# Standardization of micropropgation techniques for Aloe vera: A pharmaceutically important plant

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**Abstract**: Aloe vera (Syn *Aloe barbadensis* Mill.), a medicinal plant, has a great potential in cosmetic and drug industry due to presence of more than 200 bioactive compounds. Natural propagation of Aloe vera, by means of suckers, is very slow and insufficient to meet the increasing demand of pharmaceutical and cosmetic industries. Shoot tip was used as an explant for *in vitro* regeneration of Aloe vera. Explants were disinfested with the use of 0.1% mercuric chloride and 0.5% sodium hypochlorite, and washed thoroughly with autoclaved distilled water. Solid MS medium was used with addition of different concentrations of 6-benzyl aminopurine and α-naphthalene acitic acid. After 7 weeks of inoculation, greatest number of shoots (11.18) and highest shoot length (12.15cm) were found in MS medium supplemented with 0.5 mg $l^{-1}$ 6-benzylaminopurine (BAP) along with same concentration of α-naphthalene acitic acid (NAA). Best rooting (84.67%) was found in medium supplemented with 1.5 mg $l^{-1}$ of indole butyric acid (IBA). The rooted explants were then gradually acclimatized and shifted to green house.

**Keyword**: Aloe vera, micropropagation, *in vitro* regeneration, medicinal plant.

## INTRODUCTION

*Aloe vera* (Syn. *Aloe barbadensis* Mill). a monocotyledonous plant, belonging to family Asphodelaceae (Ali & Qaiser, 2005) and is indigenous to the Eastern and Southern Africa, the Canary Island and Spain. Its species spread to the Mediterranean basin and reached the West Indies, India, China, Pakistan and other countries in the 16th century. Certain species are now cultivated for commercial purposes, throughout India and some parts of Pakistan. It is naturalized in Pakistan, especially in the hot dry valleys of Sindh and central Punjab (Ali and Qaiser, 2005). It is a xerophyte and can be grown even in dry lands under rain fed conditions.

Aloe vera has been used in traditional medicine and cosmetics since ancient times (Davis *et al.*, 1988). More than 160 metabolites are found in *Aloe vera* leaves (Supe, 2007) and among these the most important are barbaloin and homonataloin (Nakagomi *et al.*, 1983; Groom & Reynolds, 1987). Aloe vera is used internally as laxative, antihelminthic, hemorrhoid remedy and uterine stimulant as menstrual regulator and reported to be used as wound healing (Choi *et al.*, 2001). It is reported to be used on sun damaged skin and UV damaged skin as well (Mantle *et al.*, 2001).

The common propagation of Aloe vera is conventional method by means of suckers which is inefficient to meet the industrial demand of Aloe vera leaves (Aggarwal & Barna, 2004). Current production of leaves is not enough for the fast growing demand of cosmetic and pharmaceutical industries. Further, large-scale cultivation of selected genotype is the need of time (Singh and Neelu, 2009). Sexual reproduction of Aloe vera by means of seeds is limited due to presence of male sterility (Kalimuthu *et al.* 2010). Limited number of lateral shoots from a single donor plant per year is also a limiting factor for the vegetative reproduction of Aloe vera. Because of these factors there is less availability of plant propagating material leading to low productivity of this important pant. The problem of low productivity can be minimized by using micropropagation as a possible solution to overcome the problems associated with conventional propagation techniques (Kalimuthu *et al.*, 2010). Campestrini *et al.* (2006) reproduced 4,300 plantlets from 20 explants, over a 6-month period by using *in vitro* techniques, overcoming the drawback of lack of propagating material.

Presence of plant growth regulators plays a significant role in a successful regeneration of any plant species. Among the plant growth regulators, cytokinin in culture medium is the most important factors for shoot proliferation (Aggarwal and Barna, 2004; Liao *et al.*, 2004; Mamidala and Nanna, 2009; Jafari and Hamidoghli, 2009; Hoque, 2010). Some researchers reported presence of both of auxin and cytokinin necessary for shoot proliferation (Rout *et al.*, 2001; Velcheva *et al.*, 2005). Ali *et al.* (2009) further reported the optimum combination of cytokinins and auxin critical to shoot regeneration. The role of auxins in adventitious root formation has been a focus of research in a wide range of both woody and herbaceous plants (Diaz-Sala *et al.*, 1997, Blazkova *et al.*,

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1997). The objective of research was to standardize an appropriate concentration of various plant growth regulators for a successful micropropagation of Aloe vera for disease free, true to type Aloe vera plantlets. The research was further aimed for mass propagation of this important plant.

