

Review Paper:

A review on *in vitro* propagation of miraculous physician *Aloe vera* (L.)

Sivakumar P.^{1*}, Ashok kumar K.², Rajalakshmi K.¹, Sangeetha R.¹ and Muthumalai M.¹

1. Agricultural College and Research Institute, Tamil Nadu Agricultural University, Eachangkottai, Thanjavur-614 902, Tamil Nadu, INDIA

2. Cardamom Research Station, Kerala Agricultural University, Pampadumpara, Idukki, Kerala, INDIA

*sivakumar_p@tnau.ac.in

Abstract

Aloe vera (L.) is certainly a wonder herb with all its medicinal, pharmaceutical and cosmetic properties. Conventionally it is propagated by vegetative but obviously; it does not have as much immense potential as micropropagation. Therefore, investigation of most efficient *in vitro* propagation protocol for *Aloe vera* is essential. Several researchers successfully standardized the *in vitro* regeneration protocol in *Aloe vera* using different explants and different media compositions. This review article carefully reviewed the previous scientific information and gave the suitable pathway for successful development of high regeneration protocol in *Aloe vera*.

We suggested that the shoot tip is ideal explant for *Aloe vera* micropropagation rather than the other explants tried for regeneration. BAP and NAA are the two commonly used plant growth regulators for the successful micropropagation of *Aloe vera* with high multiplication rate. The most successful concentration of media is found to be MS medium containing 0.5 mg/l BAP and 0.5 mg/l NAA. The most common constraint faced is browning of explant which is found to be overcome by using polyvinylpyrrolidone (PVP) and proline.

Keywords: *Aloe vera*, *in vitro* propagation, 2,4-dichlorophenoxyacetic acid (2,4-D), Benzyl Adenine (BA), Indole Acetic Acid (IAA), Benzyl Amino Purine (BAP), Naphthalene Acetic Acid (NAA), Polyvinylpyrrolidone (PVP), Kinetin (KIN), Proline.

Introduction

Nowadays our modern era is turning to be an herbal era where everybody has a great concern for health and is conscious about their welfare. *Aloe vera* is a perennial succulent plant belonging to the family Liliaceae. They are exclusively asexually propagated crop where the fresh side branches are used as planting material. Usually a single plant may produce merely 2-3 side shoots per year which has made the availability of quality plant material, a meager¹. *Aloe vera* was used as important substance for pharmaceutical, cosmetics and food industries^{1,2}. Despite its cosmetic and medicinal properties, *Aloe vera* has positive evidence on its soothing, moisturizing and healing properties.

In vitro propagation of *Aloe vera* has started nearly four decades ago with the work of plant embryology³. It created the momentum by the discovery of 'double fertilization' which is an exclusive feature of the reproductive biology of flowering plants. A large number of protocols for *in vitro* propagation of *Aloe* plants have been developed using various varieties of explants like shoot tip, axillary bud, stem cuttings, shoot discs, lateral shoot, apical bud and adventitious shoot. The present study briefly reviews the *in vitro* regeneration research works already carried out in *Aloe vera*.

In vitro regeneration response of *Aloe vera*

Effect of explants: Many researchers have used varied plant parts as their explants. Vegetative meristem was used as source of explants and its multiplication was found remarkably to be 1:23.2 by Natali et al⁴. Moreover, Das et al⁵ also used shoot apical meristem to which they observed 100% rooting. Interestingly, Zhang et al⁶ used bud splits as explants to which they noticed improvement of 3-4 propagative ability. In another study, lateral shoots were used as explants by Oliveria and Crocorno⁷ to which they found that the multiplication rate is 1:5. Similarly, Nayanakantha et al⁸ and Khanam and Sharma⁹ used lateral shoot as explants and obtained 100% survival rate. Lee et al¹⁰ used adventitious shoot as the explants with 100% survival of tissue cultured plants. Apical buds have been used as explant by Gupta et al¹¹ for which they obtained 85% survival rate, whereas shoot bud has been used by Sahoo and Rout¹² and they reported 80% survival rate.

