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High Frequency Regeneration Protocol of *Aloe Vera* L.

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ABSTRACT: *Guar patha (Aloe vera L.) is a succulent plant which is known for anormous medicinal properties. It propagates vegetatively in its natural state. However, propagation rate is very slow because a single plant can produce only three to four lateral shoots in a season. An efficient micropropagation protocol has been developed using shoot apical meristem as explants of Aloe vera L. The protocol involves induction, multiplication and in vitro rooting of the regenerated shoots and their acclimation under ex vitro conditions. High frequency of regenerated shoot buds from explants was achived on Murashige and Skoog (MS) medium supplemented with 3 mg/l BAP and 1 mg/l IAA. All cultures showed shoot regeneration in this medium with 27.7±1.08 shoots/explants. Cytokinine free medium was found competent for further elongation of regenerated shoots. Further elongation and 100% rooting was achived on 1 mg/l IAA. Rooted shoot were transferred to Pre acclimination chamber (PAC) filled with vermiculite, where they grew well and attained maturity. The plantlets were then successfully hardened. Cent per cent plants survived in the field conditions. It is recommended that this protocol can be used effectively for rapid multiplication of elite plants of Aloe vera L.*

Key words*: Aloe vera, BAP, IAA, Regeneration, PAC.*

Highlights

- *An efficient micropropagation protocol has been developed using shoot apical meristem as explants of* Aloe vera *L.*
- *High frequency of regenerated shoot buds from explants was achived on Murashige and Skoog (MS) medium supplemented with 3 mg/l BAP and 1 mg/l IAA.*
- *Elongation and 100% rooting was achived on 1 mg/l IAA.*
- *Cent per cent plants survived in the field conditions.*

INTRODUCTION

Aloe vera is a succulent plant indige-nous to Northern Africa and Mediterranean countries and has become naturalized almost in all parts of India (Klein *et al.* 1988). The plant has stiff gray-green lance-shaped leaves containing clear gel in a central mucilaginous pulp. *A. vera* has been used for several thousands of years in folk medicine in many cultures from ancient Egypt, Greece, and Rome to China and India (Kemper and Chiou 1999).

The genus Aloe has more than 500 species but only a few are medicinally important (Deng *et al.*, 1999). Among these, *Aloe vera* is the plant of greatest interest. Its leaves have been found to contain over 200 bioactive constituents (Waller *et al.*, 1978). *Aloe vera* contain different bioactive matherials such as saponins, anthraquinones, mucopolysaccharides, steroids, vitamins and glucomannans (Liu *et al.*, 2006, 2007).

A. vera propagates vegetatively in its natural state. However, propagation rate is very slow because a single plant can produce only three to four lateral shoots in a year. Moreover, the production of *Aloe* leaves is insufficient to meet the industry demand in India (Aggarwal and Barna 2004) and the pro-duction of cosmetics, foods and pharmaceuticals containing *A. vera* has experienced a slow increase due to limited availability of raw material with high quality (Campestrin *et al.* 2006). Therefore, there is a need for propagation methods; which can increase commercial production (Campestrini *et al.* 2006, Silva *et al.* 2007).

In this sense, the mass production of aloe plants through in vitro propagation is an alternative. Micropropagation of aloe offers several advantages

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over conventional vegetative propagation (Campestrini *et al.* 2006, Hosseini and Parsa 2007, Silva *et al.* 2007).

In vitro techniques using micropropagation and tissue culture offer a great possibility to overcome this problem. Micropropagation using stem and lateral shoot pieces of *A. vera* has already been proved successful (Natali *et al.* 1990; Roy and Sarkar 1991; Mayer and Staden 1991; Aggarwal and Barna 2004). However, source of explants, their sterilization procedure, media composition, culture conditions, phenolic browning of explants and media discoloration greatly affect shoot regen-eration from different genotypes of the same spe-cies. *A. vera* exudes lot of phenolic substances into the culture media which could decrease the survival of explants (Roy and Sarkar 1991).

