

Research Article

# Micropropagation and cytological studies of *Aloe vera* Linn

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## Abstract

*Aloe vera* Linn. is an essential medicinal plant. In this present research work, a protocol of *in vitro* regeneration and karyomorphological analysis of *Aloe vera* was developed using different concentrations and compositions of media. Shoot apices of field-grown plants were used as explant and aseptically cultured on Murashige and Skoog (MS) medium fortified with different concentrations and combinations of auxins (IAA and NAA) and cytokinins (BAP and Kn). The highest number of multiple shoot buds ( $4.36 \pm 0.07$ ) was obtained from MS + 2.0 mg/l BAP + 1.0 mg/l IAA and induced shoot buds underwent rapid elongation ( $4.24 \pm 0.06$  cm) on the same medium composition. Half strength MS media with 2.0 mg/l IBA was suitable for induction and proliferation ( $6.31 \pm 0.05$ ) of roots and 95% of plantlets were acclimatized to field conditions successfully. Somatic chromosome numbers of mother and *in vitro* grown plants were confirmed to be  $2n = 14$ . Chromosome length ranged from 4.28 - 13.74  $\mu$ m in the naturally grown plants and 4.46 - 14.1  $\mu$ m for *in vitro* grown plants. The total form percent (TF%) of mother and *in vitro* grown plants was 41.69% and 42.23%, respectively. The karyotype formula of *in vivo* grown plants was  $2n = 14 = 4L^{sm} + 6M^m + 4S^m$ , whereas that of the micropropagated plants was  $2n = 14 = 4L^{sm} + 4M^m + 6S^m$ . The frequency of the chromosome having arm more than 2:1 was 0.08 for mother plants and 0.15 for *in vitro* grown plants. Therefore, the karyotype of both plants falls into the 2B symmetrical type based on Stebbins classification (1971).

## Introduction

*Aloe vera* Linn., family Liliaceae, is a spiky cactus-like medicinal plant. The plant is a commonly looking perpetual with small, thick to some degree isolated stem 30 cm - 60 cm. The leaves have opaque green stalkless, globose, lanceolate, erect spreading instead of inward, sharp-toothed at the edge, and with saw-like teeth along their margins, up to 0.5 m long and 8-10 cm across at the base. In a transverse section, the plant shows a slightly concave appearance on the adaxial surface and a distinctly convex appearance on the lower abaxial surface [1]. The leaves are also covered with thick cuticles, beneath which epidermis and mesophyll. Roots are 30 cm - 40 cm in length [2]. The smell is nauseous and bitter.

There are 200 different types of molecules identified in *Aloe vera* [3]. Leaf extract of *Aloe vera* leaf contains about 98% water [4]. Different types of vitamins including the important antioxidant vitamins A, C and E. Vitamin B1 (thiamine), niacin, Vitamin B2 (riboflavin), choline and folic acid are also present in the *Aloe vera* leaf gel [5]. *A. Vera* has been used in pharmaceutical, food, and cosmetic industries, or in traditional medicine worldwide, [6]. Bioactive compounds of *Aloe* species have been known to have anti-inflammatory,

antitumor, antiulcer, anticancer, antibacterial, and antiviral properties [7]. Leaves of this plant species are also used to treat bacterial and fungal skin diseases [8].

Traditional propagation methods of *Aloe vera* have many downsides such as a slow propagation rate in which each plant produces three to four sprouts every year, and male sterility to use its seeds as well [9]. To meet the high demand for *A. vera* in the pharmaceutical industries and also overcome the above-mentioned problems, *in vitro* propagation has been used for rapid micropropagation of many important plants [10,11]. Hence, there have been many attempts to rapid plant multiplication and genetic improvement of disease-free plants [12]. For the establishment of most of the plant tissue, culture protocols shoot apices were used as the explant. Cytological studies in many plant species have been used for resolving a plethora of taxonomic and evolutionary problems [13,14]. The present research work aimed at micropropagation and cytological studies of *A. vera*.

