J. Indian bot. Soc. ISSN - 0019 - 4468 Vol. 94 (1 & 2) 2015 : 118-125

AN EFFICIENT PROTOCOL FOR MICROPROPAGATION OF A MEDICINAL PLANT *ALOE VERA* L. THROUGH ORGAN CULTURE

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The aim of study was to assess the effect of different auxin and cytokinin on regeneration potential of rhizomatous stem (RSL and TRS) and leaf segment explants of *Aloe vera* and techniques were standardised for the mass propagation of this wonder drug plant. Sterilized explants (TRS, RSL, and LS) were cultured on the media supplemented with different combination of IAA, NAA, IBA, 2,4-D, BAP and Kn within a range of 0.5- 3.0mg/L. Best shoot proliferation (6-7 shoots/ explants) was obtained on MS medium containing 2.5mg/L each of BAP and NAA from RSL explants and highest number of roots developed directly from LS explants in addition to callogenesis on MS medium fortified with NAA+IAA (2+1)mg/L. Optimum growth of callus was achieved from RSL and LS explants on medium containing NAA+BAP+IBA(2.5+2+0.5)mg/L. Callus in general was pale -yellow, hydrated and feathery in nature. Browning was a serious limitation in primary as well as callus culture which was minimised by ascorbic acid pre treatment. The best rooting of microshoots were obtained on shoot regenerating medium containing both BAP and NAA, 2.5mg/L each. In vitro grown plantlets were acclimatized and transferred to green house condition, regenerants were morphologically similar to mother plants.

Keywords: Growth-regulators, *Aloe vera*, Leaf segment, Microshoots, Ascorbic acid.

Aloe vera L. (*Aloe barbadensis* Mill, family - Liliaceae) comprising 500 species (Deng *et al.*1999), is a cactus like succulent, perennial, xerophytic herbs with short stem and shallow root system growing in garden as well as in wild habitat. This plant is native of North America and has restricted distribution in our locality (Muzaffarpur), it reproduces by vegetative methods through root suckers and adventitious shoot buds as the male flowers show sterility (Keizer and Cresti 1987), growth rate of these propagules is much slow. Aloe vera gel obtained from leaf pulp has been reported to have multiple beneficial properties for wound healing, including the abilities to penetrate and anesthetise tissues, arrests bacterial, fungal and viral growth, acts as an anti inflammatory agent and enhances blood flow (Klein and Penneys 1988, Haller 1990, Davis *et al*. 1994, Heggers *et al*. 1996, Yao *et al*. 2009, Sahu *et al*. 2013) Medicinal properties are due to aloin, isobarbadonin and emodin (Chopra *et al*. 1956, Naik 1998, Singh and Sood 2009) and fleshy leaves of *Aloe* have been found to contain about 200 bioactive constituents (Waller *et al.* 1978, Vogler and Ernest 1999).

Due to slow rate of natural growth, ever increasing demand for this "Potted Physician" can not be met with traditional method of propagation, hence there is need for mass propagation of this plant through *in vitro* method to fulfil the demand of pharmaceutical and cosmetic industries. It is in great demand for its medicinal and cosmetic properties by rural, urban and tribal folks (Kliein and Penneys 1988), the cultivation of this wonder drug plant is now a days encouraged by herbal experts. Keeping this in view, there is need for the systematic cultivation of rare medicinal plants including *Aloe vera* for their germplasm conservation, selection of desired genotypes and mass propagation of superior clones (Ahuja 1994, Naseem and Jha 1997, Heble *et al*. 1998, Kumar *et al.* 2010).

