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ORIGINAL ARTICLE

Efficient protocol for rapid *Aloe vera* micropropagation

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Abstract

Context: *Aloe vera* Linn. (Liliaceae) is a medicinal plant and has a number of curative properties. Vegetative propagation has not enough potential for supplying market demand. However, via *in vitro* propagation makes possible the mass production of *Aloe* plants.

Objective: The current study was conducted to investigate growth regulators' effects on proliferation of *A. vera*.

Materials and methods: In this study, for comparison of plant growth regulators' effects on proliferation, the shoot tips and auxiliary buds of *A. vera* were cultured in the Murashige and Skoog (MS) medium. Rooted plantlets were transferred to garden soil, compost, and sand in the proportion of 1:1:1, respectively, after hardening.

Results: The maximum number of shoots was obtained on the medium supplemented with 1 mg/L IAA + 4 mg/L BAP and 0.2 mg/L IAA + 0.8 BAP mg/L. Rooting was also achieved in the same media composition proliferation of shoot. The acclimatized plants showed 100% of survival. The regenerated plants looked healthy, and they were morphologically similar to that of stock plants.

Conclusion: These results suggest that *in vitro* culture may be used as a technique for rapid propagation of *A. vera*.

Keywords

In vitro culture, medicinal plant, plant growth regulators, proliferation, shoot tip

History

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Introduction

Aloe vera Linn. (Liliaceae) is an important medicinal plant (Cera et al., 1980; Gui et al., 1990; Meyer & Staden, 1991) and has been used worldwide in pharmaceutical, food, and cosmetic industries, or in traditional medicine, due to the plethora of biological activities of some of its primary and secondary metabolites (Campestrini et al., 2006). Bioactive compounds isolated from the gel of *Aloe* species have been known to have anti-inflammatory, antitumor, antiulcer, anticancer, antibacterial, and antiviral properties (Reynolds & Dweck, 1999). The *A. vera* leaves are used to treat bacterial and fungal skin diseases (Yadav & Singh, 2010). *Aloe* gel consists of 99.3% water. The remaining 0.7% is composed of solids with glucose and mannose constituting for a large part. These sugars together with the enzymes and amino acids in the gel give the special properties as a skin care product (Agarry et al., 2005). Modern use of *A. vera* was first documented in the 1930s to heal radiation burns (Collins, 1935). There is a lack of production of *Aloe* leaf to meet the industry demand (Aggarwal & Barna, 2004). So, it is necessary to undertake large-scale cultivation of *Aloe*. Natural propagation of *A. vera* is primarily by means of auxiliary shoots, and it is rather a slow way of multiplication to meet the growing demand (Natali et al., 1990b). A single plant produces 2–3 offshoots in a year which is not sufficient for undertaking commercial cultivation (Saggoo & Kaur, 2010). The presence of male sterility is also a barrier in rapid propagation (Natali et al., 1990a). Today tissue culture technology is being exploited mainly for large-scale production or micropropagation of elite planting material with desirable characteristics. The technique to tissue and organ culture is used for rapid plant multiplication, genetic improvement of crops, obtaining disease-free clones, and progressive valuable germplasm (Bhojwani & Razdan, 1992). Conventional plant breeding methods can improve both agronomic and medicinal traits. *In vitro* propagation or tissue culture of plants holds tremendous potential for the production of high-quality plant-based medicines. This can be achieved through different methods including micropropagation (Yushkova et al., 1998).

Several reports have noted rapid *in vitro* propagation of *A. vera* (Aggarwal & Barna, 2004; Hosseini & Parsa, 2007; Meyer & Staden, 1991). Scientists obtained different results applying different formulation of plant growth regulators for *in vitro* propagation of *A. vera*. The hormonal requirement for *in vitro* differentiation differs for different genotypes. The objective of this investigation was to develop a rapid, lower cost, efficient, and easy method of *A. vera* micropropagation. Thus, the present study aimed to develop a rapid and high-frequency shoot regeneration protocol for elite plants of *A. vera*. We standardized new composition of growth regulators

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for rapid and efficient micropropagation of *A. vera* using auxiliary and shoot tip explants.

