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HIGH FREQUENCY *IN VITRO* PROPAGATION OF *ALOE VERA* L. THROUGH SHOOT TIP CULTURE

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ABSTRACT: A high frequency *in vitro* plant regeneration in *Aloe vera*L. was obtained on MS medium supplemented with 2.0 mg/l BA + 0.5 mg/l NAA + 40.0 mg/l Ads using shoot tip as explants. Maximum 90% of the explants were produced multiple shoot in this medium. The average number of shoot per explant was 36.70 ± 2.25 and the average shoot length of 10.60 ± 2.70 cm were observed after 120 days of culture. Shoots rooted well when they were transferred into half strength MS + 1.0 mg/l IBA. The average number of root per shoot was 12.60 ± 0.45 and the average root length of 6.90 ± 0.40 cm were recorded in this medium after 30 days of culture whilst 90% shoots induced root.
Key Words: *In vitro* propagation, *Aloe vera*, Shoot tip, Multiplication.

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INTRODUCTION

Plant biodiversity is threatened all over the world at an unprecedented rate due to environmental perturbations, habitat loss and increased extinction rates (Lobine et al., 2015). Among them, *Aloe vera* L. is one of the economically important perennial herb, which is widely used in modern herbal practice and belongs to the family Liliaceae (Albanyl et al., 2006). It has been using worldwide in food, cosmetic and pharmaceutical industries, also in traditional medicine, due to the plethora of biological activities of some of its primary and secondary metabolites. More than 160 secondary metabolites were found in *Aloe vera* L. leaves and being used internally as laxatives.The ten main chemical constituents of *Aloe vera* include: amino acids, anthraquinones, enzymes, minerals, vitamins, lignins, monosaccharide, polysaccharides, salicylic acid, saponins, and sterols (Pankaj, 2013).It is very useful in caugh, asthma, piles, epilepsy, colic and general disability (Ujjwala, 2007). It has been used for the treatment of jaundice andas emetic & anthelmintic, gas formation in the stomach, loss of appetite, lencorrhoea, menstrual suppression, burns and scalds, rectal fissures, ulcers (Ghani,1998). It has showed amazing results on sun damaged skin and uv damaged skin (Mantle et al., 2001). The bioactive compounds isolated from the gel of *Aloe vera* L. leaves showed following properties including anti-inflammatory, anti-tumor, anti-ulcer, anti-cancer, anti-viral, and anti-bacterial (Tanabe and Horiuchi, 2006).
Vegetative propagation of *Aloe vera* is a slow multiplication process and also depends on the season. Tissue culture method could be an effective alternative to avoid those variabilities.

Therefore, it is necessary to establish a propagation method which can increase commercial production (Silva et al., 2007) and provide disease free planting materials. Moreover, genetic improvement through transformation of *Aloe vera* L. plants requires development of efficient *in vitro* cell culture systems to obtain competent materials for genetic transformation and regeneration into plantlets (Velcheva et al., 2005). Thus, the present investigation was attempted to obtain a high frequency *in vitro* regeneration of *Aloe vera* L. Using shoot tip as explants which treated with different media components to produce a commercial scale true-to-type elite clone with disease-free and uniform quality.

MATERIALS AND METHODS

Explants collection: The experiment was conducted at Plant Biotechnology and Genetic Engineering Division, IFRB, Atomic Energy Research, Establishment, Savar, Dhaka, Bangladesh. Shoot tips of field grown *Aloe vera*L. were collected from AERE Campus, Savar, Dhaka.

Surface sterilization of Explants: First of all, the explants were washed under running tap water using household detergent, Trix for 20 minutes. Subsequent sterilization was carried out under aseptic conditions in the laminar air flow cabinet. Shoots were sterilized in 0.1% mercuric chloride for 10 minutes. Rinsing was done 4 times with sterile distilled water.

Nutrient medium & growth condition: Different concentrations of BA alone or BA + NAA, BA + NAA + Ads, BA + IBA + Ads and BA + Kin + IBA with MS medium (Duchefa, The Netherlands)were used for multiple shoot formation. For rooting, MS0 and half strength MS supplemented with different concentrations of IBA, IAA and NAA were used (Murashige and Skoog, 1962). The sucrose (Merck, Germany) concentration was used 30 g/l. The pH of the media adjusted to 5.8 using HCl or NaOH prior to autoclaving (ES 315, Tommy, Japan). Cultures were incubated at 26 ± 2ºC with a 16-hour illumination of 21.8 µmol/cm^2/s provided by cool white fluorescent tubes.

Shoot development and Root induction: Shoot tips of about 2.0 cm long were excised and inoculated into MS medium with different growth regulators. For promoting healthy and strong multiple shoots,subculture was done 30 days' interval on the same medium. For root induction, healthy shoots were transferred to rooting medium.

