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Micropropagation of *Aloe barbadensis* Mill. Through *In vitro* Culture of Shoot tip Explants

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Abstract

Multiple shoots (ten per explant) in *Aloe barbadensis* were obtained from shoot tip explant cultured on MS supplemented with BAP (2.0 mg/l) and NAA (0.5 mg/1). About 95% rooting was obtained from micro-shoots cultured on half strength MS supplemented with NAA (0.5 mg/1). Well-developed rooted plantlets were successfully transferred to the soil with 70% survival.

Introduction

Aloe barbadensis Mill. (= *Aloe vera* L.), an important perennial medicinal herb belongs to the family Liliaceae. The genus comprises about 300 perennial species (Reynolds 1985). The juice from the leaves of different species yields a medicinal substance called the 'Aloe' drug. The drug contains anthracene derivatives occurring either free or in the form of glycosides, usually containing glucose (Fairbrain 1949; Capasso and Donatelli 1982). It is used as emetic and anthelmintic, and in the treatment of jaundice, loss of appetite, gas formation in the stomach, leucorrhoea, menstrual suppression, piles, rectal fissures, inflammations, ulcers, burns and scalds (Ghani 1998). As the plant is extensively used on herbal medicine and cosmetic industry, its demand is dramatically increasing. The plant is generally propagated by means of suckers arising from the base of the mother plant. The natural vegetative propagation of *Aloe* is very slow. An alternative might be the use of *in vitro* propagation for rapid multiplication of selected genotypes. *In vitro* culture of different *Aloe* species was reported by many researchers (Yagi et al. 1983; Castorena Sanchez et al. 1988; Chaudhuri and Mukundan 2001). In spite of the importance of *Aloe* in industrial fields, micropropagation of *Aloe barbadensis* in Bangladesh has not been reported so far. The present paper reports the development of a protocol on mass propagation of *Aloe barbadensis*, doable all the year round.

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Materials and Methods

The experiment was conducted at Biological Research Division in Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh, during 2003 - 2004. Young shoot tips of the suckers were collected from the field of BCSIR, Dhaka, and used as primary explants. Explants were washed under running tap water for ten to 15 min and then surface-sterilized with 0.1% $HgCl_2$ for 10 min followed by five to six rinses with sterilized distilled water. The outer leaves were discarded and inner shoot apices were inoculated either in 25 mm × 150 mm culture tubes or 100 ml/250 ml Erlenmeyer flasks containing MS supplemented with various combinations and concentrations of auxins and cytokinins. Before inoculation, the media were gelled with agar (0.6%), pH was adjusted to 5.8 and autoclaved at 121^{0}C for 15 min. The flasks containing explants were then incubated at 25 ± 0.5^{0}C, under fluorescent tube light with 16 h photoperiod. Subcultures were made at three - four weeks interval. Results were recorded for each culture before subculture. The number of replications was 25 for each experiment with three replications. From each subculture, microshoots (more than 1.0 cm long) were separated out and implanted in the rooting medium containing half strength MS with various concentrations and combinations of auxins for root induction.

For hardening, the culture tubes containing rooted shoots were kept at normal room temperature and light for 14 days. Thereafter, the rooted shoots were removed from culture tubes, washed thoroughly to free agar from roots and transplanted to small pots containing soil and sand (2 : 1 v/v). Plantlets were well-covered with a piece of polythene sheet for three weeks to ensure high humidity while watering was continued regularly. After three months the plantlets were transferred to the open field.

Results and Discussion

Shoot tip explants were cultured on MS supplemented with various concentrations and combinations of BAP, NAA and BAP alone for induction of adventitious shoots (Table 1). The best and rapid regeneration was observed on MS supplemented with 2 mg/l BAP + 0.5 mg/l NAA. This treatment yielded highest number (75) of regenerated shoots with ten shoots per culture. The average length of shoots per culture was 4.0 ± 0.16 cm. The shoot tip explants initially produced three - five shoots within three - four weeks after inoculation. Subculture in the same medium, yielded a cluster of eight - ten shoots per explant (Fig. A). Cluster of shoots were separated into pieces and each was subcultured individually on the same medium. After fourth subculture the shoot multipli-cation rate remained constant. On the other hand, regeneration of shoot buds was low (50%) on a medium containing 0.5 mg/l BAP and 0.5 mg/l NAA and the number of shoots per explant was 3.2 ± 0.81. Comparatively a lower number of adventitious shoots were observed in the medium containing BAP