The present investigation was launched, to maximize micropropagation rate of *A.vera*. by using different media, and develop a rapid, less expensive, efficient and easy method of micropropagation of *A. vera.*

MATERIALS AND METHODS

Source of Explants

Lateral shoots (suckers) of *A. vera* (one month old) were harvested from field plot, irrigated well during previous week. After removal of roots and bigger leaves, The explants were washed thoroughly with running tap water for about 1hour till all soil and other foreign materials washed off, then treated with 0.1% w/v solution of Bavistin for 30 min. Now leaving the youngest 1-2 primordial leaves, rest leaves were removed and 1-2 cm long explants each containing apical shoot and 1-2 primordia were then treated with freshly prepared 0.1% w/v aqueous solution of $HgCl_2$ for 9 min. Explants were thoroughly washed four five times with sterile double distilled water to remove any traces of the sterilant.Surface sterilized explants were inoculated on to the culture media supplemented with plant growth regulators (PGRs).

The basal medium consisted of MS salts (Murashige and Skoog 1962) supplemented with 3% sucrose and solidified with 8g/l agar. The pH of the medium was adjusted to 5.8 with 0.1N NaOH before autoclaving at 1210C for 20 min. 50ml of nutrient medium was dispensed into jam bottles. Explants devoid of contaminations were then incubated on the basal medium supplemented with different concentrations of BAP (1, 2, 3, 4,5 mg/l) alone or in combination with IAA and with 1g/l PVP for shoot amplification. Cluster of shoots amplified from initial lateral shoot explants were sub-cultured as it is without separation from the explants on the same regeneration media after one month from initial establishment stage. Shoots amplified from lateral shoot explants in shoot induction media.

Rooting of Micro Shoots

The shoot buds were transferred to shoot elongation and rooting medium supplemented with various concentrations of auxins (IAA, IBA, NAA) for further elongation and rooting.

Hardening of Regenerated Plant

Hardening was done in Pre-Acclimation chambers as described by Bhargava *et al.* (2003).Rooted shoots were transferred to Pre-Acclimation chambers (PAC). Each chamber consist of two halves of polystyrene culture vessels (Magenta box) joined together with a connector ring. Three holes of equal size, placed at an equal distance, were made on top of the upper half of the PAC to permit free flow but slow air exchange. Periodically water was poured in PACs. PACs were kept in a culture rooms maintained at 26±1^{o}C, 14h photo period. After 2 to 3 weeks acclimation in the culture room all the plants were shifted to green net shade.

RESULTS

The overall objective of the present study has been to develop a system for the mass propagation of *Aloe vera* by using different PGR contain. Manipulating the relative ratio of auxin to cytokinin has been successfully used in the current investigation. The response of the *Aloe vera* explants on different media combination of cytokinins and IAA were tested. Emergence of shoots from lateral shoot explants (suckers) was observed after 2 weeks of culture in all the hormone combinations tested except in the control medium devoid of growth hormones (Table 1 & Fig.1). Shoot buds initiated were light green to yellowish in colour and arisen either as single, or in clusters.

Callus formation did not occur at the cut end of explants. Treatment difference for the per-centage of cultures showing shoot proliferation and the number of shoots per explant was noticed within four weeks of culture. The maximum number (27.7±1.08) of shoot bud per ex-plant was observed in the presence of 3.0 mg/l BAP and 1mg/l IAA within four weeks of culture with 100% response. The response was Minimum with 1.0 mg/l BAP and 1 mg/l IAA with response of 75% (Table 1).

