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*Full Length Research Paper*

# Rapid micro-propagation of *Aloe vera* L. via shoot multiplication

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***Aloe vera* L. is a medicinal plant. Regeneration of *A. vera* in nature *(in vivo)* is too slow and insufficient to meet the industry demand. Therefore, it is necessary to use *in vitro* propagation for rapid plant production. Explant used for the *in vitro* culture was shoot tip. The shoot tip explants was disinfected with 2% NaOCl and washed thoroughly with sterile water. Then, explants were placed on solid MS medium with the addition of various concentrations of benzyladenine and α-naphthaleneacetic acid. After 8 weeks, the best proliferation of shoot per explant (9.67) and the best rooting was shown on the medium supplemented with 0.5 mg/l benzyladenine + 0.5 mg/l α-naphthaleneacetic acid. The rooted plantlets were gradually acclimatized in plastic pots containing a mixture of cocopeat and perlite (1:1) covered with transparent plastic. About 95% of the transplanted plantlets survived.**

**Key words:** *Aloe vera* L., micro-propagation, *in vitro*, plant growth regulator.

## INTRODUCTION

*Aloe vera* L. belongs to the Liliaceae, which has medicinal and cosmetic properties (Gui et al., 1990; Meyer and Staden, 1991). In nature, *A. vera* is propagated through lateral buds, which is slow, very expensive and low income practice (Meyer and Staden, 1991). *A. vera* has been cultured *in vitro* by various researchers (Natali et al., 1990; Roy and Sarkar, 1991; Abrie and Staden, 2001).

The best explants for micropropagation of *A. vera* are shoot tip and axillary bud (Meyer and Staden, 1991). Also, the presence of the plant growth regulators is necessary for this purpose (Aggarwal and Barna, 2004; Debiasi et al., 2007; Liao et al., 2004). Meyer and Staden (1991) reported axillary shoot formation using IBA, whereas Roy and Sarkar (1991) and Natali et al. (1990) obtained shoots on medium containing 2,4-D and Kn. Richwine et al. (1995) reported the induction of shoots using zeatin. Debiasi et al. (2007) and Liao et al. (2004) studied the effects of BA, IAA and NAA on bud initiation. Applying of plant growth regulators is necessary for rooting in culture (Abrie and Staden, 2001; Feng et al., 2000; Hongzhi, 2000). Acclimatization of rooted plantlets in the pots containing a mixture of sand and soil under greenhouse conditions with 70-90% moisture is suitable for the young plants survival (Natali et al., 1990; Hirimburegama and Gamage, 1995).

Genetic transformation and cloning requires a highly efficient system for regeneration of *A. vera* (Campestrini et al., 2006; Velcheva et al., 2005). The present research focuses on the influence of benzyladenine (BA) and α-naphthaleneacetic acid (NAA) on rapid *in vitro* propagation of *A. vera* plants grown in Iran.

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**Abbreviations:** BA, benzyladenine; NAA, α-naphthaleneacetic acid; MS, Murashige and Skoog; IBA, 3-indole butyric acid, 2,4-D: 2,4-dichlorophenoxyacetic acid; Kn, kinetin; IAA, 3-indoleacetic acid; ZR, zeatin riboside; 2iPR, 6-(γ, γ-dimethylallyl-amino) purine riboside; CPPU, N-(2-chloro-4-pyridyl)-N´-phenylurea.

## MATERIALS AND METHODS

### Preparation of explant

Shoot tip explants were obtained (May, 2007) from the mother plants presented in a commercial greenhouse. The explants containing 1-2 buds were washed via tap water for 30 min followed by surface sterilization using 2% (w/v) NaOCl for 30 min. The explants were thoroughly rinsed with sterile water. The surface-disinfected explants were cut into 1 cm segments, each with buds.