## MATERIAL AND METHODS

The present research was carried out in Plant Tissue Culture Lab. Department of Horticulture, PMAS-Arid Agriculture University Rawalpindi during the year 2010-2011.

***Plant material***

Healthy *Aloe vera* plants with good biomass were selected as experimental material. Shoot tip explants were selected from these healthy and good biomass plants of 2 years of age. The extra leaves were trimmed to avoid any contamination and shoots were resized to 2-3 cm.

***Surface disinfestation***

Shoot tips 2-3 cm long were placed under running tap water for about one hour to remove foreign contaminants. During washing surfactant (Tween-80) along with detergent was also added to make the contact of water deep in the plant material. Subsequently the explants were taken to laminar airflow cabinet for further sterilization. After rinsing explants were dipped in 70% ethanol for 30 seconds and then were treated with 0.1% mercuric chloride ($HgCl_2$) and 5% sodium hypochlorite (NaOCl) for 5-6 minutes simultaneously. After this treatment, explants were given 4-5 thorough washings with autoclaved distilled water to remove any trace of the surface sterilants, under aseptic conditions.

***Shoot proliferation***

The sterilized explants were inoculated singly in MS (Murashige & Skoog, 1962) medium with different concentration (0.5, 1.0, 1.5, 2.0 mg $l^{-1}$) of BAP alone or in combination with NAA. Data were recorded on the Explants showing shoot formation (%), shoot length (cm) and, number of shoots.

***Rooting of microshoots***

Newly formed shoots measuring 2-3 cm in length were excised individually from the parent explant and transferred to MS media having different concentration (0.1, 0.2, 0.5, 1.0 mg $l^{-1}$) of NAA and IBA, singly or in combination.

## STATISTICAL ANALYSIS

The experiment was one factorial set up in a completely randomized design (CRD) with three replications per treatment. Data were statistically analyzed by analysis of variance (ANOVA) technique and differences among treatment means were compared by using Duncan's multiple range (DMR) test at 5% probability level (Steel *et al.*, 1997).

## RESULTS

***In vitro culture establishment***

In the present study, the combination of these two disinfectants (5% NaOCl along with 0.1% HgCl2) showed significant reduction in microbial contamination and culture establishment of Aloe vera. NaOCl (8%) and HgCl2 (0.1%) alone were ineffective to control the contamination significantly.

***In vitro shoot proliferation***

In the present study, effect of various PGRs including Cytokinin (BAP) and Auxins (NAA) was assessed for shoot proliferation of Aloe vera. Parameter studied were shoot formation (%), number of shoots and shoot length (cm). Aloe vera explants showed signs of proliferation after two weeks of culture. Shoot multiplication was found best in MS medium supplemented by 0.5 mg $l^{-1}$ BAP and 0.5 mg $l^{-1}$ of NAA. The average number of shoots in this treatment was found 11.18 (plate 1) followed by the medium supplemented by 1.0 mg $l^{-1}$ BAP and 0.5 mg $l^{-1}$ of NAA (9.007). Shoot elongation was found the longest in the medium containing 0.5 mg $l^{-1}$ BAP and 0.5 mg $l^{-1}$ of NAA (12.15 cm) and the least shoot height was found in the hormone free medium (plate 2).

**Plate 1**: Highest number of shoots in $T_6$ with 0.5 mg $l^{-1}$ BAP along with same concentration of NAA.

***In vitro rooting***

Proliferated shoots obtained from shoot tip explants of Aloe plants took maximum 7-8 weeks from the time of

establishment to attain the size suitable for rooting (>2 cm). The shoots showed good rooting on MS medium supplemented with1.5 mg $l^{-1}$ IBA. In the present study rooting was improved with the inclusion of IBA as compare to NAA. 1.5 mg $l^{-1}$ IBA produced rooting in maximum (98.33%) where as 1.5 mg $l^{-1}$ NAA produced rooting in 86.67%.

**Plate 2**: A better shoot length of *Aloe vera* with 0.5 mg $l^{-1}$ BAP and 0.5 mg $l^{-1}$ NAA ($T_6$).

In the present study, rooting percentage showed the tendency to increase with increasing concentration of IBA, however, the higher concentration (>1.5 $mgl^{-1}$) than the optimal exerted its inhibitory effect on the number of rooted shoots. The results also indicate that with NAA the adverse effects occur at relatively low concentration as compare to IBA. The inhibiting effect of auxins might be because higher concentration of auxin induces the higher level of degradative metabolites in tissue, thus, blocking the regeneration process (Baker and Wetstein 2004).

