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CLONAL PROPAGATION OF *ALOE BARBADENSIS* MILL.: AN IMPORTANT MEDICINAL PLANT VIA APICAL MERISTEM CULTURE

B. S. BHATT, H. K. VADODARIA and R. P. VAIDYA*
Shri Hari Atmiya Centre for Post Graduate Studies, Rajkot- 360 005, Gujarat, India.
*Biology (Botany) Deptt., Shree Gyanganga College of Science and Management, Rajkot- 360 005, Gujarat, India.
E mail: s_bhavin18@yahoo.co.in

Plants and plant based products have been used traditionally by native inhabitants in India from ancient times. India continues to occupy premier position in the use of drugs of plant origin. Now a days, main problem with using herbal medicine is the net productivity of such plants in nature which don't fulfill requirements of large growing populations. Hence it is crucial to find out alternative solution for mass propagation of such plants at low cost and in a short time. One of such alternative is tissue culture through which rapid productivity of plant and plant based products can be achieved[1]. Aloe, a member of Liliaceae (Aloeaceae) family, is a rossete plant. The roots of plant known to possess important biological properties such as anti inflammatory, antipyretic, antibacterial, antiallergic etc. The demand for *Aloe barbadensis* Mill. product either as a dried root or as formulation is increasing. This call for an urgent need to mass propagate the species as its natural propagation is rather slow for commercial purposes[2]. The objective of the present communication is to develop a successful clonal propagation procedure from apical meristems of *A. barbadensis.*

Keywords: *Aloe barbadensis*; Clonal propagation; Medicinal plant.

Introduction

In recent years there has been genuine interest in natural medicines, mainly from herbal source. Even western countries are curious about medicinal plants and are trying to exploit them commercially. With the varied agro climatic zones, India has rich diversity of medicinal herbs. *Aloe barbadensis* Mill. belongs to family *Liliaceae* (*Aloeaceae*). The species don't produce any viable seeds and are propagated by vegetative means. The roots of plant known to possess important biological properties such as anti inflammatory, antipyretic, immunomodulatory, antibacterial, antiallergic etc. Alentoin acid, present in this species, used as a catharatic agent and as a laxative along with other components in many countries including India[3]. Based on these biological properties, a number of formulations are widely available in the market, for the treatment of skin disorders, wound healing, inflammation based disorders and health food. The demand for *Aloe barbadensis* product either as dried root or as formulations is increasing. Plant tissue culture offers the possibility of rapid clonal propagation and immediate conservation of invaluable germplasm[4]. Therefore, we describe here an efficient procedure for the rapid clonal propagation of *Aloe barbadensis* through apical meristem culture, which often used to produce disease free stock of plants[5-7].

Materials and Methods

Plant material and explant source: Ten-cm. long suckers with apical meristem were collected from the field (Fig. 1A). The suckers with apical meristem, after removing the mature leaves, were sterilized, by rinsing for 20 minutes in a 2% aqueous solution of 'Teepol' (Qualigen, India) followed by thorough rinsing with 0.1% (w/v) mercuric chloride for 5 minutes. Thereafter, the explants were thoroughly washed in sterile double distilled water for at least three times followed by washing with 70% (V/V) alcohol for 30 seconds and then it was thoroughly washed with sterile distilled water for at least two times. The explants were cut into four pieces prior to inoculation.

Culture medium and incubation condition: MS medium was used as a basal medium as it is the most widely used in meristem cultures[5,8,9]. The explants were placed on semi-solid basal half strength MS medium supplemented with BAP (1.5, 2.0 and 2.5 mg/l), KiN (1.5, 2.0 and 2.5 mg/l), IBA (0.01, 0.05 and 0.10 mg/l), IAA (0.01, 0.05 and 0.10 mg/l) and Ads (10, 15 and 25 mg/l) for shoot multiplication. The pH of the media was adjusted to 5.8 using 0.1 N HCl or 0.1 N NaOH before autoclaving. The basal salts and growth regulators were solidified with 0.6 % (w/v) agar (Qualigen, India) as a basal culture media. Routinely, 25 ml molten media was dispensed in to culture tubes (25 x 150 mm),

plugged with non-absorbent cotton wrapped in paper and sterilized at 121°C and 15 lb/in² pressure for 15 min. The inoculated cultures were exposed to continuous illumination with cool white fluorescent light in a photoperiodic cycle of 16 h. Since primary cultures can't be maintained for long periods[10-12], the cultures were maintained by regular subculturing at 4-week intervals.

