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Original Research Article

In vitro Multiple Shoot Induction in *Andrographis paniculata*

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A B S T R A C T

*Andrographis paniculata (*family*: Acanthanceae*) is an herbaceous plant having medicinal values like treating dysentery, cholera, diabetes, influenza, etc. *Andrographolide* is a major constituent of A. Paniculata, extracted from leaves. The phytochemical (*Andrographolid*) present in plant direct show anticancer activity again cancer cells by cell-cycle arrest at G0/G1 phase. Due to high medicinal property it's having high market demand in Homeopathy as well as in Ayurveda. Seed germination is low so multiple shoot induction is best strategies to overcome the problem. *In vitro* germinated 30 days old seedlings were used for further studies. Internodal and nodal part of the plant transfer in to shoot induction media (BAP (1-3 mg/L). Among all growth hormones treatment BAP 1.5mg/L showed highest shoot induction (4-5 shoots per plant). Well grown shoots were sub cultured on the media IAA (1-3 mg/L) and NAA (1-3 mg/L) for root initiation. Maximum roots were observed in IAA 1.5 mg/L and 2.0 mg/L.

Keywords

Andrographis paniculata, *Andrographolide*, MS, IAA, BAP

Introduction

Andrographis paniculata belongs to Acanthanceae family is annual herbaceous plant found commonly in India China and Shrilanka. Ayurvedic medicines have got enormous applications in Cosmetic, Agriculture, Pharma and food industry. The herbal preparations have contributed more specifically in these medicines. In India it is also known as Kalmegh/ Kiryat. The herb is found in variety of habitat *viz.*, Plains, hill slopes, work lands etc. as *A. paniculata* having high medicinal uses like treating dysentery, cholera, diabetes, consumption, influenza, bronchitis, swelling, itches and piles, anticancer, anti-HIV etc. Due to this species got tremendous demand.

Andrographolide is major constituent extracted from leaves. It is colourless diterpene lactone, insoluble in water but soluble in acetone and Chloroform etc. The *Andrographolide* direct shows anticancer activity again cancer cells by cell-cycle arrest at G0/G1 phase *Andrographolide* also enhanced the tumor necrosis factor-alpha production and CD marker expression, resulting in increased cytotoxic activity of lymphocytes against cancer cells, which may contribute for its indirect anticancer activity. These results suggest that *Andrographolide* is an interesting pharmacophore with anticancer and Immunomodulatory activities and hence has the potential for being developed as a cancer therapeutic agent (Rajagopal *et al.,* 2003). The conventional vegetative propagation of *A. paniculata* is too slow to meet the demand of pharmaceutical industries.

Variability among the seed derived progenies and delayed rooting of seedlings restrains propagation through seeds. Thus attempts were made by many laboratories to increase the quantity of *Andrographolide* in *A. paniculata* plant parts using different inducers. Further to meet the overgrowing demand of *A. paniculata* by pharmaceutical companies, attempts were also made to multiply *A. paniculata* through multiple shoot induction.

Materials and Methods

Seed germination

Seeds were collected from MPKV, Rahuri Maharashtra. Surface sterilization protocol followed by Deshmukh (2017). Surface sterilized seeds were dry on autoclaved filter paper then aseptically cultured on Half MS medium and incubated at 25±2ºC. 95% seeds were germinated after 18Days.

Shoot induction and elongation

The nodal and inter-nodal part was cultured on MS medium supplemented with various concentrations of BAP (0.5, 1, 1.5, 2, 3 mg/L). The induced shoots were allowed to grow for 30 days.

Rooting

After 30 days well grown shoots were aseptically transfer in to half strength MS media separately, supplemented with various concentrations of IAA (1-3 mg/L) and NAA (1-3 mg/L) for root development.

Hardening

The complete rooted plantlets with 5-6 fully expanded leaves were removed from the culture medium and the roots were washed gently under running tap water to remove agar. The plantlets were transferred to plastic pots (5 cm diameter) containing a mixture of sterilized garden soil and vermiculite in the ratio 2:1 and covered with transparent plastic bags to ensure high humidity. Each was irrigated with 1/6 MS basal salt solution devoid of sucrose and inositol every 4 days for 2 weeks. The growth chamber was maintained at 26°C ± 1°C, 80% - 85% relative humidity with 16 h photoperiod inside the culture room conditions.