Table 1
Effect of BAP alone or in combination with IAA on shoot formation of *Aloe vera*

Hormone concentration (mg/L)		*% of Cultures showing shoot proliferation*	*No. of shoots per explants from initial culturing*
BAP	*IAA*		
0	0	0	0
1.0	0	65	8.7±0.82
2.0	0	80	16.1±0.61
3.0	0	85	18.6±0.52
4.0	0	80	11.7±0.43
5.0	0	70	9.7±0.58
1.0	1	75	12.9±0.43
2.0	1	90	22.3±0.52
3.0	1	100	27.7±1.08
4.0	1	85	18.2±0.35
5.0	1	75	16.0±0.33

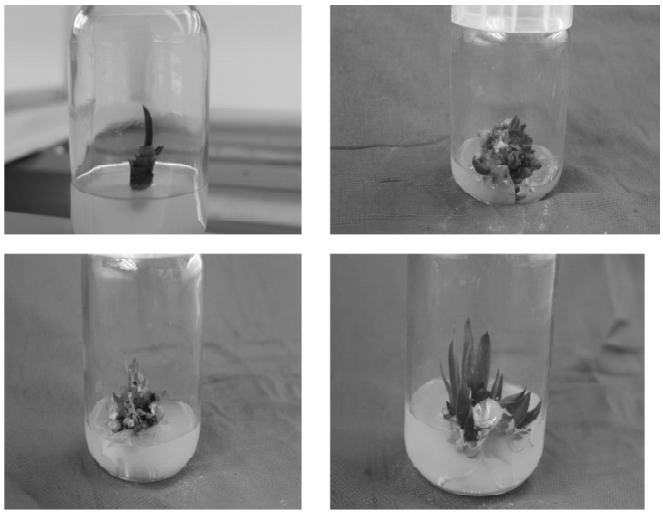

Figure 1: Different Steps of Regeneration of *Aloe Vera* L.

Table 2
Effect of auxins on root induction from *in vitro* raised shoots of *Aloe vera*

IAA (mg/L)	*IBA (mg/L)*	*NAA (mg/L)*	*Shoots*	*Plants with roots*	*Response %*
0.5			20	18	90
1.0			20	20	100
1.5			20	19	95
	0.5		20	14	70
	1.0		20	18	90
	1.5		20	12	60
		0.5	20	11	55
		1.0	20	14	70
		1.5	20	13	65

In media containing BAP alone the maximum number (18.6±0.52) of shoot bud per ex-plant was observed in the presence of 3.0 mg/l with 75% explants were responded. The number of shoot buds initiated were quite large and difficult to count however, number of shoots elongated to develop into normal plantlet were comparable among various levels of BAP.

Significant variations were observed in number of shoots produced per explants, with highest value at 3 mgL^{-1} BAP level. However, the frequency of shoots produced per explants varied from 8.7 ±0.82 (1 mgL-1 BAP) to 27.7±1.08 (3 mgL^{-1} BAP and 1 mgL^{-1} IAA). The differences in number of shoots produced per explants were mainly due to variation in number of responding explants at various levels of BAP.

Regenerated shoot were separated and transferred to MS medium supplemented with various concentrations of IAA, IBA or NAA. Rhizogenesis occurred with all these auxins but the response was significantly variable. The best rooting and elongation of the regenerated shoot buds (Fig. 1) was achieved on medium containing 1 mgL^{-1} IAA(100%) followed by 1.5 mgL^{-1} IAA (95%). On medium containing 1 mgL^{-1} IBA, 90% shoots were rooted whereas, 55- 70% shoots were rooted when transferred to medium supplemented with different concentrations of NAA.

DISCUSSION

For shoot proliferation, generally the growth regulators like cytokinins influence the process seriously. Variety of cytokinins (Kinetin, BAP, 2-ip and zeatin) has been used in micropropagation of aloe by some researchers. Wider survey of the existing literature suggests that BAP is the most reliable and useful cytokinin for shooting in higher plants. Many workers succeeded in their attempts for shoot proliferation by using BAP. Abrie and Staden (2001) Chaudhuri, Mukandhan (2001) and Aggarwal and Barna, (2004) also reported use of BAP in shoot proliferation of Aloe polyphylla and *A.vera* respectively. In agreement with these concepts in the present study also, shoot proliferation occurred only in the presence of cytokinin with particular reference to BA. However, it is in contrast to earlier reports in *Aloe vera* by Meyer and Staden (1991) and Natali *et. al.* (1990). Where in they reported that better proliferation occurred on medium containing Kn instead of BAP. It may be due to the genotypic variation.

