Research in Plant Biology, 1(5): 22-26, 2011 ISSN : 2231-5101
www.resplantbiol.com

Regular Article

In vitro propagation of *Aloe barbadensis Miller*, a miracle herb

Jayakrishna, C., Karthik, C., Barathi, S., Kamalanathan, D. and Indra ArulSelvi, P*

Department of Biotechnology, Periyar University, Salem-11, Tamil Nadu, India
*Corresponding Author: iarulselvi@gmail.com

Aloe vera **has valuable medicinal properties and is commercially used in pharmaceutical, cosmetic and food industries. An efficient micro propagation method has been developed in *Aloe vera* plants using the shoot tip explants cultured on MS medium with different phyto hormonal supplements for shoot proliferation and rooting. The shoot proliferation was found best (80%) in the MS medium containing Benzyl amino purine (BAP) 2.0 mg/L. Seventy percent of adventitious root formation was observed in half strength MS medium supplemented with Indole butyric acid (IBA). After two weeks, *in vitro* grown plants were transferred to the poly-cups containing 1:1 ratio of soil and sand respectively for hardening and then transferred to garden showed 75% of survival.**

Key words: Micro propagation, *Aloe vera,* BAP, IBA, MS medium.

Aloe barbadensis Miller (=Aloe vera L.*)* belongs to Liliaceae, which has medicinal and cosmetic properties (Gui, 1990). It is a large succulent perennial plant growing up to 1.5 meters in height, with a strong fibrous root and at-large stem supporting a rosette of narrow lance late leaves. The leaves are whitish green on both sides and bear spiny teeth on the margins. The yellow to orange drooping flowers grow in along raceme at the top of the flower stalk. The fruit is a triangular capsule containing numerous seeds **(**Kay and Thida, 2005**)**. In nature, *A. vera* is propagated through lateral buds which is slow, very expensive and low income practice (Meyer and Staden, 1991). It is a perennial succulent xerophyte, which is developing water storage tissue in the leaves to survive in dry areas of low or erratic rainfall. The innermost part of the leaf is a clear, soft, moist and slippery tissue that consists of large thin-walled parenchyma cells in which water is held in the form of viscous mucilage (Josias, 2008).

The plant contains the important antioxidant vitamins (A, C and F), B (thiamine), niacin, B2 (riboflavin), B12, choline and folic acid. The leaf pulp and liquid fraction of *A. vera* act against various microorganisms (Baby Joseph and Justin Jaj, 2010). The Chinese describe aloe's skin and the inner lining of its leaves as a cold, bitter remedy which is downward draining and used to clear constipation due to accumulation of heat; the gel is considered cool and moist. In Ayurvedic medicine of India, aloe is used internally as a laxative, antihelminthic, hemorrhoid remedy, and uterine stimulant (menstrual regulator); in combination with licorice root, to treat eczema or psoriasis (Kathi and Victoria, 1999).

Cultivation of high value medicinal plants had created new dimension in the field of agriculture. Regeneration of *A. vera* in nature (*in-vivo*) is too slow and insufficient to meet the industrial demand (Arvind, 2010). Also they experienced a

slow increase due to limited availability of raw material with high quality (Campestrin, 2006). Therefore, there is a need to develop suitable and alternative method for traditional propagation like *in- vitro* propagation for rapid plant production (Natali 1990; Roy and Sarkar, 1991; Abrie and Staden, 2001). Micro propagation using stem and lateral shoot pieces of *A. vera* had already been proved successful (Natali, 1990; Roy and Sarkar 1991; Mayer and Staden 1991; Aggarwal and Barna 2004). However; source of explants, size, age, genotype, media composition, culture conditions and phenolic content of explants and media discoloration greatly affect shoot regeneration from different genotypes of the same species. The prime objective of this study was to carry out the alternative protocol for rapid *in vitro* propagation of this medicinally important *A. vera.*

Materials and methods

Plant material

A. vera shoots with young leaves were collected from the Periyar University campus, Salem. Collected plants of *A. vera* were maintained and multiplied in the pots in the controlled condition for avoid infection. The extra leaves were removed and shoots trimmed to about 2-3cm and was used.

Sterilization of explants

The explants (shoot tips) were washed thoroughly in running tap water and then wiped thoroughly using 70% ethanol. Then it was treated with 0.1% Tween20 with constant shaking then washed with sterile distilled water for 3-4 times. After 0.25% sodium hypochlorite wash the explants was washed thoroughly with distilled water. Followed by 0.1% $HgC1_2$ treatment and finally rinsed thoroughly with sterile distilled water (3-5 times) to remove any traces of mercuric chloride and then was dried.