The *in vitro* rooted plants with good quality shoots were successfully acclimatized. Acclimatization was achieved by direct transfer of plants to pots containing the mixture of soil, sand (1:1) and placed in glasshouse having light intensity of 4000-10000 Lux, 90-95 percent humidity with temperature ranging from 26-280 C.

## DISCUSSION

For crop selection and improvement using *in vitro* techniques, culture establishment is the first and prime important stage. Contamination is a critical aspect while establishing new cultures from field grown plants because of their exposure to various microorganisms that are though not a problem in vivo, but result in contamination *in vitro* due to availability of rich culture media (Perez-Tonero *et al*., 2000). Consequently, it is necessary to remove foreign contaminants including microorganisms foregoing *in vitro* culture establishment (Zulfiqar *et al*., 2009). Surface sterilization of the explants with a vast range of disinfectants, NaOCl (sodium hypochlorite) and $HgCl_2$ (mercuric chloride) have been widely used *in vitro* due to their higher effectiveness against all types of microbes (Yildiz and Er, 2002). For the present study the combination of both the disinfactants proved to be the best to control the fungal and bacterial contaminations. Both these chemicals (NaOCl and $HgCl_2$) are oxidizing agents and damage the microorganism by oxidizing the enzymes (Rao, 2008). The ineffectiveness of NaOCl may be due to the reason that it is a mild sterilizing agent (Sirivastava *et al*., 2010). $HgCl_2$ is reported a better sterilizing agent as compared to NaOCl but is more toxic and requires special handling and is difficult to dispose (Maina *et al*., 2010). Apart from its effectiveness, mercuric chloride could be toxic to plant tissues if used in higher concentration (Muna *et al.,* 1999; Sedlak and Paprstein 2007). The efficient combine use of these two disinfectants might be due to double action of both the chemicals. Thus it can be concluded that both the disinfactants, $HgCl_2$ and NaOCl, when used in proper combination can control contamination in Aloe vera effectively.

Type of explants used, culture condition and composition of culture media play very significant role for *in vitro* shoot proliferation (Soumendra *et al.*, 2000). The data indicated a great interaction between the two growth regulators for shoot formation (%), number of shoots and shoot length of Aloe vera. The treatments without NAA were not able to produce significant number of shoots. Velcheva *et al* (2005) also reported the necessity of presence of both auxin and cytokinin for shoot proliferation. Wenping *et al.* (2004) and Liao (2004) also added the similar results regarding presence of BAP and NAA for better Aloe vera micropropagation. Further, there was a declining trend of shooting in Aloe vera with increasing the concentration of BAP (2 mg $l^{-1}$). This decline is also supported by Hashemabadi and Kaviani (2008). This decline in the shoot length of Aloe vera might be due to the inhibitory effect of BAP, which provoke a little suppression of plant growth and activity of some proteolytic enzymes (Petkova *et al.*, 2003). Jaramillo *et al.* (2008) reported that presence of BAP in the culture medium is necessary for shoot regeneration although higher concentration reduced the shoot regeneration frequency.

For *in vitro* rooting, the results showed good rooting of microshoots on MS medium supplemented with1.5 mg $l^{-1}$ IBA. Rooting was also improved with the inclusion of

IBA as compare to NAA. These results are controversial to the results obtained by Ahmed *et al.* (2007) who reported the NAA as a best auxin for rooting of Aloe vera whereas in the present study, IBA was found better for root proliferation. NAA and IBA are most commonly used auxins for rhizogenesis (Bhojwani & Razdan, 1992). By the use of IBA, many plants such as *Lycoperscicon esculemtum* (Sibi, 1982), *Hedychium roxburgii* (Tripathi & Bitallion, 1995), carnation (Werker &Leshem, 1987) rooted *in vitro*. Numerous studies also supported the usefulness of IBA as the most effective auxin in various plants rhizogenesis as compare to NAA (Benelli *et al.*, 2001; Tanimoto, 2005). They also found IBA is the preferred auxin for the induction of root formation because it is much more potent than IAA or synthetic auxins. Rooting of Aloe vera is also reported in hormone free medium by many researchers (Natali *et al.*, 1990; Aggarwal & Barana, 2004). In the present study miserable number of roots (25%) produced when not treated with either of the PGR.

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