Other studies indicate that BAP is more efficient than Kin for shoot proliferation in A. vera (Velcheva

et al., 2005; Debiasi *et al.,* 2007). According to the literature, BAP is better than other cytokinins for shoot initiation and proliferation. Velchera *et al.* (2005) concluded that efficient shoot initiation was observed in media supplemented with BAP.In our experiment the best result was observed on media supplemented with BAP in combination of IAA. Debiasi *et al.* (2007) also reported the best multiplication on medium containing BAP and IAA.

A maximum number of shoots per explant was achieved in MS medium with and 3 mg/l BAP and 1 mg/l IAA (Table 1). Other previous reports on the micropropagation of *Aloe vera* using more than one type of media for initiation and multiplication are available. In the present study a simple two step protocol was established using MS with BAP and IAA for shoot initiation, BAP for multiplication and IAA for rooting in *Aloe vera*. This protocol could be used for the massive in vitro production of the plantlets of the *Aloe vera*.

REFRENCES

Abrie A. L., van Staden J. (2001), Micropropagation of the endangered *Aloe polyphylla*. Plant Growth Regulation **33**: 19-23.

Aggarwal D., Barna K. S. (2004), Tissue culture propagation of Elite plant of *Aloe vera* Linn. *J Plant Biochem Biotech* **13**:77–79

Campestrini, L. H., Kuhnen, S., Lemos, P. M. M. Bach, D. B., Dias, P. F., 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.* **1**(5): 76-79.

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

Debiasi C., Silva C. G., Pescador R. (2007), Micropropagation of *Aloe vera* L. Rev Bras PIant Med Botucatu **9**: 36-43.

Deng X. H., Chen W. X., He Q. M., Zhu L. F. (1999), Utilization and resources protection of *Aloe vera* L. var. chinensis (Haw.) Berger. *J. Plant Resour Environ* **8**:26–30.

Hosseini R., Parsa M. (2007), Micropropagation of Aloe vera L. grown in South Iran. *Pak J Biol Sci* **10**: 1134–1137.

Kemper K. J. and Chiou V. (1999), *Aloe vera*: The Longwood Herbal Task Force and The Center for Holistic Pediatric Education and Research. **1**-29 pp.

Klein A. D., Penneys N. S. (1988), *Aloe vera.* J. Am. Acad. Dermatol. **18**: 714-720.

Liu C., Leung MYK., Koon JCM., Zhu LF., Hui YZ., Yu B., Fung KP. (2006), Macrophage activation by polysaccharide biological response modifier isolated from *Aloe vera* L. var. chinensis (Haw.). Berg Int Immunopharm **6**: 1634–1641.

Liu C. H., Wang C. H., Xu Z. L., 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 H. J., van staden J. (1991), Rapid in vitro propagation of *Aloe barbadensis* Mill. Plant Cell, Tissue and Organ Culture **26**: 167-171.

Murashige T. and Skoog F. (1962), A revised medium for rapid growth and bioassay with tobacco tis-sue cultures. *Physiol. Plant.* **15**: 473-497.

Natali L., Sanchez I. C., Cavallini A. (1990), *In vitro* culture of *Aloe barbadensis* Mill. Micropropa-gation from vegetative meristems. Plant Cell, Tissue and Organ culture, **20**: 71-74.

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

Silva C. G., Debiasi C., Pescador R. (2007), Enraizamiento in vitro e aclimatização de mudas micropropagadas de *Aloe vera* L. Rev. Bras. Pl. Med. **9**:29-35.

Velcheva M., Faltin Z., Vardi A., Eshdat Y., Peral A. (2005), Regeneration of *Aloe arborescens* via organogenesis from young inflorescences. *Plant Cell Tissue Organ Cult* **83**: 293-301.

Waller G. R., Mangiafiaco S., Ritchey C. R. (1978), A chemical investigation of *Aloe barbadensis* Miller. In: Proceedings of the Oklahoma Academy of Science **58**: 69–76.