Materials and methods

Healthy *Aloe vera* showing good biomass yield was collected as a plant material. Here the explants were further trimmed and extra outer leaves were removed to make them in suitable sizes. Shoot tip and auxiliary buds were washed under running tap water for 20 min and then treated with detergent and 4–5 drops of Tween 20 for 20 min with constant shaking. Then explants were washed 3–4 times with sterile distilled water to make the material free from detergent. Again, explants were surface sterilized with 2% (W/V) NaOCl for 20 min with constant waving. Immediately, in a laminar air flow cabinet, three consecutive rinsing were done with sterile distilled water. The MS (Murashige & Skoog, 1962) medium with 3% sugar was added as carbon source and media were solidified with 0.7% agar–agar. The pH was adjusted to 5.7 prior to autoclaving at 121 °C and 1.2 Bar for 20 min. The sterilized explants (0.5–1 cm) were cultured onto glass (9×7 cm) with 50 mL of MS medium supplemented with the plant growth regulators; 1 mg/L IAA + 4 mg/L BAP (Ujjwalw, 2007), 1 mg/L Kin + 0.1 mg/L IAA (Hosseini & Parsa, 2007), 0.5 mg/L NAA + 2 mg/L BAP (Baksha et al., 2005) and 0.2 mg/L IAA + 0.8 mg/L BAP for shoot initiation and shoot proliferation. The experiment was conducted in completely randomized design (CRD) with five replications. Two shoot per culture glass were cultured. The cultures were incubated under a 16/8 (light/dark) photoperiod. The temperature was maintained at 25 ± 1 °C. Regenerated shoots were subcultured monthly to the same fresh medium in order to increase budding frequency.

The measured traits were number of shoots/explant (NSE), shoot length (SL), root length (RL), and shoot leaf diameter (SLD). After increase in shoot elongation and rooting formation, the plantlets were taken for acclimatization. Vitroplant roots were washed in tap water followed planted in plastic pots containing with garden soil, compost, and sand in the proportion of 1:1:1, respectively. The plantlets survived to grow and were morphologically similar to mother plants. Data were analyzed using SAS statistical software (SAS, Cary, NC) and Dancan's test was used to mean comparison.

Results

The explants began to show signs of shoot proliferation after 1 week of culturing. All explants gave aseptic cultures. Plants were free from both fungal and bacterial contamination. Shoot buds initiated were light green to yellowish in color and arisen in clusters. After successful initiation of the culture (28 d after culturing), newly formed shoots were excised individually from the proliferated explants and further cultured on the same medium to increase the number of shoots for further work.

The emergence of shoots took place in 1 week. The highest shoot multiplication was found in combination of 4 mg/L BAP + 1 mg/L IAA (58.88 ± 6.05) and 0.8 mg/L BAP+0.2 mg/L IAA (48.03 ± 5.83). Also on the medium containing 1 mg/L Kin + 0.1 mg/L IAA, the average number of shoots per explant was 21.86 ± 1.67 and the average number of shoots were formed on the medium supplemented with 2 mg/L BAP + 0.5 mg/L NAA, produced 22.16 ± 3.24 (Table 1 and Figure 1A).

Shoot elongation with a maximum length was obtained on MS medium supplemented with 1 mg/L Kin + 0.1 mg/L IAA (6.37 ± 0.9 cm) and was placed in a group with 0.8 mg/L BAP + 0.2 mg/L IAA (5.98 ± 2.2 cm) (Table 1 and Figure 1B). On one hand, in the medium containing 1 mg/L Kin + 0.1 mg/L IAA, the average root length of shoots were 9.51 ± 2.3 cm (Table 1 and Figure 1C), on the other hand, in the medium containing 4 mg/L BAP + 1 mg/L IAA and 2 mg/L BAP + 0.5 mg/L NAA the minimum average roots length of shoots were produced 5.89 and 5.63 cm, respectively. The maximum SLD (0.60 cm) was observed in the MS medium supplemented with 1 mg/L Kin + 0.1 mg/L IAA (Table 1 and Figure 1D).

Plantlets in media for induction of shoots regeneration (in four treatments in present study) produce roots. Regenerated roots in this plantlets media were thick, strong, and very healthy, and the roots appeared normal and turned yellow or brown at maturity. A 100% rooting was observed in this media (Figures 2 and 3).

Acclimatization and transfer of plantlets to the soil

Plantlets with actively growing roots (5–7 cm long) were transferred to plastic pots containing garden soil, compost, and sand in the proportion of 1:1:1, respectively. All of the explants (100%) survived during and after the acclimatization in the pots in the culture room. The regenerated plants looked healthy and they were morphologically similar to that of mother plants (Figures 4 and 5).

