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Micropropagation of *Aloe vera* L. Grown in South Iran

[1,2]R. Hosseini and [1]M. Parsa
[1]Research Institute of Applied Sciences, Iranian Academic Centre for Education,
Culture and Research (ACECR), Shahid Beheshti University, P.O. Box 19835-169, Tehran, Iran
[2]Faculty of Engineering, Agricultural Biotechnology Group,
International University of Imam Khomeini, Ghazvin, Iran

Abstarct: *Aloe vera* L. is a medicinal plant grown in different parts of the world. Several papers have reported the micropropagation of this plant and its response to different combinations of hormones. In this research, we used *A. vera* plants grown in south Iran. MS culture medium with twenty three combinations of hormones were used, including some of those employed previously by other researchers. Ten media showed positive results and the best result was obtained using Kin (1 mg L^{-1})+IAA (0.1 mg L^{-1}) which has not been reported before. Produced plantlets rooted in free hormone MS medium and transferred into soil. The survival rate was 83%.

Key words: *Aloe vera*, liliaceae, micropropagation, MS medium

INTRODUCTION

The plant *A. vera* L. belongs to the Liliaceae family. It is believed to have originated from Africa (Natali *et al*., 1990). Aloe genus consists of about 300 species, all of which grow in rosette shape (www.botany.com/aloe.htm). *A. vera* has found many medicinal and cosmetic usages and has a growing demand in the market (www.aloeveraproducts.com). For example, there was a shortage of 641.5 tonnes in the aloe leaf production between years 2001-2002 to meet the industry demand (Aggarwal and Barna, 2004). However, there are two drawbacks in the traditional propagation of this plant; which are, slow propagation rate of axillary shoots and male sterility (Natalie *et al*., 1990). To overcome these problems, several groups have used *in vitro* conditions and have had success (Castorena *et al*., 1988; Gui *et al*., 1990; Natali *et al*., 1990; Meyer and Staden, 1991; Roy and Sarker, 1991; Hirimburegama and Gamage, 1995; Zhou *et al*., 1999; Aggarwal and Barna, 2004; Fattahi Moghaddam *et al*., 2004). This should be noticed that each of the mentioned groups found different results to others. The aim of this research was to optimise *in vitro* conditions and develop a rapid and easy multiplication protocol of the *A. vera* plants grown in Iran. Here, we report a new combination of growth regulators for rapid propagation of this plant.

MATERIALS AND METHODS

Aloe plants were obtained from Hormozgan province, south Iran. Meristem tips and leaf explants were both used for the micropropagation purpose. For meristem tips, axillary shoots (about 10 cm long) were removed from the mother plants and then leaves and roots removed. Only one or two innermost leaves were left. The remaining parts (about 4 cm) were washed with tap water, surface sterilized in commercial bleach, containing 1.5% (v/v) active chlorine for 20 min under sterile conditions. Then they were washed 3 times with distilled sterile water and placed in 0.12% (w/v) mercuric chloride for 5 min, then washed 3 times with distilled sterile water. The central part of the shoot, containing apical meristem and a small basal part underneath it were excised and placed in 50 mL MS medium (Murashige and Skoog, 1962) containing different growth regulators (Table 1). Culture media contained 3% (w/v) sucrose and 0.7% (w/v) agar with pH 5.8. The media were autoclaved at 120°C for 20 min. All cultures were incubated in 16:8 h (light : dark) photoperiod with light intensity of 2500 lux (provided by Mahtab, Iran, 40 W white bulbs) at 24±1°C and 40% RH. All treatments were repeated three times. For leaf explants, small leaves about 4 cm long were separated and the same procedure of surface sterilization as the meristem explants was

Corresponding Author: R. Hosseini, Research Institute of Applied Sciences, Iranian Academic Centre for Education, Culture and Research (ACECR), Shahid Beheshti University, P.O. Box 19835-169, Tehran, Iran
Tel: 0098-2129903037-8