Some workers have attempted to propagate this plant through tissue culture methods (Aggarwal and Barna 2004, Gosal and Gosal, 2009) and few reports are available on *in vitro* propagation of *A. vera* (Meyer and Staden 1991, Ahmed *et al.* 2007, Kumar *et al.* 2011). In these studies, shoots tips were cultured after complete removal of leaf primordia, this causes the complete loss of the axillary buds lying at the leaf base and resulting induction of lesser number of shoots in vitro (Singh and Sood 2009) but our studies are based on a separate line using different plant parts viz. rhizomatous stem (RSL and TRS) and leaf segments as explants. The main objective of the present investigation is to develop an efficient and

Received on February 26, 2015 Accepted on March 23, 2015

reproducible protocol for production of large number of plantlets of *Aloe vera* in a short time to meet the growing demand for pharmaceutical and herbal industries.

MATERIALS AND METHODS

Medium preparation:

Stock solution of MS medium (Murasige and Skoog 1962) and different concentration (0.5-3mg/L) and combination of phytohormones (IAA, IBA, NAA, BAP, Kn, and 2,4-D) were prepared, pH of the medium was adjusted to 5.8 prior to autoclaving at 121°C for 20 minutes and 15 ml medium was dispensed in each culture tubes (Borosilicate glass culture tube, 150×25mm).

Explants preparation:

Specimens of *Aloe vera* were taken for explants preparation from garden of University Department of Botany. Leaves and Rhizomatous stem collected from 12 months old plants during March – November, were thoroughly washed with running tap water for at least 40 minutes and were dried with blotting paper, and finally three kinds of explants set (RSL, TRS and LS,Fig.1) were prepared.

Set I : Rhizomatous stem with leaf base intact (RSL, 8-10mm).

Set II : Trimmed rhizomatous stem without leaf and root fibre (TRS, 8-10mm).

Set III : Leaf segment (LS, 8x6 mm).

For the surface sterilisation, explants were washed with liquid detergent Tween 20 (Himedia, laboratory of India) for 10 minutes with vigorous shaking. After that explants were kept in1% w/v solution of Bavistin (BASF India Limited) for 45 minutes followed by 30 minutes treatment with ascorbic acid (0.5mg/ml). After Bavistin and ascorbic acid treatment, explants were treated with 0.2% $HgCl_2$ and 0.1% NaOCl for 3-5 minutes and finally rinsed 3-4 times with sterilised distilled water.

Inoculation:

Sterilized explants (RSL, TRS, LS) were aseptically cultured on different set of MS media (Table 1 and 2) containing 3% sucrose, 0.8% Agar (Difco-Bacto) and different concentration and combination of growth regulators. Ten replicates were maintained for each experiment and were repeated twice. All cultures were incubated at 25±2°C with a relative humidity of 60-65% and 16 hour continuous photoperiod of 1000 lux irradiance provided by cool-white fluorescent tubes (Crompton Greaves, India). Callus induction lasted for 6-8 weeks, subculture of callus 4 weeks, shoot regeneration and elongation 6 weeks and *in vitro* rooting 5-6 weeks, the time of each stage was fixed.