Discussion

A number of factors, such as genotype, culture medium (including growth regulators and their combinations), physical environment, and explant developmental stage, affect

Table 1. Effects of different concentrations and combinations of BAP, IAA, Kin, and NAA on multiple shoot formation in *A. vera*.

Growth regulators (mg/l)	NSE	SL (cm)	RL (cm)	SLD (cm)
IAA(0.2) + BAP(0.8)	48.03 ± 5.83^{a}	5.98 ± 2.2^{a}	7.45 ± 2.2^{b}	0.55 ± 0.01^{b}
IAA(0.1) + Kin(1)	21.86 ± 1.67^{b}	6.37 ± 0.9^{a}	9.51 ± 2.3^{a}	0.60 ± 0.01^{a}
IAA(1) + BAP(4)	58.88 ± 6.05^{a}	5.27 ± 1.8^{b}	5.89 ± 2.5^{c}	0.51 ± 0.01^{b}
NAA(0.5) + BAP(2)	22.16 ± 3.24^{b}	5.26 ± 1.3^{b}	5.63 ± 1.6^{c}	0.55 ± 0.01^{b}

Different letters in each column indicate significant differences at a 1% level
where NSE (shoots/explant), SL (shoot length), RL (root length), and SLD (shoot leaf diameter).

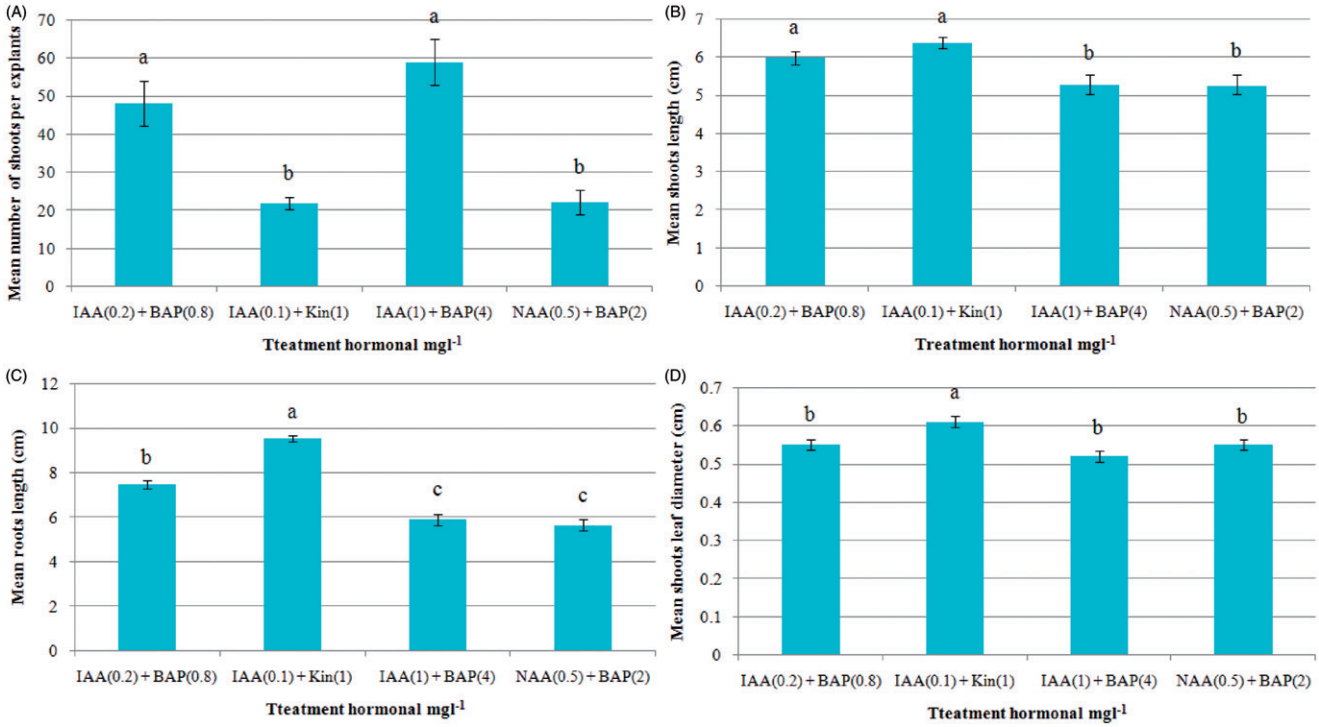

Figure 1. The effect of different concentrations and combinations of plant growth regulators BAP, Kin, IAA, and NAA on the number of shoots per explant (A), shoot length (B), root length (C), shoot leaf diameter, and (D) *in vitro* propagation of *A. vera*.