Kumari and Naseem¹³ have used rhizomatous stem and leaf segments as explants and reported 92% survival rate. But in the micropropagation of *Aloe vera*, the most commonly used explant is shoot tip.¹⁴⁻²⁸ Abdi et al²³ and Molsaghi et al²⁵ reported 100 % survival of tissue cultured plants in field and others have obtained lesser percentage of survival. Nevertheless, our observation using a shoot tip as explant was not ideal for developing regenerants. Shoot discs have been used as an explant by Saggoo and Kaur²⁹ in which they obtained 90% survival. Shibru et al²⁸ used shoot tip as explants for *Aloe vera* micropropagation and they achieved 93% survival rate of micro-propagated plants in *in vitro* condition.

In our laboratory, it was observed that a good response in shoot discs achieved multiple shoot induction on next week of inoculation, when given a transverse cut on the top and rooting on fourth week of inoculation.

Effect of hormones: *Aloe vera* has been taken for the research by various scientists. Sanchez et al³⁰ cultured vegetative meristems on the media containing 1.1 μ M 2,4-D and 2.3 μ M KIN and found it effective way of micro-propagating *Aloe vera*, whereas Meyer and Staden¹ obtained axillary bud development and adventitious bud formation from decapitated shoot explants using the MS medium supplemented with 5 μ M IBA. Those researchers noticed interesting finding that IBA supports more adventitious and axillary buds than that of NAA. Moreover adventitious buds were not formed with IAA, but axillary buds were formed. Additionally, they also observed the medium containing 2,4-D was normally inhibited the morphogenesis potential.

On the contrary to the findings of Sanchez et al³⁰, toxicity has been observed while using KIN with the explants. The main constraint to face in the *in vitro* propagation of *Aloe vera* is secretion of phenolic substances from the explants. The solution for this constraint is to use PVP in the nutrient media³¹. They have also achieved callus formation which was induced in stem segments from young axillary shoots grown on the underground rhizomatous stem using Murashige and Skoog's³² basal medium containing 1 mg/l of 2,4-D and 0.2 mg/l KIN provided the best callus induction in their experiment.

Zhang et al⁶ found that the propagation ability is increased three-four times by using the propagation media containing 4.0mg/l BA and 0.2mg/l IAA and half MS medium containing NAA (0.5-1.0mg/l) favored for better rooting. In contrast, Aggarwal and Barna³³ used BA 1 mg/l containing MS medium for micropropagation and achieved highest rate multiplication. In their research, they also observed citric acid 10mg/l and liquid medium improved the shoot multiplication rather than the use of solid medium and devoid of citric acid.

In another study, Liao et al¹⁴ investigated the important factor for bud initiation and contributed a result as that sucrose, BAP and NAA have the effect on bud initiation in the decreasing order respectively. They also concluded that best medium for bud initiation is semi-solid MS supplemented with 2.0 mg/l BA, 0.3 mg/l NAA, 30 g/l sucrose and 0.6 g/l PVP. Marfori and Malasa³⁴ used both BAP and KIN for *in vitro* multiplication of *Aloe vera*. Ujjwala¹⁵ accomplished to achieve 80% regeneration frequency and 24 numbers of shoots were obtained using shoot tip as explants on MS medium fortified with 4 mg/l BA and 1 mg/l NAA.

Several researchers worked on the micropropagation of this miracle crop and came out with varied valuable findings, one such occurred with Hosseini and Parsa¹⁶ as they obtained positive results using KIN 1mg/l + IAA 0.1 mg/l. Singh et al³⁵ selected axillary meristem for shoot proliferation which they succeeded with MS medium containing 13.32 μ M of BAP and 100 mg/l of ascorbic acid, 50 mg/l each of citric

acid and PVP with 25.0 mg/l each of arginine and adenine sulphate as additives and concluded with a most aspiring result of producing 5000 plants from a single bud within 180 days.

