A Rapid Plant Production Technique of Aloe vera. L for Commercialization

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Abstract

In recent times many techniques have been developed for mass production of high quality plants, amongst which In vitro regeneration of plants is widely used. Aloe vera which is frequently used as medicinal plant has a slow rate of growth mainly due to male sterility. Thus, In vitro culture of Aloe vera by using shoot tip as explant is used for commercial production. The process involves In vitro proliferation, In vitro rooting of the proliferated shoots and acclimatization of regenerated plantlets under ex vitro condition. The study on surface sterilization of explants revealed that 70% alcohol treatment for 1 minute followed by 0.10% $HgCl_2$ for 2 minutes and ascorbic acid (1mg/l) for 10 minutes showed minimum microbial contamination without any toxicity to the explants. Explants gave the best response on medium containing 2.0 mg/l BAP + 0.5 mg/l NAA + 20.0 mg/l $AdSO_4$, where greater number of explants (80%) were established in minimum duration (8 days). Multiple shoot proliferation and elongation were found to be the best on the medium supplemented with 4.0 mg/l BAP + 0.2 mg/l NAA + 20.0 mg/l $AdSO_4$ in terms of maximum shoots per explants (23.8) with highest shoot length (4.22 cm). Highest rooting response (90%) with maximum roots per explants (10.8) having longest root length (6.8 cm) was found to be the best on medium supplemented with 2.0 mg/l IBA and 0.5 mg/l NAA. Plantlets with developed shoot and roots were then transferred for hardening and acclimatized under the greenhouse poly-tunnels where 90.00% of survival rate was achieved.

Keywords

Aloe vera L., micro propagation, In vitro shoot induction, proliferation, rooting.

Abbreviations

$AdSO_4$ (Adenine sulphate), BAP (Benzyl amino purine), IBA (Indole butyric acid), NAA (Naphthalene acetic acid), Kn (Kinetin).

I. INTRODUCTION

Aloe vera L. is a monocotyledonous plant, belonging to the family Liliaceae. This xerophyte which grows even in dry lands under rain fed conditions have medicinal properties. Commercial *Aloes* can be obtained from wild as well as cultivated source. "*Bitter Aloe*, the sap extracted from *Aloe vera* leaves is used in various pharmaceuticals, food and cosmetic industries. They also contain a clear gel, which is used as a soothing skin remedy. Leaves are broken off and the clear gel is applied to the skin as a first aid for burns. The most important constituent of *Aloe* is "aloin". *Aloe* contains cathartic anthra-glycosides and its active principle ranging from 4.5 to 25% of Aloin. *Aloe vera* contains different bioactive compounds such as saponins, anthraquinones, mucopolysaccharides, steroids, vitamins and glucomannans [22]. These are extensively used as active ingredients in laxative, anti-obesity preparation, as antiseptic, antitumoral, anti-inflammatory, anti-oxidant, immune regulator, as moisturizer, emollient, wound healer, in various cosmetic and pharmaceutical formulations. It is a drug as well as a cosmetic. There are about more than 40 Aloe-based formulations being marketed in the global market. The traditional or conventional method of propagation of *Aloe* is primarily done by means of suckers or offshoots or lateral buds, which is a very slow, expensive and low income practice [24]. A single plant can produce only 3-4 lateral shoots in a year. Also, sexual reproduction of *Aloe vera* by means of seeds is very limited due to the presence of male sterility [19]. Because of these factors, there is less availability of plant propagating material leading to low productivity of this important plant. Thus, the natural production of *Aloe* leaves is insufficient to meet the industrial demand [2]. *In vitro* technique using micro propagation and tissue culture of *Aloe vera* offer a great possibility to overcome this problem. Hence, this technique is adopted for rapid multiplication of plants, for genetic improvement of the crop and for obtaining disease-free clones. Tissue culture or cloning of a high quality parent plant offers a viable and simple method for the large scale commercial production of *Aloe* plant.

II. MATERIALS AND METHODS

The present investigation was carried out in the Commercial Tissue Culture Laboratory of the Department of Agricultural Biotechnology, College of Agriculture, Assam Agricultural University, Jorhat.

