

IN VITRO SHOOT DEVELOPMENT IN *BLEPHARIS REPNES*

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ABSTRACT: An herb is a plant or plant part valued for its medicinal aromatic or savory qualities. In vitro shoot development of *Blepharis repnes* was achieved using thin sections of nodes and stems (1.0-1.5 mm) were cultured in MS medium containing varying concentrations of different combination of phytohormones Indole-3-butyric acid (IBA), Benzylaminopurine (BAP) & Gibberlic acid. The combination of BAP, IBA & Gibberellic acid at different concentrations showed the length of shoot development 0.6 -1.8cm with in three weeks in suitable laboratory condition.

Keywords: *Blepharis repnes*, Shoot induction, phytohormones, Indole-3-butyric acid, Benzylaminopurine, Gibberlic acid.

INTRODUCTION

Medicinal plants, herbs, spices and herbal remedies are known to Ayurveda in India. A medicinal plant is any plant which in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs (Agarwal , et.al., 2006). The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as medicinal plants (Gswami, et.al., 2002) . The World Health Organization estimates that 4 billion people, 80 percent of the world population, presently use herbal medicine for some aspect of primary health care (WHO).

The plant *Blepharis repens* a rare medicinal species, belongs to the Family Acanthacea. This leaves may contain cystoliths, calcium, carbonate. Flat branches (phylloides) are heated and tied in case of joint –ache. The phylloides are jointed like the knees. Leaves are roasted and then extract is obtained. This extract is drunk as a remedy against flatulence. Roots are employed as antidote on snake – bite. Fruits are roasted and applied on swellings. Stem powder is consumed to cure bone fracture (Colonel K.R.,Kirtikar , et.al., 1987) . The availability of the plant is very low. The importance of the shoot part will emergence for the invitro approaches (Asima Chatterjee, Satyesh Chandra Pakrashi , 1994)..

Plant tissue culture encompasses culturing of plant parts on an artificial medium under sterile conditions often to produce clones of a plant. Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation . The plants which contains high medicinal values do not survive in all agro climatic conditions, the tissue culture methods are very useful for increase the availability of the medicinal plants. IBA is a plant hormone in the auxin family and is an ingredient in many commercial plant rooting horticultural products. Auxins are a class of plant growth substance and morphogens (often called phytohormone or plant hormone). Auxins have an essential role in coordination of many growth and behavioral processes in the plant life cycle (Mantell ,et.al., 1985).

MATERIALS AND METHOD

Healthy parts of *Blepharis repens* was collected from Kolli hills, Namakkal District, Tamil Nadu. It was authenticated by State Forestry Research Institute, Kolapakkam, Chennai-48. The explants was surface sterilized with sterile distilled water and then with .1% (W/V) HgCl for 2 minutes followed by 5 washings with double distilled sterile water. Murashige and Skoogs (MS) medium was used. The medium was autoclave at 15 lbs for 15 minutes.

Then the liquid medium was dispersed equally in culture vials (10ml in each vial) an was closed with aluminium foil. The medium was inoculated with explants and incubated at (25° ± 2°C) for 12 hours. Continuous light provided by cool white fluorescent tubes. Observations were made at regular intervals. All treatments had 5 replicates and repeated thrice.

RESULTS

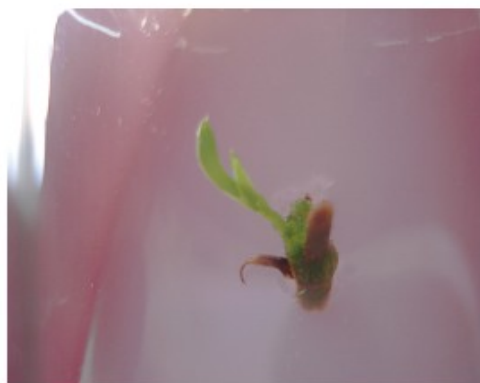
Shoot development was initiated in the stem of *Blepharis repens* on MS medium supplemented with varying concentration of different growth hormones namely IBA, BAP, GA₃ respectively. The results of the table clearly pictuarises significant and very good induction(1.8 cm ± 0.048) of shoot by the growth regulators BAP/IBA and GA₃ (Combination-2[MS+4 mg/l BAP+1.0 mg/l IBA+0.5 mg/l GA₃]) respectively. While combination -4 showed good induction (1.4 cm ± 0.016) in *Blepharis repens*. . The combination of 1,3 &5 showed moderate shoot induction (0.9 cm ± 0.005) , (0.9cm ± 0.017) & (0.9 ± 0.005) . The combination-6,7 exhibited very poor shoot initiation (0.6cm ± 0.012) & (0.5±0.02) when compared to the rest of the hormone combinations used.

Table 1: Effect of different concentration of phytohormones on shoot induction of *Blepherais repens*.

S.No	Combination	Concentration	Shoot development (cm)	Rating
1	Combination-1	MS+4 mg/l BAP+0.5 mg/l IBA+0.5 mg/l GA ₃	0.9 ± 0.063	**
2	Combination-2	MS+4 mg/l BAP+1.0 mg/l IBA+0.5 mg/l GA ₃	1.8 ± 0.048	****
3	Combination-3	MS+4 mg/l BAP+0.5 mg/l IBA+0.5 mg/l GA ₃	0.9±0.017	**
4	Combination-4	MS+4 mg/l BAP+1.5 mg/l IBA+0.5 mg/l GA ₃	1.4 ± 0.016	***
5	Combination-5	MS+3.5 mg/l BAP+1.0 mg/l IBA+0.5 mg/l GA ₃	0.9 ± 0.005	**
6	Combination-6	MS+3.0 mg/l BAP+1.0 mg/l IBA+0.5 mg/l GA ₃	0.6 ± 0.012	*
7	Combination-7	MS+3.0 mg/l BAP+1.0 mg/l IBA+1 mg/l GA ₃	0.5±0.02	*

(****) - High, (***) - Moderate, (**) – poor, (*) - Not suitable

COMBINATION-2



COMBINATION-5



DISCUSSION

The Shoot induction was experimented by providing a broad range of growth hormone combination using 3 different growth hormones with varying concentration. The induction of shoot in this combination was noted after 3 weeks period. This combinations showed the shoot development between 0.6 – 1.8cm. This particular of BAP / IBA/ GA₃ especially at (1.0 mg) seen to be the best combination of growth regulator for the regeneration of shoots in *Blepherais repens*.

Since plants of medicinal value do not survive in all agro climatic conditions. Because of long generation of plants, and difficulty of cultivating in other climatic zone by conventional breeding techniques. Such strains can be overcome through tissue approach. It's the view of its wide medicinal properties and lack of standardized tissue culture protocol & no research work has been done in *Blepherais repens*. There is an urged need to standardize the tissue culture protocol in order to improvise the quality and quantity of *Blepherais repens*. Hence the novel approach was undertaken for the shoot induction in *Blepherais repens*.

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