# *In Vitro* propagation of *Aloe vera*-A Plant with Medicinal Properties

**Arvind Kumar Bhandari, J.S. Negi, V.K. Bisht and M.K. Bharti**
Herbal Research and Development Institute - Mandal, Gopeshwar
(Chamoli), Uttarakhand, (India), Pin-246401
arvindbhandari2001@yahoo.co.in

**Abstract**
The present study aimed to evaluate the micropropagation of *Aloe vera* under *in vitro* condition. The comparative study of effects of different phytohormones concentration with hardening of plants in poly house and shade house was carried out. It has valuable medicinal properties and used commercially in Pharmaceutical, cosmetic and food industries**.** Its growth was found well in different concentrations of BA (6-Benzylaminopurine), IBA (3-Indol butyric acid), Kn (Kinetin) and adenine sulphate in present study. Explant was collected from offshoot derived of the superior genotype of *Aloe vera* at the nursery and authenticated by the botanist in Herbal Research and Development Institute Mandal. [Nature and Science 2010;8(8):174-176]. (ISSN: 1545-0740).

**Keywords:** *Aloe vera*, micropropagation, medicinal plant

## 1. Introduction

*Aloe vera* belongs to the family Liliaceae is a xerophytic medicinal plant and also grows even in rain fed condition. It is used in various pharmaceutical product, food and cosmetic industries due to its metabolites. Commercial Aloes obtained from wild as well as cultivated source. A number of biological activities have been reported by Weiner and Weiner, 1994 to substances present in, such as antiseptic (saponins and anthraquinones), antitumoral (mucopolysaccharides), anti-inflammatory (steroids and salicylic acid), anti-oxidant (Vitamins) and immune regulator (glucomannans). *Aloe vera* propagated mainly through vegetative means, as due to mail sterility, sexual reproduction was ineffective. Propagation is primarily by means of sucker or off shoots, which are separated carefully from mature plants and transplanted. The technique to tissue and organ culture is used for rapid multiplication of plants, for genetic improvement of crops, for obtaining disease-free clones and for progressive valuable germplasm (Bhojwani & Razdan, 1992). Although *Aloe vera* propagates vegetatively in it's natural state, but the process is too slow (Meyer and Staden, 1991).

## 2. Material and Methods

Shoot tip of 2.0-3.0 cm were collected from offshoot-derived elite individual of the superior genotype of *Aloe vera* on the basis of higher yield of leaf biomass. The explants first were washed thoroughly in running tap water for 15 minutes. After that they were again washed with liquid detergent and Tween 20 for 10 minutes with gentle shaking. After washing with detergent explants were again washed with running tap water to remove any traces of detergent for 15 minute and kept in 1% w/v solution of Bavistin for one hour. After that explants was shifted to the 1% v/v solution of savlon for 1-2 minutes. After these treatments shoot tip were taken inside the laminar hood for further sterilization. Here 2-3 sterile water washings are given. After these washings, explants were taken out and dipped in 70% ethyl alcohol for 30 seconds and then dip into alcohol for 20 second, explants were surface sterilized with freshly prepared 0.1% w/v aqueous solution of mercuric chloride for 5 minutes. After mercuric chloride treatment, explants were thoroughly washed for 4-5 times with sterile water to remove any traces of mercuric chloride. Medium was autoclaved at 121$^{o}$C for 20 minutes. Shoot tip explants (2-3 cm) were inoculated with MS medium containing various concentrations of BA (6-Benzylaminopurine), IBA (3-Indol butyric acid), Kn (Kinetin) and adenine sulphate.

## 3. Results and Discussion

New buds starts to appear from the axil of shoot tip of explants after 4 weeks of inoculation. Best result of growth are observed within six days. Microshoots were inoculated on MS basal medium (Murashige and Skoog, 1962) with different concentrations and combinations of BA and Kn (in combination of IBA 0.2 mg/L) for shoot proliferation. Both BA and Kn were found to give the indications of shoot proliferation after 2 weeks of incubation. It was found that BA gave better shoot proliferation than Kn (**Table 1**). In medium containing BA in different concentration, on an average each explant gave rise to 3.0-3.3 shoots (**Table 1**). Hundred percent cultures showed shoot proliferation on BA containing medium. On medium containing Kn 1 mg/L, only 90% cultures showed shoot proliferation. In medium containing higher concentration of Kn (1.0 mg/L) the average number of shoots per plant were observed 3.1±1.1. On the other hand in the medium containing less concentration of Kn (0.2mg/L) the average number of shoots per plant were observed 1.1±0.2. The shoot-tip which were cultured on medium without any phytohormone, failed to produce any new shoots. Adenine sulphate was also used to check whether it has any effect on shoot proliferation or not. It

was observed that adenine sulphate has no significant effect on shoot proliferation. In the case of adenine sulphate the average number of shoots per plant was 3.1 **(Table 2).** The percentage of shoot proliferation was also lower than control i.e. 70±25.