The plant materials required for the study were collected from a reputed nursery, located at Jorhat, Assam. Shoot tip explants were collected from healthy matured mother plant of *Aloe vera* L. for *In vitro* regeneration study. Explants were washed thoroughly under running tap water for about 10 minutes till all soil and other foreign materials washed off. Sets of twenty explants were then washed with distilled water containing a few drops of Teepol and rinsed in 70% ethanol for 30 seconds followed by initial soaking in sodium hypochlorite containing approximately 4% available chlorine for 10 minutes and then in freshly prepared mercuric chloride solution (0.1%) for 2 minutes. Finally they were washed 3-4 times with sterile distilled water before culturing.

A. *Shoot induction, proliferation and cultural condition*

Shoot tip explants were inoculated in tissue culture bottles containing modified MS [26] media supplemented with different concentration and combination of BAP (1.0-2.5 mg/l), and NAA (0.2-0.7 mg/l) for establishment. Sucrose (3%) was used as carbon source and media were solidified with Agar-agar (0.8%). As additives, ascorbic acid and adenine sulphate were used. The pH was adjusted to pH 5.7 prior to autoclaving at 121°C for 20 minutes. The bottles were then removed from the autoclave and allowed to cool. They were kept in the culture room, maintained at 25±2^{o}C temperature and 70-80% RH under 3000 lux light intensity and 12 hours dark cycles. All the initially cultured explants onto MS medium growing around 2-3 cm in length were detached individually from the parent explants. They were then transferred for shoot multiplication in modified MS media supplemented with different concentration and combination of growth regulators, BAP (3.0-4.5 mg/l) and NAA (0.1-0.5 mg/l). The best responding explants devoid of contamination were serially sub cultured on the same regeneration media for the proliferation, amplification, and shoot elongation.

B. *Rooting of micro shoots*

Regenerated shoots of 3-4 cm length were transferred to rooting media supplemented with different concentration and combination of IBA (1.0-2.0 mg/l) and NAA (0.5-2.0 mg/l).

C. *Acclimatization*

About 5-6cm tall, well rooted plantlets were taken out from the culture bottles after one month from root induction media and the root system was washed carefully to remove residues of agar attached to them. Rooted plantlets were transferred to hardening media containing coco peat and perlite (1:1) mixtures in plastic pots, and maintained under poly sheet tunnels in the Agro shade net house for hardening. The plants were watered twice in a day initially, then once in a day. Finally *In vitro* acclimatized plantlets were transferred to the soil mixture with sand and cow dung (2:1:1) and planted in earthen pots.

D. *Statistical Analysis*

The experimental design was completely randomized with three replications per treatment. Data were statistically analysed by analysis of variance (ANOVA) technique and differences among treatment means were compared by using Duncan's multiple range test (DMRT) at 5% probability level.

III. RESULTS AND DISCUSSION

In the present investigation, shoot tips were found to be the most suitable explant that show positive response for establishment under *In vitro* condition. Shoot tips gave the quickest response for regeneration of shoot after 7 days of culture. This response might be due to the different physiological state of the explants as well as the number of cells undergoing de-differentiation [34]. Shoot tips are highly regenerative since they have meristematic cells. Similar reports were made by [27], [24], [2], [23], [16], [8], [21], [10]. Therefore, shoot tip was found to be as a suitable source of explant for micro propagation of *Aloe vera* L.

To conduct the investigation, after collecting explants from mature plants, they were subjected to various surface sterilization treatments to establish contamination free healthy cultures. Among the treatments, shoot tips which were surface sterilized with 70% alcohol for 1 minute followed by 0.10% $HgCl_2$ for 2 minutes followed by ascorbic acid treatment (1mg/l) for 10 minutes gave the highest survival rate of 90.00% with minimum microbial contamination (Table 1) and proved to be the best treatment. This is significantly superior to the other treatments. This is because the most useful radical in $HgCl_2$ is probably the chloride, commonly present as bi-chloride of mercury. The two chloride atoms of mercury have high bleaching activity and tendency to mercuric ions to combine strongly with protein causes the death of organism [29]. The higher treatment of 0.10% $HgCl_2$ for more than 10 minutes recorded the death of explants. This is due to the high bleaching activity of chlorine which killed the cells. When explants were treated for only 1 minute contamination was high and when treatment duration is high, browning occurs due to death of tissue. The degree of injury or disinfection of explants depend on the sterilization procedure and also, on the type of the plant materials. *Aloe vera* tissue has low lignin content which is sensitive to disinfectants. The results were found in close conformity with the findings of [21] who sterilized the shoot tip explants with 1% solution of savlon for 1-2 minutes, then dipped in 70% alcohol for 30 second followed by 0.1%