## Materials and methods

### Preparation of plant materials

Shoot apices of field-grown plants were collected and

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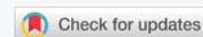
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Keywords: *Aloe vera*; Explant; *In vitro* propagation; Chromosome; Ideogram; Karyotype



thoroughly washed under running tap water. The plant materials were then cut into short pieces and surface sterilized with 1% savlon and liquid soap for 5 - 10 minutes with constant shaking. The plant parts were then washed 3 - 4 times with distilled water for complete removal of the trace of detergent and taken under a running laminar airflow cabinet and transferred to a 500 ml sterilized conical flask. After rinsing with 70% ethanol for less than 60 seconds, they were immersed in 0.1 ml HgCl<sub>2</sub> for different duration of time and after that, the plant segments were washed 4 - 5 times with double distilled water.

### Culture media and conditions for plant regeneration

The sterilized explants were cultured on an MS basal medium fortified with various concentrations and combinations of plant growth regulators (PGRs) for the induction of multiple shoot buds. The media were solidified with 0.8% (w/v) agar and P<sup>H</sup> was adjusted to 5.8 before autoclaving for 30 minutes at 121°C under 1.1 kg/cm<sup>2</sup> pressures. The culture vessels with inoculated explants were then taken to the culture room for incubation.

### Subculture for multiple shoot buds

Induced multiple shoot buds were rescued very carefully in aseptic conditions and then these were subcultured on the same media or different media for further response at an interval of certain periods.

### Rooting of *in vitro* shoot

The steps of rooting experiments were carried out in a half-strength MS medium with or without growth regulators. The elongated shoot buds at definite height (2 - 4 cm) were separated from the culture vessels and then separately moved to root media. After 2 - 3 weeks of inoculation on a suitable medium, the roots were produced from the cut ends of each micro shoot.

### Hardening and acclimatization of plantlets

The induced plantlets were taken out of the culture vessels and then washed thoroughly with running tap water for the removal of agar attached to the roots. After that, the rooted plantlets were transplanted to small plastic pots containing garden soil and compost in the ratio of 1:1 and kept at room temperature for 3 - 5 days. The soil was treated with a 0.1% agrosan (fungicide) solution.

### Statistical analysis of data

All experiments were carried out with three replicates in each treatment. Observations were recorded on the basis of percentage of response, number of shoots per explant, and number of roots per shoot bud. Means and standard deviations (mean ± SD) were calculated for each treatment. The mean values of the parameters were compared by using the SPSS (Ver. 21) software.

## Cytological studies

**Root tip collections:** The root tips of *in vivo* and *in vitro* grown plants were collected cautiously, in the early morning (9.30 a.m to 10.30 a.m) and when lengths of roots become 1 cm - 1.5 cm in size.

**Pre-treatment:** 1,4-Para-dichlorobenzene (PDB) was used for the pretreatment of root tips for 2.00 - 3.00 hours at room temperature.

**Preservation of roots:** After the above stage, the pretreated roots were washed with distilled water and kept in 1:3 (v/v) aceto-alcohol for 24 hours. And then the collected roots were transferred in 70% alcohol (v/v) for long time preservation.

**Staining of chromosomes:** The preserved roots were thoroughly washed with distilled water after that hydrolyzed in 50% HCl for 6-8 minutes at room temperature. Then treated roots were washed 4-5 times with distilled water. After that, the roots were kept in a 2% (w/v) aqueous solution of iron alum for 8-10 minutes and then the roots were stained with an aqueous solution of Haematoxylin and kept for 30 minutes. The darkly colored portions of root tips were squashed in a drop of 0.2% (w/v) acetocarmine. Then set a cover glass on the squashed root material, then the slide was warmed gently over a flame of a spirit lamp. Finally, the slides were prepared for observation under a microscope.

**Chromosome characterization:** Chromosomes were classified based on Centromeric Type (CT), Relative Length (RL), Centromeric Index (CI) and Karyotypic similarity (TF%).

**Centromeric type (CT):** The procedure proposed by Levan [15] for determining the centromeric type of chromosomes was followed. Here the l/s ratio was used as an index of centromeric type. Here, l = Length of the longer arm and s = Length of the shorter arm.

