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Studies in North Indian *Aloe vera*: callus induction and regeneration of plantlets

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Abstract

Aloe vera L. is a versatile medicinal plant with many cosmetic properties. Shoot disc explants of Aloe vera plants were cultured on MS medium with different phytohormonal supplements for callus induction. It has been concluded from the study that the best medium for callus induction is 1mg/l 2, 4-D+ 0.2mg/l Kinetin and subsequently for shoot proliferation medium is MS + 0.2mg/l BA + 0.2mg/l IBA. For rooting MS + 0.3mg/l NAA gives best result. Plants were regenerated from the callus. They were hardened, acclimatized and transferred in garden soil. Regeneration of plants from callus may help to induce variability in the Aloe germplasm for future improvement.

Key Words: *Aloe vera*, Growth hormones, MS medium, Callus induction.

INTRODUCTION

Aloe vera Linn. (=*A. barbadensis* Miller) belongs to family Liliaceae and is a perennial xerophytic plant with rosette of thick succulent leaves. Over 200 different biologically active substances have been identified from *Aloe* with many reported medicinal properties [1]. It has been shown that the plant have antiseptic, anti-tumoral, anti-inflammatory, anti-oxidant and immuno-stimulant activities [2]. Compounds from *Aloe* are also reported to show anti-bacterial [3], anti-viral [4], anti-fungal [5] and cell growth stimulatory activity [6]. The juice of plant is considered useful in gastrointestinal diseases and promotes healing [7]. This plant is an important medicinal plant with many cosmetic properties. Increased demand of *Aloe* biomass to meet the requirements of food and cosmetic industry has generated need to undertake large scale cultivation of the plant. *Aloe* is exclusively vegetatively propagated crop using lateral offshoots produced by the donor plant. A single plant produces 2-3 offshoots in a year which is not sufficient for undertaking commercial cultivation. In addition to this a higher incidence of diseases is expected due to lesions caused to donor plant after excising lateral shoots.

Raising the propagating material of *Aloe* through tissue culture has been advocated as a solution to above problems. Over the last years, a number of micropropagative protocols have been developed using a variety of explants like shoot tip [8], auxiliary buds [9], stem cuttings and leaf

explants [10]. The North Indian germplasm of *Aloe* is being evaluated at our lab. One of the local collections (PBP2) which has good gel content was selected for the study. Study has been conducted to standardize the protocol to multiply the plants through callus culture.

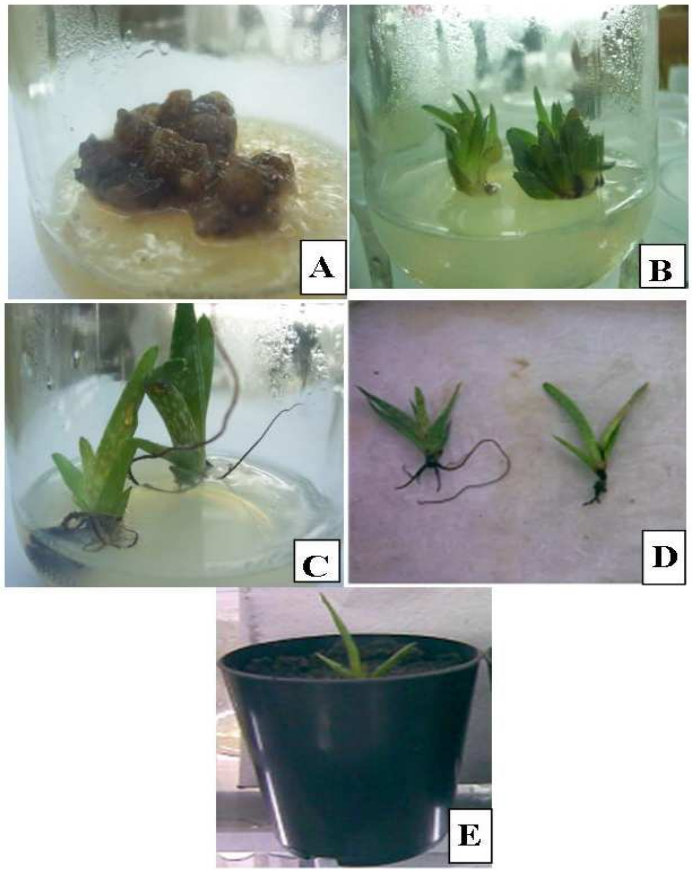

Fig 1. *In vitro* studies in *Aloe vera*

A. Callus on MS+2, 4-D (1mg/l) + Kinetin (0.2mg/l)
B. Plants regenerated on callus with MS+ IBA (0.2mg/l) + BA (1.0mg/l)
C. Plants with roots in rooting medium (MS + 0.3 mg/l NAA)
D. Plantlets with roots
E. Plants established on soil: rice husk (1:1)