aqueous solution of $HgCl_2$ for 5 minutes after which explants were thoroughly washed for 4-5 times with sterile water to remove any traces of mercuric chloride. Hosseini and Parsa sterilized axillary shoots by using 0.12% $HgCl_2$ for 5 minutes, then washed for 3-4 times with distilled water. $HgCl_2$ is an oxidizing agent that can damage the microorganisms by oxidizing the enzymes [31]. $HgCl_2$ is reported as a better sterilizing agent compared to NaOCl but is more toxic if used in higher concentration for higher duration, thus require special handling.

For establishment, optimum ratio of cytokinin and auxin played a vital role during the early stage. The shoot tip explants of *Aloe vera* L. were cultured on modified MS medium supplemented with various concentrations and combination of BAP and NAA. In MS basal medium, sucrose was added as a carbon source which is important for bud initiation. As additives, ascorbic acid and adenine sulphate were used. Ascorbic acid (2mg/l) was used as antioxidant, found to be effective in controlling the exudation of phenolic compounds from cut surface of explants and prevent them from damage. Similar reports were found from [16], [28] etc. Adenine sulphate (20 mg/l) was used because it promotes shoot initiation and proliferation. This result was found in close conformity with the findings of [11], [28], [12], [8], [21]. But they have used higher concentration of adenine sulphate (50-160 mg/l). This difference might be due to the endogenous level of adenine sulphate (a cytokinin) in the explants. Maximum establishment of shoot tip explants (80.00%) was recorded in the medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA. Shoot tips had taken 8.4 days for its establishment which was significantly lower compared to the other treatments. Similar findings were reported by [6] and [15]. But, some other scientists reported different concentrations of BAP and NAA, found to be effective for better establishment ([2], [23], [3], [13], [16] etc). This might be due to the different genotype, age and source of the explants and due to the cumulative effect of endogenous and exogenous level of cytokinin and auxin. Some scientists revealed that a suitable combination of cytokinin and auxin was needed for shoot initiation ([1], [20]). During the early stages of explants initiation, a high cytokinin to low auxin ratio favoured the response of growth of explants. With the increase in auxin concentration, the response of explants was found to be detoriated in establishment media. It might be due to the reason that at early growth stages, shoot tips contain more endogenous level of auxin, thus require less exogenous application of auxin. After 1-2 weeks of inoculation, explants had started to show some physiological changes like greening, swelling and vertical increase of explants.

Growth and morphogenesis of *In vitro* induced shoots were regulated by interaction and balance between the growth regulators supplied in the medium and growth substances produced endogenously by the cultured cells. Apart from the direct effect on cellular mechanisms, many synthetic growth regulators may modify the level of endogenous substance [14]. Some researchers indicated that the presence of both cytokinin and auxin is necessary for shoot proliferation and elongation ([33], [32], [39], [23], [37]).In this investigation, newly induced shoots from shoot tip explants were cultured on MS media supplemented with different concentrations and combinations of BAP and NAA. There was significant improvement of shoot multiplication with the increase in the level of BAP compared to the control. On the medium having 4.0 mg/l BAP and 0.2 mg/l NAA along with ascorbic acid (2 mg/l) and adenine sulphate (20 mg/l), the explants showed the best response for shoot proliferation. In case of shoot tip explants, 90.00% responded for multiple shoot initiation within a minimum average of 10.8 days having highest number of shoots (23.8) per explants with the longest shoot length (4.22 cm). This was found to be the significant over the other treatments. Similar report was given by [28], where 16.0 numbers of shoots were obtained per explants by using lateral shoots as explants on medium containing 4 mg/l BAP and 0.2 mg/l NAA. Reference [20] also reported that maximum shoots per explant (18.1 ± 0.61) and length of plantlets (2.5 ± 0.39) was obtained from lateral shoot explants on the medium containing the same concentrations of BAP and NAA as above. But, [3] reported 98.96 % shoot proliferation and 15.39 shoots per explant in a medium containing 2.0 mg/l BA, 0.5 mg/l Kn and 0.2 mg/l NAA. Reference [39] and [23] reported 95.30% shoot proliferation in a combination of BA and NAA. Whereas, [2] documented highest shoot multiplication (3.3 ± 0.9 shoots per explant) on medium supplemented with 1.0 mg/l BA and 0.2 mg/l IBA. Reference [16] reported maximum number of shoots around 9.67 shoots per explant in medium containing 0.5 mg/l BA and 0.5 mg/l NAA. This difference might be due to genotypic variation of explants reinforced by the cultural and environmental conditions and for the cumulative effect of endogenous and exogenous level of growth hormones. For multiplication, cytokinin has been utilized because it has the ability to overcome apical dominance of shoot which enhances the branching of lateral buds. Cytokinin also tends to induce adventitious bud formation. According to the hormonal control of organogenesis, a high cytokinin to low auxin ratio favours shoot differentiation and elongation [35]. Reference [40] discovered that cytokinin could release lateral buds from apical dominance. In presence of cytokinin, the dormant buds of vegetative apex are stimulated to grow and elongate. The increase in shoot length is the result of rapid elongation of cells which is due to cell division and cell differentiation. Shoot proliferation in tissue