**Relative Length (RL):** Relative Length (RL)  

$$= \frac{\text{Length of a particular chromosome}}{\text{The total length of the haploid complement}} \times 100$$

**Centromeric index (CI):** Centromeric Index (CI)  

$$= \frac{\text{The total length of the short arm}}{\text{The total length of the chromosome}} \times 100$$

**Karyotypic symmetry:** The symmetry/asymmetry of karyotype was determined by

a) TF%: According to Huziwara [16].

$$TF\% = \frac{\text{The total length of the short arms}}{\text{The total length of chromosomes}} \times 100$$

b) TF% and Stebbins qualitative classification for the determination of asymmetry: The formula of Stebbins [17] qualitative classification is given below.

Ratio of longest/shortest chromosome (l/s ratio)	The proportion of chromosomes with an arm ratio less than (< 2: 1)			
	0	0.01-0.50	0.51-0.99	1.00
<2:1	1A	2A	3A	4A
2:1 – 4:1	1B	2B	3B	4B
>4:1	1C	2C	3C	4C

**Ideogram:** For an accurate measurement of lengths at least three metaphase plates were measured. Their average arm length was used to prepare the ideogram. Karyotype XY and Image J software were used for preparing Ideogram.

## Results and discussion

### Micropropagation of *Aloe vera*

The shoot apices of *Aloe vera* were cultured on the MS basal concentrations of Kn and BAP in combination with NAA or IAA. After 30 days of culture, data were collected and also presented in Table 1 and Table 2. Shoot apices of field-grown plants were cultured on MS medium supplemented with BAP and Kn (0.5-3.0 mg/l). The explants started producing MSBs within 10-25 days of inoculation. Among the different combinations tested, the maximum explants (91%) produced MSBs in the medium containing 2.0 mg/l BAP + 1.0 mg/l IAA with the highest number of MSBs  $4.36 \pm 0.07$  per explant (Figure 1a,b). Whereas, On the contrary, the minimum number of MSBs (30%) per explant was found in 3.0 mg/l Kn and the lowest length ( $1.01 \pm 0.09$ ) was observed in the same medium within 12 - 18 days of culture. The effect of BAP with IAA was superior to that of BAP alone and in a combination of Kn with IAA and NAA. Different growth regulators, especially auxins and cytokinins played a notable role in the micropropagation of many important medicinal plants species such as *Pimpinella anisum* [18], *Scrophularia kakudensis* [19], *Euonymus verrucosus* [20]; *Valeriana jatamansi* [21].

**Table 1:** Effects of BAP individually and in combination with NAA or IAA on induction of MSBs in shoot segment of *A. vera* L.

PGRs supplement in the media (mg/l)	% of explants gave response	Time (d) requires for induction of MSBs	Average* no. of MSBs/explant ( $\bar{x} \pm SE$ )	
BAP	0.5	47	12 - 22	$2.03 \pm 0.03^m$
	1.0	56	12 - 20	$2.05 \pm 0.07^l$
	2.0	68	12 - 17	$2.08 \pm 0.08^k$
	3.0	33	13 - 25	$2.09 \pm 0.04^k$
BAP + NAA	2.0 + 0.2	50	12 - 18	$3.02 \pm 0.09^d$
	2.0 + 0.5	73	10 - 16	$2.98 \pm 0.05^e$
	2.0 + 1.0	61	12 - 14	$2.42 \pm 0.07^j$
	3.0 + 0.2	56	12 - 14	$2.78 \pm 0.06^f$
	3.0 + 0.5	43	11 - 20	$2.27 \pm 0.05^i$
	3.0 + 1.0	42	12 - 20	$2.01 \pm 0.05^n$
BAP + IAA	2.0 + 0.2	57	10 - 12	$2.84 \pm 0.08^e$
	2.0 + 0.5	72	10 - 14	$3.65 \pm 0.09^b$
	2.0 + 1.0	91	10 - 14	$4.36 \pm 0.07^a$
	3.0 + 0.2	61	11 - 14	$3.27 \pm 0.10^c$
	3.0 + 0.5	55	10 - 14	$2.77 \pm 0.11^g$
	3.0 + 1.0	49	12 - 16	$2.51 \pm 0.04^h$

d = days, MSBs = Multiple shoot buds. \*values are the means of three replicates with 15 explants. Figures in the column having the same letter(s) within a location did not differ significantly according to DMRT  $\leq 0.05$ .