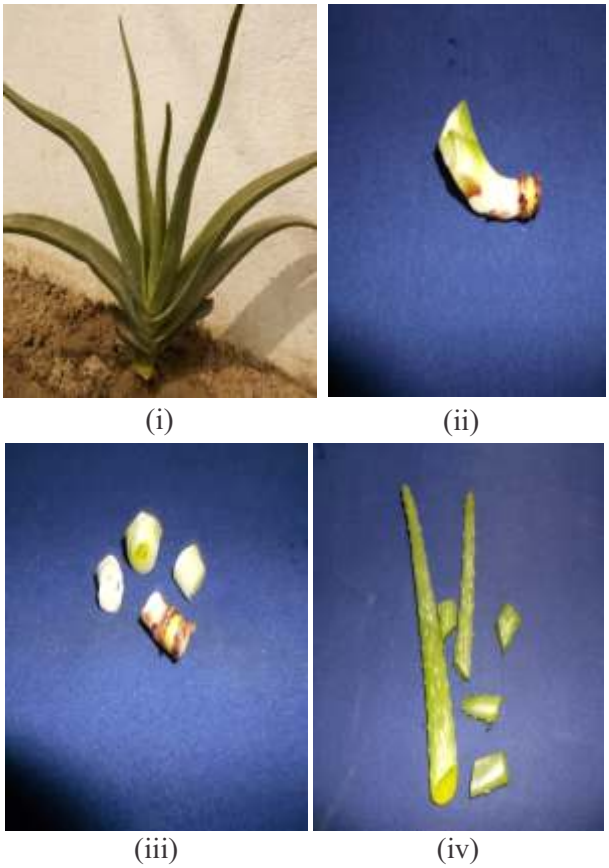

Figure 1: **i**.12 Months old *in vivo Aloe* plant **ii.** Set I explant **iii.** Set II explants **iv.** Set III explants

RESULTS AND DISCUSSION

The response in the explants (RSL, TRS, LS) became visible after 3-4 days of the inoculation, hypertrophy occurred in all the inoculated explants prior to callus formation (Table 1). MS

media fortified with different combination and concentration of growth regulators exhibited different morphogenetic responses.

Callus induction:

Callus started to form from RSL and LS explants after 12 days of inoculation on MS media having auxin (NAA, 2,4-D, IBA) and cytokinin (BAP) in different combination and concentration (Table-1). Maximum callus formation were obtained from RSL as well as LS explants on a combination of growth hormones [NAA+IBA+BAP, (2.5+0.5+2)mg/L, Fig. 2A and 2B], the callus was in general pale- yellow in colour and feathery in texture. High % of calli was also induced from LS explants on the media supplemented with 1mg/L 2,4-D and NAA+BAP+2,4-D (2+0.5+0.5)mg/L. Little work has been done on callus culture of *Aloe* species because establishment of primary cultures in case of callus induction is difficult owing to secretion of phenolic substances by explants. Groenewald *et al.* (1975) used seeds as explants for the initiation of callus but failed to obtain regeneration, however, Roy and Sarkar (1991) and Ramsay and Gratton (2000) obtained few callus mediated shoots in culture of *A. vera* L. using Polyvinylpyrolidine (PVP). Frequency of callus induction was high in case of LS explants than RSL explants (Table 1) and no response was achieved from TRS explants on any combination of hormones (Table1). Calli were subcultured after 4 weeks on the same and different combination of hormones for their conservation and further regeneration.

No result on callus formation and regeneration from LS explants has yet been reported, this is a novel attempt with regard to callus formation from leaf segments explants (Saggoo *et al.* 2010, Dwivedi *et al*. 2014, Khanam *et al*. 2014). Calli obtained from LS and RSL explants on different media (Table1) can be used as an ideal system for shoot regeneration.

Shoot induction:

Good number of shoots were directly obtained after 14 days of inoculation from RSL explants on the media having NAA+ BAP (2+2, 2.5+2.5)mg/L but after increasing the concentration of BAP and NAA above 2.5 mg/L, number of shoot proliferations were declined (Table 2). Optimum response (6.75± 0.35 shoots/ RSL explants, Fig. 2C) was obtained on BAP+NAA (2.5+2.5)mg/L. The result was more promising in RSL explants compared to TRS explants, no shoot regeneration was recorded from LS explants on any combination of hormone (Table 2). Variations of BAP concentration affected shoot proliferation in the present case, has also been reported by Naseem and Jha (1997), Singh *et al.* (2009) and Bhandari *et al*. (2010).

The type of explants had great impact on shoot proliferation (Liao *et al*. 2004, Aggarwal and Barna 2004, Velcheva *et al*. 2005), stem having axillary buds has been proven to be the most promising and efficient explants for micropropagation of *Aloe* (Natali *et al*.1990, Budhiana 2001 Meyer and Staden 1991, Liao *et al*. 2004, Aggarwal and Barna 2004, Campestrini *et al*. 2006, Debiasi *et al*. 2007), RSL and LS were found highly regenerative explants in our investigations. Shoot buds began to show proliferation within two weeks under controlled temperature (25±2°C) and 16:8, light:dark period, this was also reported by Abdi *et al*. 2013 and Khanam *et al*. 2014.

