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Influence of Plant Growth Regulators on Micropropagation of Gwarpatha [*Aloe vera* (L.) Burm.]

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A B S T R A C T

Present investigation was carried out to determine the optimum level of different plant growth regulators for rapid shoot multiplication, rooting and hardening of micropropagated plantlets of *Aloe vera*. The experiment was laid out using completely randomized design (CRD) with ten replications during 2018-19. Auxiliary shoot explants were inoculated on MS medium supplemented with various concentrations of cytokinin (BAP 0.5 – 6.0 mg/l) alone and BAP (0.5-6.0 mg/l) with auxin (NAA 0.1 – 0.6) in combination for direct shoot bud proliferation. For rooting, auxins (NAA/IBA) were used at the concentrations of 0.5-2.0 mg/l singly and 0.5-2.0 mg/l NAA with IBA at 0.5 and 2.0 mg/l in combinations. Maximum shoot bud induction was obtained when MS medium supplemented with 4.0 mg/l BAP singly and 4.5 mg/l BAP with 0.6 mg/l NAA in combination. Maximum rooting was induced at 1.5 mg/l IBA and 0.5 mg/l IBA with 2.0 mg/l NAA in combination. The rooted plantlets showed 80 per cent survival in culture room and 74 per cent in shade house during the process of hardening. Further, it is recommended that the given protocols may be used for mass multiplication of *Aloe vera* to meet the demand of farmers as well as pharmaceutical industries.

Introduction

Desert medicinal plant Gwarpatha (*Aloe vera* L. syn. *Aloe barbadensis* Miller) belongs to family *Asphadelaceae* (Souza and Lorenzi 2005). It is an ancient, semi tropical medicinal plant indigenous to Africa, Madagascar and Arabia and introduced plant in India. This genus has more than four hundred species of flowering succulent plants. *Aloe vera* is coarse looking evergreen perennial plant, grows up to 80-100 cm in height, with a strong fibrous root and at large stem supporting a rosette of narrow lanceolate leaves. The leaves are whitish green on both sides and bear spiny teeth on the margins. The

yellow to orange drooping flowers produced in an inflorescence of 90-110 cm tall, each flower is pendulous, with yellow tubular corolla 2-3 cm and hermaphrodite. It has valuable medicinal properties and is commercially used in pharmaceuticals, cosmetics, food industries, as nutraceuticals and for many herbal preparations. There are about more than 40 *Aloe* based formulations being marketed in the global market. In India, tissue culture research began nearly six decades ago with the first report on production of test tube fertilization. Plant cell and tissue culture is defined as the capability to regenerate and propagate plants from single cells, tissues and organs under sterile and controlled environmental conditions (Murashige and Skoog, 1962). Tissue culture methods have also been employed to study the basic aspects of plant growth, metabolism, differentiation and morphogenesis and provide ideal opportunity to manipulate these processes. Advancement in tissue cultural methodology led many recalcitrant plants amenable to *in vitro* regeneration and to the development of haploids, somatic hybrids and pathogen free plants (Gupta *et al.*, 2014).

In nature (*in vivo), Aloe vera* is propagated through lateral buds which are slow, very expensive and low income practice (Meyer and Staden, 1991). Sexual reproduction by seeds due to male sterility in *Aloe vera* is almost ineffective and vegetative propagation through lateral shoots only possible during growing seasons (Keijzer and Cresti, 1987). Thus large scale plantation of *Aloe vera* through *in vivo* system of plantlet multiplication is insufficient to meet the requirements of farmers and pharmaceutical industries demand (Bhandari *et al.*, 2010). There is a need to develop suitable and alternative method for large scale propagation for rapid plant multiplication to meet the demand of farmers and pharmaceutical industries (Abrie and Staden, 2001). Therefore, the present study aimed to develop an alternative protocol for rapid and high frequency *in vitro* propagation of *Aloe vera*, looking through its increasing demand for pharmaceutical industry at global level.