**Table 2:** Effects of Kn individually and in combination with NAA or IAA on induction of MSBs in shoot apices of *A. vera* L.

PGRs supplement in the media (mg/l)	% of explants gave a response	Time (d) requires for induction of MSBs	Average* no. of MSBs/explant ( $\bar{x} \pm SE$ )	
Kn	0.5	35	15 - 20	$1.95 \pm 0.04^l$
	1.0	37	14 - 16	$1.21 \pm 0.11^k$
	2.0	43	14 - 15	$2.13 \pm 0.07^g$
	3.0	30	12 - 18	$1.01 \pm 0.09^j$
Kn + NAA	2.0 + 0.2	54	10 - 14	$1.95 \pm 0.08^l$
	2.0 + 0.5	82	10 - 14	$3.12 \pm 0.15^a$
	2.0 + 1.0	63	12 - 14	$2.79 \pm 0.08^c$
	3.0 + 0.2	65	10 - 12	$2.35 \pm 0.07^e$
	3.0 + 0.5	43	11 - 14	$2.61 \pm 0.06^d$
	3.0 + 1.0	38	10 - 14	$2.13 \pm 0.05^g$
Kn + IAA	2.0 + 0.2	36	15 - 18	$1.18 \pm 0.04^m$
	2.0 + 0.5	35	13 - 15	$2.95 \pm 0.11^b$
	2.0 + 1.0	51	12 - 17	$2.27 \pm 0.07^f$
	3.0 + 0.2	40	12 - 15	$2.04 \pm 0.06^h$
	3.0 + 0.5	32	12 - 18	$1.50 \pm 0.14^j$
	3.0 + 1.0	32	11 - 15	$1.25 \pm 0.13^k$

d = days, MSBs = Multiple shoot buds. \*values are the means of three replicates with 15 explants. Figures in the column having the same letter(s) within a location did not differ significantly according to DMRT  $\leq 0.05$ .



**Figure 1:** Induction and development of multiple shoot buds in shoot apices of *A. vera* L. a) Initiation of MSBs formation after 10 days of culture, b) Development of MSBs on MS medium with 2.0 mg/l BAP + 1.0 mg/l IAA, c-d) Elongation of MSBs on MS medium containing 2.0 mg/l BAP + 1.0 mg/l IAA.

### Elongation of induced MSBs

The regenerated multiple shoot buds were individually grown in aseptic conditions on elongation media. It was found that BAP in combination with IAA was better for elongation of MSBs than that other combinations (Table 3). The MS medium fortified with 2.0 mg/l BAP + 1.0 mg/l IAA produced the maximum elongated shoot buds ( $5.21 \pm 0.11$  cm) after 30 days of culture. Figure 1c,d showed the highest elongation of MSBs. The lowest elongation ( $1.61 \pm 0.12$  cm) was found when shoot buds were cultured on the medium fortified with 3.0 mg/l BAP + 1.0 mg/l NAA. It was observed that BAP supplemented with MS media was better than Kn supplemented MS media for elongation of MSBs. The same type of enhancement of *in vitro* shoot buds elongation was also noted in many other medicinal plants including, *Alpinia galangal* [22], *Lythrum salicaria* [23], *Rhinacanthus nasutus* [24], *Securidaca longipedunculata* [25].

### Results of induction of roots in elongated shoot buds of *A. vera*

3 cm - 4 cm long micropropagated shoot buds were individually transferred to rooting media (1/2 strength MS media) with and without PGR supplement. The maximum rooting (96%) was observed when 2.0 mg/l IBA was added in half strength MS medium within the 11-12 days of inoculation and the highest numbers of roots per micro shoots were  $6.31 \pm 0.05$  and the average length of roots after 30 days of culture was  $5.81 \pm 0.17$  in the same medium composition (Figure 2a,b)(Table 4).

