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In–Vitro Regeneration of Aloe Vera (*Aloe barbadensis* Mill)

Shashi Kiran[1], Alice Tirkey[1*], Zenu Jha[2] and S.S. Porte[3]

[1]Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya, College of Agriculture, Raipur- 492012, Chhattisgarh, India
[2]Department of Plant Molecular Biology and Biotechnology, Indira Gandhi Krishi Vishwavidyalaya, College of Agriculture, Raipur- 492012, Chhattisgarh, India
[3]Department of Soil Science, Indira Gandhi Krishi Vishwavidyalaya, College of Agriculture, Raipur- 492012, Chhattisgarh, India
**Corresponding author*

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A B S T R A C T

The research was conducted in commercial tissue culture laboratory, IGKV, Raipur, C.G. from the period of March 2015 to August 2017. Present investigation was conducted to assess the suitable explant for callusing using different treatment of auxin and cytokinin (NAA and BAP) in semi-solid Murashige and Skoog (MS) media. The results revealed that the meristem shoot found to be best for callusing. Among five different treatments MS medium with 2 mg/l BAP + 2mg/l NAA was found best for multiple shoot initiation for meristem shoot. For root induction MS medium with 1.0 mg/1 IBA + 500 mg/1 activated charcoal was found best with 96% per cent rooting and 5.42 mean number of roots. Further the regenerated plants were transferred to sterilized cocopit in poly house. After 1 week, plants were transferred to sterilized earthen pots containing a mixture of sand, FYM, soil (1:2:1) for acclimatization and transfer to the open field and 85 per cent plants were survived.

Introduction

Aloe vera (Indian Aloe) is an important medicinal and miracle plant that belongs to the family Liliaceae. 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. *Aloe vera* contains different bioactive materials such as saponins, anthraquinones, mucopolysaccharides, steroids, vitamins and glucomannans (Liu *et al.*, 2006, 2007). Due to the wide spectrum of application in human health, the products of *A. vera* have showed a strong demand in India as well as in international markets. In nature, *A. vera* is propagated through lateral buds, which is slow, expensive and low income practice (Aggarwal and Barna, 2004). 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. The flowers are hermaphrodite; plant prefers light (sunny weather), requires well-drained soil and can grow in nutritionally poor soil (Dwivedi *et al.*, 2014). Pharmaceutical and cosmetic industry has great demand in *A. vera*. Global Demand for Aloe vera extracts to Reach 60720 Tonnes in 2016 (Future

market in sight, 2016). Due to slow rate of natural growth, ever increasing demand for this "Potted Physician" cannot meet 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 (Kumari and Naseem, 2015). 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 and researchers.

The hormonal requirement for *in vitro* differentiation differs for different genotypes. The objective of this investigation was to assess the suitable explant for callus formation, different concentration and combination of phytohormones (Auxin and cytokinin) for shooting and rooting response, hardening and transfer of regenerated plants to the field for uniform harvesting of the aloe leaf.

Materials and Methods

Plant material sources

The three month old suckers of *aloe vera* were obtained from field collection of herbal garden of IGKV, Raipur (C.G).

Sterilization of explant

Contamination free culture is the first step in development of the regeneration protocol for any plant species. Initially all the excised plant tissue were washed thoroughly with 7% labolene and rinsed thoroughly with sterile double distilled water. All the explants were sterilized by using three different surface sterilization treatments (Table 1). For optimization of sterilization condition each treatment after final step in all explants *i.e.* apical shoot meristem, root tip, leaf tip and spine were excide from aloe vera plants in an air sterile chamber and inoculated in to shoot proliferation medium based on Murashige and Skoog medium. The MS media of Hi-media was prepared as stoke solution of 1L by weighing 4.41gm of MS media of Hi-media by adding 30 g of sucrose followed by 0.50g PVP. The pH was maintained between 5.75 and 5.80. The solution was heated and agar 8g was added in it after that the solution was poured in bottles up to 1cm.