culture is largely due to the action of BAP. Optimum dose of BAP promotes axillary and multiple branching and thus multiple shoots were achieved. The optimum level of cytokinin is also genotype dependent [4]. Similarly, increase in NAA concentration beyond an optimal level induces callus formation and thus retard the axillary bud sprouting.

The regenerated shoots having 3-4 cm height, were excised and sub-cultured individually in the rooting media. *In vitro* rooting was achieved with the addition of auxin alone in the MS medium. The total omission of cytokinin in rooting media did not affect the differentiation of root primordial as well as the general root morphology. The highest rooting of 90.00% was observed on the medium containing 2 mg/l IBA in combination with 0.5 mg/l NAA. As a result, early rooting took place at 8.4 days having the highest number of roots (10.8) per shoot with the longest root length (6.84 cm). The result of present investigation on root induction with auxin alone are in conformity with the previous work done by [20] who found maximum roots per explants was 6.7 ± 0.31 and length of roots were 7.1 ± 0.53 in the medium containing IBA (2 mg/l) and NAA (1 mg/l). Here, auxin was used as it enhances cell division in root apical meristem. Auxin was found to be best for root induction. Among auxin, [3] reported NAA as a best auxin for rooting of *Aloe vera* while [41] found IBA best for root proliferation. NAA and IBA are most commonly used auxin for rhizogenesis [9]. Numerous studies also supported the usefulness of IBA as the most effective auxin in various plant rhizogenesis as compared to NAA ([7], [36]). They also found IBA as a preferred auxin for the induction of root formation because it is much more potent than IAA or synthetic auxin. Rooting of *Aloe vera* was also reported in hormone free medium by [27], [2]. However, roots per shoot and root length were less compared to the medium containing IBA and NAA. Auxin is a rooting hormone and the application of synthetic auxin like IBA and NAA might have increase the synthesis of IAA or could act as synergistic to IAA. Another possible reason for higher rooting and early root initiation might be due to the involvement of IBA in ethylene biosynthesis [5] and it has been suggested that auxin induced ethylene may induce adventitious root formation instead of action of auxin itself [25].

Hardening of *In vitro* regenerated plantlets is an essential operation to maximize the survival rates upon transplantation to soil. Micro propagated plantlets are relatively delicate propagules as they have grown under low light intensity and high humidity condition. They may lose water rapidly after transferring to the natural condition. Moreover *In vitro* produced plantlets are believed to have limited photoautotrophic capacity, so their energy demands must initially be met by reserves of starch accumulated during culture [38]. The benefit of any micro propagation system can however only be fully realized by the successful transfer of plantlets from tissue culture vessels to the ambient conditions [17]. The *In vitro* derived plantlets have a characteristic feature that their epicuticular layer is poorly developed. This leads to uncontrolled foliar water loss when the plants are taken out from the water vessels. *In vitro* raised plantlets also have more stomata per unit area and raised guard cells with wide opening which result in more transpiration loss and less plantlets survival [30]. However, when the plants are kept at high humidity condition they can synthesize the epicuticle layer which will enhance the survival rate during acclimatization. In this investigation, 90.00% of rooted plantlets were survived in MS medium without any growth hormones followed by 80.00% of plantlets survived in half strength MS medium, 65.00% in water agar medium, 75.00% in coco peat and perlite mixture (1:1). The plantlets transplanted into coco peat and perlite mixture were kept under shade net house polytunnels for 15-20 days. They were covered with polythene sheets to create a high humidity around the plantlets so that they could synthesize epicuticle layer. Finally, the hardened plantlets were transferred to the soil mixture with sand and cowdung (2:1:1) where 90.00% survival rate of plantlets were achieved after 30 days. The plantlets that developed epicuticular layer were able to survive under normal environmental condition. The composition of medium into which *In vitro* rooted plantlets are transplanted is important for their survival. Similar findings were reported by [3] where 82% of survivability was observed in mixture of soil, compost and sand in a proportion of 2:1:1. Reference [16] reported that 95% plantlets were successfully acclimatized in plastic pots containing a mixture of coco peat and perlite (1:1) covered with transparent plastic.