The lowest proportion (49%) of rooting was recorded in half-strength MS medium fortified with 1.0 mg/l IAA + 1.0 mg/l IBA within 14 - 18 days of inoculation. In this medium,

**Table 3:** Effects of different concentrations and combinations of plant growth regulators (PGRs) on the elongation of directly produced multiple shoot buds of *A. vera* L. when cultured on MS medium.

PGRs supplement in the medium (mg/l)		Initial* length (cm) of an individual shoot buds ( $\bar{x} \pm SE$ )	Length* (cm) of individual shoot buds after 30d of culture ( $\bar{x} \pm SE$ )
BAP + NAA	2.0 + 0.5	$1.04 \pm 0.10^j$	$2.20 \pm 0.06^v$
	2.0 + 1.0	$1.27 \pm 0.08^f$	$2.62 \pm 0.03^a$
	3.0 + 0.5	$1.01 \pm 0.08^h$	$2.54 \pm 0.10^r$
	3.0 + 1.0	$0.78 \pm 0.09^k$	$1.61 \pm 0.12^w$
BAP + IAA	2.0 + 0.5	$1.36 \pm 0.10^d$	$2.60 \pm 0.09^a$
	2.0 + 1.0	$1.74 \pm 0.05^a$	$5.21 \pm 0.11^l$
	3.0 + 0.5	$1.31 \pm 0.11^e$	$3.57 \pm 0.10^{mn}$
	3.0 + 1.0	$1.00 \pm 0.10^l$	$2.35 \pm 0.07^t$
Kn + NAA	2.0 + 0.5	$1.08 \pm 0.11^h$	$2.22 \pm 0.06^u$
	2.0 + 1.0	$1.34 \pm 0.09^d$	$3.09 \pm 0.05^e$
	3.0 + 0.5	$1.15 \pm 0.08^g$	$2.44 \pm 0.18^p$
	3.0 + 1.0	$1.00 \pm 0.11^l$	$1.74 \pm 0.12^y$
Kn + IAA	2.0 + 0.5	$1.25 \pm 0.07^f$	$2.26 \pm 0.07^t$
	2.0 + 1.0	$1.58 \pm 0.06^b$	$2.33 \pm 0.8^s$
	3.0 + 0.5	$1.57 \pm 0.05^b$	$2.33 \pm 0.11^s$
	3.0 + 1.0	$1.41 \pm 0.07^c$	$3.11 \pm 0.08^b$

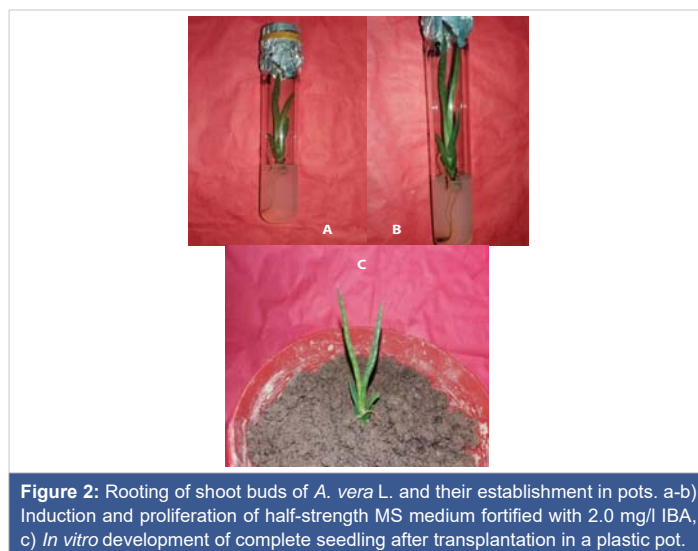
d = days, MSBs = Multiple shoot buds.\*values are the means of three replicates with 15 explants. Figures in the column having the same letter(s) within a location did not differ significantly according to DMRT  $\leq 0.05$ .

the minimum numbers of roots per micro shoot were  $1.79 \pm 0.15$  and the average length of roots after 30 days of culture was  $2.09 \pm 0.20$ .

Half-strength MS medium with different concentrations and combinations of auxins has also been reported for several plants including Greek oregano [26], *Rosa hybrid* [27], *Momordica cymbalaria* [28], *Paulownia elongata* [29], *Heliotropium Indicum* [30].

