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(RESEARCH ARTICLE)



## *In vitro* propagation of *Aloe vera* Linn from shoot tip culture

Shibru Surafel \*, Olani Gamachu and Debebe Abel

Ethiopian Institute of Agricultural Research, Melkassa Agricultural Research Center, Ethiopia

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### Abstract

*Aloe vera* Linn., plant belongs to Liliaceae family, is a plant originated in the warm, dry climates of Africa readily adaptable and grows worldwide. Slow propagation rate of axillary shoots and male sterility to use its seeds hinders its efficient propagation. Hence, the present study aimed to standardize optimum *in vitro* propagation conditions for it. Based on this purpose MS media supplemented with 0.6 mg/L BAP and 0.2 mg/L of IBA; 1.0 mg/L BAP; and devoid of hormone were selected as the optimum medium for *Aloe vera* establishment, multiplication and rooting respectively. The higher mean number of shoots, 2.90 and multiplication factor, 2.87 were registered on MS media with 0.2 mg/L IBA & 1.0 mg/L BAP respectively. During their rooting and acclimatization, the selected treatment yields 100% rooted plant and 92.59 survival percentage during acclimatization on 3:1 mixture of filter cake and sand after their fifth week. Application of this protocol helps to propagate thousands of *Aloe vera* plants from few initial mother plants in year period.

**Keywords:** *Aloe vera* Linn; MS media; BAP; IBA; *In vitro* propagation

### Abbreviations

MS- Murashige and Skoog; BAP- 6-Benzylaminopurine; IBA- 3-Indole Butyric Acid; KN- Kinetin and NAA-  $\alpha$ -Naphthalene acetic acid

### 1. Introduction

*Aloe vera* Linn., belongs to the family Liliaceae, is a xerophytic medicinal plant and grows even in rainfall condition. Although *Aloe vera* originated in the warm, dry climates of Africa, the plant is readily adaptable and grows worldwide [1]. Its gel extracts used in various pharmaceutical product, health foods, beverages and cosmetic industries due to its metabolites [2, 3]. Mexico, followed by the rest of Latin America, China, Thailand, and the USA were described as main producing countries for *Aloe* [2]. Also roots and fresh saps of *Aloe vera* also being used as traditional medicine in Ethiopia for sprain and diabetes [4]. Hence, there is a growing demand in the market. For example, there was a shortage of 641.5 tons in the *Aloe* leaf production between years 2001-2002 to meet the industry demand [5]. Propagation of *Aloe vera* by conventional methods or by means of offshoots has many drawbacks like slow propagation rate in which a single plant produces three to four offshoots every year, and male sterility to use its seeds as well [6]. To overcome this problem, plant tissue culture propagation has been applied which has been used for rapid clonal propagation of many plants [7, 8]. The technique is particularly useful for plants where the rate of multiplication is very slow. Hence, there have been many attempts to multiply the crop *in vitro* by many workers. Shoot tips have been used as the explant source in most of the *in vitro* micropropagation protocols [9-13].

\*Corresponding author

E-mail address: [surishib@yahoo.co.uk](mailto:surishib@yahoo.co.uk)

MS medium [14] was mainly used for *in vitro* propagation of *Aloe* species by different studies. For shoot proliferation, generally the growth regulators like cytokinins influence the process seriously [15, 16]. Variety of cytokinins (KN, BAP, 2-ip and zeatin) has been used in micropropagation already [17]. A 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 [15, 18]. Shoot proliferation of *Aloe polyphylla* and *A. vera* was also reported using BAP [15, 19, 20]. Among these different concentrations of BAP with 1.5 mg/L of IBA and 50 mg/L adenine sulfate were used by [13], for the *in vitro* establishment. *In vitro* multiplication was possible with different concentration of auxins and cytokinin by [9,11,20], and shoot elongation and root generation were done on MS medium with 0.5 and 1.0 mg/L of NAA [9,13] and others with combination with NAA (0.5 mg/L) [12]. Apart from the above-mentioned ways, *Aloe* gel was also reported as conventional rooting means resulting 100% rooting and the highest number of roots per culture [21]. The present study aimed at *in vitro* standardize optimum conditions for establishment, multiplication and rooting of *Aloe vera* obtained from Wondo Genet Agricultural Research Center using shoot tip as an explant.