Table I : Effect Of Surface Disinfectants On Per Cent Contamination And Number Of Healthy Culture Established In Aloe Vera Explants

[a]Medium	*No. of healthy cultures established	Per cent survival	*No. of explants contaminated	Per cent contamination
ST_0	0.00	0.00 (0.00)	20.00	100.00 (99.98)
ST_1	1.67[d]	8.33 (16.59)	18.33	91.67 (73.40)
ST_2	5.00[c]	25.00 (29.92)	15.00	75.00 (60.07)

ST_3	10.33^{b}	51.67 (45.95)	9.67	48.33 (44.04)
ST_4	18.00^{a}	90.00 (71.95)	2.00	10.00 (18.04)
SEd±	0.461		0.461	
CD at 5%	0.930		0.930	

*No. of explants inoculated = 20

[a]For media composition see Table (A).

Figures in parentheses are arcsine transformation values.

Means within columns separated by Duncan's multiple range test P=0.05

Means followed by same letter shown in superscript(s) are not significantly different.

TABLE (A)
Composition of Surface Disinfectants

Treatment time	Alcohol (%)	Exposure of alcohol (min.)	($HgCl_2$) (%)	Exposure time of $HgCl_2$ (Min.)	Ascorbic acid (mg/l)	Exposure time (min.)
ST_0	-	-	-	-	-	-
ST_1	-	-	0.10	1.00	-	-
ST_2	-	-	0.10	2.00	-	-
ST_3	70.00	1.00	0.10	1.00	-	-
ST_4	70.00	1.00	0.10	2.00	1.0	10.0
		00				0

ST = Surface Sterilization Treatment

ST_0 = Control (without any treatment)

TABLE II
Effect of Modified MS Media for Shoot Tip Establishment of Aloe vera.

[a]Medium	*Shoot tip responded for establishment	Per cent shoot tip responded	Days for establishment	Length of shoot tip (cm)		
				15 days	30 days	45 days
AVE_1	0.00	0.00 (0.00)	0.00	0.00	0.00	0.00
AVE_2	3.20^{i}	16.00 (23.43)	13.80^{a} b	0.14 h	0.54 h	1.04^{h}
AVE_3	4.60^{h}	23.00 (28.63)	14.20^{a}	0.38 fg	0.74 g	1.44^{g}
AVE_4	5.60^{g}	28.00 (31.93)	13.00^{a} bc	0.32 g	0.74 g	1.38^{g}
AVE_5	8.40^{ef}	42.00 (40.39)	11.80^{c} d	0.46 efg	1.30 f	1.50^{fg}
AVE_6	10.80^{d}	54.00 (47.31)	11.40^{c} de	0.56 de	1.42 ef	1.62^{ef}
AVE_7	11.00^{d}	55.00 (47.88)	10.60^{d} ef	0.72 bc	1.34 f	1.68^{e}
AVE_8	14.40^{b}	72.00 (58.07)	10.00^{e} fg	0.72 bc	1.96 b	2.28^{b}
AVE_9	16.00^{a}	80.00 (63.50)	8.40^{g}	1.30 a	2.32 a	3.34^{a}
AVE_{10}	13.00^{c}	65.00 (53.75)	9.60^{fg}	0.86 b	1.74 c	1.94^{c}
AVE_{11}	12.20^{c}	61.00 (51.36)	9.80^{efg}	0.66 cd	1.46 def	1.86^{cd}
AVE_{12}	7.60^{f}	38.00 (38.04)	10.60^{d} ef	0.52 def	1.54 de	1.72^{de}
AVE_{13}	9.20^{e}	46.00 (42.70)	12.40^{b} c	0.54 de	1.62 cd	1.74^{de}
SEd±	**0.440**		**0.746**	**0.067**	**0.081**	**0.078**
CD at 5%	**0.883**		**1.497**	**0.135**	**0.163**	**0.157**

*No. of shoot tip inoculated = 20

[a]For media composition see Table (B)

[b]Initial length of the shoot tip = 0.10cm

Figures in parentheses are arcsine transformation values.

