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Usage of Phytohormones for Aseptic Regeneration of Two Medicinal Important Plant Species, Adhatoda Vasica and Aloe Vera

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ABSTRACT

Two plants species Aloe vera and Adhatoda vasica belongs to family Liliaceae (Asphodelaceae) and Acanthaceae respectively. Present study was carried out to establishment of systematics protocol for ascetic, in-vitro regeneration of these plants using different concentration of phytohormones specifically cytokinin and auxin. The experimental result indicates percentages of regeneration was maximum in MS (Murashige and Skoog) medium with combination of 3mg/l BAP and 0.75mg/l NAA in Aloe barbadensis for direct shoot induction and 4mg/l BAP with 1mg/l NAA for indirect shoot regeneration, while in Adhatoda vasica highest percentage of shoot induction observed in 1 mg/l BAP and 0.2mg/l NAA. MS medium with combination of 1mg/l IBA was found to be best for the root induction in both the plant species.

Keywords: Phytohormones, MS Medium, Explant, Medicinal plant, Aseptic regeneration.

Abbreviation: IBA- Indole Butyric Acid, BAP- 6-Benzyl Amino Purine, NAA- Naphthalene Acetic Acid, MS- Murashige and Skoog.

I. INTRODUCTION

In the plant biodiversity medicinal plants is foremost intrinsic element of human healthcare from prehistoric times in traditional medicinal practitioners. (Thomson P. et al., 2014). Due to overexploitation, anthropogenic activity, climatic change, and overpopulation essential plants are become endangered or vulnerable, hence global people move towards sustainable utilisation therefore, its urgent need to protect and preserve valuable plant species. Plant tissue culture technique is an essential tool for the plant multiplication in large scale (Kumar PP. and Loh CS., 2012; Gamborg OL., 2002) which has several application and advantages (AK, B., 2018). It is a Heberlandt who first established this technique as an essential tool for in-vitro micropropagation of disease free, rare, economically, medicinally valuable plant species for the production of important commercially valued secondary metabolites. (Heberlandt 1902;

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Altpeter et.al.,2016; Debnarh et al., 2006). Knowledge of plants species with their traditional use is the key for a lot of commercially medicines which play notable role in healthcare of rural people (Thomas P. et al., 2014). Micropropagation techniques is used to develop callus, which on suitable culture media grow as complete plant after transferring it first on shooting than rooting media (Almemary, 2020). Major economically plant species are in endangered or vulnerable, for them efficient aseptic regeneration protocol is a barrier in their propagation and conservation which can be further used in transgenic plant regeneration (Chen Z., et al., 2022). The balanced concentration of plant growth regulators specially cytokinin and auxin play significant role to develop an explant whether callus, shoot or root (Maren NA, et al., 2022). Due to vast significance of plant tissue culture number of researchers in India and other countries work on to use this technique for aseptic plant regeneration and secondary metabolites production (Smetanska 2008; Ver-poorte et al.,2000; Karuppusamy 2009).

II. MATERIAL AND METHOD

Both the plant Adhatoda vasica and Aloe barbadensis Miller also called as Aloe vera being very useful for their excellent medicinal properties and low percentages of seed germination these plants were selected for standardizing micropropagation protocol by using different concentration of phytohormone specially cytokinin and auxin and to study their effect present study was carried out. Explant were selected like leaf, node, and internode were collected from mother plant and first rinsed under tap water up to 30 min to remove surface contamination, again explant was washed with tween 20 for 5-10 minutes and then with running tap water, all explant were keep in Bavistin (1% w/v) than the explant was transferred to cabinet of laminar air flow chamber for further sterilization process, Where explant was surface sterilized by 2-3 wash of distilled water followed by 70% alcohol for 3-5 minutes thrice, than 0.1% of Mercuric chloride for 3-5 minutes and again it is thoroughly washed by vigorous shaking with double distilled water for 3-4 times. After the sterilization process explant were cutted in appropriate size and inoculated in culture tube on MS media with different concentration of plant growth regulators. Inoculated conical flask and tube were kept in culture room tightly packed with cotton plug with proper labelling and kept in culture room under 16/8 hours of light and dark cycle and temperature 20-25 0C. in the MS medium with different concentration of 6-Benzylamine purine hormone in 7 level (0.5, 1.0, 1.5, 2, 2.5, 3, 4) alone and with combination of NAA (Naphthalene acetic acid) also for rooting IBA (Indole Butyric Acid) with different concentration level from 0.1 to 1.5 mg/l. The PH was maintained 5.8 using HCL and NaOH each and solidifying agent used was agar (0.8%).