Highest shoot proliferations were obtained from RSL explants (6.75±0.35 shoots/explants) and TRS explants (5.25±0.33 shoots/explants) on medium having 2.5mg/L each of BAP and NAA, similar findings were also achieved by Kumar *et al*. (2011).

Explants pre treated with ascorbic acid were found to be more responsive with regard to shoot proliferation; no such use of ascorbic acid pre treatment is on record in case of *in vitro* culture of *Aloe* (Gantait *et al*. 2014). Further $HgCl_2$ and NaOCl, when used in proper combination could control contamination in *Aloe vera* effectively compared to $HgCl_2$, as also reported by Adelberg and Naylor-Adelberg 2012 and Zakia 2013. The frequency of shoot regeneration in RSL explants on

Table 1 : Effect of different hormones on callus induction from LS, TRS and RSL explants of *Aloe vera**

MS+Growth regulators(mg/L)				Nature of callus			% Response of explants		
BAP	NAA	2,4-D	IBA	LS	RSL	TRS	LS	RSL	TRS
0.5	2.0	0.5	0.0	YF	BC	--	60.85±1.37	36.3 ±1.60	--
0.0	0.0	1.0	0.0	BF	YF	--	54.2 ± 1.43	37.3 ±1.31	--
1.0	0.5	0.0	0.5	BF	YF	--	56.5 ±4.09	35.7 ±2.90	--
0.0	0.0	0.0	1.0	WF	YF	--	49.4 ±1.80	23.15±1.30	--
2.0	2.5	0.0	0.5	YF	BF	--	65.8 ±3.50	47.05±3.17	--
0.0	0.0	2.0	1.5	BC	BC	--	62.25±2.23	25.0 ±0.63	--

*Data (Mean ± SE) pooled from 30 explants taken from three separate experiments.

[YF- Yellow Feathery, BC- Brown Compact, BF- Brown Friable, WF- White Feathery, SE- Standard Error, LS- Leaf Segment, TRS- Trimmed Rhizomatous Stem, RSL- Rhizomatous Stem With Intact Leaf Base, IAA- Indol-3-acetic Acid, NAA- Naphthalene acetic Acid, Kn- Kinetine, IBA- Indole butyric Acid, 2,4-D-2,4- Dichlorophenoxyacetic acid, BAP- 6-Benzylamonopurine.]

Table 2: Effect of different combination and concentration of phytohormones on shoot proliferation and root initiation in different sets of *Aloe* explants*

MS+Growth regulators(mg/L)					No. of shoot per explants			No. of roots per culture	
NAA	BAP	IAA	Kn	IBA	TRS	RSL	LS	LS	Microshoots**
0.5	0.0	0.0	0.5	-	0.8±0.17	1.85±0.23	--	1.25±0.17	0.4±0.11
1.0	0.5	0.0	0.0	-	1.2±0.21	1.5±0.18	--	2.1±.022	2.9± 0.2
1.5	1.5	0.5	0.0	-	2.05±0.18	2.3±0.29	--	7.4±0.29	4.9±0.21
2.0	1.5	0.5	0.0	-	2.10±0.27	1.19±0.33	--	4.95±0.34	2.50±0.32
2.0	0.5	0.0	0.0	-	1.07±0.39	2.85±0.26	--	5.1±0.15	3.6±0.35
2.5	2.5	0.0	0.0	-	5.25±0.33	6.75±0.35	--	6.5±0.35	7.12±0.33
3.0	1.0	0.0	0.5	-	2.6±0.25	3.70±0.42	--	5.8±0.38	4.5±0.24
0.0	2.0	1.0	0.0	-	2.4±0.16	3.45±0.41	--	4.75±0.23	1.5±0.23
2.0	2.0	0.0	0.0	-	4.05±0.22	4.90±0.24	--	6.65±0.4	4.35±0.25
2.0	0.0	1.0	0.0	-	1.5±0.18	2.9±0.28	--	8.15±0.16	4.0±0.36
0.0	3.0	1.0	1.0	-	3.10±0.29	3.35±0.29	--	1.3±0.16	1.2±0.17
0.0	2.0	0.0	0.0	1.5	1.3±0.14	3.2±0.25	--	4.95±0.16	5.4±0.13
3.0	0.0	2.0	1.0	3.0	1.25±0.15	1.85±0.25	--	1.4±0.22	1.05±0.23