Means within columns separated by Duncan's multiple range test P=0.05

Means followed by same letter shown in superscript(s) are not significantly different

TABLE (B)

Composition of Modified MS Media Tested for Establishment of various Explants

Media	Basal media	Ascorbic acid (mg/l)	$AdSO_4$ (mg/l)	BAP (mg/l)	NAA (mg/l)
AVE_1	MS	-	-	-	-
AVE_2	MS	2.0	20.0	1.0	0.2
AVE_3	MS	2.0	20.0	1.0	0.5
AVE_4	MS	2.0	20.0	1.0	0.7
AVE_5	MS	2.0	20.0	1.5	0.2
AVE_6	MS	2.0	20.0	1.5	0.5
AVE_7	MS	2.0	20.0	1.5	0.7
AVE_8	MS	2.0	20.0	2.0	0.2
AVE_9	MS	2.0	20.0	2.0	0.5
AVE_{10}	MS	2.0	20.0	2.0	0.7
AVE_{11}	MS	2.0	20.0	2.5	0.2
AVE_{12}	MS	2.0	20.0	2.5	0.5
AVE_{13}	MS	2.0	20.0	2.5	0.7

AVE = Aloe vera Establishment Media

MS = Murashige and Skoog medium

* All media contain MS basal + Sucrose 3%, Agar 0.8%

pH = 5.8

TABLE III

Effect of Modified MS Media for Multiple Shoot Proliferation from Established Shoot Tip Explant of Aloe vera

[a]Medium	*Shoot tip responded for multiple shooting	Per cent shoot tip responded	Days to multiple shoot initiation	8 weeks of culture		
				Shoots per explants	Shoot length (cm)	Leaves per shoot
AVS_1	0.00	0.00 (0.00)	0.00	0.00	0.00	0.00
AVS_2	4.80^{f}	24.00 (29.31)	21.20^{a}	4.20^{g}	1.68^{f}	3.40^{f}
AVS_3	5.20^{f}	26.00 (30.59)	19.80^{b}	6.40^{f}	1.80^{f}	3.60^{f}
AVS_4	10.00^{e}	50.00 (45.00)	17.80^{c}	7.60^{f}	2.34^{e}	4.00^{def}
AVS_5	12.80^{d}	64.00 (53.21)	17.40^{c}	10.40^{e}	3.06^{c}	4.60^{cde}
AVS_6	11.20^{e}	56.00 (48.46)	15.80^{d}	13.40^{d}	2.76^{d}	5.20^{abc}
AVS_7	15.60^{b}	78.00 (62.35)	14.80^{de}	14.00^{d}	2.42^{e}	5.00^{abc}
AVS_8	16.00^{b}	80.00 (63.50)	12.20^{f}	16.60^{c}	3.84^{b}	5.80^{a}
AVS_9	18.00^{a}	90.00 (71.56)	10.80^{g}	23.80^{a}	4.22^{a}	5.60^{ab}
AVS_{10}	17.60^{a}	88.00 (69.82)	12.20^{f}	19.40^{b}	3.86^{b}	4.60^{cde}
AVS_{11}	14.20^{c}	71.00 (57.51)	13.60^{e}	17.20^{bc}	3.72^{b}	4.80^{bcd}
AVS_{12}	13.60^{cd}	68.00 (55.61)	14.40^{e}	17.60^{bc}	3.56^{b}	4.00^{def}
AVS_{13}	12.60^{d}	63.00 (52.57)	14.00^{e}	16.20^{c}	3.64^{b}	3.80^{ef}
SEd±	**0.612**		**0.594**	**1.072**	**0.135**	**0.422**
CD at 5%	**1.229**		**1.193**	**2.152**	**0.271**	**0.847**

*No. of shoot tip explants inoculated = 20

[a]For media composition see Table (C).

Means within columns separated by Duncan's multiple range test P=0.05

Means followed by same letter shown in superscript(s) are not significantly different.

Figures in parentheses are arcsine transformation values.

