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Shoot organogenesis in *Justicia adhatoda* (L). Nees

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Abstract
Tissue culture methods provide a novel way for the asexual multiplication of *Justicia adhatoda* (L) Nees. Propagation of plants through tissue culture offers a unique advantage over conventional propagation methods for mass multiplication and for conserving the germplasm. Compact callus was induced from axillary bud explants of *Justicia adhatoda* and the callus showed shoot organogenesis in MS+2,4-D 0.1 mg/.l and BAP 4.0 mg/l. and root was induced in MS+ 2,4-D 0.1mg/l.

Keywords: murashige and skoog medium, explant, 2, 4-D, BAP, organogenesis, rooting

Introduction
Justicia adhatoda is a member of Acanthaceae family, an evergreen shrub. Leaves are elliptic and acuminate. Trade name of *Adhatoda Vasica* is Vasa and Vasika. This medicinal plant is known as 'Adalodakam' in Malayalam, 'Adusa' in Hindi, and 'Vasaka' in Sanskrit. The leaves contain alkaloids, vasicine, adhavasinone, vasicinone and vasnetine [1, 2]. Leaves and roots are useful in cough, asthma, bronchitis, rheumatism and as insecticides [3, 4].
The propagation of *Adhatoda vasica* is restricted due to poor seed set, low potential for seed germination and through shoot cuttings which solely rely on season for multiplication [5, 6]. Tissue culture methods are very ideal, which offers a unique advantage over conventional propagation methods for mass multiplication and for conserving the germplasm.
Some of the earlier works include shoot formation in *Justicia adhatoda* in BAP supplemented medium [7] callus induction in *Justicia gendarussa* nodal segments on M S medium containing NAA 1.0 mg/l and BAP 0.1 mg/l [8], and *in vitro* studies for shoot regeneration of *Adhatoda vasica* using nodal segment, shoot tip and leaf explants [9, 10, 11, 12, 13].
The main objectives of the study were initiation of callus and to induce organogenesis.

Materials and methods
The explants, nodal segments and tender leaves (especially 3rd – 4th from the apex) were collected from actively growing branches of healthy plants from Zamorin's Guruvayurappan College campus. The leaf explants were prepared by cutting the leaves in to 1-3 cm^2 pieces including the midrib. 1-1.5cm long nodal segments were prepared from the shoot. These were then surface sterilized with mercuric chloride (0.1%w/v) for 5-10 minutes inside in the laminar air flow chamber and later thoroughly washed 4-5 times in sterile distilled water.

Culture Medium
For the induction of callus, MS medium [14] was used as the basal medium. The concentrations of auxin and cytokinin used for both the explants were 2,4-Dichlorophenoxy acetic acid (2,4-D) 3mg/l and Benzyl Amino Purine (BAP) 0.5mg/l for callus induction and proliferation and later, 2, 4-D 0.1mg/l and BAP 4mg/l for shoot organogenesis and 2, 4-D 0.1mg/l for root induction. The pH of the prepared media was adjusted to 5.8 ± 0.1. The cultures were observed regularly at an interval of five days.
The percentage of callusing explants, color and type of callus were noted. The fresh weights were recorded at an interval of five days and the growth index (GI) was calculated using the following formula

$$\text{Growth index (\%)} = \frac{\text{Final Fresh Weight - Initial Fresh Weight}}{\text{Initial Fresh Weight}} \times 100$$

Results and discussion
Leaf Explants
In 2, 4-D-3mg/l, BAP-0.5mg/l tender leaf explants of *Justicia adhatoda* showed tissue enlargement and callusing in less than 20% on explants. The callus proliferated very slowly and did not show any differentiation.

Axillary Bud explants (nodal segments)
In 2, 4-D 3 mg/l, BAP 0.5mgl, axillary buds induced callus and proliferated actively.

Shoot organogenesis
The callus induced from axillary bud explants produced shoots, when transferred to differentiation medium with 2, 4-D 0.1mg/l and BAP 4 mg/l, within 40 Days after Inoculation (DAI). Shoot organogenesis was obtained in almost 41% of callus cultures. Shoots developed 4 leaves within 45 Days DAI. The shoot was transferred to a root inducing medium. The shoot developed adventitious root in a medium, MS+ 2, 4-D 0.1mg/l.