III.RESULT AND DISCUSSION

In the ascetic regeneration of these two medicinal plant Adhatoda vasica and Aloe vera, different explants were cultured on Murashige and Skoog medium in the combination with different concentration of BAP alone and with NAA for direct & indirect shoot induction, after this shoot were cut down aseptically and inoculated with IBA different concentration for rooting purpose, well develop plants than transferred in cup filled with sterilized soil for the hardening process of these plants. In both the plant node/nodal segment of the stem was found to be the best explant for in vitro propagation. Apical and nodal segment of the stem was also used by many researchers for in vitro propagation and achieved good result like Kaur et al., (1992), Rathore et al., (1991), Arumugam and Bhojwani (1990).

Callus Induction

In both the plant node was found to be best responding explant whereas in Adhatoda vasica internode and leaf also response well and callus

induction observed in MS medium supplemented with BAP (1mg/l) and NAA (0.2mg/l and 1mg/l) whereas in Aloe vera only nodal/stem segment explant induced into callus in MS medium supplemented with BAP (3mg/l) with NAA (0.75mg/l) and BAP(2mg/l) with IAA(0.5mg/l). (Table A). The nature of the callus of both plants were whitish yellow, soft, or hard in case of Adhatoda vasica, and friable. (Photo plate I)

Direct multiple shoot induction

In case with Adhatoda vasica direct multiple shoot regeneration observed in MS medium with concentration of BAP (2-10 mg/l) with NAA (0.5 mg/l). leaf and internode did not respond at all whereas nodal explant shows direct multiple shoot induction. similarly in Aloe vera leaf and other parts except node/stem segment found to be least responsive and when nodal explant inoculates on MS medium with BAP (3-4 mg/l) in addition NAA (0.2-1mg/l) direct multiple shooting was recorded there for node/stem segment alone is found to be best for direct shoot organogenesis. (Table B, Photo plate 1).

Indirect multiple shoot induction

In Adhatoda vasica and aloe vera after the callus induction, callus was transferred aseptically on medium for shoot induction. MS medium supplemented with varying concentration of 6-Benzyl amino purine and 1- Napthyl acetic acid. In case of Adhatoda vasica highest percentage of shoot regeneration was recorded on MS medium supplemented with BAP (1.5mg/l) and low concentration or without auxin whereas Aloe vera indirect shoot induction from callus was observed on MS medium with BAP (3mg/l) and NAA(0.75mg/l) of concentration. (Table C, Photo plate 1)

Root induction

After successful shoot organogenesis shoots were cut and separated from parent plants aseptically using sterilized scalpel on the cabinet of laminar air flow chamber and inoculated on MS medium with different level of concentration of IBA (0.1,0.2,0.3,0.5,1mg/l). The best response of root organogenesis observed in the combination of MS medium with 1mg/l of IBA in both the plants. (Table D, Photo plate I)

Table A: Callus induction on MS medium supplemented with phytohormones in Adhatoda vasica and Aloe vera.

Plant selected	Explant	MS medium + Phytohormones (mg/l)	Duration of Response (Days)	Nature of Callus	% of Response
Adhatoda vasica	Node Internode Leaf	MS+ 2,4D (2) + Kn (0.5)	- - -	- - -	- - -
	Node Internode Leaf	MS + BAP (1) + NAA (0.2)	20-25 - -	Whitish, Compact	85 ±1.5 - -
	Node Internode Leaf	MS + BAP (1) + NAA (1)	15-20	Whitish, Hard, Compact	85 ± 1.0 - -
	Node Internode Leaf	MS + BAP (1)	10-15 15-20 25-30	Yellowish green, hard, Compact	95±1.0 85±1.5 75±1.0

Plant selected	Explant	MS medium + Phytohormones (mg/l)	Duration of Response (Days)	Nature of Callus	% of Response
Aloe vera	Node/stem Leaf Root	MS + BAP (3) + NAA (0.75)	20-25 - -	Whitish green, soft, Friable	80±1.5 - -
	Node/stem Leaf Root	MS + BAP (2) + IAA (0.5)	20-25 - -	Bright green, soft, Friable	80±1.0 - -

Table B: Direct multiple shoot induction on MS medium supplemented with various plant hormones.

Plant	Types of Explants	MS Medium+ Plant Hormones (mg/l)	No. of Inoculation	% of Response	Duration of Responses (Days)	No. of Shoots
Adhatoda vasica	Node Internode Leaf	MS+ BAP (1)	10 10 10	95±1.5 - -	13-15	8-9
	Node Internode Leaf	MS+ BAP (2) +NAA (0.5)	10 10 10	85±0.5 - -	20-25	3-4
	Node Internode Leaf	MS+BAP (1) + NAA (0.5)	10 10 10	70±1.5 - -	25-30	2-4
	Node Internode Leaf	MS+BAP (10)	10 10 10	90±0.50 - -	17-20	6-7
Aloe vera	Stem/Node Leaf Root	MS+BAP (3) + NAA (0.2)	10 10 10	75±1.5 - -	45-50 - -	2-3
	Stem/Node Leaf Root	MS+BAP (4) + NAA (1)	10 10 10	90±0.5 - -	40-45 - -	3-4

Table C: Indirect Multiple shoot induction in Adhatoda vasica and Aloe vera on MS medium supplemented with Phytohormones with different concentration.

