Microshoot Formation from Shoot Tip of *Aloe vera* (L.) Under *In Vitro* Conditions

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Abstract

Aloe (*Aloe vera* L.) is an important medicinal herb which is used in drug and cosmetic industry. It is vegetatively propagated but propagation rate is slow to obtain high quality planting materials for commercial cultivation. As a result it is necessary to use *in vitro* propagation method for a large scale plant production. Therefore this study was performed to find out suitable explants for shoot induction of Aloe under *in vitro* conditions. The explants were disinfected by 20% of Clorox for 30 min. Subsequently two types of explants namely shoot tips (1.5 cm long and 0.5 cm width) and leaf bases (0.5 x 0.5cm^2) were excised from two months old plants and shoot tips were vertically dissected into four quarters. Sterilized explants were cultured on MS medium containing BAP (0, 1 and 2 mg/l) in combination with NAA (0.5 mg/l) to select suitable explants for initial culture establishment. Among these two explants, shoot tips gave the quickest response for initial shoot bud formation with the morphogenic response of 47.2% in the MS medium supplemented with 2 mg/l BAP along with 0.5 mg/l NAA. Further, shoot tips from two months and four months old plants were evaluated to select suitable mother plant for better culture establishment. Explants were placed on MS medium with 2 mg/l BAP and 0.5 mg/l NAA. The result revealed that shoot tips excised from two months old plants showed high morphogenic response within two weeks of culture.

Keywords: Aloe, In vitro culture, Shoot tips, Micro shoots

INTRODUCTION

Aloe vera (L.) is an important medicinal herb belonging to the family Liliaceae. It is originated from Africa, includes at least 300 wild species and over 200 bred varieties (Zheng *et al.*, 2005). It is widely distributed in the world and characterized by stem less, large, thick and fleshy leaves (Steenkamp and Stewart, 2007). Pharmaceutical and cosmetic industry has great demand in *Aloe vera*. The use of aloe in therapeutic is reported by several scientists (Cera *et al.*, 1980; Afzal *et al.*, 1991). A gel in the leaves makes an excellent treatment for wounds, burns and other skin disorders, placing a protective coat over the affected area, speeding up the rate of healing and reducing the risk of infection (Anshoo *et al.*, 2005). The plant is widely used in skin care, cosmetics and as nutraceuticals (Gordon and David, 2001). It is used in ayurvedic formulations as appetite-stimulant, purgative, emmenogogue and antihelminthic, for treating cough, colds, piles, debility, dyspnoea, asthma and jaundice (Joseph and Justin, 2010). *Aloe vera* is primarily propagated by means of axillary shoots and sexual propagation by seeds is ineffective. The presence of male sterility is also a barrier in rapid propagation (Natali *et al.*, 1990). Generally 3 to 4 lateral shoots per plant per year are produced in conventional systems. Due to the importance of this plant for the pharmacological, medical and cosmetic industry, the vegetative propagation of aloe has been insufficient to meet the increasing demand (Hosseini and Parsa, 2007). In this view, the mass propagation of aloe plants through *in vitro* propagation is an alternative method. The objective of the study was to select suitable explants for micro shoot formation of Aloe under *in vitro* conditions.

MATERIALS AND METHOD

This experiment was conducted at the Tissue Culture Laboratory at the Eastern University of Sri Lanka in 2013. Mother plants namely two months and four months old plants were collected from a net house and used in this experiment. Shoot tips were excised from both types of mother plants and dissected vertically into four quarters. Segments of leaf base were also excised from the two months old plants.

Sterilization of explants

Shoot tips (1.5 cm long and 0.5 cm width) and segments of leaf base (0.5 x 0.5cm^2) explants excised from two months and four months old plants as mentioned above were washed thoroughly by distilled water and then dipped in 70% ethanol for 30 sec. Thereafter they were disinfected by 20% of clorox (5.25% active ingredients) with 2-3 drops of tween 20 for 30 min. Subsequently they were washed 3-4 times with sterile distilled water to make the material free from sodium hypochlorite. After these treatments, the explants were taken inside the laminar hood for culture the explants.

Inoculation of explants

The sterilized shoot tip and leaf base explants excised from two months old plants were inoculated on MS medium containing 0, 1, 2 mg/l BAP (6-benzyl-amino-purine) and 0.5 ml/l NAA (α- naphthalene acetic acid) to select better explants for initial culture establishment. Further, shoot tips excised from two months and four months old plants were placed on MS medium with 2 mg/l BAP in combination with 0.5 mg/l NAA to choose the suitable mother plants for micro shoot formation.

