Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Conservation of Andrographis elongata - An Endemic Medicinal Plant

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ABSTRACT

Andrographis elongata is an endemic medicinal plant restricted in the Western Ghats . It is used against various diseases by the local people in old Travancore based on its high potentiality. Due to the seed metabolism and anthropological activity, this plant is now vanishing from the natural environment. In this scenario, the plant must be conserved for the future generation. Different methods adopted for the present study includes tissue culturing in MS medium, seed treatment with GA_3 and layering . Shoot initiation was observed in 0.25mg/ml NAA and 0.05mg/ml BAP combination even though further growth was arrested. In seed germination studies, the germination percentage was maximum in seeds treated with 300ppm GA_3(86%)and the saplings were well acclimatized in natural habitat. For conservation, layering was done during different months to analyze the nature of growth habit. Layering was found to be very effective in our climatic conditions. Thus habitat protection can be more effective for conserving the plant.

KEYWORDS:*Andrographis elongata*, tissue culturing, GA_3

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INTRODUCTION

Andrographis elongata is an endemic medicinal plant found in Pachamalai hills in its natural habitat.[1] *A.elongata* is medicinally important and used for the treatment of various human and veterinary diseases.[2-3] Due to the seed metabolism and anthropological activity, this plant is now vanishing from the natural environment. The indiscriminate anthropogenic activities lead to the declining of traditional culture of India. The over exploitation of nature, large number of important medicinal plants were disappearing from the natural habitat. The increasing demand for meeting the future need, the cultivation and conservation of medicinal plants must be encouraged. [4] *A.elongata* is a very slow growing species and its seed germination is very poor in natural conditions.[5] Hence alternate conservative strategies are needed for the conservation ofthis valuable medicinal plant. For conservation, different methods such as tissue culturing in MS medium, seed treatment with GA_3 and layering were adopted for the present investigation.

MATERIALS AND METHODS

Collection of Plant Material

Andrographis elongata(Vahl) T. Anderson was collected from Pachamalai hills, Salem, and locally propagated and maintained in the home garden Parassala, Thiruvananthapuram district, Kerala. A voucher specimen was identified by the taxonomy department of JNTBGRI Palode.

Direct Regeneration by Tissue Culturing

Nodal segments were collected as the explants from the healthy plants without any visible disease symptoms. Explants were thoroughly surface sterilized for inoculation.For shoot regeneration, the explants were inoculated in MS mediumsupplemented with different concentrations of BAP, NAA and 2, 4-D either alone or in combination.[6]After inoculation the explants in culture media were incubated in sterile culture room under controlled conditions of temperature, light and humidity. The culture were incubated on culture racks at 25-29ºC constant temperature illuminations provide by cool white fluorescent light (16h photoperiod) placed about 18 inches above the culture to give a intensity of 3000 lux.

Seed Treatment with GA_3

The mature pods of *Andrographis elongata* were collected and dried for 10 days at room temperature. The seeds were surface sterilized and rinsed with distilled water and was subjected to GA_3 treatment. 20 seeds were sowed in each earthern pot filled with 1 Kg of garden soil and sand mixture (1:2). The seeds were treated with different concentration of GA_3 such as 50ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm, 300 ppm, 350 ppm, 400 ppm, 450 ppm and 500 ppm for one day. The

seeds were sown and watering was done regularly.Regular observations were made to find out the germination percentage.

Layering

The nodal parts of the stem of *Andrographis elongata* was selected for the ground layering. Three months old branches were selected and made small cuts at the nodal portions and inserted in small pits with 15cm x 15cm x 20 cms. Then the pits were filled with sand, soil and coir waste at 2:1:1 proportion and watered regularly. The shooting habitat was recorded.