Materials and Methods

The present investigation was carried out at Tissue Culture Laboratory of Department of Plant Breeding and Genetics, S.K.N. Agriculture University, Jobner, Rajasthan, India during 2018-19.

Plant growth regulators

Different concentrations of plant growth regulators were incorporated singly and in combinations in the MS medium for direct shoot proliferation from auxiliary explants.

1. Plant growth regulators incorporated singly in the medium: BAP: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0 mg/l
2. Plant growth regulators incorporated in combinations: BAP: (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0 mg/l) + NAA: (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg/l) in all possible combinations.

Induction of roots

Explants were inoculated for induction of roots in the medium supplemented with different concentrations of plant growth regulators added alone or in combinations.

1. Plant growth regulators incorporated singly in the medium NAA/IBA: 0.5, 1.0, 1.5 and 2.0 mg/l.
2. Plant growth regulators incorporated in combinations:

NAA: (0.5, 1.0, 1.5 and 2.0 mg/l) + IBA: (0.5 and 2.0 mg/l)

MS basal medium without supplementation of any growth regulator was used as a control in

all the experiments. All the cultures were maintained in an air conditioned culture room at a temperature of 25 ± 2^0C under fluorescent light in a 14:10 hours' photoperiod.

Acclimatization of plantlets

After 40-50 days of culture on rooting media, the plantlets were shifted to polythene bags/earthen pots for their hardening prior to final transfer to soil in natural conditions. After removing media, these were dipped in 1% w/v solution of Bavistin to prevent any fungal infection to newly developed plants. After Bavistin treatment the plantlets were carefully planted in polythene bags/earthen pots containing 1:1 mixture of soil and vermicompost. Then the plants were shifted to shade house with indirect sunlight. After 50-60 days, shade house plants were finally transferred to the field.

Observations recorded

Observations on period of initiation of shoot, number of shoots per explant, morphogenetic response (Percent), shoots length (cm), number of days taken for root induction, number of roots, root length (cm), mean days taken for callus initiation, colour and texture of the callus are recorded following standard procedures.

Statistical analysis

Each experiment was conducted in completely randomized design and data were analyzed for means and standard error accordingly as described by Snedecor and Cochran (1972). Standard error was calculated only after value transformation for the characters where response was less than 100 per cent. The value for each replication was transformed by square root transformation as follows

$$\sqrt{Y + ½}$$

Where, Y= original value

Tests of significance were done according to Duncan's Multiple Range Test (DMRT) for different traits (Gomez and Gomez, 1984).

Results and Discussion

Effect of plant growth regulators on shoot proliferation

BAP was used as cytokinin for shoot bud proliferation in the present investigation. The medium devoid of growth regulators failed to initiate bud break or callus formation from any of the explant. BAP when incorporated singly in the basal MS medium, induced multiple shoot buds at all the levels (Table 1), however the most profuse shoot bud breaks (12.8) and shoot length (8.9 cm) were obtained at 4.0 mg/l BAP (Fig. 1) followed by 6.0 mg/l BAP (11.2). Slight to medium loose/semi compact callus was also induced at the base of auxiliary explants incubated at different levels of BAP (1.5-2.5 mg/l). Lower (0.5-1.0 mg /l) and higher (3.0-6.0) levels of BAP inhibited callus differentiation. These results were in close agreement with other reports of micropropagation of *Aloe vera* (Ahmed *et al.*, 2007 and Dwivedi *et al.*, 2014).

Eufrocinio and Malasa (2005) in *A. barbadensis* reported best multiple shoot bud induction at medium containing 1.0 mg/l BAP. However, these results were contrary to the results of present investigation with the level of BAP might be due to differences in species and explants used in the particular study. Callusing was also observed at the base of auxiliary explant in present investigation which was similar to the observations of Kawai *et al.*, 2006 and Yadav, 2008 in *Aloe arborescens* and *Aloe vera*, respectively. Auxin (BAP) combined with cytokinin

(NAA) enhanced the shoot bud multiplication considerably (Table 2). Highest shoot proliferation (12.6) was observed at medium supplemented BAP (4.5 mg/l) with NAA (0.6 mg/l) (Fig. 2). These were at par with the level of BAP (4.0 mg/l) where maximum shoot proliferation was observed upon supplementation in the medium alone. Callus formation was completely inhibited at the base of explants in the medium supplemented with combination of BAP and NAA.