Incubation of explants

Culture bottles containing four explants were labeled and sealed with parafilm strips. All explants were maintained under *in vitro* culture conditions. Observation was made at regular intervals. This experiment was repeated twice. The collected data were analyzed statistically using SAS package.

RESULTS AND DISCUSSION

Higher survival percentage of the cultures was observed in this experiment and contamination was less than 5%. It is due to the better sterilization of explants and the fungal resistant property of *Aloe vera*. In this study, explants were immersed in 20% of Clorox (sodium hypochlorite, 5.25% active ingredient) for 30 min for sterilization and shoot tips were dissected into 4 quarters instead of using entire shoot tips. This size reduction caused lower exposure of the explants' surface for microorganism. This may be one of the reasons for the less contamination. This was supported by Dey *et al.* (1997) as smaller the explants, smaller the risk of contamination. Browning of explants was observed during the incubation of cultures. Browning is usually attributed to the production of phenolic compounds released from the cut surfaces of the explants. Bhat and Chandel (1991) reported that phenolic compounds include polyphenols and tannins and they may not only prevent explants development, but also lead to death of the explants. In this study, ascorbic acid at 1 ml/l was incorporated into culture media. Due to the antioxidant property of ascorbic acid, the phenolic browning was reduced to some extent. To eliminate the blacking and browning, some antioxidants such as ascorbic acid, PVP and citric acid have already been used (Liao *et al.*, 2004; Minas, 2007).

The performance of two different types of explants (shoot tips and leaf base) was investigated in this experiment. Among these two explants, shoot tips gave the quickest response for initial shoot bud formation within two weeks of culture. The maximum response of shoot tips can be due to the fact that shoot tip has meristamatic region where cell division and differentiation

occurs (Hartmann *et al.*, 2002). Shoot tip explants cultured on MS media containing 1 mg/l and 2 mg/l BAP in combination with 0.5 mg/l NAA showed better responses within 6 days after culturing. During this period, the colour of the explants changed to dark green and little swelling was observed. 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.

The data revealed that there was a significant different ($P<0.01$) between the shoot tip explants cultured on 1 mg/l BAP and 2 mg/l BAP in combination with 0.5 mg/l NAA in terms of percentage of explants showing bud formation (Table 1). The shoot tips exhibited the morphogenic response of 47.2% in the MS medium supplemented with 2 mg/l BAP along with 0.5 mg/l NAA. The successful establishment of cultures with shoot tips has been reported by several workers in Aloe as well as many other species. In most previous research studies (Chaudhuri and Mukundan, 2001; Aggarwal and Barna, 2004; Baksha *et al.*, 2005; Hosseini and Parsa, 2007) using only shoot tips for micro propagation of *Aloe vera* has been reported.

Leaf bases fail to show any morphogenic response within two weeks of culture when supplemented on the same medium. Neither callus nor shoot buds were formed in the leaf base explants within two weeks of culture. The types of organs or tissue explants chosen affect the successful establishment of culture and their subsequent growth. Not all the tissues or organs of a plant are equally capable of exhibiting morphogenesis (Hartmann *et al.*, 1997). The differences in response among the different explants might be due to the differences in the physiological state of the explants as reported by Sreelatha *et al.* (1998). Hosseini and Parsa (2007) used leaf bases of young seedlings in their studies. Monge *et al.* (2008) used leaf base as explants for the induction of somatic embryos.

The media containing 2 mg/l BAP in combination with 0.5 mg/l NAA showed better response in terms of shoot bud production (Plate 1) (Table 1). Cytokinin breaks apical dominance thus; several shoots could proliferate from a preexisting initial shoot meristem or bud of the explants. Chaudhuri and Mukandan (2001) reported that in *Aloe vera* formation of multiple shoots *in vitro* was a function of cytokinin and auxin concentrations. The explants which were cultured on medium devoid of any phytohormone, failed to produce induction signs. The present study implies that, for shoot proliferation, generally the growth regulators like Auxin and Cytokinin influence the process seriously. Some researchers have indicated that the presence of both auxin and cytokinin is necessary for shoot proliferation (Rout *et al.*, 2001; Velcheva *et al.*, 2005).

