The Scientific Temper
Vol. 13, No. 1, January-June, 2022:pp 64-70
ISSN 0976-8653, E-ISSN 2231-6396
A Web of Science Journal
Email: letmepublish@rediffmail.com
Doc ID: https://connectjournals.com/03960.2022.13.1.64

Effect of Growth Regulators and *in vitro* Clonal Propagation of *Adhatoda vasica*

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ABSTRACT

Biotechnological tools such as plant tissue culture are paramount for the selection, multiplication and maintenance of medicinal plants. Plant tissue culture techniques proffer a desegregated approach for the production and perusal of enhanced active metabolites available in the plants. *In vitro* regeneration also empowers to execution of very large-scale multiplication of disease-resistant plants. Embracing micropropagation procedures for the creation of plantlets in high numbers as well to defend befitting germplasm is a prerequisite that needs to be tackled to develop a rapid *in-vitro* regeneration of *Adhatoda vasica*. This plant has been exercised as a putted therapy in an Unani stream of medicines for centuries. Nodal sections of *Adhatoda vasica* were cultivated on Murashige and Skoog (MS) medium with the divergent concentration of PGRs (Phytohormones) at innumerable frequencies to optimize the germination idiosyncrasy for induction, proliferation and rooting in the plant. The shoot induction tends to happen in the concentration of BAP + IBA (1.0mg/l+ 0.6mg/l). Which is supposed as the foremost concentration for shoot multiplication of excised explants in concentration of BAP+KN+IBA (2mg/l+1mg/l+0.5mg/l), For rooting the finest concentration of BAP+NAA (1mg/l+0.5mg/l) are respectively considerable. In the relevant study, nodal components were derived from wild plants and were operated as explants to prosper coherent micropropagation protocol for the aforementioned species. Murashige and Skoog (MS) medium supplemented was a worthy medium to induce and promote the growth of axillary bud.

Keywords: *Adhatoda vasica*, Nodal explants, MS medium, Micropropagation, Plant Growth Regulators.

INTRODUCTION

In the present era, medicinal plants are one of the absolute sources of drugs for the major population of the world. Indian health care system incorporates medicinal pluralism and Ayurveda is still dominating over the comparable modern era medicinal therapy, specially for the management of diversified chronic disease conditions (Wijesekera, 1991). The yet escalating global curiosity in Ayurveda has just resulted in stipulating for a giant raw material of medicinal plants and also resulted a right stage for the plant parts accessible in optimum quantities to execute herbal preparations. Herbal medicine is the utmost amazing use of plant-based bio-diversity. Bajaj and Williams (1995) revealed in their study that medicinal plants play a key function in global health care systems.

Adhatoda vasica is an evergreen shrub, mostly known as Malabar nut that relates itself to acanthaceae family; exists entirely in the diversified locations of the world

(Patel et al., 1984). It is inscribed for its pyrroloquinoline alkaloids and its derivatives such as vasicine and vasicinone. This plant is used since ancient times for its well-known medicinal value. Diseases like cough and cold were supposed to be treated by this herb in ancient Vedic period. It is dispensed in various types of medicinal forms available commercially.

Adhatoda vasica is scheduled under major medicinal plant species and is exported from India to many countries. It is rarely possible to germinate it through seeds so it is always proffered to be processed through vegetative propagation, production of secondary metabolites and conservation of germplasm. Currently, parts of plants are regularly experimented with in various medicinal firms for research due to which its demand is rising day by day. Hence endless need lead it to the state of jeopardy of its existence as an effect. This plant displays little seed germination and conventional propagation from end to end cutting is sluggish. Plant tissue and cell culture organization is being subjugated for the gathering of a variety of natural products (Sunita and Dhananjay, 2010; Gaurav et al., 2015a; Dobhal et al., 2013). Plant tissue culture is not subjected to or reliant on season and milieu. It can conquer the shortcoming of traditional propagation techniques. It has thus turned out to be a consistent routine for plant propagation, especially for the propagation of rare and endangered species (Goncalves et al., 2010; Saini et al., 2021).