These results are in accordance with the results obtained by Khanam *et al.,* (2014). They reported that a perfect combination of auxin and cytokinin is needed for optimum shoot induction. MS basal medium in combination with 4.0 mg/l BAP and 0.2 mg/l NAA was found to be the best on which explants began to show emergence of shoot buds within one week. Similar observations were also found by Nayankantha *et al.,* (2010) and Kiran *et al.,* (2017), who reported synergetic effect of BAP and NAA in shoot bud break.

Effect of plant growth regulators on root induction

Root induction was observed at all the levels of IBA and NAA added either singly or in combination with 100 per cent frequency (Table 3). Maximum number of root induction (6.6) was obtained in the medium supplemented with 1.5 mg/l IBA with shoot length (7.64 cm) followed by (5.4) 1.0 mg/l IBA (Fig. 3). In IBA supplemented media, roots were thin and long, whereas thick roots were induced in medium supplemented with NAA (Fig. 4).

In combination, maximum number of roots (5.6) was observed at 0.5 mg/l IBA with 2.0 mg/l NAA with longest root length (8.3 cm) followed by 0.5 mg/l IBA + 1.5 mg/l NAA (Fig. 5). In comparison to NAA, IBA produced higher number of roots at all the levels except at 2.0 mg/l IBA. There was significant difference among the levels of IBA and NAA for number of root induction per explant. Results of present investigation were similar to the reports of Abrie and Staden (2001) in *Aloe polyphylla* with respect to type of plant growth regulator (IBA), they observed maximum root on medium supplemented with 0.5 mg/l IBA.

However, in the present study maximum root induction was ovserved at 1.5 mg/l IBA. This might be due to difference in the species under study. Hashemabadi and Kaviani (2008) found the best root induction at MS medium supplemented with 1.0 mg/l IBA with 1.0 mg/l NAA. Khanam *et al.,* (2014) reported profuse root induction after one week of culture at 2.0 mg/l IBA and 1.0 mg/l NAA. However, these levels were not tested in the present investigation.

Acclimatization of plantlets

After 40-50 days of culture of *in vitro* developed shoots on most responsive rooting media were shifted to pots filled with mixture of 1:1 ratio of soil and vermicompost for their hardening prior to final transfer to soil, showed good percentage of survival (80 per cent) in culture room (Fig. 6). In shade house plants also showed 74 per cent survival rate (Fig. 7).

The growth and elongation of plants were less in culture room whereas, in shade house growth of plants was better and they also started to elongation and thickening of leaves in shade house. Perusal of literature on micropropagation of *Aloe* also indicated that rooting and hardening had never been encountered as a problem in *Aloe's* tissue culture. Various researchers have reported successfully *in vitro* rooting and hardening of *Aloe vera* (Kalimuthu *et al.,* 2010 and Rathore *et al.,* 2011) and corroborate present study.

Table.1 Morphogenetic effect of various concentrations of Cytokinin (BAP) added singly in the MS medium on auxiliary explant of *Aloe vera*.