Table 1: Different culture media used for *A. vera* micropropagation

	Growth regulator combination	Average no. of explants after 60 days (±SE)
M1	Kin (1 mg L^{-1})+NAA (0.25 mg L^{-1})	0.00[1]
M2	Kin (2 mg L^{-1})+NAA (0.25 mg L^{-1})	0.00[1]
M3	Kin (4 mg L^{-1})+NAA (0.25 mg L^{-1})	0.00[1]
M4	Kin (5 mg L^{-1})+NAA (1.25 mg/L)	0.00[1]
M5	Kin (0.5 mg L^{-1})+2,4-D (0.1 mg L^{-1})	0.00[1]
M6	Kin (0.5 mg L^{-1})+2,4-D (0.25 mg L^{-1})	0.33±0.33[a]
M7	Kin (1 mg L^{-1})+2,4-D (0.25 mg L^{-1})	0.00[1]
M8	Kin (1 mg L^{-1})+IAA (0.1 mg L^{-1})	10.33±3.33[b]
M9	Kin (1 mg L^{-1})+IAA (0.25 mg L^{-1})	2.00±2.00[a]
M10	Kin (1 mg L^{-1})+IAA (0.5 mg L^{-1})	4.00±2.00[ab]
M11	Kin (1 mg L^{-1})+IAA (1 mg L^{-1})	0.00[1]
M12	BAP (1 mg L^{-1})	0.00[1]
M13	BAP (2 mg L^{-1})	4.00±2.08[ab]
M14	BAP (1 mg L^{-1})+IBA (0.25 mg L^{-1})	4.66±2.91[ab]
M15	BAP (0.2 mg L^{-1})+IBA (10 mg L^{-1})	0.00[1]
M16	BAP (1 mg L^{-1})+NAA (0.1 mg L^{-1})	3.33±2.40[ab]
M17	BAP (1 mg L^{-1})+NAA (0.25 mg L^{-1})	3.00±2.08[ab]
M18	BAP (1 mg L^{-1})+NAA (0.5 mg L^{-1})	1.66±0.88[a]
M19	BAP (1 mg L^{-1})+2,4-D (0.5 mg L^{-1})	0.00[1]
M20	IBA (1 mg L^{-1})	0.00[1]
M21	IBA (2 mg L^{-1})	0.00[1]
M22	NAA (0.1 mg L^{-1})	0.00[1]
M23	Kin (1 mg L^{-1})+BAP (1 mg L^{-1})	5.00±3.61[ab]

[1]Not incuded in the ANOVA and Duncan's test. The averages with not significant results at 5% probability level have the same letter(s)

performed. Then they were cut into 0.5 cm pieces and placed on the culture media (Table 1). The plantlets rooted in hormone free MS and transferred to plastic pots containing 1/5 compost, 2/5 loam and 2/5 clay. The top of the pots was covered with transparent plastic pots, having three small holes on top. After 10 days the covering pots were removed and pots containing plantlets were transferred to the glasshouse. All experiments were complete random designs and statistical analyses were performed using SPSS software (version 10).

RESULTS AND DISCUSSION

More than 20 different culture media were used in an attempt to micropropagate *A. vera* through meristem and leaf explants. No regeneration ability was found in the leaf explants. In some media leaf explants developed callai, which with subsequent transfer into regeneration media, Kin 1 mg L^{-1}+IAA 0.1 mg L^{-1} no signs of regeneration were observed. However, meristem explants showed regeneration potential in several media (Table 1). In all cases, 30 days after culture, propagation was undetectable. Nevertheless, within 45 days, actively growing offshoots had appeared. After about 60 days, more offshoots were present and regeneration rate was more rapid (Fig. 1A). This phenomenon could be interpreted in this way that the aloe plants required a certain internal level (threshold) of a growth regulator(s) in order to regeneration could occur. Upon reaching this threshold, fast regeneration started.

