

# Studies on Organogenesis (In vitro) in Boerhaavia. Diffusa L. A Medicinal Plant of Arid Region

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**Abstract:** Callus were raised on MS Medium supplemented with 2,4- D 1.00 mg/l + BAP 0.1 mg/l from shoot segment and leaf of *B. diffusa* (Bhansali et al 1978, Biswas et al 2009) The callus shows luxuriant growth on same composition of medium for several multiplication cycle. organogenesis i.e., shoot induction were observed on MS medium containing BAP 1.0 mg/l + IAA 0.1 mg/l.

**Keywords:** callus, organogenesis, shoot induction, medium, in vitro

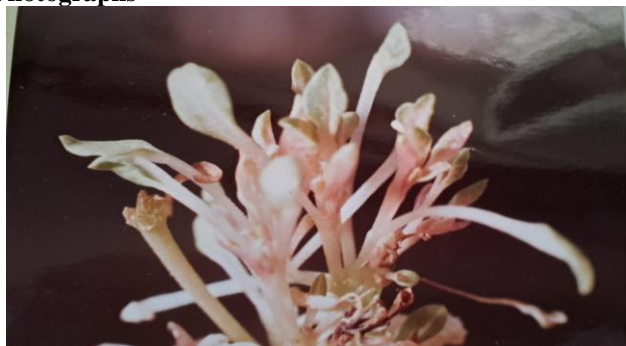
## 1. Introduction

*Boerhaavia diffusa* L is a species of flowering plants belongs to family Nyctaginaceae is a perennial herb of tropical and subtropical region of Asia, Africa, America, India, and arid region of Rajasthan. It is a commonly known as punarnava which means rejuvenates or renews the body in Ayurveda. The *B.diffusa* has many medicinal properties like antiviral, anti-inflammatory and expectorant (Bhowmik Debjit et al 2012). In Ayurvedic medicine, punarnava is used to treat disorders like intestinal colic, Kidney disorder, cough, nemorrhoids and skin disease. The green leaf of *B. diffusa* is used as a vegetable in many Asian and African countries. It can be uses as a fodder for livestock. only.

## 2. Materials and Methods

Shoot segments, nodal shoot segments and leaf were taken as a explant for callus induction and organogenesis. The explant was first washed in running tap water followed by tween 80 for 10 minutes. Then these explants were surface sterilized with 70 % ethanol for 30-50 seconds followed by 0.1% mercuric chloride for 3-4 minutes. Surface sterilized explants were washed 8-10 times with sterile water and inoculated on MS (Murashige and Skoog 1962) medium supplemented with various concentration and combination of BAP + 2,4-D and Kinetin + IAA for induction and maintenance of callus. The cultures were kept in dark for callus induction while for organogenesis the culture was kept in 12 h photoperiod at 28 +/- 2 C.

### Photographs



(A)



(B)

A & B: Shoot Induction of plant



(C)

C: Callus induction



(D)

D: Entire Plant of B.diffusa

### 3. Results and Discussion

Various explants viz. shoot segment nodal shoot segment, leaf and roots were tested for callus induction. Shoot segment found to be the best explants for callus induction followed by leaf. Various combination of BAP + 2,4- D and kinetin + NAA were tested for callus induction under in vitro condition. Best result was found on BAP 0.1 mg/l + 2,4-D 1.0 mg/l (Table-1). Tissue grows rapidly on this medium and produced cremish white, shiny, and soft callus.

For organogenesis from callus various combination of BAP and kinetin alone and with IAA&NAA were tested. Out of various experiments best results were found on BAP 1.0 mg/l + IAA 0.1 mg/l (Table-2). On this combination the best organogenesis i.e. shoot induction were taken place in callus.

It is further observed that dark condition favour callus induction compares to light condition. High temperature enhances shoot induction from callus.

**Table 1:** Callus induction in B.diffusa on MS medium from stem explants (4 week growth)

MS +Growth hormone mg/l	Response	Remark
1 BAP 0.1+NAA 1.0	+	little callusing
2 BAP 0.1+2,4-D 0.1	++	moderate callusing
3 BAP 0.1+2,4-D 1.0	+++	luxuriant callusing
4 KN 0.1+NAA 0.1	+	little callusing

**Table 1:** Callus induction in B.diffusa on MS medium from stem explants (4 week growth)

MS +Growth hormone mg/l	Response	Remark
1 KN1.0	+	Lesser number of shoot induction
2 BAP1.0	+	Lesser number of shoot induction
3 BAP0.5+IAA 0.1	++	Moderate number of shoot induction
4 BAP1.0+IAA0.1	+++	Highest number of shoot induction
5 KN1.0 +IAA0.1	++	Moderate number of shoot induction
6 KN0.5+IAA0.5	+	Lesser number of shoot induction

Abbreviation:- BAP- Benzylaminopurine, NAA- Napthalene acetic acid, 2,4 D-2,4-dichlorophenoxyacetic acid , IAA- Indole- 3-acetic acid, KN- kinetin.

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