**Table 1.** Effect of different concentrations of BA and NAA on shoot proliferation in *Aloe vera* L. after 4 and 8 weeks of culture.

| Phytohormones (mg/l) | Explants showing shoot formation (%) | Average number of shoot per single explant (after 4 weeks) | Average number shoot per single explant (after 8 weeks) |
|---|---|---|---|
| Hormone- free (control) | 0 | 0 | 0 |
| 0.5 BA + 0.5 NAA | 100 | 3.15 a | 9.67 a† |
| 0.5 BA + 1 NAA | 100 | 1.32 c | 4.65 b |
| 1 BA + 0.5 NAA | 100 | 2.00 b | 8.69 a |
| 1 BA + 1 NAA | 100 | 1.83 b | 4.74 b |
| 2 BA + 0.5 NAA | 100 | 2.83 a | 5.68 b |
| 2 BA + 1 NAA | 100 | 2.33 b | 4.55 b |

† Values followed by the same letter are not significantly different ($p<0.01$) using DNMRT.

Again, explants were sterilized using 1% (w/v) NaOCl for 2 min followed by three rinses with sterile water.

#### Culture medium

Explants were inoculated onto media composed of basal MS (Murashige and Skoog, 1962) medium supplemented with the plant growth regulators; BA (0.5, 1 and 2 mg/l) and NAA (0.5 and 1 mg/l). Sucrose (3%) was used as carbon source and media were solidified with agar-agar (0.7%). The pH was adjusted to pH 5.7 prior to autoclaving at 121°C and 102 kpa for 20 min. Four shoots per culture bottle were inoculated and 3 replicates taken. The bottles were then kept in a growth chamber under 16:8 h (light: dark) photoperiod with light intensity of 2000-2500 lux provided by Mahtab, Iran, 40 W white bulbs at 24±1°C and 70% relative humidity (RH).

Data were recorded after 4 and 8 weeks of culture. After 8 weeks, matured plantlets were washed with distilled water and transferred into plastic pots (10 cm in diameter) containing a mixture of cocopeat and perlite (1:1). The top of the pots were covered with transparent plastic and grew in a greenhouse at 24±1°C and 70% RH with periodic irrigation (3 days).

#### Statistical analysis

The experimental design was factorial with R.C.B.D basis design, which was done with unequal repetition. All experiments were carried out in three replicates. Data were subjected to ANOVA (analysis of variance) and significant differences between treatments were determined by DNMRT using the SPSS software package.

## RESULTS AND DISCUSSION

Shoot tip explants on medium with 0.5 mg/l BA + 0.5 mg/l NAA showed signs of proliferation after two weeks. New buds appeared from the axils of leaves and developed into shoots by 4th week of culture. Highest number of shoots per explant (3.15) was produced on medium containing 0.5 mg/l BA + 0.5 mg/l NAA (Table 1). On 8th week, the highest number of shoots was formed on medium with 0.5 mg/l BA + 0.5 mg/l NAA, produced 9.67. Also, on medium containing 1 mg/l BA + 0.5 mg/l NAA the aver-age number of shoots per explant was 8.69 (Table 1). Shoot elongation with a maximum height was obtained on MS medium supplemented with 0.5 mg/l BA + 0.5 mg/l NAA (Figure 1). 2 mg/l BA and 1 mg/l NAA did not show any significant effect on shoot proliferation (Table 1). The least number of shoots per explant (nil) was shown in hormone-free medium (Table 1).

In order to reflect the differences among the factors and to identify the optimum medium, ANOVA (analysis of variance) was carried out (Table 2). Two factors, namely BA, NAA and reciprocal effect of BA and NAA, had significantly different effects on proliferation of *A. vera* ($p<0.01$).

Lio et al. (2004) reported that the best medium for micropropagation of *A. vera* is that supplemented with 2 mg/l BA + 0.3 mg/l NAA. Aggarwal and Barna (2004) reported that the highest number (100%) of shoots per explant (3.3±0.9) was produced on medium containing 1 mg/l BA. Budhiani (2001) demonstrated that the best initiation and multiplication of shoot obtained on MS medium with 0.2 mg/l BAP + 0.002 mg/l NAA (1.50±1.29 shoots) and 2 mg/l BAP + 0.002 mg/l NAA (1.8±1.09 shoots), respectively. Highest number of shoot was shown on the 4th subculture, produced 12.67±0.52 shoots. In our studies, MS medium supplemented with 0.5 mg/l BA + 0.5 mg/l NAA produced the highest number of shoot (9.67) was observed only on the first subculture.