Its leaves include vasicine, a pyrroloquinoline alkaloid. In addition, this plant also contains quinazoline, vasicinone, deoxyvasicine, adhatodic acid, adhatodine, adhavasinone, vasicinol, vasicol, valine, tannins, saponins, flavonoids, steroids, glycosides, fixed oils and fats, etc (Gulfraz et al., 2006; Gaurav et al., 2016; Gaurav et al., 2018a). Leafy parts of *Adhatoda vasica* were always used to cure asthma due to efficacy as a bronchodilator and mild expectorant. According to Adnan et al., (2010), various leaf modifications are useful in treating issues like hemorrhage, dermal diseases, lesion, pain and other disease in South-East Asia.

MATERIAL AND METHODS

Explant

Materials needed for tissue culture propagation are known as explants. The explants that were taken for exploration were nodal components from the healthy mother plant of *Adhatoda vasica*. The main plant was collected from different locations of Dehradun (valley zone of Uttarakhand in North India). Post collection, the plant was sealed in small pots for its additional growth. Three months later, the growth was appreciable and then was considered to be the right time to bring it for tissue culture and so was done.

Surface sterilization of Explants

For micropropagation, surface sterilization plays a very crucial role. Mihaljevic et al; 2013 suggested the use of chemicals like $Ca(OCl)_2$, C_2H_5OH, NaClO, etc. $HgCl_2$ is used to surface sterilization of explant. (Yadav and Singh, 2018). Surface sterilization of *Adhatoda vasica* begins with cutting off the nodal segments up to a scale of two centimeters and washing under the tap of running H_2O for 4 minutes to 10 min along with a single drop of Tween-20 emulsifier. Encountering it with 0.05% (w/v) bavistin for 10 min In order to extirpate excessive phenolic compounds, antioxidant intro was given by 0.1% (w/v) polyvinyl pyrrolidone (PVP) for 10 min. These explants were then washed with seventy percent of C_2H_5OH for a minute, Anti-microbial $HgCl_2$ .04% (w/v) for 50 to 60 sec. Sterilized distilled water was used again and again for the extirpation of traces of chlorox. Dissection of nodal parts was done to one and a half centimeters before transfer in full strength MS medium with various concentrations of growth regulators.

Medium and Parameters

For the induction of shoot, multiplication and rooting, Murashige and Skoog media was used along with variable concentrations of plant growth regulators in the experimental setup. The medium pH at 5.8 before in cooperation with Agar using 0.1 N NaOH and 0.1N HCl. In every flask approx 30 ml of medium was poured by introducing many growth hormones after Autoclaving kept at room temperature (Sharma et al., 2016: Rautela et al., 2018).

In vitro shoot and its development

MS + 6-benzylaminopurine (BAP) + Indole-3-butyric acid (IBA), Murashige and Skoog full strength + BAP+ Indole-3-acetic acid (IAA) of discrete application plus BAP + IBA+ kinetin for shoot development. Charcoal was also added in tiny concentrations to overcome the phenolic exudates as they were turning brown to the explants. Some flasks were used without growth hormone as they served as a control for a few cases. This was done after shoot proliferation Activity was observed frequently after 20 to 22 days of culture.

Rooting

For induction, microshoots about 2-5 cm long were taken. Half strength medium with concentrations of Napthalene Acetic Acid (NAA) and BAP in various combinations. Parameters were recorded within 2 weeks of culture. The cultures without plant growth hormones served as reins for both shooting and rooting

Statistics

Parameters were taken in regular intervals. For every flask, triplicates were inoculated for rooting and multiplication After 15 days of culture rooting was observed. ANOVA was used for analytical purposes.