*Data (Mean±SE) pooled from 30 explants taken from three separate experiments.
** Roots emerged from shoots on same SRM after 25 days of culture.

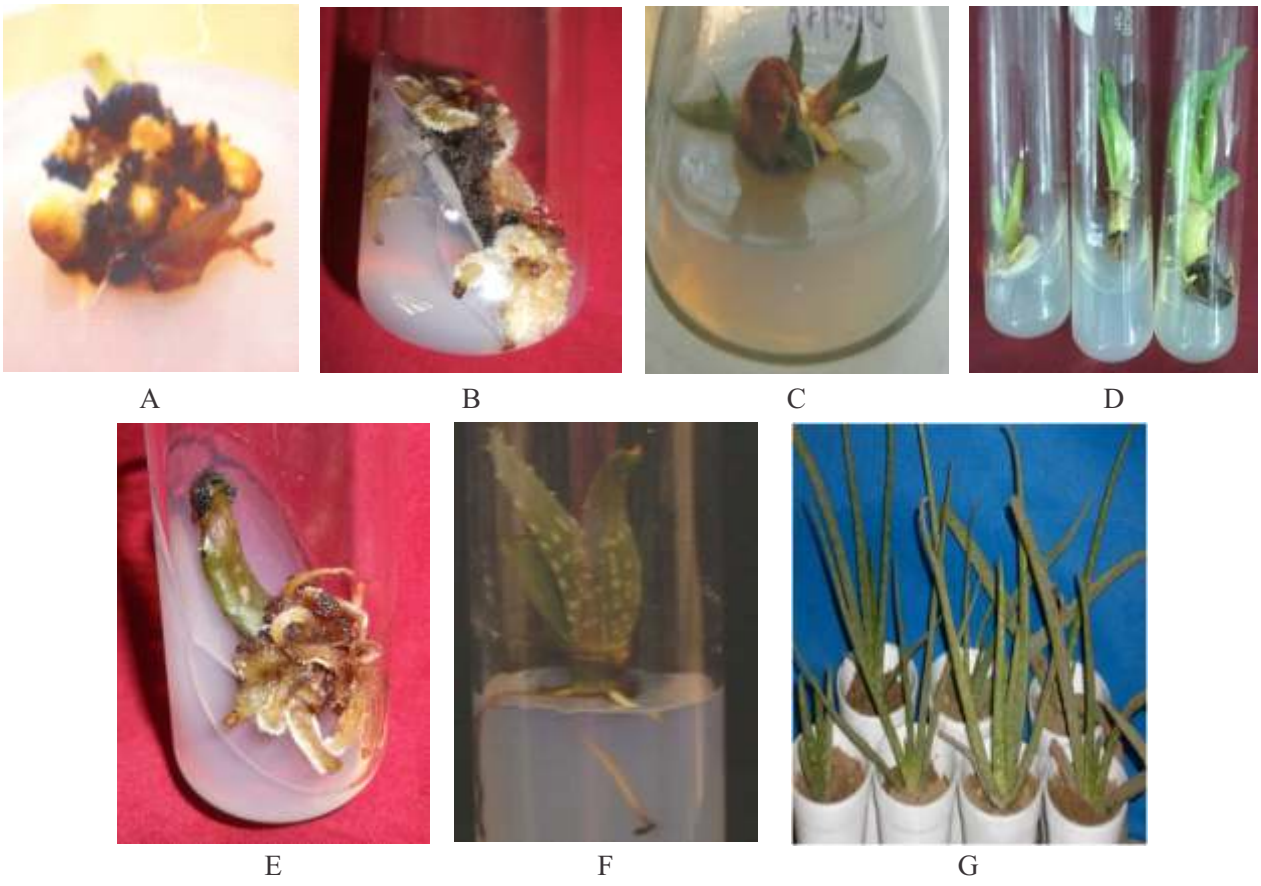

Figure 2: (A-G)

