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Cost effective protocol for *in vitro* propagation of *Adhatoda vasica* Nees. Using modified tissue culture media

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Abstract
The present investigation was intended to devise a cost effective protocol for *in vitro* propagation of important medicinal plant *Adhatoda vasica* Nees. Thus, the in depth cost analysis of both protocols viz. conventional tissue culture method using MS media and modified protocol using modified tissue culture media/ cost effective media were taken into consideration. The different parameters that were studied here are: cost of electricity, cost of media and cost of other important ingredients. Thereafter, the comparative assessment of both the protocols was made. It was found that the modified method was very much cost effective in terms of cost per plantlet.

Keywords: *Adhatoda vasica* Nees, medicinal, modified, propagation

Introduction
Adhatoda vasica is an Ayurvedic medicinal plant which is a home remedy for several diseases and human requirements. It is mentioned in Vedas as an herbal remedy for treating cold, cough, whooping cough, chronic bronchitis and asthma, as sedative expectorant, antispasmodic and anthelmintic. It is a licensed drug and is mentioned in the India Pharmacopoeia (Pharmacopoeia of India, 1966) [7]. The drug is employed in different forms such as fresh juice, decoction, infusion and powder; also given as alcoholic extract and liquid extract or syrup. The leaf juice is confirmed to cure diarrhoea, dysentery and glandular tumor. The powder is reported to be used as icepack on rheumatic joints as counter-irritant on inflammatory swelling, on fresh wounds, urticaria and in neuralgia (Wealth of India, 1985) [10].
Adathoda vasica Nees. Is listed under top 36 Medicinal Plant Species in High Trade & consumed in volumes exceeding 100 MT per year as per 2007 survey cum study on Demand and Supply of Medicinal plants in India by National Medicinal Plants Board, New Delhi through Foundation for Revitalisation of Local Health Traditions (FRLHT), Bangalore. It is also listed under Major medicinal plant species exported from India (Handa, 1992) [5]. The demand of this important plant mostly comes across from the natural habitat. This plant show low seed germination and conventional propagation through cutting is slow (Wealth of India, 1985) [10]. This leads to rapid depletion of plant material due to over exploitation. Pant tissue and cell culture system are being exploited for the accumulation of the variety of natural products (Sunita and Dhananjay, 2010) [9]. Hence it was felt that a cost effective method should be available for mass propagation of this important medicinal plant Adhatoda vasica Nees.

Materials and Methods
In the present investigation, an important medicinal plant *Adhatoda vasicawas* studied in great detail for the development of protocol for cost effective *in vitro* micropropagation. For each experiment explants were collected from healthy plants growing in the Botanical Garden of Govt. Motilal Vigyan Mahavidyalaya. Shoot tips, axillary buds and nodal buds were used as explants. The explants were surface sterilized and inoculated into Modified as well as Murashige and skoog (MS)medium supplemented with different combination of growth regulators. The regenerated shoots were subcultured every three weeks in the same medium. The responses of the explants were studied at weekly intervals for several parameters. The Experiments were also carried out to check the effect of cost effective substitutes such as Nutrients, Carbon source, tap water and growth regulators and *in vitro* hardening under ordinary conditions like natural light and temperature in order to reduce the production cost without compromising the quality of micropropagules.

Results and Discussion
The production cost of *in vitro* raised plantlets is extremely influenced by composition of culture medium. It was estimated that the cost of media preparation can account for 25-35% of the micro propagated plant production (Raghu, *et al*., 2007) [8]. The main components of most plant tissue culture media are mineral salts, sugar, water, organic supplements, growth regulators and gelling agent (Gamborg *et al*., 1968; Gamborg and Phillips, 1995) [3-2].
The gelling agents such as agar contribute 70% of the costs. Other ingredients in the media - salts, sugar and growth regulators, have minimal influence on production cost. Thus, low cost alternatives are needed to reduce the production cost of tissue-cultured plant (George, 1993; Anonymous, 2004) [4, 1], However, variety of unusual low cost options are available to substituteexcessive gelling agents, sugars, water and growth regulator.
In the present investigation, to minimize the cost of culture medium, the constituents and other supplements of culture medium were framed in such a way that it favors the process economics without negotiating the quality and quantity of plantlets. Earlier cost effective *in vitro* propagation of several plant species with unambiguous commercial value viz.,

medicinal, ornamental, horticulture, floriculture etc. have reported. Most of these studies confirmed the suitability of low cost medium for the single plant species.

However, a cost effective medium as suitable as MS (Murashige & Skoog, 1962) [6] for variety of species is needed to ensure the regeneration of *in vitro* plantlets of desired quantity and quality at low cost. Thus, we report here a novel cost effective medium for *in vitro* propagation Adhatoda vasica Nees.

