco selected by the somatic hybridization between *Nicotiana tobaccum* and *N. rustica*. pp.273-278, (1993)

4. Campbell, R.A. and Durzan, D.J. Induction of multiple buds and needles in tissue cultures of *Picea glauca*. **Can.J. Botany** 53: 1652-1657, (1975).

5. Chalupa, V. Control of root and shoot formation and production of trees from poplar callus. **Biol. Plant** 16: 316: 320, (1974).

6. Das, T. and Mitra, G. C. Micropropagation of *Eucalyptus tereticornis* S.M. **Plant Cell, Tissue and Organ culture**. 22: 95-103, (1990).

7. Jain, S.K. 1996. "**Ethnobiology in relation to human welfare"**. Deep Publications, New Delhi.

8. Molinar, F.,.Mackay, W.A., Wall M. M., and Cardenas, M. Micropropagation of agarita (*Berberis trifoliata* Moric.) **Hort. Science** 31: 1030-10, (1996).

9. Murashige, T. and Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue culture. **Physiol. Plants** 15: 473-497, (1962).

10. Paulsamy, S., Padmavathy S. and Vijayakumar, K.K. Conservation of an endemic medicinal plant, *Berberis tinctoria* Lesch. in Nilgiris through micropropagation. **Ancient Science of Life** XXIV(1): 22-26 (2004).

11. Ray, S.S., Sahoo, S. and Das, R. *In vitro* propagation of endangered medicinal plants. In: S. Sahoo, D.B.Ramesh, Y.R.Rao, B.K.Debata and V.N. Misra (eds.). "**Conservation and Utilization of Medicinal and Aromatic Plants"**. Allied Publishers Limited, New Delhi, (2001).

12. Steinitz, B., Kusek, K, M., Tabib, Y., Paran, I. and Zelcer, A. Pepper (*Capsicum annum* L.) regenerants obtained by direct somatic embryogenesis fail to develop a shoot. **In vitro Cellular and Developmental Biology – Plant** 39(3): 296-303, (2003).

13. Subbaiah, M.M. and Minocha, S.C. Shoot regeneration from stem and leaf callus of *Eucalyptus teretecornis*. **Plant Cell Rep**. 9: 370-372, (1990).

14. Wali, S.A. and Siddique, B.A. Standardization of efficient *in vitro* plant regeneration system for black night shade (*Solanum nigrum* L.). **Indian J. Trop. Biodiv**. 11: 69 – 73, 2003).