Shoot proliferation

The explants were cultured on Murashige and Skoog (MS) medium supplemented with different hormones of concentrations from 10 - 40 µg/L of BAP alone. The pH of the medium was adjusted to 5.8 and autoclaved for 15 min at 121 lbs. Then the surface sterilized explants were inoculated in the culture medium and incubated at 24 ±0.2°C for 16 hours photo period and were sub cultured in order to increase budding frequency. After 4 weeks of incubation, the regenerated shoot was transferred to increased hormonal concentration for shoot elongation. Serial sub-culturing with certain time interval was used for multiple shoot formation.

Rooting of micro shoots

Newly formed shoots measuring 2-3cm in length were excised individually from the parent explants and transferred to rooting media with different IBA+NAA (1.5+2.0 µg/L) concentrations. The development pattern of roots was observed throughout the entire culture period.

Acclimatization

After 20 days of root formation on rooting media, the plantlets were shifted for acclimatization. Pots were kept ready filled with garden soil, compost and sand in the proportion of 1:1:1 ratio respectively. Then the plants were transplanted into the pots with special care. The plant was kept in surface chamber for initial periods and then, to the glass house having 80% humidity and 31˚C temperature for 10 days. Then it was taken to shade house with less humidity. After 45 days, the plants were transferred to the soil.

Results

The explants began to show the signs of shoot proliferation after 6 days of culturing. All explants gave aseptic culture. Plants were free from fungal as well as bacterial contamination. New buds

appeared from the axils of leaves and developed into shoots, only after 4 weeks of culturing (Fig.1A). Shoot tip explants grown on medium with different concentrations of BAP and the multiplication of shoots was found best on MS medium with 2 mg/L BAP and 4.0 mg/L BAP and the emergence of shoots took place in 2 weeks. (Fig.1B) However, the higher concentration of BAP showed low response compared to low concentration of BAP. Shoot proliferation on medium with 4mg/L BAP + 1mg/L KIN also showed a better response (Table 1, Fig1B). Medium having 50 µg/L concentrations of IBA and combination of Auxin and cytokinin (NAA+ IBA) was found to be the best medium for shoot and root proliferations in *A. vera* (Table 2, Fig. 1C and ID). The small rooted shoots were transferred from *in vitro* conditions to plastic pots and placed under net to keep the environment wet and shade. Survival of plantlets was observed after one month (Fig. IE).

Figure 1. *In vitro* propagation of *Aloe barbadensis* A: Inoculated explants showing multiple shoot formation after 6 days of incubation; B: Shoot Elongation; C: Root Induction; D: Branch roots of multiple shoots; E: Acclimatization

Discussion

The present study implies that, for shoot proliferation, generally the growth regulators like Auxin and Cytokinin influence the process seriously. The regeneration of shoots from the explants were observed within 7 days of incubation and the roots were observed after 7 days of incubation under controlled conditions i.e. temperature at 24±2°C and 16 hours photo period with light. Similar result was also reported in *A. vera* by Arvind *et.al.* (2010). The multiple shoots were observed in the MS medium containing 2.0 mg/L and 4.0 mg/L BAP without any additional supplements of other hormones and

charcoal. MS medium with growth hormones, produced shoots within 3 days of transfer. However, medium without growth regulator produced shoots after 5 days (Coudhary and Mukundan, 2001). Maximum shoots were obtained at the concentration of BAP 2.0 - 4.0mg /L. Similar results were given by Meyer and staden report (1991). An interesting result was observed in combination of KIN and IAA, in average produced 10 shoots and roots. The highest number of roots per culture was found in MS medium containing 1.5 mg/L IAA. The IAA induced roots were able to produce many lateral roots which can survive as *ex vivo* plants in acclimatized conditions with soil and sand. The acclimatised plants showed highest percentage of plant survival.

Table1. Effect of different concentration of BAP and NAA on multiple shoot formation in *Aloe vera.*

Growth regulators (mg/L)	% of explants producing shoots	Average of branches/shoots	Mean of shoots length (cm)
BAP			
2.0	80	32	1.43 ± 0.30
4.0	70	15	1.13 ± 0.11
BAP+NAA			
2.5 + 0.5	70	12	1.26 ± 0.20
3.0 + 0.5	75	16	1.70 ± 0.60

The experiment consists of 14 explants and repeated for three times and their mean values are calculated

Table 2. Effect of various Concentrations of IBA and NAA in half strength MS on rooting of micro shoots in *Aloe vera.*

Hormone con. (mg/L)	% of shoot rooted	Average of roots formed	Mean of root length (cm)
IBA			
2.0	70	10	1.26 ± 0.20
2.5	70	08	1.16 ± 0.57
IBA + NAA			
1.5 + 1	60	09	1.56 ± 0.51
2.0 + 1	65	12	1.66 ± 0.76

The experiment consists of 14 explants and repeated for three times and their mean values are calculated.