A novel cost effective medium has been standardized using conventional and non-conventional media constituents and other supplements.

Generally, formulation of culture medium for *in vitro* propagation studies consists of macro and micronutrients, vitamins, sucrose, growth regulators and gelling agent. In the present investigation, during the course of media formulation, several changes were made using both conventional and non-conventional media components and other supplements to devise a new cost effective medium.

Proportional cost breakdown of nutrients, water, carbon source, growth regulators, gelling agent and other supplements reveals that there is a vast difference in production cost of conventional culture medium upto Rs.60/ liter for semi-solid medium and Rs.25/ liter (for liquid medium) and modified protocol Rs.23.60/ liter for semi-solid medium and 6.10/ liter for liquid medium (Table 1). The three main factors that have made the biggest difference viz, gelling agent (agar-agar), carbon source (Sucrose), and distilled water.

In conventional medium, detached analysis of different component of culture medium reveals that, agar is the most expensive ingredient of the culture medium as it account 58% of the total media cost, followed by distilled water (16.6%) and sucrose as carbon source (16.6%) while rest of the component are reasonably cheap as altogether account 8.8% of the total medium cost, despite the fact that the plant growth regulator & basal medium (inorganic and organic nutrients) are the vital component of *in vitro* propagation. However, integration of low cost and cost effective alternative in modified culture medium has significantly reduced the cost of medium (Table).

In modified medium, the overall cost reduction in semisolid medium was workout to be 70% while, elimination of gelling agent has dramatically reduced the cost of liquid medium. The overall cost reduction was workout to 85% in modified liquid medium.

Table 1: Proportional cost breakdown of Conventional and Modified protocol for comparative analysis

Conventional Protocol (As per MS Medium)			**Modified Protocol (As per MM Medium)**		
Constituents of Culture medium	Rate (`/ gm)	Cost/liter (aprox.)	Constituents of Culture medium	Rate (`/ gm)	Cost/liter (aprox.)
Inorganic Nutrient		1.25	Inorganic Nutrient		1.25
Organic Nutrients		0.75	Organic Nutrients		0.25
Double distilled water	10.0/ lt.	10.0	Rain water (Avg. max. cost 0.10/ ltr.)		0.10
Sucrose (Loba Chemicals)	330/ kg	10.0/ 30 gm	Market Sugar	50/ kg	1.5/30 gm
Agar (Loba, Chemicals)	3500/ kg	35.00/ 10gm	Agar (Loba, Chemicals)	3500/kg	17.5/ 5 gm
PGRs (Avg. max. cost)		3.00	PGRs (Avg. max. cost)		3.00
Total Cost	Semi-solid medium` 60.00/ ltr.		Semi-solid medium` 23.60/ ltr		
	Liquid medium` 25.00/ ltr.		Liquid medium` 6.10/ ltr		

Afterwards, the major cost cutting approach was abolition of cooling facility through air conditioner from the micropropagation system. This was achieved by propagating selected plant species *in vitro* under normal room temperature. This has becomes possible during the month of October to February which is usually enriched relatively low temperature range which permits the in vitropropagation of selected medicinal plant i.e Adhatoda vasica without artificial cooling facility. This approach has bizarrely condensed the electrical consumption as well as electrical cost. In modified approach, the overall reduction in energy consumption was workout to be 60%. This has resulted in significant reduction of electrical cost by 60% of the total cost of electricity.

The present study demonstrated a cost effective in vitromicropropagation technique for Adhatoda vasica Nees. In addition to this, the detailed and precise cost analysis of media cost and electrical cost and cost reduction in modified protocol over conventional protocols were also carried out.

The comparative cost analysis of both conventional & modified medium reveals that, the overall the overall cost reduction in semisolid modified medium was workout to be 65-70% and the significant cost reduction in modified liquid medium was workout to 80-85% over conventional medium. This was achieved mainly by adopting some low cost alternatives and options such as market sugar, rain water, and low concentration of gelling agent (5.0mg/l Agar). Moreover, the successful *in vitro* propagation of the selected medicinal plant on modified medium proving its stability and suitability for *in vitro* studies.

The present study reveals that in case of electrical consumption and the cost of electricity, a remarkably reduction in the electrical consumption as well as electrical cost was obtained in modified approach. The overall reduction in energy consumption was workout to be 60% over conventional approach. This has resulted, a significant reduction in electrical cost by 60% of the total cost of electricity in modified approach. This was resulted mainly due to better planning and adoption of energy saving strategies.

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