A. Feathery yellow callus induced from RSL explant of *Aloe* on medium having NAA+IBA+BAP (2.5+0.5+2)mg/L, 35 days old cuture **B.** Creamish-white colour callus obtained from LS-explant on medium supplemented with NAA+IBA+BAP (2.5+0.5+2)mg/L, 20 days old culture **C.** *In vitro* direct shoot induction from RSL explants on BAP+NAA (2.5+2.5)mg/L, 21 days old culture **D.** Different stage of shoot elongation and root induction from microshoot on medium(SRM) having NAA+BAP (2.5+2.5)mg/L (3– 6 weeks old) **E.** Root fibres emerging from LS explant turned into yellow feathery callus on medium fortified with NAA+IAA(2+1)mg/L,28 days old cuture **F.** Roots emerging from microshoots on SRM having NAA+BAP(2.5+2.5)mg/Lon subculture; mark healthy shoot (15 days old subculture) **G.** Plastic pots containing acclimatized *in vitro* grown plantlet, 60 days old

2.5mg/L each of BAP and NAA was about 82-85%. Proliferated shoots were healthy, green and attained a height upto 7cm in about 6 weeks (Fig. 2D) and shoots were morphologically similar to mother plants.

Root induction:

Rooting of microshoots (4-6cm) obtained from TRS and RSL explants, was achieved subsequent to caulogenesis on the same shoot regeneration medium (SRM, Table 2) after 25 days of inoculation, the best rooting percentage (92%) was recorded on medium having BAP+NAA (2.5+2.5)mg/L and similar finding was also noted by Hashemabadi and Kaviani (2010). Excellent growth of roots as well as shoots was encountered on the same hormonal composition of SRM (Table 2, Fig. 2F) on subculture. In the present study, rooting of microshoots was strongly affected by the concentration and type of growth regulators, plant genotype and culture conditions, as also supported by Bhojwani and Razdan (1992) and Hashemabadi and Kaviani (2010). Promising number of roots also emerged from shoots when the medium was supplemented with combination of hormones; IBA+BAP, NAA+IAA+BAP (Table 2). Profuse root fibres (8.15±0.16/culture) developed directly from LS explants besides callusing on MS medium

supplemented with NAA+IAA (2+1)mg/L (Table 2, Fig. 2E), no shoot regeneration was recorded in culture and entire root fibres turned into callus on the same medium after 23 days of inoculation. Root derived callus can further be exploited for regeneration of callus mediated shoots as well as extraction of active constituents.

In vitro grown plantlets (Figs. 2D,F) were successfully transferred into small plastic pots containing sterilized soil mixture (soil+sand+vermiculite, 1:1:1, Fig. 2G) and left for 10 days in culture room and finally transferred to green house for acclimatization. In these conditions, about 85- 90% plantlets survived and plantlets were morphologically identical to mother plants.

CONCLUSION

It can be concluded that an efficient protocol for *in vitro* cloning of *Aloe vera* using rhizomatous stem (RSL) can be established to fulfil the growing demand and also help in conservation and sustainable growth of this highly medicinal plant. Protocol for callus mediated shoot regeneration can be employed by using leaf segment (LS) as explant.

Author is thankful to UGC, New Delhi for providing research fellowship and Head, University Department of Botany, B R A Bihar University for providing necessary facilities for this experiment.

REFERENCES

Abdi G, Hedayat M, and Modarresi M 2013 *In vitro* micropropagation of *Aloe vera* – Impacts of plant growth regulators media and type of explants. *J Biol Environ Sci* **7 (9)** 19-24.