Other pervious report on the micropropagation of *A.vera* using more than one type of medium for initiation and multiplication are available (Aggarwal, *et al.,* 2004). In the present study a simple two step protocol was established using MS with BAP for shoot initiation and multiplication and IAA for rooting in *A. vera.* This protocol could be used for the massive *in vitro* production of the plantlets of the *A. vera.*

References

Abrie AL, Staden JV. (2001). Micro-propagation of the endangered *Aloe polyphylla*. *Plant Growth Regul*. Vol-33: 19-23.

Arvind Kumar Bhandari, J.S.Negi, V.K.Bisht and M.K.Bharti. (2010). In Vitro propagation of Aloe vera-A plant with medicinal properties. Nature and Science ;8(8):174-176.

Aggarwal D, Barna KS, Lion MM, Chacin P. (2006). A Methodology for the propagation in edge *Aloe vera* L.Rev. Fac. Agron.

Aggarwal D, Barna KS., 2004, Tissue culture propagation of Elite plant of *Aloe vera* Linn. J. Plant Biochemistry and Biotechnology., 13:19-23

Arvind Kumar Bhandari, Negi JS, Bisht VK and Bharti MK. (2010). *In Vitro* propagation of *Aloe vera*-A Plant with Medicinal Properties. *Nature and Science*. Vol-8(8):174-176.

Baby Joseph and Justin Jaj. (2010). Pharmacognostic and Phytochemical Properties of *Aloe vera. International Journal of Pharmaceutical Sciences Review and Research*.vol- 4:2.

Campestrini LH, Kuhnen S, Lemos PMM, Bach DB, Dias PF, Maraschin M. (2006). Cloning protocol of *Aloe vera,* as a study-case for "Tailor-Made" biotechnology to small farmers. *Journal of Technology Management and Innovation*. Vol-1(5):76-79.

Coudhary S and Mukundan.U (2001). *Aloe vera* L. Micropropagation and characterization of its gel. *Phytomorphology*. Vol- 51: 155-157.

Davood Hashemabadi and Behzad Kaviani. (2008). Rapid micro-propagation of *Aloe vera* L. via shoot Multiplication. *African Journal of Biotechnology*. Vol. 7 (12): 1899-1902.

Gui YL, Xu TY, Gu SR, Lin SQ, Zhong Z, Sun GD and Zhang Q (1990).Studies on stem tissue culture and organogenesis of *Aloe vera*. *ActaBot. Sin*. Vol-32: 606-610.

Hosseini R and Parsa M. (2007). Micropropagation of *Aloe vera* L. grown in South Iran. *Pakistan Journal of Biological Sciences.* Vol-10 (7): 1134-1137.

Josias H Hamman. (2008). Composition and Applications of *Aloe vera* Leaf Gel. *Molecules.* Vol-13*:* 1599-1616.

Kathi J Kemper and Victoria chiou. (1999). Aloe vera (*aloe vera*). The Longwood Herbal Task Force and The Center for Holistic Pediatric Education and Research. Vol- 2: 29.

Kay Thi Mya, Thida Myint, Myo Han and Khin Maung sein. (2005). Meristem Culture *Gf Aloe vera* L. *Jour. Myan.Acad. Arts & Se.* Vol. III. Nb.f(ii) Botany.

Meyer HJ and Staden.J. (1991). Rapid *in vitro* propagation of *Aloe barhadensis* Mill. *Plant cell, Tissue and organ culture.* Vol-26: 167-171.

Meyer HJ and Staden JV. (1991). Rapid *in vitro* propagation of *Aloe barbadensis* Mill. *Plant Cell, Tissue and organ Culture.* Vol-76(1): 83-86.

Natali L, Sanchez IC, Cavallini. (1990). In *vitro* culture of *Aloe barbadensis* Mill. Micropropagation from vegetative meristems. *Plant Cell, Tissue and Organ culture*. Vol-20:71-74.

Roy SC and Sarkar A. (1991). *In vitro* regeneration and micropropagation of *Aloe vera* L. *Scientia Horticulturae*. Vol-*47:* 107-113.