MATERIALS AND METHODS

Shoot tip explants obtained from succulent garden at Botanic Gardens, Punjabi University Patiala were washed thoroughly in running tap water for 20 min and thereafter kept in detergent for 10 min. Explants were again washed with running tap water to remove any trace of detergent for 20 min and then treated with 1% Bavistin and 0.25% Dithane M-45 for 20 min. Further explants were taken inside laminar air flow and surface sterilization of explants was done with freshly prepared 0.1% $HgCl_2$ for 5-7 min. Then materials were thoroughly washed for 3-4 times with autoclaved distilled water.

After surface sterilization, the explants were trimmed and extra outer portion of stem disc were removed with a sterilized scalpel. Prepared explants were carefully inoculated in autoclaved

culture bottles containing MS medium with different phytohormonal supplements [11]. Twenty explants were used for each concentration. The cultures were kept in culture room under 16 h lights at 27±1°C.

RESULTS AND DISCUSSION

Shoot discs explants were cultured on different combinations of auxins and cytokinins. Among all the combinations best response was observed in MS medium supplemented with 2, 4-D (1.0mg/l) and Kinetin (0.2mg/l) where in 90% of the explants callus induction was noticed (Table1). On this medium formation of pale white colored callus was observed between 20-25 days of inoculation which subsequently reached a stationary phase. No callus induction was observed on rest of the explants even after one month.

Table 1: Effect of supplementation of MS medium with different growth regulators on callus induction in *Aloe vera*

MS + Growth regulators (mg/l)				Callus formation (%)
2,4-D	NAA	Kn	BAP	
0.025	-	1.0	-	0
0.25	1.0	-	-	25
1.0	0.2	-	-	90
1.0	1.0	-	-	15
-	-	-	2.0	0

Table 2: Effect of different growth regulators on shoot proliferation

MS+Growth regulators (mg/l)			Explants with shoot proliferation	Number of shoots/explant
IBA	BA	IAA		
0.2	0.2	-	100%	1-2
0.2	0.8	-	100%	2
0.2	1.0	-	100%	5+
0.3	0.3	-	100%	1-2
-	2.2	0.1	100%	1-2

The various callii obtained were sub cultured on shoot proliferation medium. The callus on sub culturing showed signs of proliferation after two weeks. On almost all the callii, bud appeared and developed into shoots by 20th day of culture. Higher number of shoots per callus (5-7) was observed on medium containing 0.2mg/l IBA+1.0mg/l BA (Table 2).

The proliferation of callus into leaf/shoot was slow in remaining combinations of IBA, BA and IAA where 1-2 shoots/callus were produced. It is found that MS medium supplemented with 2mg/l BA+0.3mg/l NAA as the best medium for micro propagation of *Aloe vera* [12]. Presence of both auxins and cytokinins is necessary for shoot proliferation [13]. Studies conducted by different workers clearly indicated that BA is more effective than NAA for shoot proliferation in *Aloe vera* [14, 15], IBA [16] and acetic acid [17] are also reported to be helpful in shoot proliferation in *Aloe*. The regular maintenance of callus by sub culturing on fresh callus inducing media again also showed its differentiation into shoots but it took nearly 40-45 days.

The *in vitro* raised shoot tips were cultured on rooting media where MS medium was supplemented with different concentrations of NAA. All the combinations showed induction of

roots. Maximum numbers of roots (5-8) per plant were obtained in plantlets cultured on MS+0.3mg/l NAA. The plantlets cultured on MS medium supplemented with 2mg/l to 10mg/l NAA showed induction of only one root per shoot. Earlier, reported rooting in hormone free medium [18, 19]. Present observations do not support these observations as no rooting was observed in the shoot tips cultured on MS medium alone.

After sufficient rooting, fully regenerated plants were removed, washed and transferred to plastic pots containing soil and rice husk (1:1) for hardening. For 10 days plantlets were kept in culture room for acclimatization. These plants were watered manually everyday. Then these plants were transferred to green house for 15 days. After that the plants were transferred in natural soil. The plantlets survived to grow and were morphologically similar to mother plants. Some of the scientists have proposed a mixture (1:1) of soil and sand, cocopeat and perlite [8], soil and farmyard manure [18] and soil, sand and perlite or vermiculite [19].

CONCLUSION

The present study reveals the importance of growth regulators in callus initiation, stem proliferation and plant rooting for successful use of the tissue culture for plant propagation. The plants regenerated through callus induction may help in generation of somaclonal variants that may enrich the germplasm for further utilization of this wonderful medicinal plant.

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