S.N.	BAP (mg/l)	Days taken in shoot initiation	Morphogenetic response (%)	Number of shoot buds induction per explant		Shoot length (cm)	Days to callus initiation	Colour of callus	Texture of callus
				6 weeks after inoculation	8 weeks after inoculation				
1.	0.5	18.1	100	2.1 ± 0.18 **f**	3.8 ± 0.25 **g**	3.32 ± 0.36 **e**	-	-	-
2.	1.0	16.1	100	2.2 ± 0.25 **f**	4.1 ± 0.28 **fg**	4.55 ± 0.38 **d**	-	-	-
3.	1.5	14.6	100	2.6 ± 0.16 **f**	4.4 ± 0.16 **fg**	5.33 ± 0.36 **d**	22.1 [40]	Yellowish green	Loose
4.	2.0	15.3	100	3.4 ± 0.16 **e**	5.2 ± 0.33 **ef**	6.68 ± 0.40 **c**	24.6 [20]	Yellowish green	Loose
5.	2.5	15.9	100	3.6 ± 0.27 **e**	6.1 ± 0.28 **de**	8.3 ± 0.40 **b**	28.5 [10]	Light green	Semi compact
6.	3.0	15.3	100	3.8 ± 0.25 **e**	6.8 ± 0.25 **d**	8.55 ± 0.38 **b**	-	-	-
7.	3.5	14.2	100	5.2 ± 0.25 **d**	8.4 ± 0.45 **c**	8.9 ± 0.42 **b**	-	-	-
8.	4.0	12.5	100	8.4 ± 0.16 **a**	12.8 ± 0.39 **a**	11.16 ± 0.37 **a**	-	-	-
9.	4.5	14.4	100	6.2 ± 0.25 **c**	9.2 ± 0.44 **c**	4.92 ± 0.37 **d**	-	-	-
10.	5.0	11.5	100	6.3 ± 0.26 **c**	8.4 ± 0.45 **c**	2.05 ± 0.27 **f**	-	-	-
11.	5.5	12.2	100	7.1 ± 0.18 **b**	10.4 ± 0.34 **b**	1.62 ± 0.14 **f**	-	-	-
12.	6.0	13.2	100	6.2 ± 0.25 **c**	11.2 ± 0.77 **b**	1.47 ± 0.14 **f**	-	-	-

Values followed by same letters in each column are not significantly different ($p<0.05$) using DMRT

[ ] = Value in parenthesis represents percentage of response (-) = No Response

Table.2 Morphogenetic effect of various concentrations of Cytokinin (BAP) and Auxin (NAA) added in combinations in the MS medium on auxiliary explant of *Aloe vera*

