ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 04, pp.14840-14842, April, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

AN EFFECTIVE PROTOCOL FOR THE IN VITRO CONSERVATION OF AN IMPORTANT MEDICINAL PLANT, *Aloe vera*

***Umesh, B.T. and Febina Ferose**

*MES College, Marampally, Aluva

ARTICLE INFO

Article History:
Received 04th January, 2015
Received in revised form
22nd February, 2015
Accepted 20th March, 2015
Published online 30th April, 2015

Key words:
Micropropagation,
Aloe vera, MS Medium,
contamination free cultures.

ABSTRACT

Aloe vera belongs to the family Liliaceae, commonly called as 'Burn plant'. In this study the basal medium used for the culture is Murashige and Skoog with sucrose 3% and 0.08% Agar, Growth hormones, 6-benzyl amino purine (BA), 3-Indole butyric acid (IBA), Kinetin (Kn), Adenine Sulphate etc. were added to the basal medium either singly or in various combinations. For shoot proliferation, BA (0-2 mg/L) and Kn (0-1 mg/L) at different concentrations in combination with IBA (0.2 mg/L), and agar (0, 0.8%) used. *Aloe barbadenesis* propagates vegetatively in its natural state, but propagation is too slow for commercial plant production. Shoot with young leaves was collected from the elite Micro shoots were inoculated on MS basal medium with different concentrations and combinations of BA and Kn (in combination of IBA 0.2 mg/L) for shoot proliferation. In both BA and Kn the plants were free from both fungal as well as bacterial contamination to some extent. After 15 days of inoculation, rooting was 100% in hormone- free medium. In case of hormone- free medium, roots were more thick and elongated, while the roots on hormone supplemented medium were thin and less elongated

INTRODUCTION

Aloe vera belongs to the family Liliaceae, commonly called as 'Burn plant'. It is extensively used as active ingredients in laxative, anti-obesity preparation, as a moisturizer, emollient, wound healer, in various cosmetic and pharmaceutical formulations (Abrie and Staden, 2001). It is a drug as well as a cosmetic. It contains Anthraquinones (aloin, *Aloe* - emodin), Resins, Tannins, Polysaccharides etc. The principal constituent of aloin is water-soluble crystalline glycoside barbaloin (Chaudhuri and Mukundan, 2001, Gui and Xu, 1990). The technique of tissue and organ culture is used for rapid multiplication of plants, for genetic improvement of crops, for obtaining disease- free clones and for preserving valuable germplasm (Bhojwani and Razdan, 1992). One of the major applications of plant tissue culture is micropropagation or rapid multiplication. Compare to conventional propagation, micropropagation has the advantage of allowing rapid propagation in limited time and space (Gamborg and Phillips, 1998). Although *Aloe barbadenesis* propagates vegetatively in its natural state, but propagation is too slow for commercial plant production (Meyer and Staden, 1991). To overcome slow propagation rate, micro propagation will be a very useful technique for mass multiplication of *Aloe* (Roy and Sarkar, 1991). The sources of contaminated cultures usually are difficult to determine. Bacteria which contaminate plant cultures may originate from explants, laboratory environments, operators, mites and thrips, or ineffective sterilization techniques.

Bacteria are associated with plants as epiphytes or endophytes. Explants from field-grown plants, diseased specimens or from plant parts which are located close to or below the soil may difficult or impossible to disinfect due to both endophytic and epiphytic microbes. Contaminants of greenhouse-grown plants are mostly those associated with soil and may originate from irrigation water (Kumar and Kumar, 1996).

