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DEVELOPMENT OF RAPID MICROPROPAGATION METHOD OF *ALOE VERA* L.

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ABSTRACT

An efficient micropropagation method has been developed using shoot tip explants in *Aloe vera* L. The process involves subsequent *in vitro* morphogenesis and rooting of the *in vitro* proliferated shoots and transplantation of regenerated plants under *ex vitro* condition. Shoot proliferation was found best in MS medium containing BA 2.0 mg/l, KIN 0.5 mg/l and NAA 0.2 mg/l. Maximum 98.96% shoots were proliferated in this media composition. This media composition is best comparing to other treatment used in this study. Highest shoot number per explant was also achieved in the same medium within 5 weeks. In case of adventitious rooting, MS medium containing NAA 0.2 mg/l and NAA 0.5 mg/l was found to be the best. Maximum 80.25% rooting and highest number of root per culture (6.71) was obtained in this media composition. After transplanting the 20 days old rooted shoots into mixture of garden soil, compost and sand (2:1:1), 80% of survivability after 5 weeks was an achieved. Regenerated plants after acclimatization were transferred to soil and they showed 82% survival. The regenerated plants were morphologically similar to the mother plants.

Key words: Shoot tips, MS medium, shoot proliferation, acclimatization, *Aloe vera* L.

INTRODUCTION

Aloe vera is an important medicinal plant that belongs to the family Liliaceae. The flowers are hermaphrodite, plant prefers light (sunny weather), requires well-drained soil and can grow in nutritionally poor soil. Pharmaceutical and cosmetic industry has great demand in *A. vera*. The use of aloe in therapeutic is reported by several scientists (Cera et al., 1980; Afzal et al., 1991; Davis et al., 1988). A gel in the leaves makes an excellent treatment for wounds, burns and other skin disorders, placing a protective coat over the affected area, speeding up the rate of healing and reducing the risk of infection

(Facciola 1990; Hart et al., 1988, Anshoo et al., 2005). There is a lack of production of aloe leaf to meet the industry demand (Aggarwal and Barna, 2004). So, it is necessary to undertake large-scale cultivation of aloe. Natural propagation of *Aloe vera* is primarily by means of axillary shoots and it is rather a slow way of multiplication to meet the growing demand (Natali et al., 1990). The presence of male sterility is also a barrier in rapid propagation (Natali et al., 1990). *In vitro* technique offer a possibility to solve these problems. Several reports have been noticed rapid *in vitro* propagation of *Aloe vera* (Meyer and Staden, 1991; Aggarwal and Barna, 2004; Gui et al., 1990; Hosseini and Parsa, 2007; Fattachi et al., 2004; Tripathi and Bitallion, 1995; Albanyl, 2006). Scientists obtained different results applying different formulation of plant growth regulators. The hormonal requirement for *in vitro* differentiation differs for different genotypes. The objective of this investigation was to develop a rapid, less expensive, efficient and easy method of micropropagation of *A. vera*. We standardized new composition of growth regulators for rapid and efficient micropropagation of *A. vera* using shoot tip explant.

MATERIAL AND METHODS

Plant material

Healthy *Aloe vera* showing good biomass yield were collected for plant material. Shoots with young leaves were collected from the elite plants. The extra leaves were removed and shoots were trimmed to size of 2-3 cm for further work. The research was conducted in the Department of Botany, Rajshahi University from the period of March 2006 to August 2007.

Sterilization of explant

The shoot tips were washed thoroughly under running tap water and then treated with 1% Savlon and 4-5 drops of Tween-80 for about 15-20 minutes with constant shaking. Then explants were washed 3-4 times with sterile distilled water to make the material free from Savlon. Subsequently the explants were transferred to laminar airflow cabinet and transferred to 250/500 ml sterilized conical flask. After rinsing in 70% ethanol for 1-3 minutes the explants were immersed in 0.1% $HgCl_2$. The flask with explants was constantly shaken during sterilization. The sterilized explants were inoculated singly in culture tubes containing MS (Murashoge and Skoog, 1962) medium with 3% sugar and 0.8 % agar. The cultures were maintained at 25±2 °C with light intensity varied from 2000–3000 lux. The photoperiod was generally 14 hours light 10 hours dark.

Shoot proliferation

Thee explants were cultured on MS nutrient medium supplemented with different concentration (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l) of BA and KIN alone or in combination of BA, KIN and NAA. Percentage of explants showing shoot proliferation, number of total shoots per explant and length of the longest shoots were considered as parameters

for evaluating this experiment. At this stage, the proliferating cultures were subcultured again in the same initial medium in order to increase budding frequency. After another 5 weeks of incubation the proliferating cultures were transferred to different media for shoot elongation.