Induction of rooting: Micropropagated shoots (2-3 cm) were excised from the parent culture and transferred to half-strength MS semi-solid medium with 20 g/l sucrose[13] with different concentrations (0.01, 0.10 and 0.50 mg/l) of IAA or IBA or NAA(0.01, 0.10 and 0.50 mg/l). One excised shoot was cultured in each tube (25 x 150 mm) containing 15 ml of medium. The incubation conditions were the same as those used for shoot multiplication. The pH of the medium was adjusted to 5.8 prior to autoclaving.

Acclimatization and hardening: Shoots which formed roots were removed from culture tubes, washed gently with sterilized distilled water and planted in small polyethylene bags containing a sterile mixture of sand : soil : decomposed cow-dung manure (1:1:1; v/v/v). To prevent the potting mixture from desiccation, adequate amount of half strength MS basal salt solution without sucrose was added and kept at 28±2°C and 80% humidity for acclimatization before subsequent transfer to soil[14, 15]. After two weeks, the plantlets were transplanted into bigger pots and kept in the open under partial shade for one week before being fully exposed to natural light.

Observation of cultures: Each experiment contained 15 replicates per treatment and each experiment was repeated thrice. The data pertaining to clonal propagation, callus growth, shoot bud regeneration, root induction were statistically analyzed of the mean of 15 replicates and their standard error (S.E.).

Results and Discussion

Multiplication of shoots: For shoot multiplication, half strength MS media supplemented with combinations of cytokinins and auxins. The result showed that the inclusion of auxins into the culture medium enhanced the rate of multiplication as compared to the medium containing cytokinin alone. The medium with BAP, adenine sulfate and IBA helped in the rapid multiplication of shoots. The rate of shoot multiplication was the maximum on the medium containing 2.5 mg/l BAP + 2.0 mg/l KiN + 0.1 mg/l IBA + 0.01 mg/l IAA + 15 mg/l Ads (Table 1) and a maximum of 8.8 shoots were produced per explant within 4 weeks of initial culture (Fig. 1B). The shoot multiplication rate declined on medium containing lower concentration of BAP and KiN. The frequency of multiplication increased up to 6 fold after 8th sub-culture (Fig. 1C).

Rooting medium: The success of propagation of plants depended largely on a good root system of the micropropagated shoot for better field establishment and growth. Three auxins NAA, IAA and IBA were tested alone or in combinations at 0.05, 0.1, 0.25 and 0.5 mg/l concentration in the medium supplemented with half strength MS along with 20g/l sucrose for rooting of the micropropagated shoot of *A. barbedensis*. There was no root formation in the media devoid of auxins. About 90-95% of the excised shoots were rooted within 7-8 days on medium containing different concentrations of auxins (Table 2) (Fig. 1D & 1E). At higher concentration of IAA, IBA, and NAA, the rate of root initiation declined rather callus developed at the basal ends. IBA induced poor rooting and the maximum percentage of rooting (93.2%) and the maximum number of root per shoot were observed on media containing 0.5 mg/l NAA. The average number of roots/shoot significantly varied with different concentrations of IAA, IBA and NAA.

Acclimatization and field establishment : The general schedule followed for acclimatization included transfer of thoroughly washed rooted plantlets from agar culture to small earthen pots containing sterile mixture of sand: soil: cow dung manure at the ratio of 1:1:1 and kept in the culture room for acclimatization before subsequent transfer to soil. After two weeks, the plantlets were transplanted into bigger pots and kept in the open under partial shade for one week before being fully exposed to natural light. About 90-95% of the micropropagated and regenerated plantlets were grown normally (Fig. 1F).

The present study revealed that there was distinct effect of growth regulators of shoot multiplication of *A. barbadensis* and BAP in combination with adenine sulfate was found to be the most suitable cytokinin for regeneration of shoot buds from apical meristem and shoot multiplication. Inclusion of auxin like IAA and IBA in the culture medium enhanced the rate of multiplication and shoot growth. The rate of shoot multiplication was the maximum on medium containing 2.5 mg/l BAP + 2.0 mg/l KiN + 0.1 mg/l IBA, 0.01 mg/l IAA + 15 mg/l Ads and maximum of 8.8 shoots were produced per single explant within 4 weeks of initial culture. Similar trends were reported for several medicinal herbs using cytokinins and auxins at low concentrations[16-20].