RESULTS AND DISCUSSION

There is always a risk of interaction with microbes. To make the explants microbes free, Various .sterilizers were used such as $HgCl_2$, NaOcl. As per Leifert et al., (1992); Monokesh et al., (2013) 70% ethyl alcohol for 1.5 min followed $HgCl_2$ (0.1% + 1-2 drops of Tween 20 for 10 minutes) proved most efficient for the greatest survival percentage in nodal part and shoot tip. The process of decontamination results in 90 percent microbes-free cultures and Polyvenyl Pyrrolidone has averted discoloration because of phenolic compounds in the plant (Staba et al., 1980). It has been seen that charcoal proved more effective to reduce phenolics.

Regeneration of shooting:

Plant regeneration and its growth both are synchronized by plant hormones, most physiological processes depend on several phytohormones (Wang and Irving, 2001; Gaurav et al., 2015b; Gaurav et al., 2018b). Various types of attentiveness of cytokinin (BAP) and auxins, (IBA) are used in a wide range of concentrations and many are mixed for the rebirth of the shoot. Best with MS medium supplemented with BAP (1.0mg/l) + IBA (0.6mg/l) (Table-1) 67% of created shoot was observed. 3.67 ± 0.33 cm of the usable shoot with an average length of the shoots 2.20 ± 0.06 cm of the plantlet. The second reading was on MS medium supplemented with BAP (1.2 mg/l) + (0.8 mg/l) in which the average number of usable was 3.67 ± 0.34 with an average of 1.90 ± 0.06 cm. BAP+IBA combination highlighted the best results rather than the BAP+IAA combination.

Table-1: Different concentrations of BAP+IBA with full MS medium for the shoot induction of *Adhatoda vasica*

S.No.	MS+BAP+IBA mg/L	Shoot induction in %	Shoot count for each explant (Mean + SE)	Mean Length (Mean + SE)
1	MS	0.0	0.0	0.0
2	0.5+0.2	33%	1.67±0.32	1.20±0.06
3	0.7+0.4	41%	2.67±0.32	1.57±0.09
4	1+0.6	67%	3.67±0.33	2.20±0.06
5	1.2+0.8	58%	3.67±0.34	1.90±0.06
6	1.4+1	50%	2.67±0.33	1.70±0.06
7	1.6+1.2	41%	2.67±0.33	1.37±0.09
8	1.8+1.4	33%	1.33±0.31	1.07±0.09

Ten repeats/ treatment; go over three times, means are computed by Ducan's multiple range test at the significance level of 5%

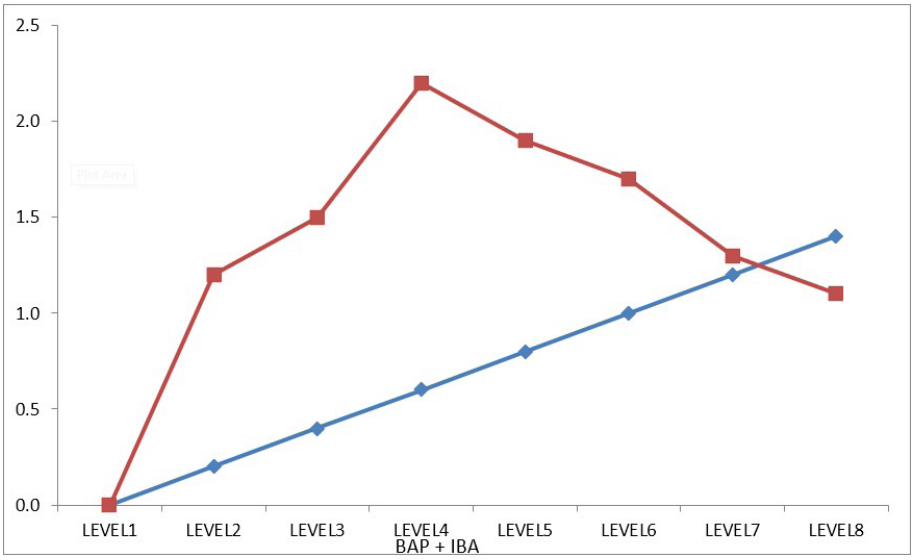

Figure-1: Effect of growth hormones (Level 1 to Level 8 is at various combinations of BAP 0.5 to 1.8 mg/l and IBA 0.2 to 1.4 mg/l) induction of shoot bud of *Adhatoda vasica*

Shoot Multiplication and proliferation

According to khosh-khui and sink, (1982), a higher rate of multiplication can be observed in *in vitro* regeneration.