Adelberg J and Naylor-Adelberg J 2012 Effects of cytokinin on multiplication and rooting of *Aloe barbadensis* during micropropagation on agar and liquid medium. *J Med Active Plants* **1** 1–5.

Aggarwal D and Barna KS 2004 Tissue culture propagation of elite plant of *Aloe vera* L. *J Plant Biochem Biotech*. **13** 77- 79.

Ahmed S, Kabir AH, Ahmed MB, Razvy MA and Ganesan S 2007 Development of rapid micropropagation method of *Aloe vera* L. *Sjemenarstvo* **24 (2)** 121-128.

Ahuja PS 1994 Role of plant tissue culture in the improvement of medicinal and aromatic plants. In: *Proceedings of xvii Plant Tissue Culture Conference*, BHU India eds V S Jaiswal and Uma Jaiswal pp-1.

Bhandari AK, Negi JS, Bisht VK and Bharti MK 2010 *In vitro* propagation of *Aloe vera*- A plant with medicinal properties. *Nature and Science* **8** 8.

Bhojwani SS and Razdan MK 1992 *Plant Tissue Culture: Theory and Practice*. Elsevier Amsterdam London, New York Tokyo.

Budhiana E 2001 Micropropagation of *Aloe vera* L through shoot multiplication /Top/ S1-final project / jbptitbbi-s1-2004-estibudhia-93.

Campestrini LH, Kuhnen SP, Lemos MM, Bach DB, Dias DB and Maraschin M 2006 Cloning protocol of *Aloe vera* L as a study-case for "Tailor-Made" biotechnology to small farmers. *J Technol Manag Innov* **5** 76-79.

Chopra RN, Naya SL and Chopra IC 1956 *Glossary of Indian medicinal plants* (New Delhi India:CSIR publication).

Davis RH, DiDonato JJ, Hartman GM and Haas RC 1994 Anti-inflammatory and wound healing activity of a growth substance in *Aloe vera. Journal of the American Pediatric Medical Association* **84** 77

Debiasi C, Silvia C and Pescadore R 2007 Micropropagation of *Aloe vera* L. *Rev. Bras. Pl. Med. Botucatu* **9** 36-43.

Deng XH, Chen WX, He QM and Zhu LF 1999 Utilization and resources protection of *Aloe vera* L var *chinensis* (Haw) Berger. *J Plant Resour. Environ* **8** 26–30.

Dwivedi NK, Indiradevi A, Asha KI. Nair RA and Suma A 2014 A protocol for micropropagation of *Aloe vera* L. (Indian *Aloe*)- a miracle plant. *Research in Biotechnology* **5(1)** 01-05.

Gantait S, Sinniah U and Das P 2014 *Aloe vera*:

a review update on advancement of *in vitro* culture. *J. Acta agriculture Scandinvica, section B- Soil and Plant Science*. **64** 1-12.

Groenewald EG, Koeleman A and Wessels DCJ 1975 Callus formation and plant regeneration from seed tissue of *Aloe pretoriensis. Z Pflanzenphysiol* **75** 270-272.

Haller JS 1990 A drug for all seasons: medical and pharmacological history of *Aloe*. *Bull NY Acad Med* **66** 647-659.

Hashemabadi D and Kaviani B 2008 Rapid micropropagation of *Aloe vera* L. Via shoot multilication. *African J Biotech* **7(12)**1899-1902.

Hashemabadi D and Kaviani B 2010 *In vitro* proliferation of an important medicinal plant *Aloe*-A method for rapid production. *Australian J of crop science* **4(4)** 216-222

Heggers JP, Kucukcelebi A, Listengarten D, Stabenau JKoF, Broemeling LD Robson MC and Winters WD 1996 Beneficial effect of *Aloe* on wound healing in an excisional wound model. *Journal of Alternative and Complementary Medicine* **2** 271–277.