The number of plantlets producing roots, the number of days required for root initiation and the average root growth in the micro shoots of *A. barbadensis* depended on auxin, sucrose and mineral concentrations in the culture medium. Root formation did not take place in any of the micro shoots when the shoots were cultured

Fig. 1 A. Aerial view of an elite plant *A. barbadensis.* B: Shoot multiplication and elongation of *A. barbadensis* in ½ MS + 2.5 mg/l BAP + 2.0 mg/l KiN + 0.1 mg/l IBA + 0.01 mg/l IAA + 15 mg/l Ads after 3 - 4 weeks of culture. C: Multiplication of *A. barbadensis* in ½ MS + 2.5 mg/l BAP + 2.0 mg/l KiN + 0.1 mg/l IBA + 0.01 mg/l IAA + 15 mg/l Ads increased up to 6 fold after 8th sub-culture. D, E: Rooting in the *in vitro* derived shoots of *A. barbadensis* after 8-d of culture in ½ MS + 0.5 mg/l NAA + 2% sucrose. F: *In vitro* Raised plantlets of *A. barbadensis* grown in polybags.

Table 1. Effect of cytokinins and auxins on shoot multiplication of *Aloe barbadensis* after 4 weeks of culture.

½MS + Growth regulators (mg/l)					% of culture with multiple shoots	Number of shoots/culture (Mean ± S.E)*
BAP	KiN	IBA	IAA	Ads		
0	0	0	0	0	0	0
1.5	0	0.01	0	0	42.5	4.2 ± 0.7
2.0	0	0.05	0	10	56.7	4.6 ± 0.8
2.5	0	0.1	0	10	62.3	5.2 ± 0.6
0	1.5	0	0.01	15	33.2	3.7 ± 0.6
0	2.0	0	0.05	10	46.8	4.4 ± 0.7
0	2.5	0	0.1	15	50.4	4.5 ± 0.8
1.5	1.0	0.01	0.01	10	68.6	5.6 ± 0.8
2.0	1.5	0.05	0.05	15	82.4	7.2 ± 0.6
2.5	**2.0**	**0.1**	**0.01**	**15**	**90.8**	**8.8 ± 0.8**
2.0	**2.5**	**0.1**	**0.1**	**15**	**85.0**	**7.8 ± 0.5**
2.5	2.5	0.1	0.1	25	65.2	5.4 ± 0.8

*Mean of 15 replicates per treatment; experiment repeated thrice.

Table 2. Effect of IAA, IBA and NAA on rooting of micropropagated shoots of *Aloe barbadensis* cultured on ½ MS supplemented with 2% (w/v) sucrose.

½ MS + Growth regulators (mg/l)			% of shoot rooted	Average number of roots/shoot (Mean ± S.E)*	Days to rooting
IAA	IBA	NAA			
0.00	0.00	0.00	0	0	0
0.01	0.00	0.00	38.4	3.1 ± 0.2	09-10
0.10	0.00	0.00	56.2	4.8 ± 0.3	08-09
0.50	**0.00**	**0.00**	**89.6**	**3.4 ± 0.6**	**07-08**
0.00	0.01	0.00	0	0	0
0.00	0.10	0.00	0	0	0
0.00	**0.50**	**0.00**	**12.4**	**1.6 ± 0.4**	**08-09**
0.00	0.00	0.01	52.7	2.8 ± 0.3	10-11
0.00	0.00	0.10	41.4	2.6 ± 0.4	09-10
0.00	**0.00**	**0.50**	**93.2**	**8.2 ± 0.3**	**09-10**

*Mean of 15 replicates per treatment; experiment repeated thrice.

on media devoid of growth regulators. The effectiveness of NAA on rooting in microshoots was reported[21]. The maximum percentage of rooting and the maximum number of roots per shoot were observed on media containing low concentrations of NAA (0.5 mg/l). Similar results were obtained in case of *Saussurea lappa*[22]. The media containing auxins stimulated the induction of roots as reported earlier in other medicinal plants[17,20,23]. This study demonstrates a successful clonal propagation system for *Aloe barbadensis:* an important medicinal plant via apical meristem culture.

(Abbreviations: BAP - 6-benzyl aminopurine; KiN - Kinetin; IAA - Indole-3-Acetic Acid; IBA - Indole-3-Butyric Acid; NAA - α – Naphthalene Acetic Acid; MS - Murashige and Skoog; Ads - Adenine sulphate; °C - Degrees centigrade; cm - Centimeter; g/l - Gram (s)/liter; mg/l - Milligram/liter; mm - Millimeter; % - Percent/ percentage; lb/in^2 - Pound per inch^2).

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