Shoot proliferation

Three explants were cultured on MS nutrient medium supplemented with five different concentration of BAP Table 1. The explants showing shoot proliferation on basis of number of total shoots per explants and length of the longest shoots were considered as parameters. The data was recorded for shoot proliferation in suitable medium and further sub culturing of proliferated shoots.

Rooting of micro shoots

Newly formed shoots measuring 2-3 cm in length were excised individually from the parent explant and transferred to rooting media after 7 sub culture. For rooting four types of growth regulators MS (half) + 0.5 mg/l IBA + 500mg/l activated charcoal, MS (half) + 0.5 mg/l IBA, MS (half) + 0.5 mg/l IAA + 500mg/l activated charcoal, Basal MS (half) medium +500 mg/l activated charcoal were used in different concentration in addition to MS medium and observation was recorded for rooting response.

Acclimatization

Regenerated plantlets of 6-8 weeks old with well-developed roots were removed from culture vessels and washed thoroughly with the tap water to remove the Agar media. The roots were treated with 0.2% bavistin for 30

to 45 seconds and transferred to sterilized cocopit in green house with automatically controlled relative humidity, proper misting and irrigation and air exhaustion. After one week, plants were transferred to sterilized earthen pots containing a mixture of sand, FYM, soil at a ratio of 1:2:1. The potted plants were kept in the conventional net house for acclimatization before transfer to the open field. Plants were watered at two days interval.

Results and Discussion

Sterilization, explants response, shoot differentiation and rooting

Among the treatment given good response for surface sterilization of explants was seen in explants when dipped in 1.0% bavistin for 30 minutes followed by 0.1% $HgCl_2$ for 45 minutes followed by washing with double sterilized water followed by dipping in 2% NaOCl for 20 min. then dipping in 70% ethanol for 30 second. This sterilization procedure gave contamination free explants. The four explants *viz.*, apical shoot bud, meristem root tip, leaf tip and spine of 1.5 cm were subjected to the MS medium supplemented with different concentration of BAP + NAA for their response.

Apical shoot bud, leaf tip, spine dose not showed any response, whereas, apical shoot showed multiplied. Further these responsive explants, were used for the standardization of protocol. The size of explants at time of culture was found critical for shoot initiation. Similarly, Gupta *et al.*, (2014) studied various explants such as apical, nodal segments and leaves were tested for understanding *in-vitro* response in the nutrient media. Among the 3 explants, the apical bud explants gave the best results and were used for further experiments (Kumari *et al.*, 2015). MS medium with 2 mg/l BAP +.2mg/l NAA was found best for multiple shoot initiation for apical shoot bud. As they produced 5.0 mean number of shoots/explant with 92.85% shoot initiation response. The similar results were obtained by Kumari *et al.*, (2015), Ahmad *et al.*, (2007), Gupta *et al.*, (2014) and Mehta (2013). However, MS medium supplemented with 4 mg/l BAP + 0.2 mg/l NAA also produced good number of multiple shoot with 3.69 mean number of shoots/ explants.

Among other treatment except the treatments mentioned above using MS medium containing 0.2mg/l NAA in the combination unit 3, 5, 8 mg/l BAP were found to be less responsive for meristem shoot explants with mean 2.6, 2.45 and 1.66 mean number of shoots/explant respectively under study.

For root induction, it was observed that basal MS medium devoid of phytohormones also induce rooting but percentage of rooting and number of roots per shoot were observed. IBA was found best for induction of roots followed by IAA. IBA supplemented at 1.0 mg/l induced highest frequency of rooting. The best result was obtained by using MS medium with 1.0 mg/1 IBA +500 mg/1 activated charcoal.