Three to four centimeters long shoots were excised individually from the proliferated shoot clumps and cultured on rooting medium. The shoots inoculated on hormone free (medium lacking IBA) and IBA supplemented medium showed rooting response with in a week. However the response was improved in hormone free medium. After 15 days of inoculation, rooting was 100% in hormone free medium (**Table 3**). The number of roots per shoot was 2.8±0.2 on hormone free medium. In both the cases roots were without any branches and normal in appearance. Average number of roots per plant was found 2.2±1.2 in medium containing hormones.

After 15 days of culture of microshoots on rooting medium, which result in the sufficient rooting of shoots, the plantlets were transplanted to plastic pots containing garden soil and Farmyard manure (1:1) for their hardening. In first 10 days the plantlets were kept in poly house. To maintain the appropriate humidity level (80±2%), plants were carefully watered with the help of manual sprinkler every 2 hours. Temperature of the poly house was sustained at 30$^{o}$C with humidity level of nearly 90%. Plantlets that were transferred to the plastic pots in poly house showed 90% survival and under shade house (50%) it was found 80% (**Table 4, Fig 1**). After keeping plantlets for initial ten days in poly house, the plantlets were transferred to the shad house under less humidity and temperature controlled condition and indict sun light. In shade house plants started to elongate and leaves also start to thicken. In shade house plants were watered twice in a day i.e. morning and evening. Among the survived plants, some plants showed the symptoms of leap tip necrosis during shade house condition.

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**Table 1-** Effect of different combinations of cytokinin on shoot proliferation after four weeks of culture

| **Phytohormones** | **(mg/L)** | **% of shoot formation (n = 5)** | **Number of shoot per *explants* (n = 10)** |
|---|---|---|---|
| Control | - | Nil | 1 |
| 6-Benzylaminopurine (BA) | 0.2 | 100±2 | 2.5 ± 0.5 |
| | 0.4 | 100±5 | 2.7 ± 0.6 |
| | 0.6 | 100±10 | 2.9 ± 0.7 |
| | 0.8 | 100±15 | 3.1 ± 0.8 |
| | 1.0 | 100±20 | 3.3± 1.1 |
| Kinetin (Kn) | 0.2 | 40 ± 40 | 1.0±0.2 |
| | 0.4 | 50 ± 35 | 1.3±0.4 |
| | 0.6 | 60 ± 30 | 1.6±0.5 |
| | 0.8 | 70 ± 25 | 2.5±0.8 |
| | 1.0 | 90 ± 20 | 3.1±1.1 |

*Combination with IBA 0.2 mg/L*

**Table 2-** Effect of adenine sulphate on shoot proliferation in *Aloe vera* after four weeks of culture

| Adenine sulphate (mg/L) | % of shoot formation (n = 4) | Number of shoot per *explants* (n = 8) |
|---|---|---|
| Control | 100±0.0 | 2.8 ± 0.6 |
| 50 | 90±5.0 | 3.0 ± 0.8 |
| 100 | 80±10.0 | 3.1 ± 1.0 |
| 150 | 70±20.0 | 3.2 ± 1.3 |

*Combination with BA 0.2 mg/L and IBA 0.2 mg/L*

**Table 3-** Effect of IBA on root induction in *Aloe vera* after 15 days of culture

| IBA (mg/L) | % of root formation (n = 4) | Number of roots per *explants* (n = 8) |
|---|---|---|
| 0 | 100±0.0 | 2.8 ± 0. 2 |
| 0.2 | 98±5.0 | 2.6 ± 0. 4 |
| 0.4 | 96±7.0 | 2.4 ± 0. 6 |
| 0.6 | 94±10.0 | 2.2 ± 0. 8 |
| 0.8 | 92±15.0 | 2.0± 1.0 |
| 1.0 | 90 ±20.0 | 1.8± 1.2 |

**Table 4-** Survival rate of plantlets of *Aloe vera* at different stage of hardening

| Stage of transplantation | Number of plants transplanted | Percentage of survival |
|---|---|---|
| Poly House ($I^{st}$ stage) | 75 | 90 |
| Shade House ($II^{nd}$ stage) | 75 | 80 |

**Figure 1** Plant of *Aloe vera* growing in open