RESULTS AND DISCUSSION

Direct Regeneration Through Tissue Culture

The morphogenetic response of the nodal segments of *A.elongata* was inoculated in MS medium supplemented with different combination of growth regulators at different concentrations were recorded in Table -1, and 2

Table1.The combined effect of growth regulators in direct regeneration of *A.elongata*

Explant	**Medium**	**Growth Regulators**	**Concentration (mg/ml)**		**Response**
			2,4-D (mg/ml)	Kinetin (mg/ml)	
Nodal segment	MS medium	2,4-D and Kinetin	0.05	0.25	No Response
			0.1	0.2	No Response
			0.15	0.15	No Response
			0.2	0.1	No Response
			0.25	0.05	No Response
Nodal segment	MS medium	NAA and BAP	NAA(mg/ml)	BAP(mg/ml)	
			0.05	0.25	No Response
			0.1	0.2	No Response
			0.15	0.15	No Response
			0.1	0.1	No Response
			0.25	0.05	Shoot initiation

In the 1st set, the nodal segments inoculated in MS medium supplemented with a combination of different concentrations of 2, 4- D and Kinetin treated, no response is noted in this experiment (Table - I). The second set of nodal segments treated with a combination of NAA and BAP in various concentrations. The shoot initiation noted in the combination of 0.25mg/ml NAA and 0.05 mg/ml BAP treated nodes after two weeks(Table –I).

In the3rd, 4th and 5thsets of nodal segments treated with only BAP,NAA and 2, 4-Drespectively at different concentrations. There was no response obtained.

Table 2. Effect of BAP, NAA and 2, 4-D in direct regeneration of *A.elongata*

Explant	Medium	Growth Regulators	Concentration (mg/ml)	Response
Nodal segment	MS medium	BAP only	0.05	No Response
			0.1	No Response
			0.15	No Response
			0.2	No Response
			0.25	No Response
Nodal segment	MS medium	NAA only	0.05	No Response
			0.1	No Response
			0.15	No Response
			0.2	No Response
			0.25	No Response
Nodal segment	MS medium	2,4-D only	0.05	No Response
			0.1	No Response
			0.15	No Response
			0.2	No Response
			0.25	No Response

The experimental trials were repeated more than 10 times. But the result is found to be negative. In short, the direct regeneration of *A.elongata* is successful from the nodal segments treated with the combination of NAA (0.25mg/ml) and BAP (0.05mg/ml) only (Table- I). Eventhough shoot initiation occurs, after two weeks of inoculation further growth was found to be ceased.The present findingsof conservation through tissue culture methods of *A.elongata correlated* withthe earlier studies in*A. paniculata*and*A. neesiana*.[7-10] From these studies MS medium was found to be very effective for direct regeneration of *Andrographis species.*

Seed Treatment with GA_3

GA_3 (Gibberellic acid) is an effectivephytoregulator promoting seed germination. The seed germination started within 6 days after GA_3treatment. The GA_3 treatment enhanced the percentage of seed germination in a concentration dependent manner. The maximum percentage of seed germination is found in the seeds treated with 300 ppm and 350 ppm GA_3 *ie,* 86% and 81% respectively. The germination percentage is found to be decreasing at higher concentrations (400 ppm, 450 ppm and 500 ppm) . The seed germination was 25% in control. The germination results are depicted in Table-III. The seedlings were well acclimatized our natural habitat. The present study correlated with the earlier findings in *A.elongata*.Thus Gibberellic acid is found to be a good germination promoter substantiates the earlier findings of in*A.lineata*, and *A. paniculata*.[11-12]

Table 3. Effect of GA_3 on seed germination of *A.elongata*

Sl.No	Gibberellic acid (concentration in ppm)	% of germination
1	Control	25
2	50	41
3	100	53
4	150	59
5	200	65
6	250	79
7	300	86
8	350	81
9	400	76
10	450	64
11	500	59

Layering

The vegetative propagative method such as Layering was experimentally tried in this plant for conservation at different seasons such as June- July, August- September, October- November, December- January and March- April.The percentage of shooting was maximum in June- July (98%), followed by December- January (80%) and August- September (75%) (Table- IV). From the observation it is clear that rain plays an active role in layering. Layering is found to be very effective in our climatic condition for conserving this plant.

Table 4.Layering of *A.elongata*in different seasons

Season	Number of nodes for layering	% of shooting
June- July	10	98
August- September	10	75
October- November	10	60
December- January	10	80
March- April	10	55

CONCLUSION

In tissue culturing Auxin – Cytokinin combinations (0.25mg/ml NAA and 0.05mg/ml BAP)initiate shoots even though further growth was arrested. So as a next step of conservation, seed treatment with different concentrations of GA_3also showed significant result. The germination percentage was maximum in seeds treated with 300ppm GA_3 (86%).Layering method showed highly significant data. Hence layering was found to be very effective in our climatic conditions. Thus habitat protection can be more effective for conserving the plant for future generation.

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