### Acclimatization and establishment of *in vitro* grown plantlets of *A. vera*

For the establishment of *in vitro* raised complete seedlings, the culture vessels with well-developed rooted plantlets were taken out of the culture room and followed several steps for acclimatization. Then the plantlets were planted in small plastic pots (Figure 2c) containing garden soil and compost in the ratio of 1:1 and kept at room temperature for 3 - 5 days and the survival percentage of the regenerated



**Table 4:** Effects of different concentrations and combinations of plant growth regulators (PGRs) on the development of roots in elongated multiple shoot buds of *A. vera* L. when cultured on half-strength MS medium.

PGRs supplements in the media (mg/l)		Days to root induction	% of micro Shoot Produced roots	Average*number of roots/macro shoot ( $\bar{x} \pm SE$ )	Average*length (cm) roots after 30d of culture ( $\bar{x} \pm SE$ )
1/2 MS		15 - 25	58	$2.65 \pm 0.11^j$	$2.13 \pm 0.08^v$
1/2 MS + IBA	0.5	15 - 22	61	$3.80 \pm 0.06^f$	$2.76 \pm 0.04^s$
	1.0	12 - 18	70	$4.50 \pm 0.07^c$	$3.14 \pm 0.07^q$
	2.0	11 - 12	96	$6.31 \pm 0.05^a$	$5.81 \pm 0.17^n$
	3.0	12 - 17	65	$4.01 \pm 0.11^e$	$2.15 \pm 0.11^k$
1/2 MS + IAA	0.5	12 - 18	63	$2.24 \pm 0.01^k$	$2.22 \pm 0.03^w$
	1.0	13 - 17	79	$5.05 \pm 0.08^b$	$2.50 \pm 0.04^u$
	2.0	11 - 14	81	$3.51 \pm 0.05^g$	$3.18 \pm 0.06^q$
	3.0	12 - 115	53	$3.50 \pm 0.12^g$	$2.02 \pm 0.04^y$
1/2 MS + NAA	0.5	18 - 22	50	$2.05 \pm 0.09^j$	$2.52 \pm 0.11^u$
	1.0	18 - 20	54	$2.58 \pm 0.10^j$	$3.30 \pm 0.02^o$
	2.0	15 - 20	43	$2.86 \pm 0.04^i$	$2.53 \pm 0.16^i$
	3.0	18 - 20	34	$3.07 \pm 0.12^h$	$2.42 \pm 0.24^y$
1/2MS + IBA + IAA	1.0 + 0.5	10 - 14	85	$4.35 \pm 0.03^d$	$3.18 \pm 0.05^p$
	1.0 + 1.0	13 - 16	82	$5.12 \pm 0.06^b$	$2.81 \pm 0.05^s$
1/2MS + IBA + NAA	1.0 + 0.5	11 - 14	76	$3.89 \pm 0.10^f$	$2.91 \pm 0.05^t$
	1.0 + 1.0	14 - 18	49	$1.76 \pm 0.15^m$	$2.09 \pm 0.20^z$

d = days, MSBs = Multiple shoot buds.\*values are the means of three replicates with 15 explants. Figures in the column having the same letter(s) within a location did not differ significantly according to DMRT  $\leq 0.05$ .

plantlets under natural conditions was about 95%. *In vitro*, grown plantlets were morphologically uniform having a normal phenotype.

### Karyomorphological analysis of *A. vera*

**Karyotype of naturally grown plants of *A. vera*:** The somatic cells had 14 chromosomes each and the chromosome length ranged from 4.46 to 14.10  $\mu\text{m}$  (Table 5). Among the 7 basic chromosomes, 2 were submetacentric and 5 were metacentric (centromeric formula = 2 sm + 5 m). All the submetacentric were long (Figure 3a). Among the 5 metacentric chromosomes, 2 were medium and 3 were small (Figures 3b,c). For this chromosome characteristic, the appropriate karyotype formula was  $n = 7 = 2L^{sm} + 2M^m + 3S^m$ . The total length of haploid complement was 62.96 and TF% 41.69. The total length of long arms and that of short arms were 36.73  $\mu\text{m}$  and 26.26  $\mu\text{m}$ , respectively. None of the chromosomes had secondary constrictions. The ratio of the longest and shortest chromosome was more than 2:1 and the frequency of the chromosome having arm more than 2:1 was 0.29 and the karyotype falls into the 2B category of Stebbins (1971) chart of chromosome asymmetry. A similar type of chromosome morphology and the karyotype is shown in different plant species like *Santalum album* [31], *Plumbago auriculata* [32], *Thespesia lampas* [33].