Figure 2. *In vitro* plantlet *A. vera* having healthy shoots and roots.

Figure 3. *In vitro* regenerated *A. vera.*

adventitious shoot regeneration from tissue cultured explants (Qu et al., 2000).

The present study attempted to optimize the growth regulator and their concentration for efficient direct shoot regeneration from auxiliary and shoot tip explants of *A. vera.* Meristem culture is a very useful approach giving high-quality plantlets with high virus-free rates (Debiasi et al., 2007). Using auxiliary buds as the explants has been proven to be the most successful and efficient micropropagation procedure for *A. vera* (Meyer & Staden, 1991). The most researchers proposed the use of shoot tip and apical meristem for micropropagation of *A. vera* (Ahmed et al., 2007; Baksha et al., 2005; Kalimuthu et al., 2010; Natali et al., 1990a; Roy & Sarkar, 1991). Velcheva et al. (2005) showed that the young inflorescence of *Aloe barbadensis* is a reliable explant for *in vitro Aloe* micropropagation. Immature inflorescences and flowers, mature ovaries and seeds, rhizome discs, apical meristems, and leaf bases were used as explants for embryogenic callus induction (Garro Monge et al., 2008). BAP, Kin, IAA, and NAA were selected for shoot regeneration in the present study as they are among the growth regulators used most often for the shoot organogenesis.

In our study, further proliferation on MS medium containing 4 mg/L BAP + 1 mg/L IAA and 0.8 mg/L BAP + 0.2 mg/L IAA indicates that it is an appropriate combination of medium for producing multiple shoots from single explants. The present findings are in agreement with the result of Ujjwalw (2007) who achieved maximum

Figure 4. Adaptation of micropropagation plantlets *A. vera* and transferring them into soil.

Figure 5. Acclimatized *A. vera* in pot conditions.

regeneration on the MS medium supplemented with 4 mg/L BAP + 1 mg/L IAA. Shoot proliferation occurred in the presence of cytokinin with particular reference to BAP. According the to results of Kalimuthu et al. (2010), the best medium for shoot induction from shoot tips of *A. vera* was MS supplemented with 1.5 mg/L BAP + 50 mg/L Adenine sulfate. BAP alone in concentration 2.0 mg/L has been also found effective for shoot proliferation in *A. vera* (Ahmed et al., 2007). It was also reported that the highest shoot multiplication in *A. vera* was found in the MS medium containing 2.2 μM BA. In this media composition, regeneration of a new shoot in 80% of its cultures in 6 weeks is shown (Tanabe & Horiuchi, 2006). Zhou et al. (1999) suggested that the number of regenerated buds *A. vera* could be increased by used MS media with 3 mg/L at BA. In a previous study, Velcheva et al. (2005) observed MS medium enriched with 19.6, 22.2 μM BA, and 3.92 μM ancymidol, promoted organogenesis enabling 87.3% of the explants to regenerate 6.04 ± 1.79 shoots/explant. Hirimburegama and Gamage (1995) found that high rates of shoot proliferation were obtained from auxiliary and apical buds of *A. vera* cultured on MS media supplemented with 0.18 mg/L IAA+2.25 mg/L BA. The presence of the plant growth regulators, particularly cytokinin in the culture medium, is the most important factors for shoot proliferation (Abrie & Staden, 2001; Aggarwal & Barna, 2004; Chaudhuri & Mukundan, 2001; Hoque, 2010; Liao et al., 2004; Mamidala & Nanna, 2009). Cytokinin could interact with other growth regulators to stimulate the vegetative growth of plants (Maxwell & Kieber, 2004). The combination of IAA with cytokinin (BAP or Kin) promoted shoot formation in various plant species as observed by Yasmeen and Rao (2005), Guo et al. (2007) and Jawahar et al. (2008). In the plant physiology process, cytokinin influences cell division in order to broaden the area of the tissues and plantlet height (Davies, 2004). Wider survey of the existing literature suggests that BAP is the most reliable and useful cytokinin for shooting in higher plants. Many workers succeeded in their attempts for shoot proliferation by using BAP (Barna & Wakhlu, 1994; Sebastin & Barna, 2003). Maximum number of shoot multiplication and proliferation was recorded in the medium fortified on MS medium with 2.5 mg/L BAP (Gantait et al., 2010). The beneficial effect of BA on the ability to maintain continuous shoot multiplication was noted already (Aggarwal & Barna, 2004; Natali et al., 1990b; Richwine et al., 1995). Abrie and Staden (2001) and Chaudhuri and Mukundan (2001) also reported the use of BAP in shoot proliferation of *Aloe polyphylla* and *A. vera,* respectively. However, for the media supplemented with Kin, the maximum number of shoots per explant was 21.86 ± 1.67 in the medium fortified with 1 mg/L Kin + 0.1 mg/L IAA. The indications of shoot proliferation BAP gave better than Kin. However, it is in contrast to earlier reports in *A. vera* by Meyer and Staden (1991) and Natali et al. (1990a) that reported better proliferation occurred on the medium containing Kin instead of BA. Baksha et al. (2005) reports the best and rapid regeneration was observed on MS supplemented with 2 mg/L BAP + 0.5 mg/L NAA. This treatment maximum number of shoots per explant was 10.1 ± 0.50, while in this study in same combination (2 mg/L BAP with 0.5 mg/L NAA) maximum numbers of shoots per explant was 22.16 ± 3.24. This might be due to genotypic variation of explants reinforced by the cultural and environmental conditions.

