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A protocol for micropropagation of *Aloe vera* L. (Indian Aloe) – a miracle plant

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Emerging axillary shoots of *Aloe vera* (Indian Aloe) produced new plants and roots simultaneously when cultured in MS medium supplemented with BAP 1.5 mg l^{-1}. Each explant produced on average 14 shoots and roots simultaneously within 8 weeks. Explant multiplication could be continued even after a year by transferring each divided shoot explant to the same medium. Regenerated plantlets could be successfully transferred to the soil where they grew well within 4-6 weeks with 83% survival.

Key words: *Aloe vera*, axillary shoot, micropropagation, protocol

Aloe vera (Indian Aloe) is an important medicinal and miracle plant that belongs to the family Liliaceae. The plant prefers sunny weather, requires well-drained soil and can grow in nutritionally poor soil. Pharmaceutical and cosmetic industry has great demand for *A. vera*. Its therapeutic use was reported earlier by several scientists (Cera *et al*., 1980; Afzal *et al*., 1991; Davis *et al*., 1998). The gel in the leaves provide 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. There is a lack of production of Aloe leaf to meet the industry demand and so it is necessary to undertake large scale cultivation of Aloe (Aggarwal & Barna, 2004). Poor natural propagation by means of axillary shoots and the presence of male sterility are the two major barriers in rapid propagation of *A. vera* (Natali, 1990). *In vitro* propagation offers a possibility to solve these problems. Several studies have reported rapid *in vitro* propagation of *A. vera* (Meyer Staden, 1991; Aggarwal & Barna 2004; Ahmed, 2007; Gantait, 2010; Mukesh Kumar 2011; Bhaksha *et al*., 2005). Scientists had obtained different results applying formulation of plant growth regulators with MS media. The objective of this investigation was to develop a rapid, less expensive, efficient and easy method of micropropagation of *A.vera*. We standardized new composition of growth regulators for rapid and efficient micropropagation of *A. vera* using young axillary shoot.

Materials and Methods

The explants of *A. vera* (IC281112, augmented from Jodhpur, Rajasthan) maintained in pots under green house condition at NBPGR Regional Station, Thrissur served as the explant source for the present *in vitro* plant regeneration experiments. Axillary shoots of the stored *A. vera* were used as experimental material. They were trimmed to 1-2 cm length and washed with 0.15% Bavistin for 5 minutes and wiped with ethyl alcohol (70%). They were surface sterilised with 0.1% mercuric chloride solution for 5 minutes followed by three rinses in sterile water. Outer scale leaves were removed asceptically and explants were inoculated in MS (Murashige & Skooge, 1962) medium with different concentration of BA. Cultures were maintained at 22 ± 2°C under 16/8 h photo

period, provided by cool white fluorescent tubes. *A. vera* was further cultured on this medium with various concentrations of BA (0.5 mg l^{-1} to 2.5 mg l^{-1}) and combination of BA and IBA (0.5mg l^{-1}) (Table 1) and Kinetin (1 mg l^{-1} and 2 mg l^{-1}). Data were recorded after 15, 30, 45 and 60 days for multiple shoot induction, number of leaves and rooting, respectively. Murashige and Skooge's (1962) medium containing sucrose 3% and agar 0.6% was used as basal medium. The cytokinin (0.5- 2.5 mg l^{-1}) was added to basal alone. The pH of the medium was adjusted to 5.75 by using 0.1 N NaOH or 0.1N HCl before autoclaving. After 8 weeks, the culture was developed to new explants. Proliferated plantlets with well developed leaves and root systems were maintained to hardening. Based on hardening, explants were transferred to plastic cups containing sterilised sand. Every day hardened materials were moistened with a very diluted MS broth. After 15 days, they were transferred to earthen pots containing soil and sand under green house conditions for 3-4 weeks for acclimatisation. The survival rate was 83% and the plants established well in 4-6 weeks of growth.

Results

Establishment of aseptic cultures were difficult, but once a healthy culture was established, there was no further contamination. Under given conditions and over a culture period of 60 days explants from all the treatments produced multiple explants and roots simultaneously. The rate of explants multiplication was significantly different according to the various concentration of cytokinins supplemented. Cytokinin level produced a significant response upon the number of explants formed per plant and also showed influence on production of leaf numbers and rooting. Treatment with BA 0.5 mg l^{-1} induced comparable number of shoots (Fig-1). Multiplication rate in the treatment with BAP at 1.0 mg l^{-1} was intermediate between treatments with 0.5 mg l^{-1} and 2.5 mg l^{-1} (Fig-2). The best explants multiplication with growth was observed at 1.5 mg l^{-1} where maximum of 14 explants could be observed per tube (Table-1, Fig-3). Treatment with 2 mg l^{-1} gave 7.4 shoots on an average (Fig-4). Average number of 5.4 shoots was obtained on MS media with 2.5 mg l^{-1} (Fig-5). No shoot enhancement was obtained on medium containing Kinetin (Fig-6) compared to MS medium (Fig-7). Explant cultures were thus obtained on this selected medium by regular subculturing at 6 weeks intervals. However, root growth and elongation could be enhanced by supplementing IBA (0.5) mg l^{-1} with MS medium (Fig-8). Plantlets were hardened after 8 weeks and transferred to potting soil for acclimatization (Fig-9, 10).