S. N.	(BAP + NAA) (mg/l)	Days taken in shoot initiation	Morphogenetic response (%)	Number of shoot buds per explant		Shoot length (cm)
				6 weeks after inoculation	8 weeks after inoculation	
1.	0.5 + 0.1	18.2	100	2.2 ± 0.25 **x**	3.8 ± 0.249 **ab**	3.15 ± 0.33 **qrstuv**
2.	1.0 + 0.1	17.1	100	2.3 ± 0.21 **wx**	4.2 ± 0.249 **aaab**	3.31 ± 0.33 **pqrstu**
3.	1.5 + 0.1	16.2	100	2.6 ± 0.16 **vwx**	4.4 ± 0.163 **zaaab**	4.09 ± 0.39 **mnopqr**
4.	2.0 + 0.1	15.3	100	4.2 ± 0.25 **pqrs**	5.2 ± 0.2 **xyzaa**	5.24 ± 0.43 **jklm**
5.	2.5 + 0.1	15.8	100	4.3 ± 0.21 **opqrs**	6.4 ± 0.34 **stuvw**	6.99 ± 0.22 **efgh**
6.	3.0 + 0.1	15.2	100	4.4 ± 0.34 **nopqr**	6.6 ± 0.221 **rstuv**	7.5 ± 0.41 **def**
7.	3.5 +0.1	13.8	100	4.6 ± 0.27 **mnopq**	7.6 ± 0.34 **opqr**	7.95 ± 0.43 **cdef**
8.	4.0 + 0.1	12.4	100	5.4 ± 0.27 **klm**	8.8 ± 0.327 **jklm**	9.16 ± 0.43 **a**
9.	4.5 + 0.1	12.1	100	6.4 ± 0.22 **hij**	9.6 ± 0.427 **ghijk**	4.93 ± 0.37 **jklmn**
10.	5.0 + 0.1	11.3	100	7.2 ± 0.2 **efgh**	10.2 ± 0.416 **fghi**	1.95 ± 0.23 **vwx**
11.	5.5 + 0.1	12.3	100	7.2 ± 0.29 **efgh**	9.2 ± 0.359 **ijklm**	1.67 ± 0.18 **wx**
12.	6.0 + 0.1	13.2	100	8.4 ± 0.31 **cd**	10.4 ± 0.221 **fgh**	1.58 ± 0.14 **x**
13.	0.5 + 0.2	18.4	100	3.2 ± 0.25 **tuvw**	5.2 ± 0.249 **xyzaa**	2.69 ± 0.26 **stuvwx**
14.	1.0 + 0.2	16.9	100	2.4 ± 0.16 **wx**	5.6 ± 0.267 **vwxy**	3.48 ± 0.29 **opqrst**
15.	1.5 + 0.2	15.9	100	3.2 ± 0.25 **tuvw**	6.2 ± 0.327 **tuvwx**	4.26 ± 0.39 **mnopq**
16.	2.0 + 0.2	15.1	100	3.2 ± 0.25 **tuvw**	7.2 ± 0.2 **qrst**	5.74 ± 0.38 **ijkl**
17.	2.5 + 0.2	14.6	100	3.6 ± 0.27 **rstu**	7.4 ± 0.221 **pqrs**	7.0 ± 0.26 **efgh**
18.	3.0 + 0.2	13.9	100	4.2 ± 0.2 **pqrs**	7.8 ± 0.327 **nopq**	7.84 ± 0.42 **cdef**
19.	3.5 + 0.2	13.3	100	5.2 ± 0.2 **lmno**	7.2 ± 0.359 **qrst**	7.58 ± 0.36 **def**
20.	4.0 + 0.2	12.9	100	6.2 ± 0.25 **ijk**	8.6 ± 0.34 **klmn**	9.83 ± 0.45 **ab**
21.	4.5 + 0.2	11.9	100	6.6 ± 0.36 **ghij**	9.2 ± 0.389 **ijklm**	5.72 ± 0.38 **ijkl**
22.	5.0 + 0.2	11.1	100	7.2 ± 0.2 **efgh**	9.4 ± 0.306 **hijkl**	2.39 ± 0.24 **yuvwx**
23.	5.5 + 0.2	13.3	100	8.4 ± 0.4 **cd**	10.2 ± 0.249 **fghi**	1.92 ± 0.24 **vwx**
24.	6.0 + 0.2	13.1	100	9.8 ± 0.44 **a**	12.2 ± 0.291 **e**	1.57 ± 0.16 **x**