Rooting of microshoots

Newly formed shoots measuring 2-3 cm in length were excised individually from the parent explant and transferred to rooting media. Three types of growth regulators (NAA, IBA, IAA) were used in different concentration (0.1, 0.2, 0.5, 1.0 mg/l) in addition to MS semi-solid medium. The observations on development pattern of roots were made throughout the entire culture period. Data were recorded after 7-8 weeks of culture.

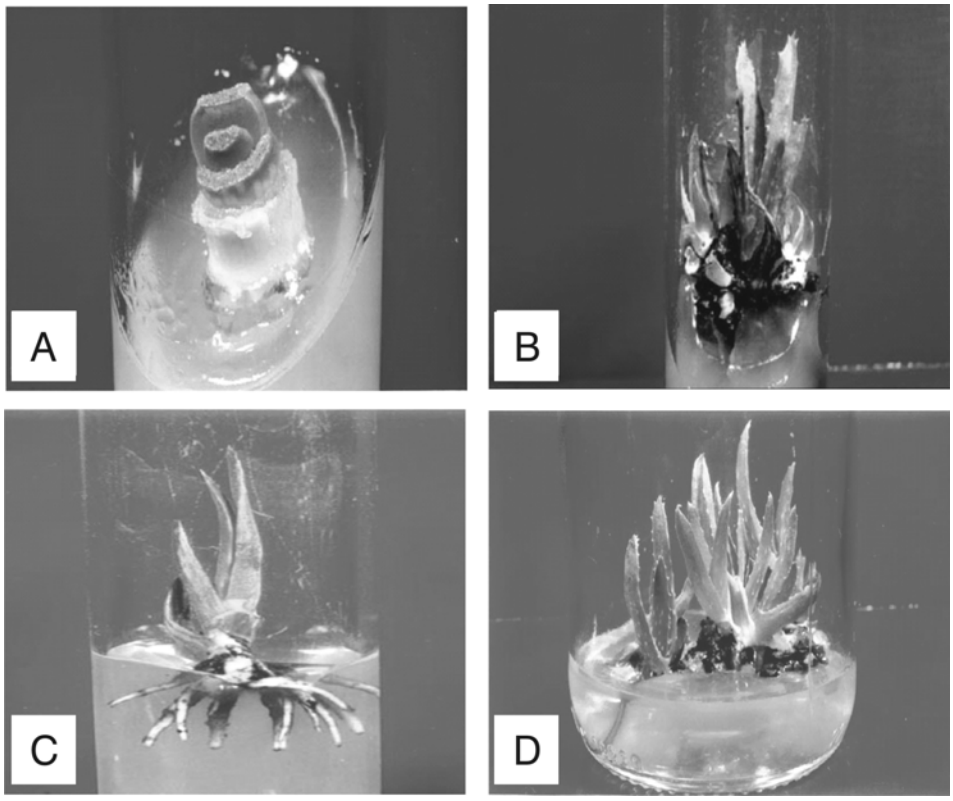
Acclimatization

After 15 days of culture on rooting media, the plantlets were taken for acclimatization. Pots (9×6 cm) were kept ready filled with garden soil, compost and sand in the proportion of 2:1:1 respectively. The plans were then transplanted in to the pots with special care. After planting the plants were thoroughly watered and kept under plastic house having 80% humidity and 31 °C temperature for ten days. Then the plants were shifted to shade house with less humidity and indirect sunlight. After 45 days, the plants were transferred to the soil. The plants were watered periodically and upper layer of the soil mulched occasionally whenever necessary.

RESULT AND DISCUSSION

The explants began to show the signs of shoot proliferation after two weeks of culturing. All explants gave aseptic cultures. Plants were free from both fungal as well as bacterial contamination. After successful initiation of the culture (4-5 weeks culturing), newly formed shoots were excised individually from the proliferated explant. Multiplication of shoots was found best on MS medium in combination of BA 2.0 mg/l, KIN 0.5 mg/l and NAA 0.2 mg/l. (Table 1 and Figure 1) and the emergence of shoots took place in 2 weeks. In this media composition, the percentage of shoot proliferation and the number of shoots per explant was 98.96 and 15.39, respectably (Table 1). But the length of the longest shoot (4.92 cm) was maximum in MS medium supplemented with BA 2.0 mg/l and NAA 0.5 mg/l. These variations were also reported by other authors (Islam, 2001; Hossain et al., 1991). The other good combinations of growth regulators in proliferating shoot were BA 2.0 mg/l and KIN 0.2 mg/l (95.30%), followed by BA 2.0 mg/l (90.91%). MS medium containing BA and NAA was found to be the best medium in Aloe micropropagation (Wenping et al., 2004; Liao, 2004). It was also reported that the highest shoot multiplication in *Aloe vera* was found in MS medium containing BA 1.0 mg/l and IBA 0.2 mg/l (Aggarwal and Barna, 2004). BA alone in concentration 2.0 mg/l has been also found effective for shoot proliferation in *Aloe vera*