**Karyotype of *in vitro* grown plants of *A. vera* L:** The chromosome number of *in vitro* raised plants was found to be  $2n = 14$  in the somatic cells (Figure 4a) and the individual chromosome length ranged from 4.28  $\mu\text{m}$  to 13.74  $\mu\text{m}$  (Table 6).

Of the 7 genomic chromosomes, 2 were sub metacentric and 5 were metacentric. The centromeric formula was  $2s^m + 5m$  (Figures 4b,c).

All the submetacentric were long. Three of the metacentric chromosomes were medium and 2 were small. Thus the appropriate karyotypic formula was  $n = 2L^{sm} + 3M^m + 2S^m$ . TF% was 42.23 and the total length of the haploid complement set was 62.63. Chromosomes with secondary constrictions were absent. The total length of long arms was 36.20  $\mu\text{m}$  and that of short arms was 26.47  $\mu\text{m}$ . The ratio between longest and shortest chromosomes was more than 2:1 and the frequency of chromosomes with arm ratio was more than 0.29. Thus karyotype fell in the 2B type. The same types of results are shown in various types of plant sp species namely, *Brachystemma* and *Craspedolobium* [34], *Lonicera* [35].

**Comparative analysis of karyotype of mother plants and micropropagated plants:** The somatic chromosome

**Table 5:** Length, Arm ratio, Relative length, Centromeric index, Centromeric type, TF% and Centromeric formula of mitotic metaphase chromosome of *in vivo* grown plants of *A. vera* L.

Chromosome pair	Long arm (l) $\mu\text{m}$	Short arm (s) $\mu\text{m}$	Total length (l+s = T) $\mu\text{m}$	Arm ratio (l/s)	Relative length (RL) %	Centromeric Index (CI) %	Centromeric Type (CT)	Chromosome Type	TF%	Centromeric formula
1	9.65	4.45	14.10	2.17	22.4	31.54	sm	L	41.69	$2s^m + 5m$
2	8.82	3.55	12.37	2.48	19.65	28.72	sm	L		
3	5.18	5.18	10.36	1	16.44	50	m	M		
4	4.45	4.45	8.90	1	14.14	50	m	M		
5	3.52	3.52	7.04	1	11.17	50	m	S		
6	2.88	2.88	5.76	1	9.13	50	m	S		
7	2.23	2.23	4.46	1	7.08	50	m	S		

**Table 6:** Characterization of chromosomes of *in vivo* grown plants of *A. vera* L. on the basis of Length, Arm ratio, Relative length, Centromeric index, Centromeric type and TF%.

Chromosome pair	Long arm (l) $\mu\text{m}$	Short arm (s) $\mu\text{m}$	Total length (T) $\mu\text{m}$	Arm ratio (l/s)	Relative length (RL) %	Centromeric Index (CI) %	Centromeric Type (CT)	Chromosome type	TF%	Centromeric formula
1	9.22	4.52	13.74	2.04	21.92	32.88	sm	L	42.23	$2s^m + 5m$
2	8.64	3.61	12.25	2.39	19.56	29.44	sm	L		
3	5.27	5.27	10.54	1	16.83	50	m	M		
4	4.41	4.41	8.82	1	14.07	50	m	M		
5	3.86	3.86	7.72	1	12.33	50	m	M		
6	2.66	2.66	5.32	1	8.48	50	m	S		
7	2.14	2.14	4.28	1	6.82	50	m	S		