## 2. Material and methods

### 2.1. Explant preparation

*Aloe vera* L. plant obtained from Wondo Genet Agricultural Research Center was planted in Melkassa Agricultural Research Center. All necessary plant management were given in order to conventionally propagate the plant under greenhouse condition to obtain enough amount of planting materials to start the experiment. A total of twenty shoots tip explants were collected from the offshoot-derived from the mother plant. The explants were thoroughly washed in running tap water and detergent; thereafter kept in 1% (w/v) solution of Bavistin for an hour, and finally washed thoroughly three times with sterile distilled water. Explants were trimmed to 3-4 cm, sterilized with 1% Sodium hypochlorite solution for 10 minutes, and washed 3 to 4 times with sterile distilled water.

### 2.2. Basal media composition

MS medium supplemented with 100 mg/L activated charcoal, 100 mg/L ascorbic acid, 50 mg/L PVP, 25 mg/L L-arginine, 50 mg/L adenine sulfate, 3% sucrose, 0.8% agar with the pH 5.8 was used. The medium was autoclaved at 121°C for 20 min. Shoot tip explants (1-2 cm) were inoculated on MS medium containing four different concentration of hormones: BAP (0.4 mg/L, 0.6 mg/L, 0.8 mg/L and 1 mg/L) with 0.2 mg/L of IBA for shoot initiation. One shoot per culture bottle was inoculated and 5 replicates were maintained.

MS medium supplemented with 3% sucrose, 0.2% phytigel with the pH of 5.8 containing five different hormone concentrations: (0.2, 0.2), (0.2, 0.4), (0.2, 1.0), (0.0, 1.0) mg/L IBA and BAP respectively, and (0.1, 1.0) mg/L IAA and kinetin were used to culture the initiated shoots and in subsequent multiplication sub cultures. The established shoots spliced into two and cultured in each multiplication treatments. In order to avoid the carryover of the remaining hormone from the establishment period, explant obtained from one treatment was distributed evenly to all treatments of multiplication. Two spliced explants per culture bottle were inoculated for the first sub culture and 3-6 explants were inoculated in every four weeks of subsequent sub cultures.

A similar media used for multiplication but with different hormone concentration was used for rooting of micro shoots. Hormone treatments used for this stage includes 0.2 mg/L IBA alone, 1.0 mg/L NAA alone, 0.5 mg/L NAA and BAP, and hormone free. Five shoots per culture bottle were inoculated with ten replications. When plantlets become ready for acclimatization, plantlets were washed in tap water followed by a dip in 1% (w/v) RIDOMIL GOLD® for 10 min and planted on a plastic seedling tray containing 3:1 oven sterilized filter cake and sand respectively.

### 2.3. Data collection and analysis

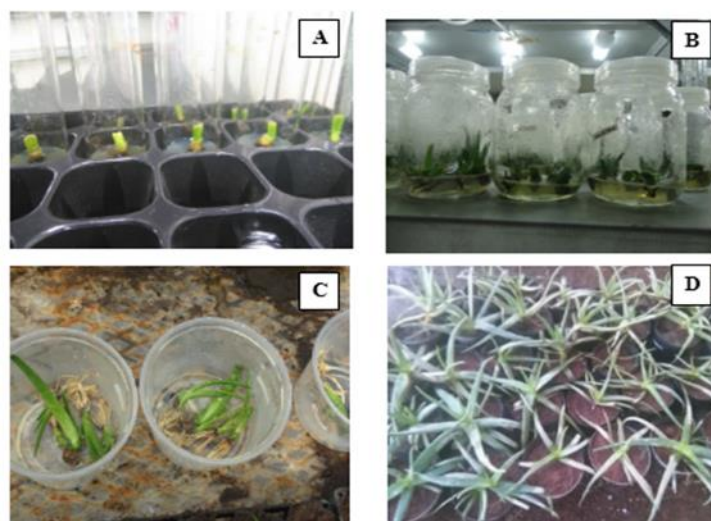
At each stage, all necessary data have been taken for instance at establishment stage data like number of survived and sprouted plants were counted every week, for multiplication during the subcultures and for rooting every week and during planting the number of rooted explant and the number of survived plants were counted respectively. The cultures were incubated under 16 hrs photoperiod with 2000-2500 lux at 25±1 °C and 60±5% Relative Humidity (RH). The number of shoots and multiplication factors (which explains, to how many parts a cultured explants divided into the next culture; that means a very vigorous shoot was divided into two to four parts based on its vigorous. In the other way, two or more adventitious shoots were cultured as one based on their size) were taken during subculture for multiplication stage; while the number of rooted explants in each week, shoot length, root density and root length were taken before acclimatization. During acclimatization, the number of survived plants from each treatment and replication were counted each week. All the count data were transformed using square root and analyzed by R software [22].