The process of tissue culture technique begins from the selection of mother plants having the desired characteristics. The explants excised from the two months old (young) plants showed higher morphogenic response within two weeks of culture. This may be due to the physiological status and the age of mother plants where explants were taken. The explants excised from young plants, being juvenile may contain a higher proportion of actively dividing cells As a result it exhibited quick morphogenic response within two weeks of culture in the presence of the exogenous and endogenous hormonal activity. Meanwhile, the explants excised from the four months old plants had no response within two weeks. Growth of any tissues or organs whether in culture or as part of the intact plant, is ultimately influenced by physiological conditions within the tissues (Khanna, 1999). Generally, immature organs and meristematic tissues which contain undifferentiated cells are more suitable for plant morphogenesis (Hoque and Mansfield, 2004).

CONCLUSION

The results revealed that the suitable type of explants for rapid morphogenic response of aloe was shoot tips which exhibited the morphogenic response of 47.2% in the MS medium supplemented with 2 mg/l BAP and 0.5 mg/l NAA. The explants derived from two months old mother plants responded well rather than those derived from four months old plants. It showed that the physiological stage of the mother plant is important in the successful *in vitro* propagation of *Aloe vera*.

REFERENCES

Afzal M, Ali M, Hassan RAH, Sweedan N and Dhami MSI (1991) Identification of some prostanoids in *Aloe vera* extracts. Planta Medica *57:* 38-40.

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 micro propagation method of *Aloe vera* (L.). Seed Science Journal 24: 121-128.

Anshoo GS, Singh S, Kulkarni A, Pant S and Vijayaraghavan R (2005) Protective effect of *Aloe vera* L. gel against sulphur mustard-induced systemic toxicity and skin lesions. Indian Journal of Pharmacology 6: 23-29.

Baksha R, Jahan MAA, Khatun R and Munshi JL (2005) Micro propagation of *Aloe barbadensis Mill* through *in vitro* culture of shoot tip explants. Plant Tissue Cult Biotech 15: 121–126.

Bhat SR and Chandel KPS (1991) A novel technique to overcome browning in tissue culture. Plant Cell Report 10: 358-361.

Cavallini A, Natali L, Cionini C, Sessoli O and Sanchez IC (1993) *In vitro* culture of *Aloe Barbadensis Mill.*: Quantitative DNA variation in regenerated plants. Plant Science 91: 223-224.

Cera LM, Heggers J, Robson MC, Hafstrom WJ (1980) The therapeutic efficacy of *Aloe vera* cream in thermal injuries: Two case reports. Journal of the American Animal Hospital Association 16:768-772.

Chaudhuri S and Mukundan U (2001) *Aloe Vera* (L.) Micro propagation and Characterization of its gel. Phytomorphology 51(2): 155-157.

Dey PM and Harborne JB (1997. Plant biochemistry. (Eds. Dey PM and Harborne JB) 2nd Edition. Academic Press, UK. 523p.

Facciola S, (1990) Cornucopia - A Source Book of Edible Plants. Kampong Publications. ISBN: 0-9628087-0-9.

Garro-Monge G. Gatica-Arias AM and Valdez-Melara M (2008) Somatic embryogenesis, plant regeneration and acemannan detection in aloe (*Aloe barbadensis Mill.*). Agronomía Costarricense 32: 41–52.

Gordon MC and David JN (2001) Natural product drug discovery in the next millennium. Pharm Biol 39: 8-17.

Hartamann HF, Kester DE, Dauies FDJR and Geneve RL (1997) Plant Propagation- Principles and Practices, 6th Ed. Prentice Hall of India Private Ltd., New Delhi. Pp. 549-611.

Hartmann HF, Kester DE, Dauies FD and Geneve RL (2002) Plant Propagation- Principles and Practices, Prentice hall of India Ltd., New Delhi. 549-675.

Hoque MD and Mansfield JW (2004) Effect of genotype and explant age on callus induction and subsequent plant regeneration from root derived callus of Indica rice genotypes. Plant Cell Tissue and Organ Culture 78: 217-223.

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

Joseph B and Justin Raj S (2010) Pharmacognostic and phytochemical properties of *Aloe vera Linn.* An overview. Int J of Pharma Sci Review and Res 2: 106-110.

Khanna VK (1999) Plant Tissue Culture Practice, Kalyani Publishers, New Delhi.

Liao Z, Chen M, Tan F, Sun X and Tang K (2004) Micro propagation of endangered Chinese aloe. Plant cell Tiss Org Cult 76: 83-86.