Multiple shoot data were recorded on explants after 15 days on MS medium containing various concentrations of BAP, IBA and Kinetin. Variable shoot count was recorded on all culture media. The maximum number of 5.66±0.34 shoots for each explant and with a mean length of the shoot 2.06±0.07 cm was recorded on MS medium containing 2.0mg/l BAP in combination with 1.0mg/l IBA and 0.5mg/l Kinetin (Table-2). This suggests that this plant required cytokinins to proliferate the high number of shoots per explants.

Table-2: Different concentrations of BAP, IBA and Kinetin in Full MS medium for initiation of the shoot, shoot growth multiplication of *Adhatoda vasica*.

S.No.	BAP+IBA + Kinetin mg/L	Report percentile	Count of Shoot for each explant (Mean + SE)	Mean Length (Mean + SE)
1	MS	0.0%	0.0	0.0
2	0.5+0.5+0.2	41%	1.33±0.31	1.20±0.06
3	1+0.5+0.3	50%	3.33±0.33	1.40±0.06
4	1.5+1+0.4	66%	3.66±0.33	1.60±0.07
5	2+1+0.5	75%	5.66±0.34	2.06±0.09
6	2.5+1.5+0.6	58%	4.66±0.33	1.90±0.06
7	3+1.5+0.7	41%	2.66±0.33	1.60±0.06
8	3.5+2+0.8	41%	2.33±0.33	1.60±0.06

10 replicates/treatment, done three times, Means are calculated by Ducan's multiple range test at the significance level of 5%