Studies conducted by Velcheva et al. (2005) and Debiasi et al. (2007) indicated that BAP is more effective than NAA for shoot proliferation. Hashemabadi and Kaviani (2008) suggested supplementation of the MS medium with various concentrations of BAP and NAA for rapid micropropagation of *Aloe* via shoot multiplication. Abrie and Staden (2001) cultured *Aloe* plantlets on the medium containing BAP alone, or with combination NAA. Nayanakantha et al. (2010) report in the medium containing 4 mg/L BAP + 0.2 mg/L NAA + 1 g/L PVP + 10 mg/L citric acid, the average number of shoots per plant was 18.7 ± 1.2. Liao et al. (2004) also reported that a combination of BAP and NAA enhanced the multiple shoot proliferation from shoot tip explants of *A. barbadensis*.

In this study, roots regenerated in the same media for induction of shoots regeneration. Velcheva et al. (2005) believed that phytohormones present in the culture medium of *A. sarborescens* during shoot induction and elongation play a major role during the rooting stage. Rooting of plantlets was achieved on hormone free MS (Aggarwal & Barna, 2004; Hosseini & Parsa, 2007; Natali et al., 1990a). The highest

number of roots per culture was found in the MS medium containing 0.2 and 0.5 mg/L NAA, also obtained by Ahmed et al. (2007). According to the results of Kalimuthu et al. (2010), microshoots rooted in 12 d of culture on the MS medium fortified with 1.0 mg/L of NAA. In an optimal medium, Chinese *Aloe* buds grow very well, rooted quickly on 1/2 strength MS with 0.2 mg/L NAA and 2.0 g/L PVP (Liao et al., 2004). NAA and IBA are most commonly used for root induction (Bhojwani & Razdan, 1992). Rooting response of microshoots is reported by Baksha et al. (2005), 95% rooting was obtained from plantlets cultured on half strength MS supplemented with 0.5 mg/L NAA.

Conclusion

Plant medicinal *A. vera* needed a different protocol to obtain optimum shoot initiation, shoot multiplication, root induction, and elongation. The maximum number of shoot tip and auxiliary bud of *A. vera* were obtained on shoot multiplication media containing 4 mg/L BAP+1 mg/L IAA and 0.8 mg/L BAP + 0.2 mg/LIAA. Roots regenerated in the same media for induction of shoots regeneration were thick, strong, and very healthy and the roots appeared normal and turned yellow or brown at maturity. A 100% rooting was observed in these media. Different results found by some groups may be due to the genotypes of the mother plants used. The other reasons could be the physiological status and the age of mother plants that explants are taken from.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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