### 3. Results and discussion

Data analysis for *Aloe vera* explant survival rate during establishment stage indicated that 80% of cultured explants on 0.4 mg/L BAP with 0.2 mg/L IBA were spouted; for the remaining three treatments 100% of cultured explants were spouted. Since there is no statistical difference between treatments, the optimum medium selected based on the least amount of hormone concentration used in the medium which is MS media supplemented with 0.6 mg/L BAP and 0.2 mg/L of IBA was selected as initiation media (Table 1). Many scholars avoid the establishment culture and merge the first two stages as one [11, 12]. However, since the *Aloe* culture had huge phenolic extraction and browning mainly during the initial stages, it is advisable to evaluate the establishment stage separately (Figure 1A) [23, 24]. In addition to lateral shoot tips, basal portions of inflorescence could also have utilized as a primary explant source for *in vitro* regeneration and multiplication via organogenesis/ callus formations [19, 23].

**Table 1** Weekly survival and sprout percentage of initiated *Aloe vera*

Treatments	Concentration of BAP (mg/L)	Weekly survival percentage (%)					Weekly sprout percentage (%)				
		1	2	3	4	5	1	2	3	4	5
1	1 (0.4)	100	80	80	80	80	20	40	60	60	80
2	2 (0.6)	100	100	100	100	100	80	100	100	100	100
3	3 (0.8)	100	100	100	100	100	60	80	100	100	100
4	4 (1.0)	100	100	100	100	100	40	100	100	100	100



**Figure 1** Procedure within *Aloe vera* tissue cultures

A-*Aloe* shoot tips being established on initiation media. B-*Aloe vera* culture on multiplication media treatments. C- Rooted cultures being treated to be acclimatization. D-Acclimatized seedlings within greenhouse conditions.

The analysis of variance results to evaluate the multiplication treatments using two parameter indicated significant variations for their number of shoots and multiplication factors ( $P=0.006$  and  $0.0005$  respectively). MS media with 1.0 mg/L BAP alone showed the highest means for numbers of shoots per explants (2.90), whereas treatment with 0.2 IBA and 1.0 BAP had the highest multiplication factor (2.87) during mean comparison among hormone combinations used for *Aloe vera* multiplication (Table 2 and 3). In contrast to the present findings, [11] reported a better micropropagation rate with a combination of two cytokinins (BAP with KN) in comparison to when BAP alone used. Both [9] for *Aloe barbadensis* and [12] for *Aloe vera* also indicated highest numbers of shoots (10 shoots per culture and 9.67) with 2 mg/L and 0.5 mg/L BAP along with 0.5 mg/L NAA respectively. Lower numbers in comparison to the above-mentioned studies.

**Table 2** Mean comparison of among numbers of shoots and multiplication factors/ratio between treatments

Parameter	Treatments (mg/L)	Mean	Mean comparison
No. of shoots	1 (0.2 IBA + 0.2 BAP)	2.26	B
	2 (0.2 IBA + 0.4 BAP)	2.19	B
	3 (0.1 IAA + 1.0 KN)	2.34	B
	4 (0.2 IBA + 1.0 BAP)	2.54	AB
	5 (1.0 BAP alone)	2.9	A
Multiplication factor/ratio	1 (0.2 IBA + 0.2 BAP)	2.36	B
	2 (0.2 IBA + 0.4 BAP)	2.27	B
	3 (0.1 IAA + 1.0 KN)	2.55	AB
	4 (0.2 IBA + 1.0 BAP)	2.87	A
	5 (1.0 BAP alone)	2.81	A