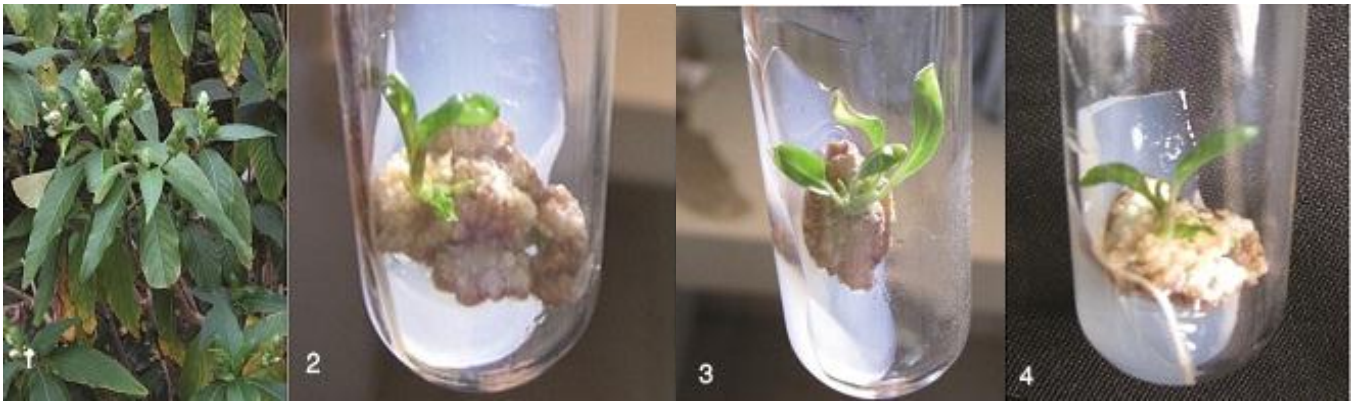

Fig 1: *Adhatoda vasica* habit, **2:** Shoot organogenesis on 40 days after inoculation, **3:** Shoot with 4 leaves on 45 days after inoculation **4:** Rooted plantlet

Table 1: Rate of callus proliferation in *Justicia adhatoda*

Explants	Initial weights (g)	Final weights (g) 30 DAI	Growth index (%) **
Tender leaf	0.044	0.082*	86.3%
Axillary Bud	0.063	0.204*	223.8%

***** Mean of 25 Replicates

** Growth index = $\frac{\text{Final fresh weight - Initial fresh weight}}{\text{Initial fresh weight}} \times 100$

Table 2: Overall Responses of callusing and organogenesis in *Justicia adhatoda*

Explant	Medium for callusing	% of explants responded	medium for shoot organogenesis	% of explants responded
Leaf	2, 4-D (3mg/l) BAP (0.5mg/l)	18%	2, 4-D (0.1mg/l) BAP (4mg/l)	-
Shoot	2, 4-D (3mg/l) BAP (0.5mg/l)	43%	2, 4-D (0.1mg/l) BAP (4mg/l)	41%

Conclusions

1. MS medium with a higher concentration of 2, 4-D along with a lower concentration of BAP was found to be favorable for callusing in *Justicia adhatoda*.
2. When 2, 4-D was reduced and BAP was increased in the differentiation medium, shoot organogenesis observed.
3. The callus culture can be effectively exploited for secondary metabolite production in *Justicia adhatoda* which contain highly valuable alkaloids, which would avoid the destruction of plants in their natural habitatsand thereby conservation of germplasm and shoot organogenesis through plant tissue culture offers a reliable method for mass multiplication.

References

1. Shalaka DK, Parameswaran S. Micropropogation and Organogenesis in *Adhatoda vasica* for the estimation of vasicine. Pharmacognosy Magazine. 2009; 5(20)359-363.
2. Rashmi PA, John R, Mathewa L. Isolation and characterisation of vasicine from *in vitro* cultures of *Justicia adhatoda*. International Journal of Pharma and Bio Sciences. 2012; 3(3):B58-B64.
3. The wealth of India Raw Materials Series, National Institute of Science Communication and Information Resources, (Publ) CSIR, Dr KS Krishnan Marg, New Delhi-11. 2000, 1.
4. Panda H. Medicinal Plants Cultivation & Their Uses. (Publ). Asia Pacific Business Press Inc. 2002, 157-158
5. Anand Y, Bansal YK. Synthetic seeds: a novel approach of *in vitro* plant formation in vasaka (*Adhatoda vasica* Nees.) Plant Biotechnol. 2002; 19(3):159-162.
6. Maurya S, Singh D. *In vitro* Callus Culture of *Adhatoda vasica*: A Medicinal Plant, Annals of Biological Research. 2010; 1(4):57-60.
7. Nath S, Buragohain AK. Micro propagation of *Justicia adhatoda* - A woody medicinal plant by shoot tip culture. Indian Journal of biotechnology. 2005; 4(3):396-399.
8. Agastian, Williams P, Ignacimuthu LS. *In vitro* propagation of *Justicia gendarussa* Burn F. A Medicinal plant. Indian Journal of Biotechnology. 2006; 05(2):246-248.
9. Abhyankar G, Reddy VD. Rapid micropropagation via axillary bud proliferation of *Adhatoda vasica* from nodal segments. Indian J Exp Biol. 2007; 45:268-271.
10. Khalekuzzaman M, Rahman MS, Rashid MH, Hossain MS. High frequency *in vitro* propagation of *Adhatoda vasica* Nees through shoot tip and nodal explants culture. J Bio Sci. 2008; 16:35-39.
11. Azad MA, Amin MN. *In vitro* regeneration of plantlets from internode explants of *Adhatoda vasica* Nees. Plant Tissue Cult. 1998; 8:27.
12. Amin MN, Azad MAK, Begum F. *In vitro* plant regeneration from leaf derived callus cultures of *Adhatoda vasica* Nees. Plant Tissue Cult. 1997; 7(2):109-115.
13. Mandal J, Laxminarayana U. Indirect shoot organogenesis from leaf explants of *Adhatoda vasica* Nees; Springerplus. 2014, 3:648.
14. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*. 1962; 15:473-495.