**Table 7:** Comparison of chromosome characters between *in vivo* and *in vitro* grown plants of *A. vera* L.

Chromosome characters	<i>In vivo</i> grown plant	<i>In vitro</i> raised plant
Somatic chromosome number	$2n = 14$	$2n = 14$
Range of Individual Chromosome Length ( $\mu\text{m}$ )	4.46 - 14.1	4.28 - 13.74
Total Haploid Chromatin Length (TCL)	62.96	62.63
The total length of haploid complement	Long arm( $\mu\text{m}$ )	36.73
	Short arm( $\mu\text{m}$ )	26.26
The ratio of longest/Smallest Chromosome	3.16:1	3.21:1
Range of Relative Length ( $\mu\text{m}$ )	7.08 - 22.40	6.82 - 21.92
The Total Form Percent (TF%)	41.69	42.23
Centromeric Index (CI)	31.54 - 50	32.88 - 50
Karyotype Formula	$n = 7 = 2L^{sm} + 2M^m + 3S^m$ $2n = 14 = 4L^{sm} + 4M^m + 6S^m$	$n = 7 = 2L^{sm} + 3M^m + 2S^m$ $2n = 14 = 4L^{sm} + 6M^m + 4S^m$
Karyotype class	2B	2B



number, chromosome size and details of the karyotype of *in vivo* and *in vitro* grown plants of *A. vera* were presented in (Figures 3,4) (Table 7). Chromosome sizes of the mother plant ranged from 14.1 µm to 4.46 µm long with a gradual decrease in length from the longest to the shortest chromosome. Whereas, chromosome sizes of *in vitro* raised plants ranged from 13.73 µm to 4.28 µm long with a gradual decrease in length from the longest to the shortest chromosome. Short arm and long arm chromosomes of naturally grown plants and *in vitro* grown plants were almost similar. The longest short arm chromosomes of *in vivo* and micropropagated plants were 4.45 µm and 4.52 µm, respectively, while the shortest short arm chromosomes of both plants were 2.23 µm and 2.14 µm, respectively. In addition, the shortest long arm chromosomes of *in vivo* and *in vitro* grown plants were 2.23 µm and 2.18 µm respectively, whereas the longest long arm chromosomes of mother plants and *in vitro* grown plants were 9.65 µm and 9.22 µm respectively.

The total length of the haploid complement of field-grown plants and *in vitro* grown plants was 62.96 and 62.63 respectively. The centromeric index of mother plants and *in vitro* grown plants was 31.54 to 50 and 32.88 to 50 respectively. The total form percent of mother and *in vitro* grown plants was 41.96 and 42.23, respectively. The chromosome complement of both plants consisted of four submetacentric and 10 metacentric chromosomes, (Figures 3,4) (Table 7). Therefore, the karyotype formula of *in vivo* grown plants was  $2n = 14 = 4L^{sm} + 4M^m + 6S^m$ . Whereas, the karyotype formula of *in vitro* grown plants was  $2n = 14 = 4L^{sm} + 6M^m + 4S^m$ . As shown in Figures 3,4, it can be seen that no satellite was found in both karyotypes. The result also showed that the proportion of the largest chromosome total length and the smallest chromosome total length (R) of the mother plant and *in vitro* plants were 3.16 and 3.21, respectively. The karyotype of both plants fell in the 2B type.

## Conclusion

The present investigation has shown that the response of *A. vera* seems to be dependent more precisely on the types of explant as well as on the PGRs treatments. MS medium fortified with 2.0 mg/l BAP + 1.0 mg/l IAA gave the best response to shoot induction. The highest elongation ( $5.21 \pm 0.11$  cm) of multiple shoot buds was noted on MS medium with 2.0 mg/l BAP + 1.0 mg/l IAA and half strength MS medium with 2.0 mg/l IBA was most suited for root initiation. The plantlets obtained survived and grew normally in the outside environment. The results of the karyomorphological analysis showed that growth medium compositions, plant growth regulators, light length, culture time and subcultures did not affect the *in vitro* grown plants. Their generated plants were morphologically similar to the mother plants of *A. vera*. There were very minute karyotypic variations between selected *in vivo* and *in vitro* plant species. The Karyomorphological

study of the present investigation will be helpful to understand the number and morphology of chromosomes which are essential for future research in cytogenetics.

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