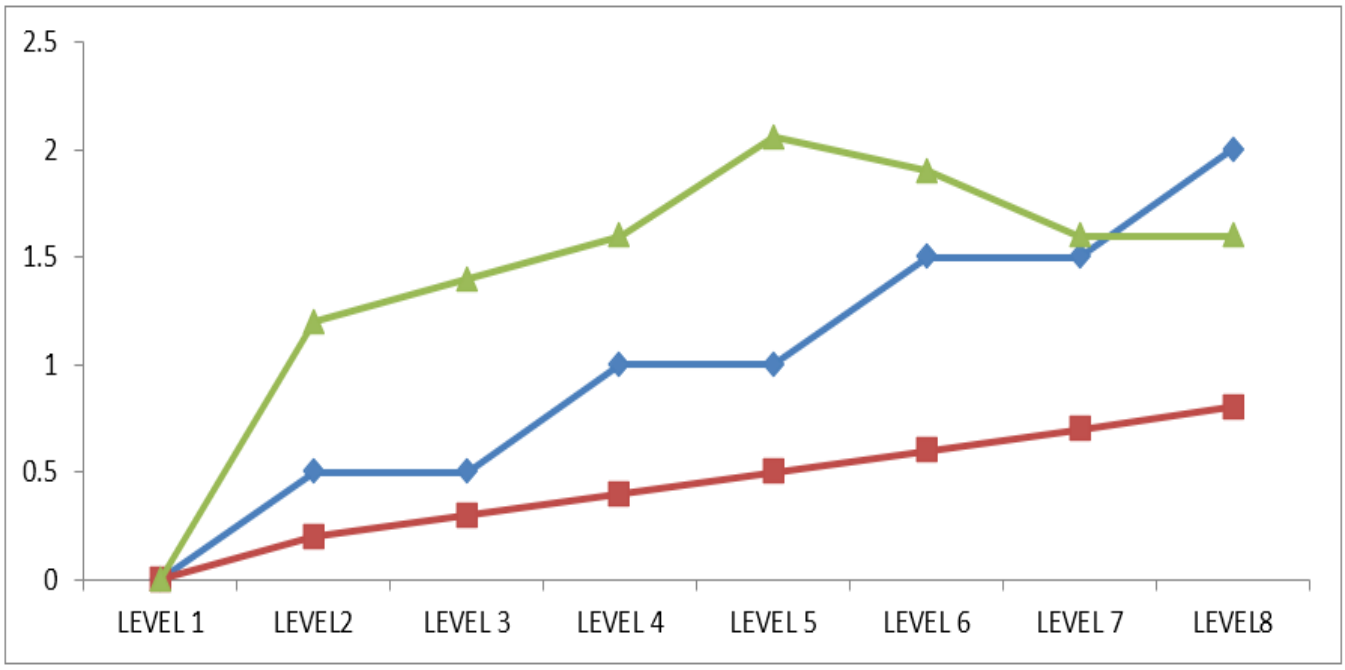

Figure-2: Results of Phytohormones from the first level to eighth level of many concentrations of BAP 0.5 to 3.5 mg/l, IBA 0.5 to 2.0 mg/l and kinetin o.2 to 0.8 mg/l) on shoot multiplication of *Adhatoda vasica.*

In Vitro rooting

To reborn roots in ½ strength MS medium, auxins were taken in various combinations. Here NAA was comparatively better than IBA & IAA. Positive results were reflected in combinations of NAA and BAP. Rooting was greatly seen in 1.0 mg NAA and 0.5mg BAP in half strength of MS medium along with the huge count of roots for each micro shoots as, 6.33± 0.34 which takes only 11 to 15 days for the beginning of root primordial along mean length of root2.80± 0.07.

Table-3: Different concentrations of BAP and NAA in ½ MS medium for root induction from regenerated shoots of *Adhatoda vasica*.

S.No.	BAP+NAA mg/l	Report percentile	Count of root for each explant (Mean + SE)	Average length (Mean + SE)
1	½ MS	0	0	0
2	0.1+0.1	25%	2.33±0.33	1.60±0.06
3	0.5+0.3	41%	5.33±0.33	2.20±0.06
4	1+0.5	66%	6.33±0.34	2.80±0.07
5	1.5+0.7	58%	4.33±0.33	2.40±0.06
6	2+0.9	50%	4.33±0.33	1.90±0.06
7	2.5+1.1	50%	3.33±0.32	1.60±0.05
8	3+1.3	41%	3.33±0.32	1.60±0.05

10 replicates/treatment, done three times, Means are evaluated by Ducan's multiple range tests at the significance level of 5%.

