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IN VITRO ROOT CULTURE OF *BOERHAAVIA DIFFUSA* L. AND STUDY OF THE "PUNARNAVOSIDE" PROFILE

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In vitro root culture was done from the third leaf segments of *Boerhaavia diffusa* L. by adding different levels of auxins namely IAA, IBA, NAA and 2, 4-D on MS (Murashige and Skoog) medium containing 3% sucrose. MS basal supplemented with 0.25 mg/l IAA and 0.25 mg/l IBA showed the maximum amount of regenerated roots. Chemical analysis of the natural and cultured roots showed the presence of the alkaloid "punarnavoside". Roots cultured in 0.5 mg/l IAA showed 0.15% of the alkaloid as compared to 0.04% in the natural roots whereas roots cultured in 0.25 mg/l IAA + 0.25 mg/l IBA showed 0.03% accumulation of the alkaloid "punarnavoside".

Keywords : *Boerhaavia diffusa* ; "Punarnavoside" ; Root culture.

Introduction

Boerhaavia diffusa L. a herbal medicinal plant is the source of many important ayurvedic drugs with potent biopharmaceutical importance. It belongs to the Nyctaginaceae family, and is commonly known as Punarnava. The root extract of this plant finds application as anti-hepatotoxic[2] and anti-viral agents[3]. It is reported to have cured corneal ulcers and night blindness. Pharmacological studies have demonstrated that punarnava possesses diuretic and anti-inflamatory activities[4]. Due to extensive use and exploitation, the availability of the plant have become scanty thus making the extraction of alkaloid difficult. *In vitro* root culture provides a novel way of meeting this need. The present study is an attempt to find out the ideal media for *in vitro* root culture and to find out the alkaloid accumulation in the cultured roots.

Material and method

Segments of the third leaf of *Boerhaavia diffusa* was used as explants, which were thoroughly washed with detergent (tween 20) and then sterilized with 0.1% mercuric chloride for 2.30 min. Under aseptic condition, the sterilized leaves (1 cm^2) were inoculated and cultured on MS media[5] containing 3% sucrose and different growth regulators (IAA, IBA, NAA, 2- 4D). The pH was adjusted at 5.7 and 0.8% agar was added. The inoculated explants were cultured under 1000 lux intensity with 16 hr/8 hr light/dark period and inclubated at 25±2°C. After four weeks of culture the *in vitro* roots were taken out, measured, weighed, air dried and crushed to powder. This powder was used for chemical analysis.

In order to isolate punarnavoside, natural roots, leaves, cultured roots were collected, air dried and crushed to powder. The powder was mixed with 70% ethanol at room temperature. The alcoholic extract was concentrated under reduced pressure. The dark green syrup was successfully fractionated by extra action with hexane, chloroform, and n-butanol. The n-butanol fraction was collected at 45°C to give a brown amorphous powder which was subjected to silica gel chromatography after mixing with different ratios of chloroform and methanol. The mixure residue from 9:1 and 8:2 $CHCl_3$: MeOH eluent was rechromatographed over silica gel. A fraction soluble in 92:8 eluent contained punarnavoside which was then

subjected to Thin Layer Chromotography (TLC).

Results and Discussion

The present investigation was undertaken to find out the *in vitro* efficiency of four different auxin (IAA, IBA, 2, 4-D, NAA) on root initiation and growth from the leaf explants of the potential herbal medicinal plant *B.diffusa*. MS basal was supplemented with different hormones (Table 1).

The explants grown in MS basal without hormonal supplementation showed no response. The NAA supplementation (0.25-1 mg/l) also showed no response. The media

Table 1. Effect of IAA and IBA on weight, length, number of roots regenerated and per cent regeneration after four weeks of culture.

MS basal supplementation (mg/l)	Weight (mg)	Length (cm)	Mean no. of roots/explant	Per cent regeneration
IAA				
0.5	1300	12.3	20	86.6
1	1020	7.5	15	93.3
2	830	6.6	12	53.3
3	700	4.6	10	73.3
4	500	3.2	7	63.3
5	350	2.2	4	63.3
IBA				
0.5	960	3.12	30	93.3
1	700	2.05	23	86.6
2	500	1.70	15	73.3
3	100	1.00	14	73.3
4	75	0.5	10	66.6
5	50	0.6	5	63.3
IAA + IBA				
0.25+0.25	1850	16	35	86.6
0.5+0.5	1450	10	20	73.3
1+1	700	3	18	66.6
1.5+1.5	100	2.3	20	53.3
2+2	50	2.05	16	40
2.5+2.5	50	1.5	10	33.3

Table 2. Effect of different supplementation on punarnavoside accumulation.*

MS basal with supplementation (mg/l)	Percentage of punarnavoside accumulation
IAA (0.5)	0.15
IAA (1.0)	0.05
IAA (2.0)	0.02
IAA (0.25)+IBA(0.25)	0.03

* Punarnavoside accumulation in natural roots were found to be 0.04%.