Figure 1. Different stages of *in vitro* organogenesis in *Aloe vera* L.
Slika 1. Različite faze organogeneze in vitro Aloe vera L



A. An initial shoot tip explant (Aloe vera) cultured *in vitro*
A. početni vršak izboja biljke uzgojen in vitro

B. Shoot proliferation on MS medium after 5 weeks of explant
B. Razmnažanje izboja na supstratu MS 5 tjedana nakon eksplantacije

C. Microshoots showing rooting after 7-8 weeks of culture
C. Mikroizboji koji pokazuju ukorjenjavanje nakon 7-8 tjedana uzgoja

D. Complete in vitro plantlet having healthy shoots and roots
D. Potpuna in vitro bilčica sa zdravim izbojima I korijenima

(Tanabe and Horiuchi, 2006). Similar result was also found in the present investigation. A number of plants like white clover (Barna and Wakhlu, 1994), hybrid willow (Bhojwani, 1980), chickpea (Barna and Wakhlu, 1994) has been successfully multiplied on MS medium containing BA. Some scientists also reported the use of BA in shoot

Table 1. Effect of different concentration and combination of growth regulators on shoot proliferation from *in vitro* grown shoot tip explants of *Aloe vera* 35 days after explantation

Tablica 1. Djelovanje različitih koncentracija i kombinacija regulatora rasta na razmnažanje izboja in vitro izgojenih vršaka izboja Aloa vera nakon 35 dana uzgoja

Growth regulators (mg/l) *Regulator rasta (mg/l)*		% of explants showing proliferation *% razmnoženih eksplatanata*	No. of shoots per explant *Broj izboja po eksplatantu*	Average length of the longest shoot (cm) *Prosječna dužina najduljeg izboja (cm)*
BA	0.2	25.30	1.70	3.60
	0.5	40.23	3.54	4.30
	1.0	60.42	4.63	2.20
	1.5	85.73	6.85	1.85
	2.0	90.91	8.81	1.25
	2.5	75.57	6.09	1.98
	3.0	45.66	3.82	3.31
KIN	0.2	20.51	1.60	3.21
	0.5	35.33	3.10	2.39
	1.0	50.81	3.60	1.95
	1.5	65.72	4.28	1.70
	2.0	75.93	5.13	1.04
	2.5	55.11	3.44	1.60
	3.0	40.02	2.87	2.05
BA + KIN	0.5 + 0.2	45.31	4.12	3.18
	0.5 + 0.5	50.89	4.30	2.33
	0.5 + 1.0	45.92	4.21	3.09
	1.0 + 0.2	60.20	5.25	2.45
	1.0 + 0.5	65.03	5.41	2.86
	1.0 + 1.0	55.81	4.76	2.67
	1.5 + 0.2	70.32	6.23	2.54
	1.5 + 0.5	75.51	6.92	3.32
	1.5 + 1.0	60.74	5.39	3.03
	2.0 + 0.2	80.15	9.18	2.44
	2.0 + 0.5	90.51	10.46	1.96
	2.0 + 1.0	65.02	5.94	2.81
	2.5 + 0.2	55.93	4.93	3.69
	2.5 + 0.5	50.32	4.40	2.09
	2.5 + 1.0	40.23	4.09	2.39
BA + NAA	1.0 + 0.1	70.23	7.41	3.12
	1.0 + 0.2	75.84	8.18	2.62
	1.0 + 0.5	50.53	5.13	3.41
	2.0 + 0.1	80.02	9.76	3.93
	2.0 + 0.2	95.30	12.43	3.01
	2.0 + 0.5	70.81	7.15	4.92
	2.5 + 0.1	75.21	8.37	3.32
	2.5 + 0.2	80.58	9.59	2.91
	2.5 + 0.5	60.11	5.88	3.72
BA+KIN+NAA	1.0+0.2+0.1	65.63	6.35	3.01
	1.0+0.5+0.1	70.86	7.69	2.39
	1.0+0.2+0.2	55.56	5.13	3.87
	1.0+0.5+0.2	75.01	8.70	2.60
	2.0+0.2+0.1	80.90	10.21	2.59
	2.0+0.5+0.1	95.77	12.85	2.60
	2.0+0.2+0.2	70.31	7.88	2.95
	2.0+0.5+0.2	98.96	15.39	1.50
	2.5+0.2+0.1	70.30	7.52	3.11
	2.5+0.5+0.1	55.99	5.33	3.20
	2.5+0.2+0.2	65.13	6.10	3.10
	2.5+0.5+0.2	75.44	8.58	2.99

proliferation of *Aloe polyphylla* and *Aloe vera* respectively (Abrie and Staden, 2001; Chaudhuri and Mukundan, 2001). Acetic acid also helped in the enhancement of shoot proliferation in *Aloe vera* in the present study. Similar result was also reported in *A. vera* by several authors (Hosseini and Parsa, 2007).