Heble MR, Benjamin BD, Roja PC and Chadha MS 1998 Plant tissue culture for secondary products: potential, priorities and application; In *Proceedings of The National Seminar on Plant Tissue Culture,* pp111-126 (eds SN Tata and AM Wadhwani) New Delhi India: ICAR publication.

Keijzer CJ and Cresti M 1987 A comparison of anther tissue development in male sterile *Aloe vera* L and male fertile *Aloe ciliaris*. *Ann Botany* **59** 533 -542.

Klein AD and Penneys NS 1988 *Aloe vera. J Am Acad Dermatol* **18** 714-720.

Khanam N and Sharma GK 2014 Rapid *in vitro* propagation of *Aloe vera* L with some growth regulators using lateral shoots as explants. *World journal of Pharma and Pharmaceutical Sciences* **3 (3)** 2005-2018.

Kumar M, Singh S and Singh S 2011 I*n vitro* morphogenesis of a medicinal plant –*Aloe vera* L. *Asian Journal of Plant Science and Research* **1(1)** 31-40.

Kumar A, Ahmad S and Naseem M 2010 *In vitro* plant regeneration from organ cultures of *Gmellina arborea Roxb. J Indian bot Soc* **89** (**1and 2)** 197-203.

Liu CH, Wang CH, Xu ZL and Wang Y 2007 Isolation chemical characterization and antioxidant activities of two polysaccharides from the gel and the skin of *Aloe barbadensis* Miller irrigated with seawater Process. *Biochem* **42** 961–970.

Meyer HJ and Staden JV 1991 Rapid *in vitro* culture of *Aloe barbadensis* Mill. *Plant Cell Tiss Org Cult* **26** 167-171.

Murashige T and Skoog F 1962 A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* **15** 473–479.

Natali I, Sanchez IC and Cavallini A 1990 *In vitro* culture of *Aloe barbadensis* Mill micropropagation from vegetative meristem. *Plant Cell Tiss Org Cult* **20** 41-47.

Naseem M and Jha KK 1997 Rapid clonal multiplication of *Cleome gynandra* DC. through tissue culture. *Phytomorphology* **47 (4)** 405-411.

Ramsay MM and Gratton Y 2000 Propagation of *Aloe polyphylla* Schonl Expillanus from callus derived from leaf tissue. *Bot. Gardens Micropropagation News* **2** 61- 63.

Roy SC and Sarkar A 1991 *In vitro* regeneration and micropropagation of *Aloe vera*. *Scientia Hort* **47** 107-114.

Saggoo M and Kaur R 2010 Studies in North Indian *Aloe vera*: callus induction and regeneration of plantlets. *Archives of Applied Science Research* **2 (2)** 241-245.

Sahu PK, Giri DD, Singh R, Pey P, Gupta S, Shrivastava AK, Kumar A, and pey KD 2013 Therapeutic Medicinal Uses of *Aloe vera*: A Review. *Pharmacology and Pharmacy* **4** 599-610.

Singh B and Sood N 2009 Significance of explants preparation and sizing in *Aloe vera* L-A highly efficient method for *in vitro* multiple shoot induction. *Scientia Horticulturae* **122** 146-151.

Vogler BK and Ernst E 1999 *Aloe vera*: a systematic review of its clinical effectiveness. *British Journal of General Practice* **49** 823-828.

Waller GR, Mangiafiaco S and Ritchey CR 1978 A chemical investigation of *Aloe barbadensis* Miller In: *Proceedings of the Oklahoma Academy of Science* **58** 69–76.

Yao H, Chen Y, Li S, Huang L, Chen W and Lin X 2009 Promotion proliferation effect of polysaccharide from *Aloe barbadensis* Miller on human fibroblasts *in vitro*. *International Journal of Biological Macromolecules* **45(2)** 152-156.

Zakia S 2013 Standardization of micropropgation techniques for *Aloe vera*: A pharmaceutically important plant. *Pak J Pharm Sci* **26** 1083-1087.