Other studies indicate that BA is more efficient than NAA for shoot proliferation in *A. vera* (Velcheva et al., 2005; Debiasi et al., 2007). According to the literature, BA is better than other cytokinins for shoot initiation and proliferation. Velchera et al. (2005) concluded that efficient shoot initiation was observed in media supplemented with BA. However, ZR or 2iPR containing media gave only a moderate response while inclusion of CPPU and Kn in the media gave very low or no initiation, respectively. Debiasi et al. (2007) also reported the best multiplication on medium containing BA and IAA.

Some researchers have indicated that the presence of both auxin and cytokinin is necessary for shoot proliferation (Roy and Sarkar, 1991; Rout et al., 2001; Velcheva et al., 2005). The suitable ratios of cytokinin to auxin for the propagation of buds depend on the species of aloe used, as generally being 10:1 for *Aloe arborescens* Miller (Wu, 2000), and 2:0.3 for chines aloe (Liao et al., 2004).

| | | | | |
|---|---|---|---|---|
| BA (mg/l) | 0.5 | 1 | 2 | 1 |
| NAA (mg/l) | 0.5 | 0.5 | 0.5 | 1 |

**Figure 1.** Shoot proliferation of *Aloe vera* L. after 8 weeks of culture on MS medium supplemented with various concentrations of BA and NAA.

**Table 2.** Statistical analysis the effect of different concentrations of BA and NAA on proliferation of *Aloe vera* L.

| Source of variance | Sum of squares | Degree of freedom | Mean square | F-value |
|---|---|---|---|---|
| NAA | 50.938 | 1 | 50.938 | 111.7389** |
| BA | 13.857 | 2 | 6.929 | 15.1987** |
| NAA × BA | 12.121 | 2 | 6.060 | 13.2941** |

**significantly different ($p<0.01$).

Our results showed the best ratio of BA to NAA for buds initiation, shoot proliferation and rooting was 1:1 or 2:1.

The shoots showed good rooting on MS medium supplemented with 0.5 mg/l BA + 0.5 mg/l NAA and 1 mg/l BA + 0.5 mg/l NAA (data not presented). Some researchers reported rooting in hormone-free medium (Natali et al., 1990; Aggarwal and Barana, 2004) while some others showed the presence of plant growth regulators is necessary (Abrie and Staden, 2001; Meyer and Staden, 1991; Velcheva et al., 2005). In the present study, rooting percentage was improved in the presence of low concentrations of BA and NAA. Our findings support those of Liao et al. (2004) and Budhiani (2001). In the previous studies, Aggarwal and Barna (2004) as well as Barna and Walkhlu (1995) reported rooting (100%) in *A. vera* and rose in hormone-free medium. Our results do not support these observations. The current study revealed that there is a negative correlation between rooting and BA concentration in the medium. This supports the results obtained by Velcheva et al. (2005) and Dubois and Vries (1996).

Type of explant used in *in vitro* conditions affects plant proliferation. In this work, shoot tip was used. Most researchers propose shoot and meristem for micropropagation of aloe (Natali et al., 1990; Meyer and Staden, 1991; Liao et al., 2004; Aggarwal and Barna, 2004).

The plantlets were successfully acclimatized in plastic pots containing a mixture of cocopeat and perlite (1:1) covered with transparent plastic. The result of acclimatization showed that 95% of plantlets survived to grow under greenhouse conditions and were morphologically similar to mother plants (Figure 2). A mixture of light soil with good drainage is suitable for acclimatization of this plant. Researchers have proposed a mixture of soil and sand (1:1) or soil, sand and perlite or vermiculite (1:1:1) for hardening of *A. vera* (Hirimburegama and Gamage, 1995; Natali et al., 1990).

## Conclusion

In conclusion, type and concentration of plant growth regulators, present in the media during shoot initiation, shoot proliferation and plant rooting, played a significant role in plant regeneration depending on the genotype of the mother plant and explant used. This project indicated

**Figure 2.** Regenerated plant of *Aloe vera* L. growing in plastic pots after 30 days of hardening.

that micropropagation can be a useful tool in the proliferation of *A. vera*.

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