25.	0.5 + 0.3	18.6	100	2.4 ± 0.16 **wx**	5.4 ± 0.163 **wxyz**	2.97 ± 0.36 **qrstuvw**
26.	1.0 + 0.3	17.4	100	2.6 ± 0.16 **vwx**	5.8 ± 0.249 **uvwxy**	2.85 ± 0.30 **rstuvw**
27.	1.5 + 0.3	16.5	100	3.4 ± 0.27 **stuv**	6.4 ± 0.163 **stuvw**	3.41 ± 0.34 **opqrst**
28.	2.0 + 0.3	15.7	100	3.6 ± 0.27 **rstu**	6.8 ± 0.249 **qrstu**	5.73 ± 0.48 **ijkl**
29.	2.5 + 0.3	14.1	100	4.6 ± 0.16 **mnopq**	6.4 ± 0.267 **stuvw**	5.89 ± 0.64 **hijk**
30.	3.0 + 0.3	14.3	100	5.2 ± 0.25 **lmno**	8.4 ± 0.221 **lmno**	6.85 ± 0.44 **fghi**
31.	3.5 + 0.3	13.5	100	5.3 ± 0.34 **lmn**	9.6 ± 0.371 **ghijk**	7.05 ± 0.26 **efgh**
32.	4.0 + 0.3	12.5	100	6.2 ± 0.33 **ijk**	10.6 ± 0.306 **fg**	7.74 ± 0.36 **cdef**
33.	4.5 + 0.3	11.6	100	6.4 ± 0.43 **hij**	10.8 ± 0.416 **f**	5.64 ± 0.48 **ijkl**
34.	5.0 + 0.3	11.7	100	7.4 ± 0.37 **efg**	12.2 ± 0.467 **e**	3.0 ± 0.38 **qrstuvw**
35.	5.5 + 0.3	12.7	100	8.4 ± 0.27 **cd**	12.3 ± 0.359 **de**	2.37 ± 0.29 **tuvwx**
36.	6.0 + 0.3	11.9	100	9.8 ± 0.29 **a**	12.1 ± 0.476 **e**	1.53 ± 0.14 **x**
37.	0.5 + 0.3	18.1	100	2.2 ± 0.25 **x**	5.1 ± 0.233 **yzaa**	2.99 ± 0.36 **qrstuvw**
38.	1.0 + 0.4	17.2	100	2.4 ± 0.16 **wx**	5.2 ± 0.249 **xyzaa**	3.75 ± 0.37 **nopqrs**
39.	1.5 + 0.4	16.1	100	3.4 ± 0.27**stuv**	5.2 ± 0.2 **xyzaa**	4.68 ± 0.19 **klmno**
40.	2.0 + 0.4	15.2	100	3.6 ± 0.27 **rstu**	5.6 ± 0.306 **vwxy**	5.7 ± 0.31 **ijkl**
41.	2.5 + 0.4	14.9	100	4.6 ± 0.16 **mnopq**	6.8 ± 0.249 **qrstu**	7.76 ± 0.48 **cdef**
42.	3.0 + 0.4	14.5	100	5.2 ± 0.25 **lmno**	8.2 ± 0.359 **mnop**	7.57 ± 0.41 **def**
43.	3.5 + 0.4	12.7	100	5.2 ± 0.25 **lmno**	9.2 ± 0.249 **ijklm**	7.81 ± 0.53 **cdef**
44.	4.0 + 0.4	13.1	100	6.8 ± 0.33 **fghi**	9.8 ± 0.389 **fghij**	8.04 ± 0.61 **cdef**
45.	4.5 + 0.4	11.5	100	6.4 ± 0.27 **hij**	10.1 ± 0.482 **fghi**	4.27 ± 0.34 **mnopq**
46.	5.0 + 0.4	11.2	100	7.4 ± 0.34 **efg**	10.8 ± 0.442 **f**	2.96 ± 0.33 **qrstuvw**
47.	5.5 + 0.4	12.9	100	8.4 ± 0.45 **cd**	10.8 ± 0.291 **f**	2.21 ± 0.30 **tuvwx**
48.	6.0 + 0.4	13.7	100	8.5 ± 0.43 **ab**	11.6 ± 0.163 **bc**	2.04 ± 0.27 **uvwx**
49.	0.5 + 0.5	17.9	100	2.4 ± 0.27 **wx**	5.2 ± 0.249 **xyzaa**	2.26 ± 0.22 **tuvwx**
50.	1.0 + 0.5	17.6	100	2.4 ± 0.16 **wx**	5.6 ± 0.163 **vwxy**	3.74 ± 0.37 **nopqrs**
51.	1.5 + 0.5	16.7	100	3.6 ± 0.27 **rstu**	6.6 ± 0.163 **rstuv**	4.47 ± 0.39 **lmnop**
52.	2.0 + 0.5	15.5	100	4.2 ± 0.25 **pqrs**	6.2 ± 0.2 **tuvwx**	5.27 ± 0.35 **jklm**
53.	2.5 + 0.5	14.2	100	5.2 ± 0.25 **lmno**	7.1 ± 0.277 **qrst**	6.05 ± 0.5 **ghij**
54.	3.0 + 0.5	13.7	100	5.4 ± 0.16 **klm**	7.4 ± 0.306 **pqrs**	8.24 ± 0.50 **cde**
55.	3.5 + 0.5	13.6	100	6.4 ± 0.27 **hij**	8.4 ± 0.371 **lmno**	7.87 ± 0.35 **cdef**

