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# IN-VITRO CULTURE STUDIES FOR CALLUS AND ROOT GENERATION OF *BOERHAAVIA DIFFUSA* LINN

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***ABSTRACT :*** *Leaf and stem explants excised from young plant of B.diffusa were cultured on Murashige and Skoog (MS) medium containing agar (0.8%), sucrose (2.5%) and varied concentration of Indole butyric acid (IBA), Napthyl acetic acid (NAA), 2,4 – dichloro phenoxy acetic acid (2,4-D) and Picrolam. Leaf explants has given better response for both rooting and callus formation. IBA in a concentration of 5µM has shown maximum regeneration (69.7%) with induction period of 7 days. The developed roots were similar to that of naturally grown roots with little anatomical changes. For callus formation 20µM 2, 4-D has given maximum amount and percentage response 979.5% with an induction period of 8 days. Picrolam (10µM) has shown 36.6% response and the average weight of callus was less as compared with 2,4-D. The callus obtained was friable and opaque in nature.*

## INTRODUCTION

Punarnava consists of whole plant of *Boerhaavia diffusa* Linn family Nyctaginaceae. It is mainly cultivated at high altitudes mainly in hot Himalayan valleys.

The chief active constituents are punarnavoside, an anti-fibrinolytic glycoside (0.03 – 0.05%), boeravine, flavones, isoflavones, sterols, boeraviones, hypoxanthine 9-L arabinofuranoside, large amount of potassium nitrate and lignanes. The plant has anti-fibrinolytic and anti-inflammatory properties, it is used for its diuretic, hepatoprotective properties and in the treatment of menorrhagia and loss of appetite[2-4].

*B.diffusa* being a valuable plant of Indian system of medicine, the present study was undertaken to establish its in vitro culture requirements which will provide a lead for improving and establishing the biosynthetic pathway of its active principles.

## MATERIALS AND METHODS

The plant of punarnava was obtained from medicinal garden of B.R.N. College of Pharmacy, Mandsaur and identified by Agriculture College, Mandsaur. Leaf and stem explants were collected from nature plants.

Surface sterilization of explants (both leaf and stems) were done first with an antifungal agent bvastin (0.2%) for 7-8 min, followed by 0.1% mercuric chloride treatment of 2 -3 min. The explants were then washed thrice with sterile distilled water; the explants of 1*1 cm size were cultured on to the MS medium containing 2.5% sucrose[5]. The medium was solidified using 0.8% agar. The medium was supplemented with different hormones like IBA and NAA in varying concentration for rooting purpose. Various concentrations of

2,4-D and picrolam were for callus generation. The pH of the medium was adjusted to 5.75 before autoclaving at 121 $^0$C, 15 lbs for 15 min. The cultures were incubated at 25 $^0$C under white fluorescent light with 12 hrs photoperiod and RH of 55-60%[6-9]. Each treatment included 15 replicates. The percent response, fresh weight and dry weight were determined after 4 weeks. The table 1 & 2 shows the results of various hormones and their different concentration tried for the generation of callus and roots of *B. diffusa* in Murashige and Skoog medium.

## RESULTS AND DISCUSSION

With 2,4-D as plant growth regulator, leaf explants have given a better callus formation as compared to stem explants. The best results in terms of percent response and dry weight basis were obtained at concentration of 20µM, the induction period was 8 days and the callus obtained was friable and opaque in nature. When leaf and stem explants were cultured on MS medium supplemented with picrolam ranging form concentrations 1 – 30 µM, callus was maximum in 10 µM but the induction period was 12 days and also the % dry weight was less than 2,4- D as plant growth regulator.

For root generation, amongst various concentrations of IBA and NAA tired, 5µM IBA has generated maximum roots in terms of number, fresh wt and dry weight basis, stem explants have shown poor results for root regeneration. The roots grown have shown a negative geotropic development. The cultured roots exhibited normal development without gross morphological and anatomical change.

## CONCLUSION

In conclusion, the optimized media requirement for callus culture of *B. diffusa* is by using 20µM 2, 4- D in MS media while MS media with 5µM IBA has generated a large biomass of roots using leaf explants. Further estimation of active constituents and by using suitable precursor in this optimized media; an improvement in the yield of active principles of *B. diffusa* can be achieved.