In this study, we found 10 media to be effective in *A. vera* micropropagation (Table 1). The combination of Kin and NAA caused no response on axillary shoot generation (M1-M4), but in contrast, BAP along with NAA induced axillary shoot production (M16-M19). This showed that BAP could act better with NAA than Kin for the micropropagation purpose. An interesting result was observed in M23 medium, in which the combination of two cytokinines, in average produced 5 plantlets. This combination had improved micropropagation rate in comparison with when BAP alone was used (M13). Our results did not support some of the previous findings obtained by other research groups. For example, M11 medium (Table 1) which was amongst the best media used by Fattahi Moghaddam *et al.* (2004) (10.33±0.58 plantlets produced per explant) was not found to be the same in our study. Other results contradicting with what we found, are those of Natali *et al.* (1990) and Fattahi Moghaddam *et al.* (2004) (M6), Meyer and Staden (1991) and Fattahi Moghaddam *et al.* (2004) (M20). However, our findings support those of Aggarwal and Barna's (2004), namely the explants in the MS medium containing BAP (1 mg L^{-1}) + IBA 0.25 (mg L^{-1}) produced 4.66±2.91 plantlets in our study and 3.3±0.9 in the Aggarwal and Barna's study (2004). The main difference between the two results is the evaluation period, which was 60 days for this research and 4 weeks for Aggarwal and Barna's study (2004). ANOVA analysis of the average number of off shoots produced between the 10 media, was not significant (p-value of 0.295). However using Duncan's test, some significant differences were found between averages (Table 1). Rooting of plantlets was achieved on hormone free MS (Fig. 1B) and upon transferring the plants into soil (Fig. 1C and D) the survival rate was 83%.

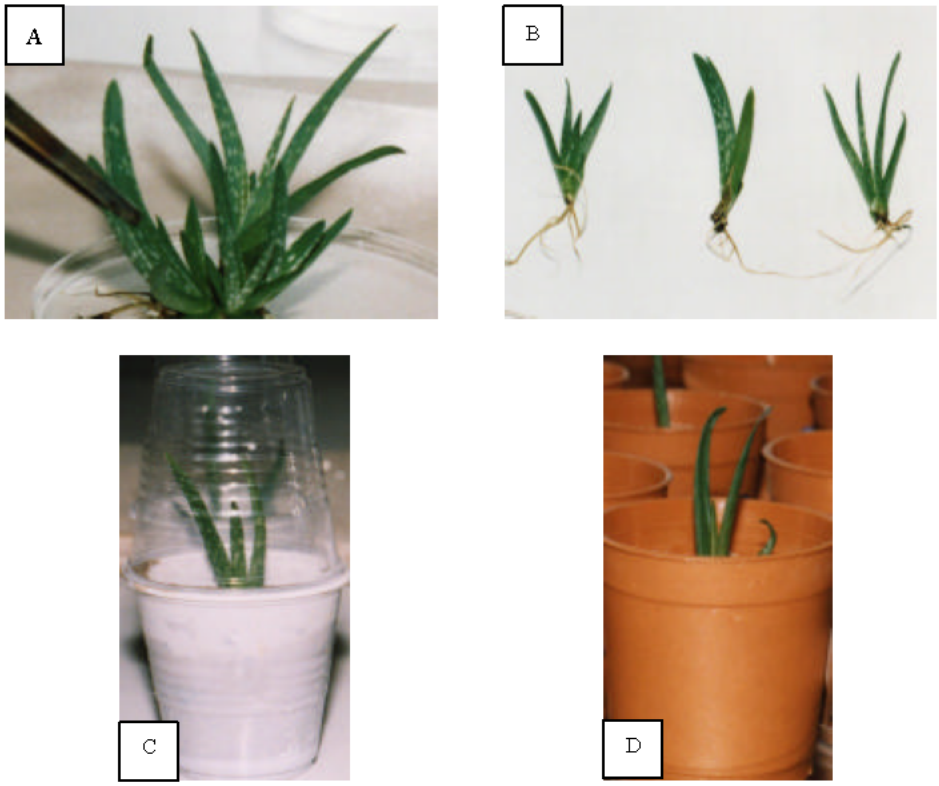

Fig. 1: Different stages of *A. vera* micropropagation. (A) micropropagated shoots, (B) rooted shoots in hormone free MS medium, (C) adaptation of micropropagated plantlets by transferring them into soil and (D) adapted plantlets into bigger pots without cover

In conclusion, it seems noteworthy that different results found by some groups may be due to the genotypes of the mother plants used. We used aloe plants growing in south Iran, whereas other groups used plants from other parts of the world. It has been proposed that *A. vera* has originated from Africa (Natali *et al*., 1990) and has been distributed to some parts of the world. It could be suggested that this plant may have evolved differently in each region and consequently respond to the growing conditions adversely. The other reason could be the physiological status and the age of mother plants that explants (offshoots) are taken from. It would be interesting to collect *A. vera* plants from different regions of the world, in one place, apply identical micropropagation experiments on all and compare the results together.

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