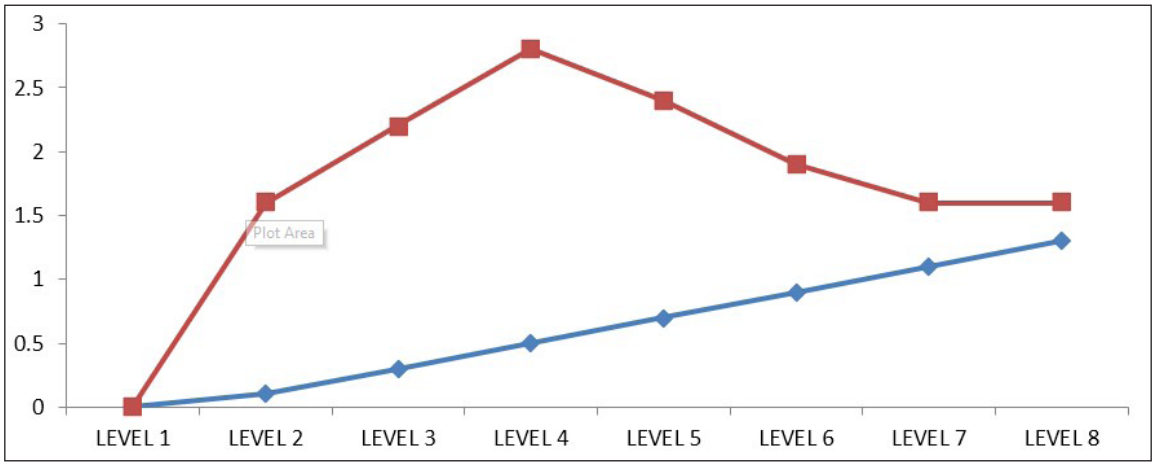

Figure-3: Different concentrations of Phytohormones from the first Level to eighth level in the various concentration of BAP 0.1 to 3.0 mg/l and NAA 0.1 to 1.3 mg/l) on rooting of *Adhatoda vasica.*

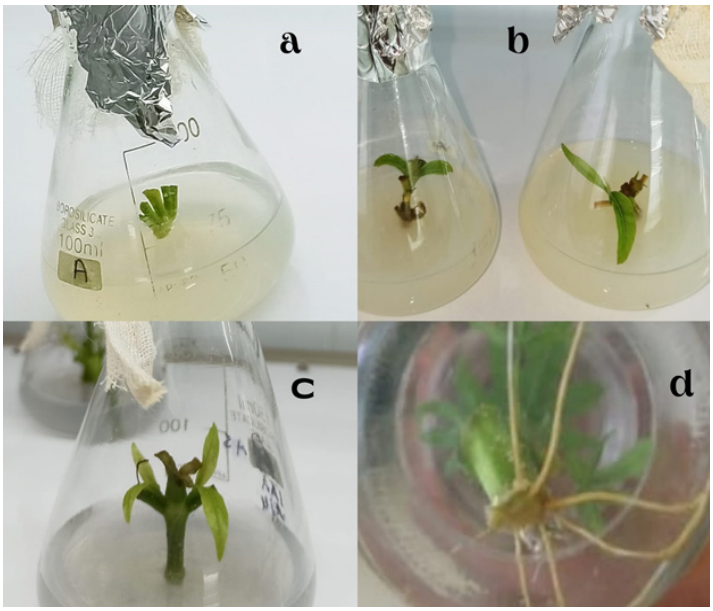

Figure-4: Micropropagation from nodal explants of *Adhatoda vasica:* (a) nodal explant taken from mother plant; (b) shoots propagated on MS medium supplement with BAP, IBA; (c) shoot multiples formed on MS medium prepared with BAP, IBA, KN after first subculture; (d) Formation of roots

CONCLUSION

Micropropagation practices used in regard to decorative, medication purposes in addition to valuable herbaceous seedlings have been platformed in the best way. This method for plant germplasm maintenance is really an influential instrument. The ability to generate plants directly for explants is fundamental to colonial development of elite germplasm via micropropagation for the present study young shoots of *Adhatoda vasica* were occurring in the season of November- February. Nodal explants and shoot tips were used as the source of explants material for micropropagation. The timeframe in which explants are collected is among the most crucial factors driving the development of laboratory-growing cells. Once explants were treated with BAP + IBA at various concentrations, maximum shooting and length increase occurred after 12-16 days of incubation. Employing 1.0 mg/l 6-benzyl aminopurine (BAP) through association to 0.6 mg/l Indole butyric acid, overall effectiveness increases to shoot induction, which was reported as observed, with maximal shoot proliferation 3.67 ± 0.33 and the average length of the shoot 2.20+ 0.06 cm (IBA). During shoot proliferation, various amounts including BAP, IBA, and Kinetin have been utilized. The efficient cytokinin in triggering shoot proliferation development was discovered to be BAP, IBA, and Kinetin. MS medium with a low dose of BAP (2.0 mg/l), IBA (1.0 mg/l), as well as kinetin (0.5 mg/l) in shoot proliferation plus halfway resilient MS medium with NAA (0.5 mg/l) and BAP (1.0 mg/l) in root development in plants generates a good response. The main observation in the study was made that root and shoot proliferation was greatly enhanced. Both the escalation and expansion are pretentious by growth regulator sort and concentrations as mentioned in modus operandi.

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