Fig-1

Fig-2

Fig-3


Fig-4


Fig-5

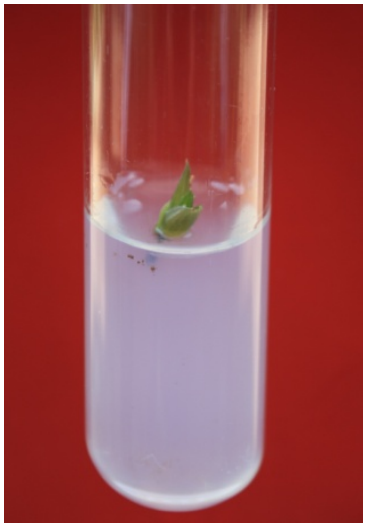
Fig-6


Fig-7


Fig-8


Fig-9


Fig-10

Fig.1. Multiple shoot induction (3.2) from the explant in MS medium with 0.5 mg l^{-1} BA;
2. Multiple shoot induction (4.2) from rhizome bud explant in MS medium with 1.0 mg l^{-1} BA;
3. Multiple shoot induction (14.0) from rhizome bud explant in MS medium with 1.5 mg l^{-1}BA;
4. Induction of 7.4 shoots in MS medium with 2.0 mg l^{-1} BA;
5. Multiple shoot induction (5.4) from rhizome bud explant in MS medium with 2.5 mg l^{-1} BA;
6. No shoot induction in MS medium with 1.0 mg $l^{-1}$and 2.0 mg l^{-1} Kinetin;
7. Average shoots (1.1) from MS basal medium;
8. Vigorous rooting of shoots in MS medium supplemented with 0.5 mg l^{-1}IBA;
9. Shoots transferred to plastic cups with sterile sand for hardening;
10. Hardened plants in pots in shade house.

Discussion

Growth regulators, mainly cytokinins are the most important factors affecting the shoot proliferation. A range of cytokinins BA, kinetin 2-ip, and seating has been used in micropropagation research work (Bhojwani & Razdan, 1992). A wider survey of existing literature suggests that BA is the most reliable and useful cytokinin. In the present study also, shoot proliferation occurred in the presence of cytokinin. Signs of shoot proliferation were showed after 10 days of culturing. Multiplication of shoot was best on MS medium with 1.5 mg l^{-1} (Table-1, Fig-1).

The percentage of shoot proliferation and number of shoots were 90 and 14, respectively. BA variations affecting shoot proliferation were also reported by Bhandari *et al*. (2010), Gantait *et al.* (2010) and Mangal Singh *et al.* (2009). Abrie and Staden (2001) and Chaudhuri and Mukundan (2001) had also reported the use of BA in shoot proliferation of *A. polyphylla* and *A. vera,* respectively. It was also reported that highest shoot proliferation in *A. vera* was found in MS medium containing BA and IBA (Aggarwal & Barna, 2004; Mukesh Kumar *et al.*, 2011 and Meyer & Staden, 1991). This is in contrast to earlier reports in *A. vera* by Natali *et al.* (1990), where better proliferation occurred on medium containing Kinetin instead of BA. Baksha *et al.* (2005) also reported that the enhancement of shoots was observed by using BA and NAA. Rooting response of microshoots was also reported with the use of growth regulators such as NAA and IBA in medium (Bhojwani & Razdan, 1992). In the present study, healthy rooting was observed in IBA (0.5 mg l^{-1}) medium. Healthy roots (number >10 and length >6 cm) were obtained in medium with IBA (0.5 mg l^{-1}) in 8 weeks of time. Highest root response in *A. vera* was reported in hormone free medium (Bhandari *et al*., 2010; Aggarwal & Barna, 2004). Highest shoot proliferation was also reported in MS medium containing BA 1 mg l^{-1} and IBA 0.2 mg l^{-1} (Mukesh Kumar *et al*., 2011) while highest percentage of root induction (80) was observed in MS medium supplemented with IBA (0.5 mg l^{-1}) (Table-1). Similar result was reported by Abrie and Staden (2001) in *A. vera.* Based on hardening, explants were transferred to plastic cups containing sterilised sand and every day moistened with 10 times diluted MS broth. After two weeks they were transferred to earthen pots containing soil and sand under green house conditions for 3-4 weeks for acclimatisation. The survival rate was 83% and the plants established well in 4-6 weeks of growth.

The present experiment demonstrated a very simple one step protocol for the rapid propagation of the medicinally important herb *A. vera*, which can be applied as a part of *in vitro* conservation of germplasm too.

Table 1: Effect of different concentrations of BA (0.5-2.5mg l^{-1}) and Kinetin in MS basal solid medium on shoot multiplication from the explants and rooting of the induced shoot buds in *Aloe vera*.

Conc. of BA & Kinetin (mg l^{-1})	Average No. of shoots/ explants	% of regeneration	Average No of leaves/explant	Rooting %
MS basal	1.1	50	3	40
MS+0.5	3.2	56	12	63
MS +1.0	4.2	62	10	80
MS +1.5	14.0	92	52	90
MS +2.0	7.4	60	28	60
MS+ 2.5	5.4	62	21	40
MS+kn(1mg l^{-1})	Nil			
MS+kn(2mg l^{-1})	Nil			

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