Acclimatization: Comparatively healthy rooted plants were taken out from the culture vessels to remove of agar by gentle washing under running tap water. The rooted plantlets were then transferred to earthen pots (Figure 4) containing a mixture of soil and compost (2:1). The pots kept in a shade and also misted twice a day and subsequently transferred to the large pots and gradually acclimatized to outdoor condition.

Statistical Analysis: The experiments were set in completely randomized design. Observations on culture were carried out daily. Finally, data werecollected on different parameters at day 90 for multiple shooting and at day 30 for rooting of shoots. The mean values and standard deviations of shoot formation, average number of shoot/explants & shoot length were calculated using computer software (MS Excel Worksheet).

RESULTS AND DISCUSSION

The plant regenerated from nodal segment is considered to beone of the most promising ways for multiplying a selected variety true to its type (Alam et al., 2015). Shoot tip explants enlarged and became swollen within 7 to 10 days of culture. Explants were grown up and produced new shoot buds and leaves within 20 to 30 days of culture on medium containing MS + 2.0 mg/l BA + 0.5 mg/l NAA + 40.0 mg/l Ads. On the other hand, the rest of the media explants responded poorly. Direct axillary shoot proliferation from the explants tissue was observed in this study. However, shoot proliferation differed according to media components used (Table 1).

For multiple shoot formation,MS medium supplemented with 2.0 mg/l BA + 0.5 mg/l NAA + 40.0 mg/l Ads was found to be the best, in which 90% explants produced multiple shoots (Figure 1) culture after 1 month. The average number of shoot per explant was 36.70 ± 2.25 and the average shoot length of 10.60 ± 2.70 cm were observed in this medium (Figure 2) whilst best results of 10 shoots and 4.0 ± 0.16 cm shoot lengths using MS + 2.0 mg/l BA + 0.5 mg/l NAA was reported (Baksha et al., 2005). This is partially in agreement with our study but present study showed more improved results in terms of multiple shoot formation using Ads in the medium. In contrast, MS + 4.0 mg/l BA + 1.0 mg/l IAA (Ujjwala, 2007), MS + 10.0 mg/l BA + 160.0 mg/l Ads + 0.1 mg/l IBA (Chaudhuri et al., 2001) and MS + 2.0 mg/l BA + 0.3 mg/l NAA + 0.6 mg/l PVP (Liao et al., 2004) used those media combinations to obtain best results on multiple shooting in *Aloevera* L. plants.

In this study, the addition of 40.0 mg/l Ads in the media was found most effective for shoot multiplication and shoot elongation, suggests that Ads has influence to promote *in vitro* shoot proliferation in *Aloe vera* plants. The occurrence of good response of Ads was also mentioned by many authors with different plants (Mathan et al., 2009). These might be due to genotypic variation of explant tissue reinforced by cultural and environmental conditions. Among the plant growth regulators, KN and BA promoted shoot proliferation but BA proved better (Aggarwal and Barna, 2004).

Same results also found in our study, in which poor response, succulent shoot with stunted root and also callusing tendency at the base of explants tissue were observed in kin supplemented media. At the highest concentration (4.0 mg/l) of BA alone or in combination with other media component did not increase shoot proliferation.

We often might have observed that enhanced level of BA had a stimulating effect of shoot bud proliferation but almost all plant species have enough levels of exogenous growth regulators for their regeneration. This is also supported on *in vitro* propagation of bulbs and corms (Hussey, 1982). A cluster of shoots was separated into pieces and each was sub cultured individually on the same medium and observed that after 4th subculture the shoot multiplication rate remained constant. This indicates that up to this level of subculture is optimum for shoot proliferation in *Aloe vera* L. plant in this study. Further increase in the subcultures had no effect on the number of multiple shoot formation which might be due to the effect of maximum potentiality of explant tissue to grew up at up to those levels of 4th sub cultures. Similar observation was also described (Baksha et al., 2005).

The rooting responses differed among the auxins used (Table 2). IBA was found most responsive for root induction and 1.0 mg/l IBA was found optimum, in which 90% shoot rooted (Figure 3) within 15 days of culture. The average number of roots per shoot was 12.60 ± 0.45 and the average root lengths of 6.90 ± 0.40 cm were observed in this medium. Similar observations were reported (Agarwal and Barna, 2004, Baksha et al., 2005) in *Aloe vera* plants. In this study, shoots were also rooted in MS medium devoid of plant growth regulators, which is same to reported. Shoots also responded to roots in IAA supplemented media, which is also supported (Ujjawala, 2007). IBA was superior to IAA or NAA for rooting of many other *in vitro* raised plants has been reported (Roy and Kabir, 2007, Litz and Jaiswal, 1990). For rooting, previous report also stated that the IBA is superior over other auxins (Amin et al., 1992, Grewal et al., 1994).About 90% of the plants were resumed new growth (Figure 5) within 30 days of acclimation period.