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**Table 1: Response of different concentration of growth regulators supplement in MS media on callus formation by stem and leaf explant of *B. diffusa* Linn.**

| Explant | Growth regulator | Conc (mM) | Result % | response | Fresh wt. (gms) Means ± S.E.M | Dry wt. (gsm) Mean ± S.E.M |
|---|---|---|---|---|---|---|
| Leaf | 2,4-D | 0.1 | -- | -- | -- | -- |
| Leaf | 2,4-D | 1.0 | -- | -- | -- | -- |
| Leaf | 2,4-D | 2.0 | -- | -- | -- | -- |
| Leaf | 2,4-D | 5.0 | -- | -- | -- | -- |
| Leaf | 2,4-D | 10 | Callus with roots | 30.2 | 0.24 ± 0.015 | 0.025 ± 0.002 |
| Leaf | 2,4-D | 15 | Callus with roots | 35.6 | 0.32 ± 0.025 | 0.041 ± 0.009 |
| Leaf | 2,4-D | 20 | Callus | 75.9 | 0.71 ± 0.031 | 0.11 ± 0.003 |
| Leaf | 2,4-D | 25 | Callus | 40.1 | 0.029 ± 0.024 | 0.027 ± 0.011 |
| Leaf | 2,4-D | 30 | -- | -- | -- | -- |
| Stem | 2,4-D | 0.1 | -- | -- | -- | -- |
| Stem | 2,4-D | 1.0 | -- | -- | -- | -- |
| Stem | 2,4-D | 5.0 | Callus | 15.2 | 0.035 ± 0.0100 | 0.004 ± 0.009 |
| Stem | 2,4-D | 10.0 | Callus | 18.7 | 0.032 ± 0.007 | 0.003 ± 0.001 |
| Stem | 2,4-D | 15.0 | Callus | 24.3 | 0.071 ± 0.012 | 0.015 ± 0.002 |
| Stem | 2,4-D | 20.0 | Callus | 22.0 | 0.045 ± 0.008 | 0.009 ± 0.002 |
| Stem | 2,4-D | 25.0 | -- | -- | -- | -- |
| Stem | 2,4-D | 30.0 | -- | -- | -- | -- |
| Leaf | Picrolam | 0.1 | -- | -- | -- | -- |
| Leaf | Picrolam | 1.0 | -- | -- | -- | -- |
| Leaf | Picrolam | 5.0 | Callus | 27.0 | 0.092 ± 0.012 | 0.012 ± 0.005 |
| Leaf | Picrolam | 10.0 | Callus | 35.6 | 0.131 ± 0.057 | 0.045 ± 0.008 |
| Leaf | Picrolam | 20.0 | Callus | 23.1 | 0.061 ± 0.014 | 0.034 ± 0.006 |
| Leaf | Picrolam | 30.0 | -- | -- | -- | -- |
| Stem | Picrolam | 0.1 | -- | -- | -- | -- |
| Stem | Picrolam | 1.0 | -- | -- | -- | -- |
| Stem | Picrolam | 5.0 | -- | -- | -- | -- |
| Stem | Picrolam | 10.0 | Callus | 13.8 | 0.061 ± 0.012 | 0.010 ± 0.004 |
| Stem | Picrolam | 20.0 | Callus | 11.2 | 0.024 ± 0.009 | 0.008 ± 0.005 |
| Stem | Picrolam | 30.0 | -- | -- | -- | -- |

**Table 2: Response of different concentration of growth regulators (Auxins) supplemented in MS medium on root formation from stem and leaf explant of *B. diffusa* Linn.**