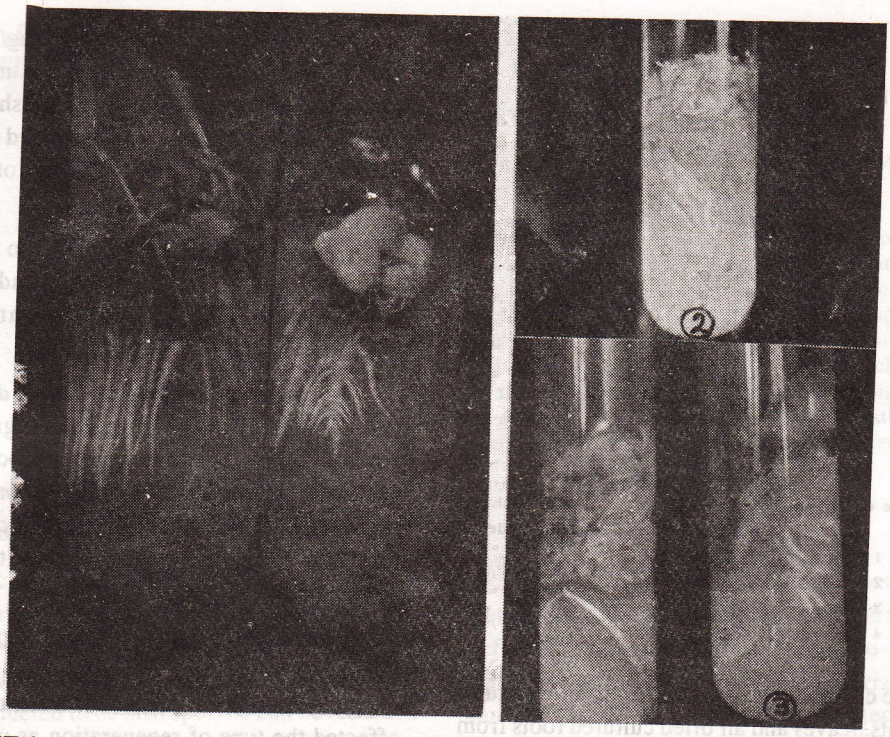

PLATE 1 : Growth of roots observed after three weeks of culture in media supplemented with 0.5 mg/l of IAA

PLATE 2 : Heavy growth of roots observed after three weeks of culture in media supplemented with 0.25 mg/l of IAA + 0.25 mg/l of IBA

PLATE 3 : Development of lateral roots in media supplemented with 0.5 mg/l IBA after three weeks of culture.

supplemented with 2, 4-D (0.5-1 mg/l) showed callusing. The highest callus growth was observed in the media containing 0.5 mg/l 2, 4-D. After four weeks of regeneration, the calluses showed fine hair roots, with negligible alkaloid content. The regeneration percentage was highest (93.3%) in MS basal supplemented with 1 mg/l IAA and 0.5 mg/l IBA. (Table 1).

Higher concentration of IAA led to the formation of short, thick roots and a decrease in root number (Table 1). Roots developed in higher concentration of IAA led to the depletion of the alkaloid contnet. Highest number of roots among IAA concentration was recorded at 0.5 mg/l of IAA, with the number of roots being 20 and average weight being 1.3 and length being 12.3 cm (Plate 1).

Root generation was found maximum in IAA (0.25 mg/l) along with IBA (0.25 mg/l) with average weights being 1.85 gm and average length 16 cm. Highest number of roots (35) was also observed in this combination (Plate 2) (Table 1). IBA was found less effective than IAA in inducing root regeneration (Table 1). Lateral roots were found well developed in 0.5 mg/l IBA (Plate 3). Increase in the IBA concentration led to the development of many small roots and also increase in callus growth.

Plate 4 : TLC plate run in 1:2, Hexane, EtODAC Solvent System Showing presence of Punarnavoside
Spot 1 : From roots of B. diffusa
Spot 2 : From roots of B. diffusa
Spot 3 : From leaves of B. diffusa
Spot 4 : From tissue cultured roots.

The chemical analysis of the air dried natural roots, leaves and air dried cultured roots from the media supplemented with 0.25 mg/l IAA + 0.25 mg/l IBA revealed the presence of the alkaloid punarnavoside in the cultured roots (Plate 4). The quantity of alkaloid accumulation was 0.3%. This amount was less than the percentage of alkaloid content 0.15% present in the cultured roots of 0.5 mg/l of IAA but it was more than the percentage of alkaloid content (0.02%) derived from the cultured roots of media supplemented with 2 mg/l of IAA (Table 2).

In the present study it was observed that quantity of auxin supplemented showed pronounced effect on the morphology of regenerated roots. The presence of IAA and to some extent IBA showed good growth of roots.

The presence of IBA in the media showed good growth of roots decreased and callusing occured. Lateral rooting was observed in clutures containing 0.5 mg/l IBA and 1 mg/l IBA. Rooting in media supplemented with 0.5 mg/l of IAA showed a very good response but the combined effect of 0.25 mg/l of IAA and 0.25 mg/l of IBA showed the best growth of roots.

The presence of IAA and to some extent combined effect of IAA and IBA showed influence on the biosynthetic potential.

It appeared that in *Boerhaavia diffusa* regeneration of roots from leaf segments cultured *in vitro* is an autonomous process as observed in *Haplopappus revnil*[6]. *In vitro* rhizogenesis was observed in *Nicotiana tobacum* from the fourth leaf with the application of IBA[7].

From these results it can be suggested that quantity and nature of auxin supplemented to the cultures of leaf explants affected the type of regeneration as well as secondary metabolite production .

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