Table 1. Effects of different concentrations and combinations of plant growth regulators in MS media for multiple shoot formation from shoot tip explants in *Aloe vera*L. at 120 days.

Different concentrations and combinations of plant growth regulators (mg/l)	% of shoot forming explants	Average number of shoot/ explants (Mean ± SE)	Average length of shoot/ explant (cm) (Mean ± SE)
BA (1.0)	50	2.10 ± 0.20	4.30 ± 0.10
2.0	60	4.20 ± 0.45	4.50 ± 0.25
3.0	50	3.60 ± 0.30	3.60 ± 0.30
4.0	40	2.90 ± 0.60	3.50 ± 0.45
BA + NAA (1.0 + 0.5)	60	7.20 ± 0.40	4.60 ± 0.20
2.0 + 0.5	70	12.60 ± 0.90	6.45 ± 0.30
3.0 + 0.5	60	5.90 ± 0.20	3.90 ± 0.40
4.0 + 0.5	40	3.60 ± 0.10	3.70 ± 0.30
BA + NAA +Ads (1.0 + 0.5 + 40.0)	60	10.40 ± 0.65	5.25 ± 0.20
2.0 + 0.5 + 40.0	90	36.70 ± 2.25	10.60 ± 2.70
3.0 + 0.5 + 40.0	60	14.10 ± 1.20	6.10 ± 0.90
4.0 + 0.5 + 40.0	40	7.25 ± 0.75	4.90 ± 0.40
BA + IBA + Ads (1.0 + 0.1 + 40.0)	40	2.50 ± 0.20	2.10 ± 0.25
2.0 + 0.1 + 40.0	50	7.20 ± 0.65	5.70 ± 0.40
3.0 + 0.1 + 40.0	30	3.60 ± 0.30	3.10 ± 0.40
4.0 + 0.1 + 40.0	30	2.20 ± 0.20	2.50 ± 0.20
BA +Kin + IBA (1.0 + 0.5 +0.1)	30	2.20 ± 0.20	2.10 ± 0.25
2.0 + 0.5 + 0.1	50	5.10 ± 0.70	5.20 ± 0.70
3.0 + 0.5 + 0.1	30	3.90 ± 0.20	3.30 ± 0.60
4.0 + 0.5 + 0.1	30	3.40 ± 0.10	3.20 ± 0.10

Table 2.Effects of IBA, IAA and NAA on half strength MS media in root induction of *in vitro* raised shoots of *Aloe vera* L. at 30 days.

Name of hormones	Concentrations (mg/l)	% of root inducing shoots	Average number of root induced/shoot (Mean ± SE)	Average root length/shoot (cm)(Mean ± SE)
MS0	0	40	3.50 ± 0.25	4.20 ± 0.40
IBA	0.5	50	5.70 ± 0.40	4.60 ± 0.25
	1.0	90	12.60 ± 0.45	6.90 ± 0.40
	1.5	70	6.90 ± 0.30	4.50 ± 0.60
	2.0	50	5.20 ± 0.20	3.80 ± 0.30
IAA	0.5	10	2.10 ± 0.20	3.10 ± 0. 45
	1.0	10	2.40 ± 0.25	2.50 ± 0.30
	1.5	30	2.90 ± 0.40	2.30 ± 0.40
	2.0	30	2.20 ± 0.45	2.10 ± 0.25
NAA	0.5	40	3.40 ± 0.70	3.60 ± 0.30
	1.0	60	8.70 ± 0.30	4.20 ± 0.45
	1.5	60	4.25 ± 0.20	3.25 ± 0.20
	2.0	50	2.90 ± 0.10	2.90 ± 0.25

Figure (1-5): *In vitro* plant regeneration of *Aloe vera* L. through shoot tip culture 1. Shoot initiation on MS + 2.0 mg/l BA + 0.5 mg/l NAA + 40.0 mg/l Ads. 2. Multiple shoot formation in the same medium at subculture four. 3. Root induction on half strength MS + 1.0 mg/l IBA. 4. *In vitro* raised plants resumed new growth in earthen pot. 5. *In vitro* raised plants in earthen pots.

The ultimate survival rate of the transferred plantlets to soil was 85% and their growth in such condition was satisfactory, where rapid shoot length was also observed. It was also revealed that regenerated plants were morphologically similar to the mother (control) plant.

The protocol described a repeatable and long term *in vitro* regeneration of *Aloe vera*L. Plants using shoot tips as explants. The protocol established in this study is also useful for conservation, commercial cultivation and genetic improvement of this crop.

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