****Corresponding author: Umesh, B.T.***
MES College, Marampally, Aluva.

MATERIALS AND METHODS

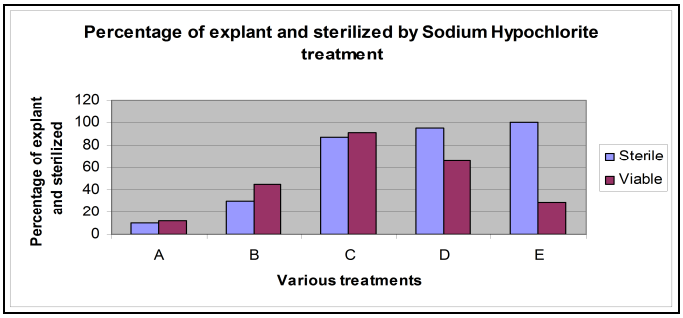
Shoot with young leaves was collected from the elite plants. The extra leaves were removed and shoot was trimmed to size of 2-3 cm for further work. Explants were surface sterilized. The basal medium used for the culture is Murashige and Skoog medium (MS, 1962) with sucrose 3% and 0.08% Agar, Growth hormones,6-benzyl amino purine (BA), 3-Indole butyric acid (IBA), Kinetin (Kn), Adenine Sulphate were added to the basal medium either singly or in various combinations (Natali *et al*., 1990). The concentrated stock solutions of all the ingredients were prepared and pH of the medium was adjusted to 5.8 by using 1N KOH or 1N HCl. After adjusting the pH, agar is added to the medium at the rate of 0.08% w/v for solidification of the medium. After sterilization of explants, explants were inoculated to culture bottles containing MS medium with

0.2 mg/L BA and 0.2 mg/L IBA. For shoot proliferation, BA (0-2 mg/L) and Kn (0-1mg/L) at different concentrations in combination with IBA (0.2 mg/L), and agar (0.08%) used. After 28 days of culture period of the explants with newly formed shoots were taken out under strict aseptic conditions and were excised from the parent plant with help of sterile scalpel blade and sterile forceps and inoculated into new bottles containing solid medium with different set of growth hormones as mentioned earlier. Newly formed shoots were excised individually from the parent explants and transferred to rooting media. Three types of rooting media were used. One MS basal media without hormone and other MS basal media with hormone (IBA 1 mg/L). 3 - 5 shoots per culture bottle were used and 5 replicates were used per treatment. Data were recorded after 15 days of culture. All cultures were incubated under 16 hr photoperiod with light intensity of 2000-2500 lux (36W and temperature of 25 ± 1^0C).

Establishment of Contamination free Culture- An Alternative Safer Sterilization for Explants of *Aloe vera*

Different concentrations of Sodium Hypochlorite were prepared in 2.5% (A), 3.75% (B), 5% (C), 6.25% (D), 7.5% (E) with distilled water and 5 drops of Tween 80 in one litre of solution. Treated explants were innoculated in MS medium having different concentration of growth hormone both for shoot proliferation and root proliferation as described in following tables:

86.66% and 66.66% respectively and percentage of survived explants were 12.5%, 45.8% and 91.7% . Based on the results, treatment E which has the highest concentration among the treatments, no contaminations were observed. At the same time, only 29% of explants regenerated in treatment E.



Micro shoots were inoculated on MS basal medium with different concentrations and combinations of BA and Kn (in combination of IBA 0.2 mg/L) for shoot proliferation. In both BA and Kn were found to give the Plants were free from both fungal as well as bacterial contamination to some extent. Explants started to show signs of proliferation after two weeks of culture. New buds starts to appear from the axil of leaves of shoot explants and buds develop into shoots by 4 weeks of culture. It was found that BA gave better shoot proliferation than Kn. Hundred percent cultures showed shoot proliferation on BA containing medium. Adenine sulphate was also used to check whether it has any effect on shoot proliferation or not. It

Different concentration of hormones for root and shoot initiation

Shoot initiation			Root initiation	
Hormone			Media	Concentration of hormone
BA(mg/l)	NAA(mg/l)	Kn(mg/l)		
2	0.2	0.5	MS without hormone	O
2	0.2	1.0	MS + IBA	4.41g/l+1.0mg/l
1.5	0.2	1.5	MS + IBA	2.205g/l+0.5mg/l
2	0.2	1.5	-	-
0.5	0.2	0.25	-	-

RESULTS AND DISCUSSION

After 5 to 10 days, tubes contained bacterial contamination and browning.