Plant	Types of Explants	MS medium + Hormones (mg/l)	No of inoculation	% of Response	Duration of response
Adhatod	Nodal Callus	MS + BAP (1.5)	10	90±1.5	8-9

Plant	Types of Explants	MS medium + Hormones (mg/l)	No of inoculation	% of Response	Duration of response
Aloe vera	Node/Stem Segment Callus	MS + BAP (3) + NAA (0.75)	10	90±1.0	40-45

Table D: Root formation on MS medium in addition with different concentration of IBA

Plant	MS medium + Hormone conc, (mg/l)	No. of inoculation	% of Response
Adhatoda vasica	MS+ IBA (1)	5	50±1.5
	MS+ IBA (0.2)	5	-
	MS+ IBA (0.1)	5	-
Aloe vera	MS+ IBA (1)	5	55±1.0
	MS+ IBA (0.2)	5	-
	MS+ IBA (0.1)	5	-

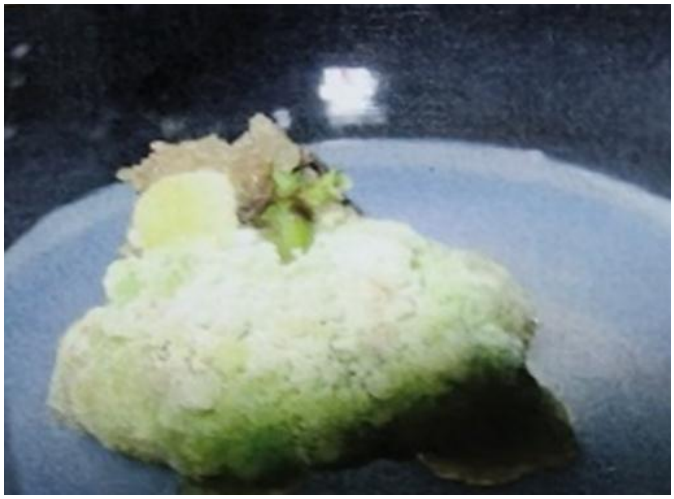
IV. CONCLUSION

With the present study of in vitro propagation and multiplication of two medicinally important plant Adhatoda vasica and Aloe vera it is possible to propagate using nodal segment on MS media with different concentration of phytohormones specially BAP alone or with combination of NAA, IBA and IAA. The data of standardise protocol of different hormone concentration indicate their response for in vitro culture of these plant which can improve the aseptic regeneration and disease-free plantlets in the short period of duration and it is also helpful to maintain the nature equilibrium. BAP and NAA combination is found to be best for shoot organogenesis whereas IBA (1mg/l) responded well for root formation. Plant tissue culture is well established technique for the multiplication of essential plant with medicinal properties also play important role for enhancing commercial gardening and cultivation, and for the production of secondary metabolites of industrial value.

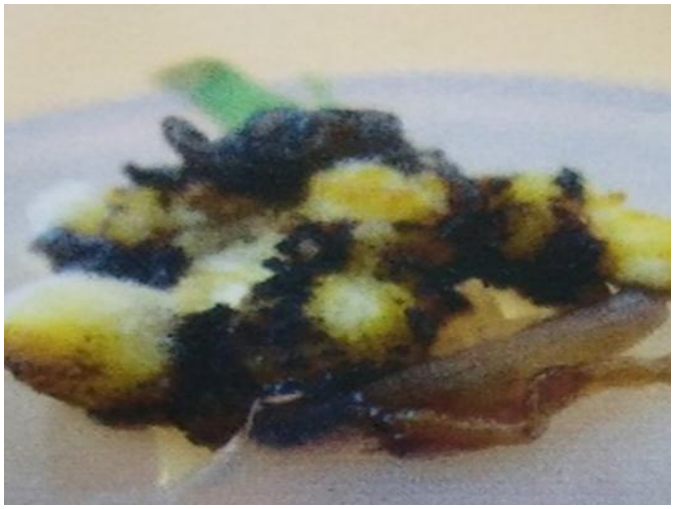
Photo Plate I

A

B



C



D

E

F

G

H

I

J

Photo Plate I: A- Adhatoda vasica (C-Callus induction,E-Direct shoot formation,G-Indirect shooting,I-Rooting),B- Aloe vera(D- Callus Induction, F- Direct shooting, H-Indirect shoot formation,J-Rooting).

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