| Explant | Growth regulator | Conc (mM) | Result % | response | Fresh wt. (gms) Means ± S.E.M | Dry wt. (gsm) Mean ± S.E.M |
|---|---|---|---|---|---|---|
| Leaf | IBA | 0.1 | -- | -- | -- | -- |
| Leaf | IBA | 1.0 | -- | -- | -- | -- |
| Leaf | IBA | 2.0 | Roots with little callus | 12.1 | 0.231 ± 0.0124 | 0.021 ± 0.003 |
| Leaf | IBA | 5.0 | Roots | 69.7 | 0.546 ± 0.068 | 0.081 ± 0.011 |
| Leaf | IBA | 10 | Roots | 31.0 | 0.253 ± 0.017 | 0.033 ± 0.007 |
| Leaf | IBA | 20 | Roots | 19.2 | 0.113 ± 0.013 | 0.017 ± 0.005 |
| Leaf | IBA | 30 | -- | -- | -- | -- |
| Stem | IBA | 0.1 | -- | -- | -- | -- |
| Stem | IBA | 1.0 | -- | -- | -- | -- |
| Stem | IBA | 5.0 | Roots | 13.2 | 0.076 ± 0.012 | 0.018 ± 0.005 |
| Stem | IBA | 10.0 | Roots | 11.5 | 0.069 ± 0.014 | 0.015 ± 0.006 |
| Stem | IBA | 15.0 | -- | -- | -- | -- |
| Stem | IBA | 20.0 | -- | -- | -- | -- |
| Leaf | NAA | 0.1 | -- | -- | -- | -- |
| Leaf | NAA | 1.0 | Roots | 11.5 | 0.074 ± 0.007 | 0.019 ± 0.004 |
| Leaf | NAA | 5.0 | Roots | 22.6 | 0.095 ± 0.015 | 0.026 ± 0.007 |
| Leaf | NAA | 10.0 | Roots | 19.8 | 0.088 ± 0.012 | 0.023 ± 0.006 |
| Leaf | NAA | 20.0 | -- | -- | -- | -- |
| Leaf | NAA | 30.0 | -- | -- | -- | -- |
| Stem | NAA | 0.1 | -- | -- | -- | -- |
| Stem | NAA | 1.0 | Roots | 8.2 | 0.033 ± 0.008 | 0.010 ± 0.002 |
| Stem | NAA | 5.0 | Roots | 15.7 | 0.045 ± 0.007 | 0.015 ± 0.003 |
| Stem | NAA | 10.0 | Roots | 6.4 | 0.035 ± 0.004 | 0.011 ± 0.003 |
| Stem | NAA | 20.0 | -- | -- | -- | -- |
| Stem | NAA | 30.0 | -- | -- | -- | -- |

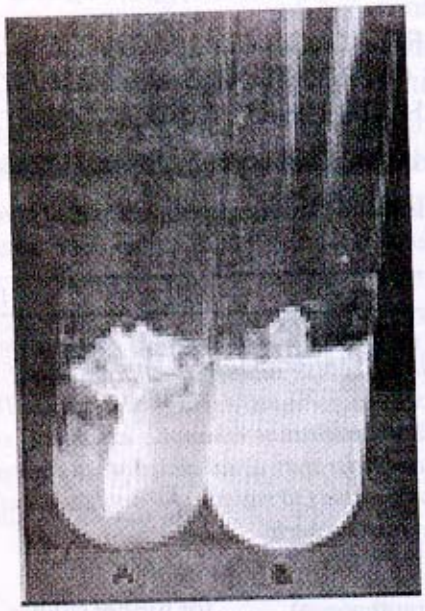

**Fig No.1 (A&B):** Callus cultured in 20μM 2,4-D and 10μM Picrolam

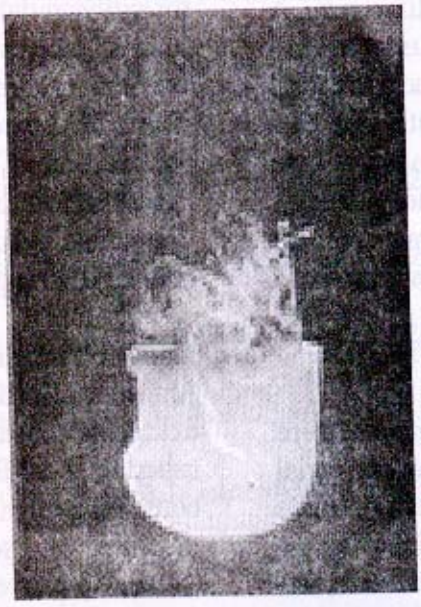

**Fig No.2 :** Roots cultured in 5μM Indole butyric acid