Percentage of contamination in culture tubes

CULTURE TUBES	1	2	3	4	5
Contamination after 5 days	35%	49%	38%	56%	40%
Contamination after 9 days	67%	70%	70%	79%	85%

In the earlier studies *Aloe vera* has been sterilized using the mercuric chloride which is harmful for both the explants and environment. This method is easy and new for explants sterilization for in vitro culture of *Aloe vera*. The number of contaminated explants kept increasing until the fourth week in A and B treatments (Sodium Hypochlorite 2.5% and 3.75%). In treatments C and D few contaminations were seen but less contamination were seen in treatment E. After two weeks of observation, all explants gave axenic cultures. By adopting safer sterilization process, the explants showed positive result ie; shoots initiation. Percentages of dead explants due to contamination effect in treatments A, B and C were 95.45%,

was observed that adenine sulphate has no significant effect on shoot proliferation in *Aloe vera*. Three to four centimeters long shoots were excised individually from the proliferated shoot and cultured on rooting medium. The shoots inoculated on hormone –free (medium lacking IBA) and IBA supplemented medium showed rooting response within a week of inoculation. However, the response was better in hormone- free medium. After the 15 days of inoculation, rooting was 100% in hormone- free medium. In case of hormone- free medium, roots were more thick and elongated, while the roots on hormone supplemented medium were thin and less elongated. There was no difference in colour of roots. In both the cases colour of roots was creamish yellow. The micro shoots inoculated on solid medium showed better rooting response.

Contamination in culture tubes **Micropropagated plant**

Effect of hormones on MS media for shoot and root initiation

Shoot proliferation				Root proliferation		
BA (mg/l)	NA (mg/l)	Kn (mg/l)	% of response	MS with out hormone	MS + IBA	% of response
2	0.2	0.5	20%	MS only	-	100%
2	0.2	1.0	45%	-	4.4g/l+1.0mg/l	10%
1.5	0.2	1.5	15%	-	2.205g/l+0.5mg/l	8%
2	0.2	1.5	0	-	-	-
0.5	0.2	0.25	10%	-	-	-

REFERENCES

Abrie, A. and Staden, J.V. 2001. Micropropagation of endangered *Aloe polyphylla*, Plant Growth Regulation, 33(1): 19-23.

Bhojwani, S. S. and Razdan, M. K. 1992. Plant Tissue Culture: Theory and Practice. Elsevier, Amsterdam, London, New York, Tokyo.

Chaudhuri, S. and Mukundan, U. 2001. *Aloe Vera* L.—Micropropagation and Characterization of its gel, Phytomorphology, 51(2): 155-157.

Gamborg, O.L. and Phillips, G.C. 1998. Plant cell, Tissue and Organ Culture: Fundamental methods, Narosa publishing house New Delhi.

Gui, Y.L. and Xu, T.Y. 1990. Studies on stem tissue culture and organogenesis of *Aloe vera*, Acta Botanica Sinica,32(8): 606-610.

Kumar, A. and Kumar, V.A. 1996. Plant Biotechnology and Tissue Culture Principles and Perpectives, International Book Distributing Co, Lucknow

Meyer, H.J. and Staden, J.V. 1991. Rapid in vitro propagation of *Aloe barbadensis* Mill., Plant cell, Tissue and Organ Culture, 26:167-171.

Natali, L., Sanchez, I.C. and Cavallini, A. 1990. In vitro culture of *Aloe barbadensis* Mill.: Micropropagation from vegetative meristems, Plant Cell, Tissue and Organ Culture, 20 :71-74.

Roy, S.C. and Sarkar, A. 1991. In vitro regeneration and micro propagation of *Aloe vera*, Scientia Horticulturae, 47(1-2).

****