alone. Further increase in the concentration of BAP had no effect on the number of multiple shoots. At the highest concentration (4.0 mg/l) of BAP with (0.5 mg/l) or without NAA did not increase shoot proliferation. In contrast, in *Crinum macowanii* (Amaryllidaceae), the enhanced level of BAP had a stimulating effect on the total number of regenerated plantlets (Slabbert et al. 1993). The results reported in this paper confirm that some plant species have enough levels of endogenous hormones and do not require any extra amount of exogenous growth regulators for their regeneration (Hussey 1982). The lateral buds developed into shoots 10 - 15 days after inoculation. Liao et al. (2004) also reported that a combination of BAP and NAA enhanced the multiple shoot proliferation from shoot tip explants of *Aloe barbadensis*. The present findings are in agreement with those observed in other plant species such as *Asparagus* sp. (Yang 1977), *Ruscus hypophyllum* (Jha and Sen 1985), *Rehum emodi* (Lal and Ahuja 1989). Liao et al. (2004) showed the same results in MS supplemented with 2.0 mg/l BAP, 0.3 mg/l NAA and 0.6 mg/l PVP. In contrast, the best shoot multiplication medium reported by Chaudhuri et al. (2001) is MS supplemented with 10 mg/l BAP + 160 mg/l Ads + 0.1 mg/l IBA. This might be due to genotypic variation of explants reinforced by the cultural and environmental conditions.

Table 1. Effects of different concentrations and combinations of BAP and NAA on multiple shoot formation in *Aloe barbadensis*.

Growth regulators (mg/l)	% of explant producing shoots	Number of shoots/ explant	Mean length of shoots (cm)
BAP			
0.5	20	2.1 ± 0.31	1.0 ± 0.10
1.0	25	2.4 ± 0.25	1.8 ± 0.24
2.0	30	3.2 ± 0.52	2.2 ± 0.51
4.0	25	3.0 ± 0.50	2.5 ± 0.50
BAP + NAA			
0.5 + 0.5	50	3.2 ± 0.81	2.8 ± 0.31
1.0 + 0.5	60	4.6 ± 0.52	3.2 ± 0.50
2.0 + 0.5	75	10.1 ± 0.50	4.0 ± 0.16
4.0 + 0.5	60	4.8 ± 0.28	2.8 ± 0.52

Each treatment consists of 25 replicates.

Root formation was induced in *in vitro* regenerated shoots by culturing them on half strength of MS supplemented with 0.5 to 1.5 mg/l of either of the three hormones IBA, NAA or IAA (Table 2). Root formation was not observed when shoots were cultured on a medium lacking auxin. Among the three types of

auxins NAA was found to be the best for root induction. In the medium with 0.5 mg/l of NAA, roots began to emerge from the tenth day of culture (Fig. B) and within a period of 23 - 28 days frequencies of root formation were 95%. The highest number of roots per shoot was 4.8 ± 0.53 with an average length of 3.5 ± 0.35 cm. The roots that developed in the medium containing higher concentration (1.0 - 1.5 mg/l) of auxins were poor in quality. The use of low dosage of NAA (0.5 mg/l) was found optimal, probably due to the genotypic and explant

