

**Conservation of an endemic medicinal plant, *Berberis tinctoria* Lesch.
In Nilgiris through micro propagation**

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ABSTRACT: *Berberis tinctoria* Lesch. Is an endemic plant to high hills of Nilgiris having lot of medicinal properties. For its better conservation through mass multiplication, attempts have been made to standardize tissue culture technology. The results of the study exhibited that the basal medium containing BAP and NAA each at 0.5 mg/l was found to be the optimum for callus formation. Shoot proliferation was highly effective in the basal medium supplemented with BAP at 0.5 mg/l. The root initiation was maximum in the basal medium containing the NAA at 1.0 mg/l and the plantlet establishment was successful in the hardening medium composed by vermiculite and soil in the ration of 1:1.

INTRODUCTION:

The plant species, *Berberis tinctoria* of the Berberidaceae family is an evergreen shoal species endemic to the high hills of Nilgiris and has certain medicinal value. The roots are used for curing jaundice, and the leaf parts are used for the purpose of cancer treatment to some extent by the tribal and local people (1). Further, due to evergreen nature and bushy appearance, the plant has also been recommended for controlling surface fire through raising vegetation in the grasslands of Nilgiris (2). Over exploitation for its medicinal importance and the existence of poor reproductive potential, reduced its ecological importance in Nilgiris (3). Hence, in order to perpetuate the species in Nilgiris, attempts were made in the present study to develop strategies for mass multiplication and establishment of plantlets in soil through the application of tissue culture technology.

MATERIALS AND METHODS:

Healthy individuals of *B. tinctoria* were collected from Nilgiris and maintained in mist chamber as mother plants for further experiment. The leaf components of mother plants in form of discs were used as explants and they were sterilized for aseptic conditions. The basal medium (4) containing 30g/l of sucrose and gelled with 8 g/l difco-bacto agar supplemented with and without 6-benzyl amino purine (BAP), and a-naphthalene 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 $24 \pm 1^{\circ}\text{C}$ 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 from the basal medium with optimum level of NAA. For hardening, pot culture experiment was conducted by using a mixture of vermiculite and soil in the ratio of 1:1 and the survivability rates were determined after 40 days of hardening. Five replicates were maintained for the culturing experiment of callus formation and subculturing experiments of shoot proliferation and root initiation. For hardening experiment triplicate samples were maintained.

RESULTS AND DISCUSSION:

It was noted that 10 days after explant inoculation, callus formation was taking place with different degree according to the concentration of BAP and NAA in basal medium. Further, the callus initiation begun, at the cut ends which gradually extended to the entire leaf surfaces of the explant within a week of culture.

The response of various combinations of BAP and NAA to callus formation, BAP to shoot proliferation and NAA to root initiation for the study species, *B. tinctoria* is given in Table 1. The percentage of leaf discs producing callus ranged between 75 to 100 across the various combinations of BAP and NAA. All combinations of BAP and NAA induced higher degree of callus formation over the pure basal medium which lacks BAP and NAA. However, the combination of BAP and NAA at 0.5 mg/l each, induced callus in all inoculated explants (Figure 1a). It is already reported that the basal medium supplemented with

BAP and NAA is highly effective for the callus formation in a species of *Berberis* (5)

The subculturing for shoot proliferation indicates that the shoot forming calli were found in all basal media containing BAP at various concentrations and the shoot primordial appear within a period of 3 weeks from the date of subculturing. The BAP at 0.5mg/l was found to be the optimum at which all calli (100%) initiate shoots (Figure 1b). The importance of BAP for shoot proliferation, was already discussed and documented well.(6and 7).

It was further observed that root initiation started after 10 days of subculturing on the basal medium containing NAA at different concentrations. In addition, it was noted that a maximum of 90% shoots subcultured on the medium containing the NAA at 1.0mg/l, produced roots, and the number of roots produced were also higher (5/shoot) in the medium supplemented with the same concentration of NAA. It was already reported that the NAA at 1 mg/l level in the basal medium is highly effective for root initiation in the species, *Berberis trifoliata* (5).

The hardening experiment indicates that a high survivability (78% of plantlets was established in the hardening medium containing the vermiculite and soil in the ratio of 1:1 (Figure 1c). The hardening medium composed by vermiculite and soil was already found to be successful in many cases (8and 9)

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Table – 1

Responses of various combinations of BAP and NAA on callus formation, BAP on shoot proliferation and NAA on root initiation for the species *B. tinctoria*