Mayer HJ and Staden JV (1991). Rapid *in vitro* propagation of *Aloe barbadensis Mill.* Plant Cell Tissue and Organ Culture 26: 167-171.

Minas GJ (2007) A rapid protocol for *in vitro* micro propagation of three types of gardenia (*Gardenia* spp. Ellis) for quality produce flowering pot plants. Acta Hort 755: 81-86.

Natali L, Sanchez IC and Cavallini A (1990) *In vitro* culture of *Aloe barbadensis Mill* micro propagation from vegetative meristems. Plant Cell Tissue Organ Cult 20: 71–74.
Roy SC and Sarkar A (1991) *In vitro* regeneration and micro propagation of *Aloe vera* (L.). Sci Hortic 47: 107–113.
Sreelatha U, Nair RS and Rajmohan K (1998) Factors affecting somatic organogenesis from leaf explants of Anthurium species. *J Ornamental Hort* 1: 48-54.
Steenkamp V and Stewart MJ (2007) Medicinal applications and toxicological activities of Aloe products. Pharmaceut Biol 45(5): 411-420.
Velcheva M, Faltin Z, Vandi A, Eshdat Y and Perl A (2005) Regeneration of *Aloe arborescens* via organogenesis from young inflorescences. Plant Cell Tiss Org Cult 83: 293-301.
Zheng M, Sh YUX, Li Y, Wu H and Zhang SZ (2005) Karyotype analysis of species and 2 varieties in *Aloe (L.)*. J Wuhan Bot Res 23(6): 535-540.

Figure

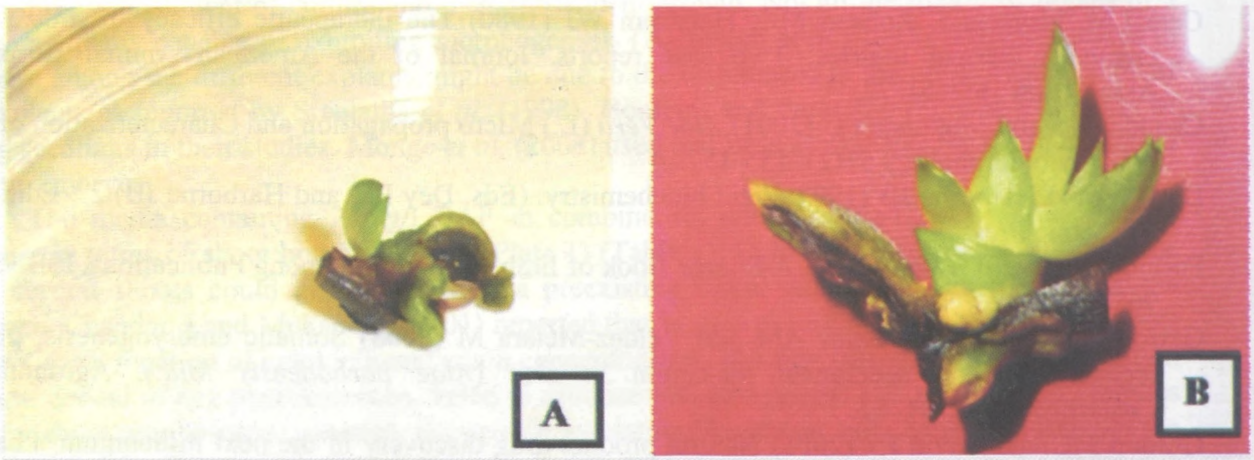

Plate 1: Micro shoot formation on explants cultured on MS medium containing 1 mg/l (A) and 2 mg/l (B) BAP in combination with 0.5 mg/l NAA after 4 weeks of culture (x3).

Table

Table 1: Effect of BAP on micro shoot formation in *Aloe vera* (L.) after 4 weeks of culture

Explant type	Phytohormone (mg/l)	Explants showing shoot bud initiation (%)	Numbers of microshoots
shoot tip	Hormone-free (Control)	0	0
	1 BAP + 0.5 BAP	20.6 ± 0.5	1.3 ± 0.33
	2 BAP + 0.5 BAP	47.2 ± 1.0	1.7 ± 0.33
Leaf base	Hormone-free (Control)	0	0
	1 BAP + 0.5 BAP	0	0
	2 BAP + 0.5 BAP	0	0
	t test	P<0.01	P>0.05

Data based on 36 explants