56.	4.0 + 0.5	11.7	100	7.6 ± 0.16 **def**	9.4 ± 0.371 **hijkl**	7.94 ± 0.38 **cdef**
57.	4.5 + 0.5	12.5	100	7.6 ± 0.27 **def**	9.8 ± 0.327 **fghij**	9.77 ± 0.63 **ab**
58.	5.0 + 0.5	11.2	100	7.4 ± 0.27 **efg**	10.6 ± 0.34 **fg**	4.81 ± 0.35 **jklmn**
59.	5.5 + 0.5	12.7	100	8.1 ± 0.28 **cde**	12.2 ± 0.163 **cd**	2.28 ± 0.23 **tuvwx**
60.	6.0 + 0.5	13.5	100	9.6 ± 0.37 **ab**	12.0 ± 0.221 **a**	1.89 ± 0.19 **vwx**
61.	0.5 + 0.6	18.7	100	2.8 ± 0.25 **uvwx**	4.8 ±0.133 **yzaa**	3.09 ± 0.32 **qrstuv**
62.	1.0 + 0.6	17.3	100	3.2 ± 0.13 **tuvw**	5.4 ± 0.163 **wxyz**	4.03 ± 0.36 **mnopqr**
63.	1.5 + 0.6	16.4	100	4.2 ± 0.25 **pqrs**	5.8 ± 0.327 **uvwxy**	4.99 ± 0.38 **jklmn**
64.	2.0 + 0.6	15.6	100	3.8 ± 0.25 **qrst**	5.1 ± 0.233 **yzaa**	7.16 ± 0.31 **efg**
65.	2.5 + 0.6	14.7	100	5.1 ± 0.31 **lmnop**	6.2 ± 0.2 **tuvwx**	7.6 ± 0.42 **def**
66.	3.0 + 0.6	14.4	100	4.8 ± 0.29 **mnop**	6.8 ± 0.249 **qrstu**	8.74 ± 0.54 **bcd**
67.	3.5 + 0.6	13.9	100	5.8 ± 0.29 **jkl**	8.2 ± 0.359 **mnop**	8.27 ± 0.59 **cde**
68.	4.0 + 0.6	13.4	100	6.0 ± 0.26 **ijkl**	8.4 ± 0.306 **lmno**	8.91 ± 0.64 **bc**
69.	4.5 + 0.6	12.2	100	9.8 ± 0.42 **a**	12.6 ± 0.427 **a**	9.02 ± 0.65 **bc**
70.	5.0 + 0.6	14.2	100	6.6 ± 0.4 **ghij**	9.6 ±0.371 **ghijk**	6.76 ± 0.37 **fghi**
71.	5.5 + 0.6	13.7	100	8.4 ± 0.43 **cd**	10.4 ± 0.267 **fgh**	2.64 ± 0.29 **stuvwx**
72.	6.0 + 0.6	13.6	100	8.8 ± 0.33 **bc**	10.8 ± 0.327 **b**	1.9 ± 0.23 **vwx**

Values followed by same letters in each column are not significantly different ($p<0.05$) using DMRT

Table.3 Effect of various concentrations of Auxins (IBA and NAA) added singly and in combinations in the MS medium on rooting in auxiliary explants of *Aloe vera*