Results and Discussion

Explants (nodal and inter nodal) were inoculated in MS media supplemented with BAP (1-3 mg/L) for shoot multiplication. At concentrations of BAP 1.5, 2mg/L showed highest shoot multiplications. 3-4 Shoots per plant are obtained within 30 days.

Purkayastha (2008) stated *in vitro* culture of nodal explants obtained at High frequency direct shoot proliferation was induced in nodal explants cultured on Murashige and Skoog′s medium supplemented with 6-benzylaminopurine (BAP). Amongst the various cytokinins tested (BAP, kinetin, thidiazuron and 2-isopentyl adenine), BAP proved to be the most effective.

The shoot forming capacity of the nodal explants was influenced by the BAP concentration (1–12.5 μM), and the optimal response was observed at 10 μM BAP, which induced an average of 34 shoots in 94% of the cultures within 4 wk. Significant differences were recorded in terms of average number of shoots per explant (8.6–34.1) among the different concentrations of BAP investigated. Concentrations of all cytokinins tested reach a level that can be considered above the optimum level, as marked by a reduced frequency of shoot proliferation.

Roots initiation

Well grown shoots were exiced and sub cultured on media IAA (1-3 mg/L) and NAA (1-3 mg/L) for roots initiation. Maximum roots were observed in IAA 1.5 mg/L. Roots were grown within 30-35 days.

Basu *et al.,* (2011) developed an efficient protocol for micropropagation of *Andrographis paniculata* from nodal region of explant. They observed highest rate of multiple shoot (4-6) on MS fortified with 4.0 mg/L BAP and 0.5 mg/L NAA. The well-developed shoots were rooted on MS supplemented with IBA (2.0 mg/L). The root extract of *Andrographis paniculata* showed effective *in-vitro* anti-inflammatory activity (95.8± 0.20 %). Maximum number of root induction (5.857 ± 0.470) was observed in medium fortified with 2.0 mg/L IBA.

The root induction gradually decreased with increasing concentration of auxin; proving ideal concentration for root induction was most suitable. It is stated that most of roots are grown in IAA and NAA medium concentration. Roots were observed at IAA (1.5 mg/L, 2.0 mg/L and 2.5 mg/L).

Hardening

Plantlets with well grown shoots and roots were transferred to soil containing mixture of soil and coco peat at the 2:1 ratio. Minute quantity i.e. upto 300-500µl of Panchagavya is added to the soil for well growth and development of plants.

Jindal *et al.,* (2015), observed that in vitro rooted plantlet were excised carefully from culture bottles to avoid breaking of roots and washed with tap water for the hardening plantlets developed from in vitro rooting, plantlets were transferred to poly bags containing sand, farm yard manure (FYM), soil, coco peat in 1:1:1:1. After 60-65 days under greenhouse condition. These plantlets were transferred to the nursery.

The average 95% seed germination was observed in half MS media with mean 18 days. Early appearance of shoot initiation was observed in MS supplemented with 1.5 and 2mg/L BAP in 15 days. Half of MS medium supplemented with 1.5mg/L IAA gave promising response and showed 93.33 percent rooting response in *A. paniculata*. Average 90 % plants were survived during primary hardening.

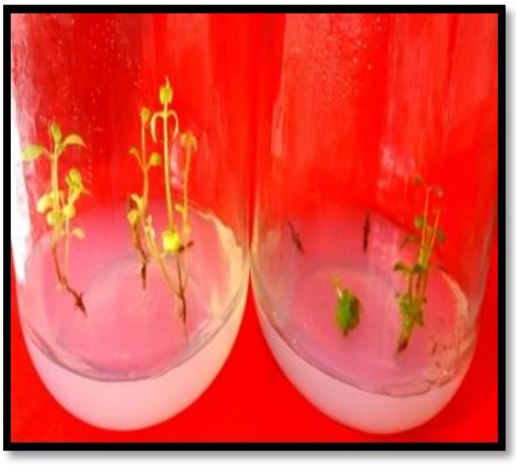

Plate.1 Shoot induction (BAP 1.5mg/L)

Plate.2 Shoot induction (BAP 2mg/L)

Plate.3 Roots initiation in IAA (1.5 mg/L) after 15 days

Plate.4 Hardening

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