TABLE C

Composition of Modified MS Media Tested for Multiple Shoot Proliferation

Media	Basal media	Ascorbic acid (mg/l)	$AdSO_4$ (mg/l)	BAP (mg/l)	NAA (mg/l)
AVS_1	MS	-	-	-	-
AVS_2	MS	2.0	20.0	3.0	0.1
AVS_3	MS	2.0	20.0	3.0	0.2
AVS_4	MS	2.0	20.0	3.0	0.5
AVS_5	MS	2.0	20.0	3.5	0.1

AVS_6	MS	2.0	20.0	3.5	0.2
AVS_7	MS	2.0	20.0	3.5	0.5
AVS_8	MS	2.0	20.0	4.0	0.1
AVS_9	MS	2.0	20.0	4.0	0.2
AVS_{10}	MS	2.0	20.0	4.0	0.5
AVS_{11}	MS	2.0	20.0	4.5	0.1
AVS_{12}	MS	2.0	20.0	4.5	0.2
AVS_{13}	MS	2.0	20.0	4.5	0.5

AVS = Aloe vera Shooting Media
MS = Murashige and Skoog medium
* All media contain MS basal + Sucrose 3%, Agar 0.8%
pH = 5.8

TABLE IV
Effect of modified MS Media for In vitro Rooting from Regenerated shoots of Aloe vera

[a]Medium	*Shoots responded for rooting	Per cent rooting	Days to root initiation	5 weeks of culture	
				Roots per shoot	Root length (cm)
AVR_1	0.00	0.00 (0.00)	0.00	0.00	0.00
AVR_2	4.00^{f}	40.00 (39.23)	14.40^{bc}	3.40^{h}	2.36^{h}
AVR_3	7.00^{cde}	70.00 (56.91)	13.60^{cd}	5.40^{fg}	3.02^{g}
AVR_4	6.60^{de}	66.00 (54.38)	12.80^{de}	6.80^{de}	4.22^{de}
AVR_5	5.00^{f}	50.00 (45.00)	15.00^{ab}	3.80^{h}	2.56^{h}
AVR_6	6.20^{e}	62.00 (52.02)	15.60^{a}	4.60^{gh}	3.10^{g}
AVR_7	4.20^{f}	42.00 (40.33)	14.60^{abc}	4.20^{h}	3.06^{g}
AVR_8	8.00^{abc}	80.00 (63.73)	12.00^{e}	6.40^{ef}	3.58^{f}
AVR_9	7.20^{bcde}	72.00 (58.71)	10.80^{f}	7.20^{de}	3.59^{d}
AVR_{10}	6.80^{de}	68.00 (55.88)	9.80^{fg}	7.80^{cd}	4.12^{e}
AVR_{11}	7.40^{bcd}	74.00 (59.44)	10.40^{fg}	8.60^{bc}	5.28^{c}
AVR_{12}	9.00^{a}	90.00	8.40^{h}	10.80^{a}	6.84
AVR_{13}	8.20^{ab}	82.00 (65.35)	9.60^{g}	9.20^{b}	5.76 b
SEd±	**0.483**		**0.486**	**0.551**	**0.186**
CD at 5%	**0.970**		**0.976**	**1.107**	**0.374**

*No. of Shoots inoculated = 10
[a]For media composition see Table (D).

Figures in parentheses are arcsine transformation values.
Means within columns separated by Duncan's multiple range test P=0.05
Means followed by same letter shown in superscript(s) are not significantly different.

TABLE D
Composition of Modified MS Media Tested for Rooting of *In vitro* Regenerated Shoots

Media	Basal media	IBA (mg/l)	NAA (mg/l)
AVR_1	MS	-	-
AVR_2	MS	1.0	-
AVR_3	MS	1.5	-
AVR_4	MS	2.0	-
AVR_5	MS	-	1.0
AVR_6	MS	-	1.5
AVR_7	MS	-	2.0
AVR_8	MS	1.0	0.5
AVR_9	MS	1.0	1.0
AVR_{10}	MS	1.5	0.5
AVR_{11}	MS	1.5	1.0
AVR_{12}	MS	2.0	0.5
AVR_{13}	MS	2.0	1.0

AVR = Aloe vera Rooting Media
MS = Murashige and Skoog medium
* All media contain MS basal + Sucrose 3%, Agar 0.8%
pH = 5.8

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