Proliferating shoots obtained from shoot tip explants of Aloe plants took maximum 7-8 weeks from the time of establishment to attain the size suitable for rooting (> 2 cm). The highest percentage of shoots that induced roots (80.25%) was observed in MS medium supplemented with NAA 0.2 mg/l, followed IBA 0.2 mg/l (Table 2). Effect of IAA in rooting was very poor. The highest number of root per culture was found in MS medium containing NAA 0.2 mg/l (Table 2). Similar result was also obtained by Liao (2004). NAA and IBA are most commonly used for root induction (Bhojwani and Razdan, 1992). By the use of IBA, many plants such as *Lycoperscicon esculemtum* (Sibi, 1982), *Hedychium roxburgii* (Tripathi and Bitallion, 1995), *carnation* (Werker and Leshem, 1987) rooted *in vitro*.

Table 2. Effect of different concentration of growth regulators in MS medium for induction of roots from *in vitro* grown micro shoots 7-8 weeks after of explantation.

Tablica 2. Djelovanje različitih koncentracija regulatora rasta u MS mediju za induciranje korijena iz in vitro uzgojenih mikroizboja 7-8 tjedana nakon eksplantacije

Growth regulators *Regulator rasta*	Concentration (mg/l) *Koncentracija (mg/l)*	% of micro shoots rooted % ukorijenjenih mikroizboja	No. of root per micro-shoots *Broj korijena po mikroizbojima*	Average length of roots (cm) Prosječna dužina izboja (cm)
MS_0	-	-	-	-
NAA	0.1	40.36	2.98	2.05
	0.2	80.25	6.71	2.71
	0.5	60.24	4.91	2.55
	1.0	50.12	3.88	2.11
IBA	0.1	30.91	2.77	1.66
	0.2	60.85	4.51	2.33
	0.5	40.64	2.75	1.80
	1.0	20.17	1.78	1.02
IAA	0.1	-	-	-
	0.2	-	-	-
	0.5	-	-	-
	1.0	10.36	1.09	0.56

- Indicates no response
- *Indukcija nije utvrđena*

Plantlets with actively growing roots were transferred to pots containing three different types of soil mixture. The highest 82% of survivability was recorded in mixture of garden soil, compost, and sand in proportion 2:1:1 where rapid shoot length was also observed. It was also revealed that regenerated plants were morphologically similar to the mother (control) plant. That was expected knowing that the method of micropropagation used in this investigation (axillary bud method) does not usually produce somaclones.

RAZVOJ METODE BRZE MIKROPROPAGACIJE ALOJE VERE (*ALOA VERA* L.)

SAŽETAK

Razvijena je djelotvorna metoda mikrorazmnožavanja aloje vere (*Aloa vera* L.) upotrebom vršnih izboja biljke. Postupak uključuje sljedeću in vitro morfogenezu i ukorjenje in vitro razmnoženih izboja te transplataciju regeneriranih biljaka u ex vitro uvjetima. Razmnažanje se pokazalo najbolje u MS supstratu koji je sadržavao BA 2,0 mg/l, KIN 0,5 mg/l i NAA 02 mg/l. Razmnožen je maksimum od 98,96% izboja u tom sastavu supstrata. Najveći broj izboja po eksplantu također je postignut u istom supstratu u 5 tjedana. Kod adventivnog ukorjenjavanja najboljim se pokazao MS supstrat koji je sadržavao NAA 0,2 mg/l + NAA 0,5 mg/l. Maksimum od 80,25% ukorjenjenja i najveći broj korijena po eksplantu (6,71) dobiven je u supstratu istog sastava. Nakon presađivanja 20 dana starih ukorijenjenih izboja u mješavinu vrtnog tla, komposta i pijeska (2:1:1) postignuto je 80% preživljavanja nakon 5 tjedana. Regenerirane biljke su nakon aklimatiziranja presađene u zemlju uz preživljavanje od 82%. Regenerirane biljke bile su morfološki slične matičnim biljkama.

Ključne riječi: vršni izboji, supstrat MS, aklimatiziranje, Aloa vera L.

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