Studies conducted by Hashem and Kaviani¹⁸ revealed that use of activated charcoal increased the plantlets length and proposed that sucrose is better than any other carbon source. They obtained best shoot proliferation with medium supplemented with 0.5 mg/l BA + 0.5 mg/l NAA and best rooting is observed with medium supplemented with 1mg/l IBA and 1 mg/l NAA. Similar findings were also reported in *Aloe vera* by Zakia et al²⁴. An efficient micropropagation method has been developed using shoot tips as explants cultured in MS medium containing BAP 2.0 mg/l which showed 80 % survival, but they also observed seventy percent of adventitious root formation was observed in half strength MS medium supplemented with IBA²⁰.

Adventitious root induction was made possible by enrichment of 0.5 mg/l NAA and 0.2 mg/l IBA in Murashige and Skoog (MS) medium and more over to avoid the hindrance of accumulating phenolic compounds, the use of PVP is recommended.¹⁰

Nayanakantha et al⁸ were able to standardize an effective protocol using mainly lateral shoots as explants and obtained shoot induction with MS medium supplemented with 4mg/l BAP + 0.2mg/l NAA + 1g/l PVP. They experienced inefficiency while using 1g/l PVP in controlling the browning and with the MS medium supplemented with 4mg/l BAP + 0.2mg/l NAA + 1g/l PVP + 10mg/l citric acid and 0.5g/l activated charcoal, the browning was minimized and more over achieved 100% of the survival of rooted plantlets after acclimatization.

Abdi et al²³ conducted a vast research on *in vitro* propagation of *Aloe vera* using different explants (with and without sheath), different media (MS, B5, SH) and different hormone combinations of which they concluded that no successful regeneration has been obtained without using any hormones and with media supplemented with BAP alone or KIN alone. The highest favorable shoot proliferation was obtained with MS medium supplemented with 0.2 mg/l NAA and 4 mg/l BA and rooting response was obtained using B5 medium supplemented with 2 mg /l NAA.

This review recommends that both auxin and cytokinin are necessary for shoot induction but as a contrast with this the previous research reports^{14,17,33,36,37} used different combinations of auxins but had not tried BAP. The interesting study demonstrates that among cytokinins, BAP leads as the best performer than the KIN. Moreover, callus induction has occurred with media containing NAA (0.4 mg/l). High volume of callus has made possible when transferring to MS medium containing 2, 4-D (0.2 mg/l) but since the calli produced is non-organogenic, shoot proliferation from callus is not possible.

The previous studies used shoot tips as explants^{11,16,33,36,37,41} while some used leaf as explants. But this study uses a little different change in explants, that is, they retained some leaf base surrounding the explants which proved to protect the explant and so the explants performed better and it promoted multiple shoot induction.

Molsaghi et al³⁴ provided a micro propagation protocol which recommended a combination of 4 mg/l BAP and 1mg/l IAA along with MS for the highest rate of shoot multiplication. The plants showed 100% survival and they looked healthy and morphologically alike as the mother plant. Khanam and Sharma⁹ agreed with the importance of the miracle herb and had a view to produce rapid effective *in vitro* method to propagate the crop of the day. They used lateral shoots i.e. suckers from matured, disease free 5-6 year old plant and renovated protocol using the MS medium

along with 4.0 mg/l BAP and 0.2 mg/l NAA for shoot induction and for root induction, the best performing combination is found to be 2.0 mg/l IBA and 1mg/l NAA.

Gupta et al¹¹ conducted research on this herbal crop and reported that the highest shoot multiplication in *Aloe vera* was found in MS medium containing BA 1mg/l and IBA 0.2mg/l. In contrast, Kumari and Naseem¹³ reported that BAP is ideal cytokinin for shoot proliferation when combined with NAA and also observed that best rooting is attained when using 2.5 mg/l of NAA in MS medium. They also encountered browning during culturing time and they came over by using ascorbic acid treatment. Ahirwar and Mehta²⁶ obtained maximum number of shoots in MS medium containing BA 2 mg/l and KIN 0.2 mg/l. The recent *in vitro* regeneration procedures followed in *Aloe vera* are summarized in table 1.