This produced good roots with 96% per cent rooting and 5.42 mean number of roots, whereas, 2.26 mean number of roots/shoot were obtained with 89.06% per cent rooting on half strength MS medium with 1.0 mg/1 IAA + 500 mg/1 activated charcoal. When apical shoot buds were culture using MS medium with 1.0 mg/1 IBA +500 mg/1 activated charcoal development of root was observed on explant. This result coincides with the findings of Mehta (2013) and among the three types of auxins NAA was found to be the best for root induction. MS medium containing BA and NAA was found to be the best medium in Aloe micropropagation (Gupta *et al.*, 2014) (Tables 2 and 3).

Different stages of apical shoot bud

Table.1 Effect of different combination of hormones for shoot initiation

Treatment code	Hormone combination	Number of explant Inoculated	No. of culture contaminate	No. of explant response	No. of explants shoot initiated	No. of shoot initiated/plant Range	Mean	% Response
T_1	3mg/l BAP+0.2 NAA	16	1	3	12	1-4	2.6	80 %
T_2	5mg/l BAP+0.2 NAA	16	3	2	11	2-3	2.45	84.6%
T_3	8mg/l BAP+0.2 NAA	16	1	5	10	1-2	1.66	66.66%
T_4	2mg/l BAP+0.2 NAA	16	2	1	13	5-7	5.0	92.85%
T_5	4mg/l BAP+0.2 NAA	16	1	2	13	2-5	3.69	86.66%

Table.2 Effect of different treatments on rooting response

Treatment Rooting hormones	No. of culture inoculated	No. of plant contaminated	No. of plant responded	No. of plant root responded	Mean	Plant Response in %
MS (half) + 0.5 mg/l IBA + 500mg/l activated charcoal	64	1	1	62	5.42	96.87%
MS (half) + 0.5 mg/l IBA	64	10	12	40	1.71	74.07%
MS (half) + 0.5 mg/l IAA + 500mg/l activated charcoal	64	2	9	53	2.26	84.12%
Basal MS (half) medium (MSO)	64	2	9	50	1.95	80.64%
Basal MS (half) medium +500 mg/l activated charcoal	64	1	9	54	2.15	84.37%

Table.3 Effect of different treatments on rooting response

Media	Rooting percentage	Mean no. of roots/explant	Type of root response
Basal MS (half) medium +500 mg/l activated charcoal	92.18%	2.15	Long thick root, Tap root.
MS (half) + 0.5 mg/l IBA + 500mg/l activated charcoal	96.87%	5.42	Bunchy type, hairy type, fibrous root.
MS (half) + 0.5 mg/l IAA + 500mg/l activated charcoal	89.06%	2.26	Long few no. long thin type.
MS (half) + 0.5 mg/l IBA	87.5%	1.71	Bunchy type, thin root.

Acclimatization of plants and transfer of regenerates to field

Regenerated plantlets of 6-8 weeks old with well-developed roots were removed from culture vessels, washed thoroughly with the tap water to remove the Agar media.

The roots were treated with 0.2% bavistin for 30 to 45 seconds and transferred to sterilized cocopit in poly house. After 1 week, plants were transferred to sterilized earthen pots containing a mixture of sand, FYM, soil at a ratio of 1:2:1. The potted plants were kept in the net house for acclimatization before transfer to the open field. Plants were watered at two days interval. More than 85 per cent of the potted plants survived after one month of transfer and could be successfully transferred to the field. The same media as that of polybag were used by Ahmed *et.al.* (2007).

In the present investigation, using different concentrations and different explants, it has been concluded that *aloe vera* can be successfully regenerated using *in-vitro* techniques, if the proper concentrations of plant growth regulators and initial explant tissues is made. The high frequency plant regeneration system for *in vitro* production of aloe vera through meristem shoot tip explants has been developed during the present investigation. Various factors affecting the plant regeneration system has been examined and standardized and opens avenues for large-scale production of genetically stable planting materials. Commercial micropropagation of aloe vera could be successfully performed utilizing apical shoot bud as explants for uniform harvesting of the aloe vera.

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