Experiment for callus formation		Experiment for shoot proliferation		Experiment for root initiation		
Contents of BAP and NAA (mg/1) in MS medium	* Per cent leaf discs producing callus	Content of BAP (mg/1) in MS medium	** Shoot forming callus (%)	Content of NAA (mg/1) in MS medium	**Percentage of shoots producing roots	No.of roots per shoot
0.5+0.1	80 2.6	0.5	100 6.5	0.5	60 3.9	3 0.5
1.0+0.1	80 5.8	1.0	80 4.3	1.0	90 7.9	4 0.4
2.0+0.1	85 8.3	2.0	90 5.4	2.0	70 6.8	3 0.2
3.0+0.1	90 6.2	3.0	80 5.8	3.0	80 6.8	4 0.3
4.0+0.1	90 9.9	4.0	70 5.1	4.0	60 4.0	3 0.2
0.5+0.5	100 6.1		10 1.1		40 3.2	1 0.2
1.0+0.5	95 6.0	MS medium without BAP		MS medium without BAP		
2.0+0.5	85 7.2					
3.0+0.5	90 5.1					
4.0+0.5	80 4.1					
0.5+1.0	90 6.1					
1.0+1.0	85 6.1					
2.0+1.0	85 5.0					
3.0+1.0	85 3.9					
4.0+1.0	90 4.6					
0.5+2.0	95 6.1					
1.0+2.0	75 4.7					
2.0+2.0	80 3.8					
3.0+2.0	85 5.9					
4.0+2.0	85 6.8					
Ms medium without BAP NAA	75 6.6					

* Mean value of 5 replication each cultured with 20 leaf discs

** Values are arrived at by the observations made on 10 test tubes in each replication

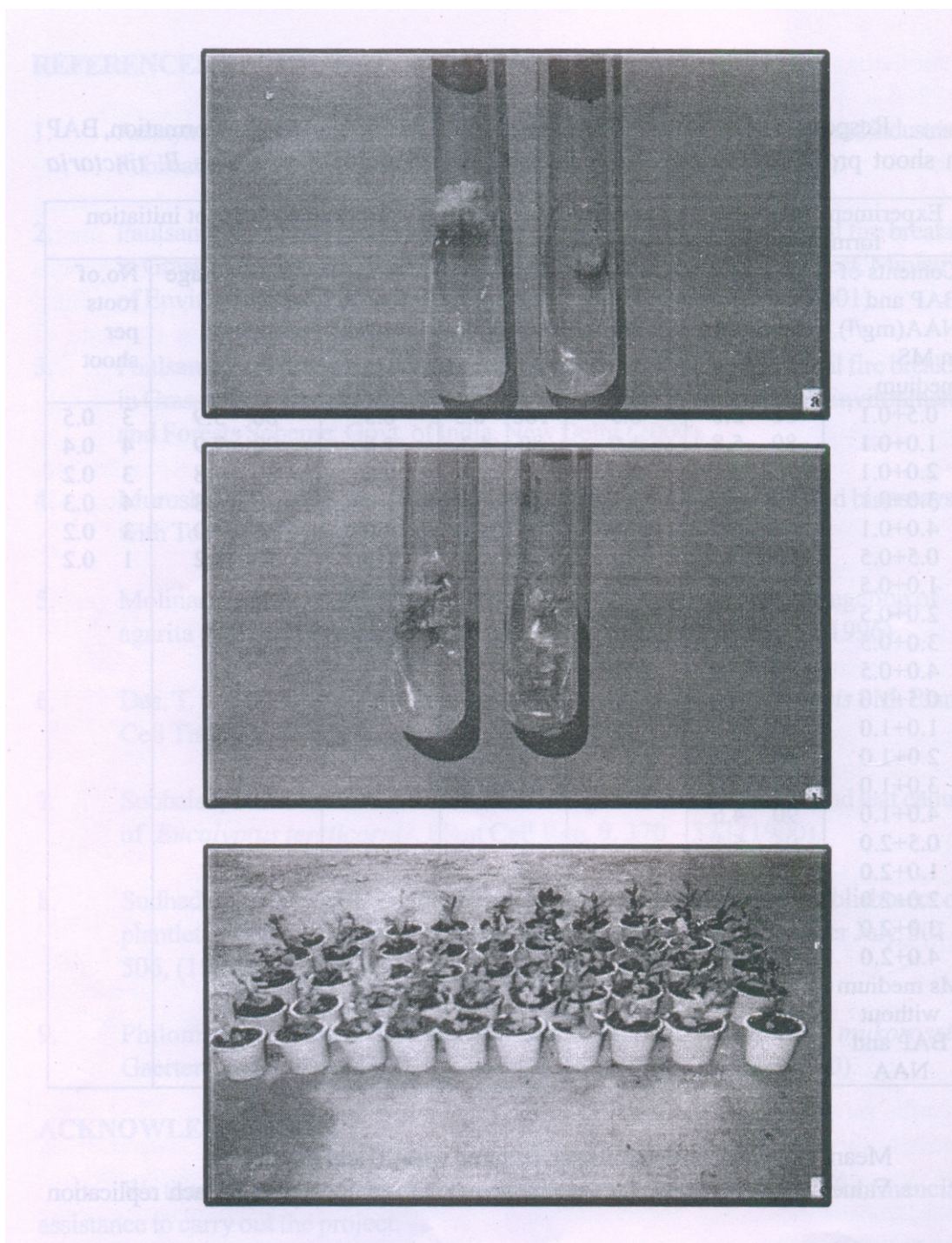


Fig .1 (a) Massive formation of callus observed in the basal medium supplemented with BAP and NAA at 0.5 mg/l each. (b) Active proliferation of shoots in the basal medium containing BAP at 0.5 mg/l and (c) Higher survivability of plantlets observed in the hardening medium composed by vermiculite and soil in the ration of 1:1