Auxins (mg/l)	Days taken in root initiation	Rooting response (%)	Number of roots/explant	Root length (cm)	Number of shoot buds/explant
IBA 0.5	25.2	100	4.7 ± 0.153 **bc**	2.74 ± 0.142 **ef**	1.2 ± 0.13 **c**
IBA 1.0	24	100	5.4 ± 0.163 **b**	3.92 ± 0.213 **cd**	1.2 ± 0.13 **c**
IBA 1.5	21.2	100	6.6 ± 0.400 **a**	7.64 ± 0.330 **a**	1.1 ± 0.1 **c**
IBA 2.0	22.4	100	2.2 ± 0.133 **e**	1.81 ± 0.142 **g**	1.3 ± 0.15 **c**
NAA 0.5	26.2	100	4.6 ± 0.306 **bc**	5.64 ± 0.279 **b**	2.1 ± 0.28 **b**
NAA 1.0	25.4	100	4.1 ± 0.539 **cd**	4.55 ± 0.318 **c**	2.4 ± 0.22 **ab**
NAA 1.5	20	100	3.6 ± 0.340 **d**	3.24 ± 0.207 **de**	2.4 ± 0.22 **ab**
NAA 2.0	20	100	3.4 ± 0.221 **d**	2.49 ± 0.291 **fg**	2.7 ± 0.15 **a**
(IBA + NAA)					
0.5+0.5	22.4	100	4.2 ± 0.291 **bcd**	3.1 ± 0.317 **d**	1.3 ± 0.15 **d**
0.5+ 1.0	16.8	100	4.5 ± 0.522 **abc**	4.2 ± 0.384 **cd**	1.8 ± 0.2 **cd**
0.5+1.5	14.2	100	4.8 ± 0.467 **ab**	5.6 ± 0.45 **b**	2.1 ± 0.23 **bc**
0.5+2.0	13.6	100	5.8 ± 0.742 **a**	8.3 ± 0.448 **a**	2.3 ± 0.26 **ab**
2.0+0.5	15.6	100	2.8± 0.327 **d**	6.2 ± 0.429 **b**	2.2 ± 0.25 **ab**
2.0+1.0	12.8	100	3.9 ± 0.458 **bcd**	5.8 ± 0.404 **b**	1.8 ± 0.2 **cd**
2.0+1.5	10.9	100	3.4 ± 0.340 **bcd**	5.5 ± 0.390 **b**	2.5 ± 0.22 **ab**
2.0+ 2.0	10.6	100	3.1 ± 0.379 **cd**	5.0± 0.377 **bc**	2.8 ± 0.13 **a**

Values followed by same letters in each column are not significantly different ($p<0.05$) using DMRT

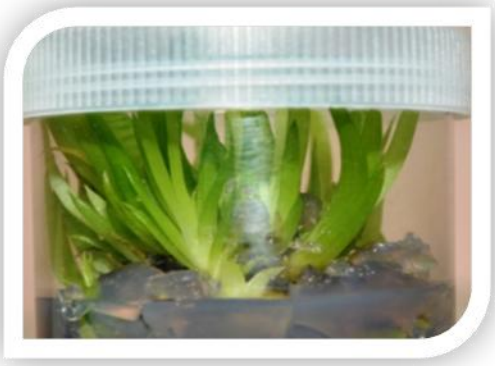

Figure.1 Shoot bud induction in MS media supplemented with 4.0 mg/l BAP

Figure.2 Shoot bud induction in MS media supplemented with 4.5 mg/l BAP + 0.6 mg/l NAA

Figure.3 Root induction in MS media supplemented with 1.5 mg/l IBA

Figure.4 Root induction in MS media supplemented with 0.5 mg/l NAA

Figure.5 Root induction in MS media supplemented with 0.5 mg/l IBA + 2.0 mg/l NAA

Figure.6 Hardening of *in vitro* rooted *Aloe vera* plantlets

Figure.7 Fully hardened plantlets of *Aloe vera* grown in net house

In the present investigation we did not investigate all the factors which in one way or other way block or stimulate micropropagation potential in *Aloe vera*, thus in the light of these factors we feel the prolonged and intensive *in vitro* efforts are needed to exploit the micropropagation in *Aloe vera.*

However, results of the present study may be recommended to develop protocols for mass multiplication of *Aloe vera* to meet the demand of farmers and pharmaceutical industries.

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