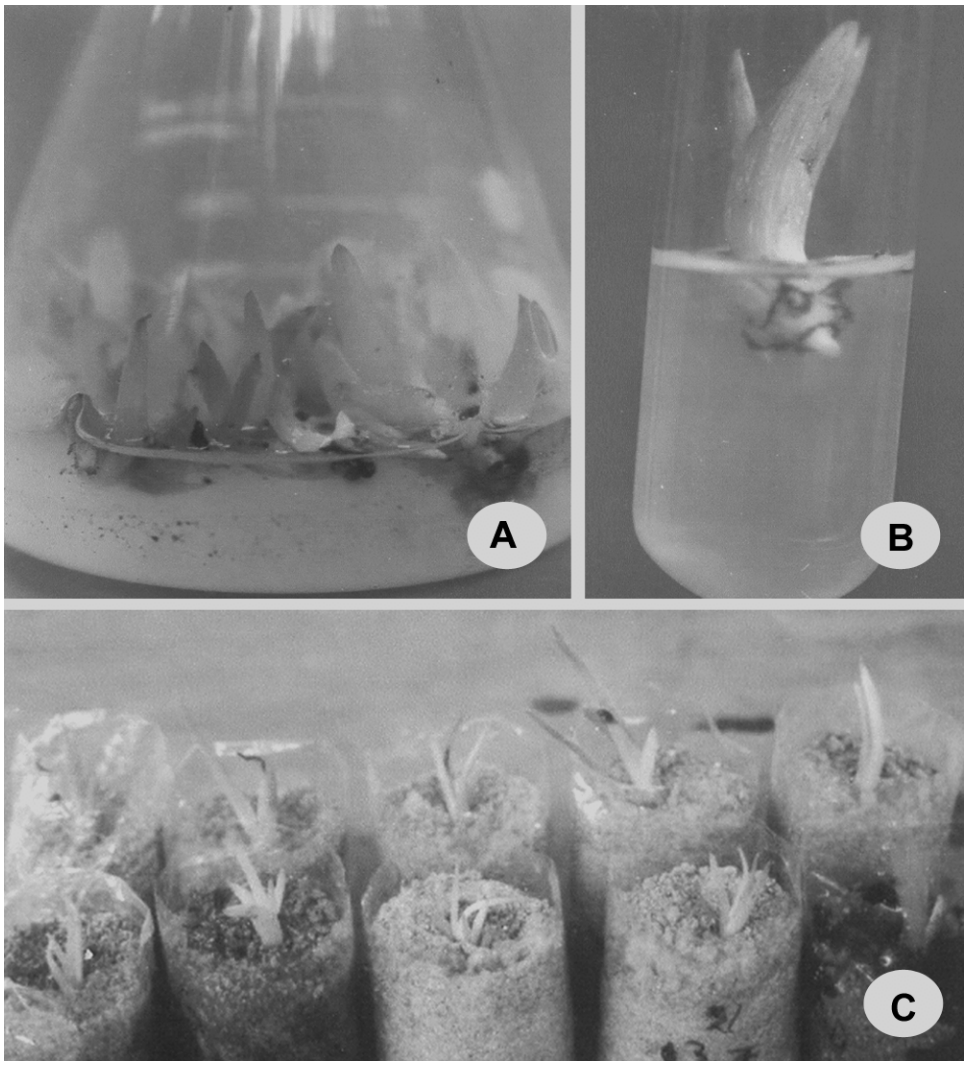

Fig. A. Regeneration of multiple shoots on MS + 2 mg/l BAP + 0.5mg/l NAA. Fig. B. Root induction in half MS + 0.5 mg/l NAA. Fig. C. Hardened plantlets transferred to soil.

specificity. The superiority of NAA over other auxins has also been reported in other plant species such as *Caphaelis ipecacuanha* (Jha and Jha 1989), *Plantago ovata*

(Wakhlu and Barna 1989), *Rehum emodi* (Lal and Ahuja 1989). Plants that were transferred directly to field did not survive. The plantlets with well-developed roots were transferred to polythene bags and the acclimated plants were finally transferred to soil with 70% survival (Fig. C). For successful micropropagation axillary buds or shoot tip cultures were preferred as pre-existing meristems were capable of developing into shoots with clonal fidelity. The protocol described in this paper is recommended for high frequency regeneration as well as for conservation of this important medicinal plant.

Table 2. Effects of various concentrations of IBA, NAA and IAA in half strength MS on rooting of microshoots in *Aloe barbadensis*.

Auxin conc. (mg/l)	% of shoot rooted	Number of roots/ shoot	Mean length of roots (cm)
IBA			
0.5	66	2.5 ± 0.22	1.5 ± 0.21
1.0	70	2.8 ± 0.41	1.8 ± 0.51
1.5	75	3.2 ± 0.51	2.2 ± 0.25
NAA			
0.5	95	4.8 ± 0.53	3.5 ± 0.35
1.0	85	3.0 ± 0.32	2.2 ± 0.21
1.5	80	3.2 ± 0.26	2.0 ± 0.51
IAA			
0.5	35	2.0 ± 0.15	1.0 ± 0.20
1.0	40	2.2 ± 0.35	1.5 ± 0.31
1.5	32	2.2 ± 0.55	1.9 ± 0.55

Each treatment consists of 25 replicates.

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