Table 1
***In vitro* regeneration responses of *Aloe vera* (L.)**

S.N.	Explant	Medium	Regeneration and or survival response
1.	Vegetative meristem ⁴	MS+ 2,4-D 1.1 µM + Kinetin 2.3 µM	High morphogenetic ability; Multiplication rate: 1:23.2
2.	Shoot tip ¹	MS + IBA 5 µM	Adventitious bud formation
3.	Bud split ⁶	MS + BAP 4mg/l +IAA 0.2 mg/l	Propagation ability is increased 3-4 times.
4.	Axillary branch ³³	MS+ BAP1mg/l + IBA 0.2mg/l	100% Survival
5.	Shoot tip ¹⁴	MS+ BAP 2 mg/l + IAA 0.3 mg / l + PVP 0.6 g/l	92 Survival
6.	Shoot tip ¹⁵	MS+ BAP 4 mg/l + NAA 1 mg / l	Multiplication rate: 1:24.4
7.	Shoot tip, axillary shoot ¹⁶	MS+ BAP 1mg/l + Kinetin 1mg/l	83% survival
8.	Shoot tip ¹⁷	MS+ BAP 0.5mg/l + NAA 0.5 mg /l	95% survival
9.	Lateral Shoot ⁷	MS+ BAP 2mg/l + Glycine 2mg/l + 2mg/l myo-inositol	Multiplication rate: 1:5.3
10.	Lateral shoot ⁸	MS+ BAP 4mg/l + NAA 0.24mg/l + PVP 1g/l	100% Survival
11.	Shoot disc explant ²⁹	MS+ BAP 0.2 mg/l + IBA 0.2 mg/l	90% Survival
12.	Shoot apical meristem ⁵	MS+ BAP 35.5 µM + IBA 9.8µM + AS81.4 µM	100 rooting
13.	Shoot tip ¹⁸	MS+ BAP 0.5 mg/l + NAA 0.5 mg/l	Multiplication ratio - 1: 9.67 and 100% Survival rate
14.	Shoot tip ²⁰	MS+ BAP 2 mg/l	75% survival
15.	Adventitious shoot ¹⁰	MS+ BAP 0.2mg/l +NAA0.5mg/l +PVP1g/l	60 survival rate
16.	Shoot tip ²¹	MS+ BAP 6 µM +MT10 µM	Multiplication rate: 1:4.7
17.	Shoot tip ²²	MS+ BAP 1mg/l +Kinetin1mg/l +NAA0.15 mg/l	80 – 95 % Shoot proliferation
18.	Shoot tip ²³	MS+ BAP 4mg/l +NAA0.2mg/l	100% Survival
19.	Lateral shoot ⁹	MS+ BAP 4.0 mg/l +NAA 0.2mg/l	100% survival
20.	Shoot tip ²⁵	MS+ BAP 4 mg/l + IAA 2.5 mg/l	100% Survival
21.	Apical buds ¹¹	MS+ BAP 2mg/l +NAA. 0.5 mg/l	85% survival
22.	Shoot bud ¹²	MS+ BAP 2.0 mg/l + NAA 0.5 mg/l +AS 40 mg/l	80% survival
23.	Rhizomatous stem Leaf segments ¹³	MS+ BAP 2.5 mg/l + NAA 2.5 mg / l	92% survival
24.	Shoot tip ²⁷	MS+ BA 2mg/l + NAA 0.5 mg/l + Ads 40.0 mg/l	90 % survival
25.	Shoot tip ²⁸	MS media with 0.2 mg/l IBA + 1.0 mg/l BAP	93% survival

Conclusion

Everyone in this world aspires to lead a happy and prosperous life. But the humankind ignores the fact that it would occur only on the lap of nature. As time rolls on, he has enlightened that admiration and conservation of nature would lead to greater heights. The beauty of nature lies in the diversified herbal plant which serves as a source of healthy life. The micropropagation studies on this *Aloe* are reviewed in this study. Notable advances on *in vitro* propagation of *Aloe vera* have been achieved.

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