**Table 3** ANOVA summary of effect of different concentrations of plant hormones on number of shoots and their multiplication ratio in *Aloe vera*

Sources of variations	Number of Shoots					Multiplication ratio				
	DF	SS	MS	F value	Pr (>F)	DF	SS	MS	F value	Pr (>F)
Treatments	4	0.174	0.044	3.693	0.006**	4	0.195	0.049	5.172	0.0005**
Residuals	230	2.714	0.012			230	2.168	0.009		*

DF-Degree of freedom, SS- sum of squares, MS- mean square, CV (8.66 and 7.65) for both number of shoots and multiplication ratio after data (n+1)0.25 transformed, mean numbers by Duncan's new multiple range test.

\*\* Significance code (alpha level) for 0.01, \*\*\* for 0.001

After a couple of sub-cultured (three times) on the same multiplying media, the *Aloe* plantlets were transferred to rooting media. Based on the statistical analysis MS-medium without the addition of any hormones, that is the fourth treatment, showed the maximum number of rooted plantlets (5.00) and maximum percentage of survival during acclimatization in the greenhouse (Table 4 and 5). Numbers of rooted plants and their survival count after the final week 6 were 100% and 92.59% respectively. The same acclimatization soil mixture used for all treatments, which is 3:1 sieved and oven sterilized mixture of filter cake and sand respectively. Hosseini and Parsa (2007) also reported the same rooting of *Aloe* as achieved on hormone- free medium [11]. To the contrary, other studies indicated MS media with 0.5-1.0 mg/L NAA and IBA resulting in high rate of rooting and numbers of roots per shoots [9, 13, 19]. Even haven't observed any roots when shoots were cultured on half strength MS medium lacking auxin. Finally, the greater survival rate during *Aloe vera* hardening in the greenhouse in the present study was in line with those of who reported in the range of 83-95% survival [11-13, 19, 24, 25].

**Table 4** ANOVA summary of effect of different concentrations of plant hormones on rooting/number of weekly rooted explants in *Aloe vera*

Sources of variations	DF	SS	MS	F Value	Pr (> F)
Treatment	3	0.103	0.034	3.777	0.018*
Residuals	38	0.347	0.009		

\*CV= 6.52

**Table 5** Mean comparison of among number of weekly rooted explants between treatments

Treatment (mg/L)	Mean	Mean comparison
1 (0.2 IBA alone)	4.34	A
2 (1.0 NAA alone)	4.65	A
3 (0.5 NAA + 0.5 BAP)	2.51	B
4 (Hormone free)	5	A

**Table 6** Weekly rooting and survival percentage of plants *in vitro* and in greenhouse

Treatment	Weekly percentage of plants rooted						Weekly count of survival plant						Survival percentage
	1	2	3	4	5	6	0	1	2	3	4	5	
1	2.22	35.6	77.1	85.7	88.6	88.6	41	38	33	31	31	31	75.61
2	2.5	45.7	84	88	92	93.3	32	23	16	13	13	13	40.63
3	0	8.89	35	40	47.5	55	52	50	39	34	33	33	63.46
4	12.5	40	88	96	96	100	27	27	26	24	25	25	92.59

#### 4. Conclusion

Mass propagation of *Aloe vera* can be attained by this protocol. In which the initial planting material is properly established on MS media supplied by 0.6 mg/L BAP in six weeks, whereas the established explants is multiplied at 2.9 multiplication rate on MS media having 1.0 mg/L BAP in four weeks. MS media devoid of hormone is used to properly roots all explants in six weeks and gives 92.6% survival during acclimatization. Therefore by application of the obtained protocol one can multiply thousands of *Aloe vera* plants and supplied for different farms.

#### Compliance with ethical standards

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##### Disclosure of conflict of interest

The authors declare that they have no conflict of interests. Shibru Surafel conducted the set-up of the experiments and conducted the experiments, including statistical analyses and mainly wrote the manuscript. Olani Gamachu also participated in conducting the experiment and in writing of the manuscript. Debebe Abel participated in the writing of the manuscript, and management of the tissue culture laboratory.

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