ISSN: 2690- 912X

Research Article

Journal of Genetic Engineering and Biotechnology Research

Optimizing Callus Formation in Aloe Vera L. Exploring the Impact of Different Hormonal Concentrations

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Submitted: 2024, Dec 11; **Accepted:** 2025, Jan 17; **Published:** 2025, Jan 27

Citation: Sohrabi Sedeh, S. S. (2025). Optimizing Callus Formation in Aloe Vera L. Exploring the Impact of Different Hormonal Concentrations. *J Gene Engg Bio Res, 7*(1), 01-04.

Abstract

One of the most important advantages of tissue culture is helping to reproduce plants in a short period of time, especially plants whose reproduction is very slow in the traditional way. Moreover, Tissue culturing enhances crop resilience to future climate change and soil health by enabling the propagation of stress-tolerant, high-yielding, and disease-resistant plant varieties. Aloe vera is a very widely used medicinal plant that is propagated very slowly through grafting and the use of tissue culture can be a huge advantage for surviving of these fascinating plant species. But due to the presence of phenolic compounds in the plant structure, the establishment of tissue culture in the environment will be difficult. In this research, with the help of PVP, to solve this problem, leaf explants were exposed to different concentrations of two types of growth hormones, auxin and cytokinin, in order to identify the most suitable culture medium. Undoubtedly, achieving the best protocol for the micropropagation of this unique plant will be very valuable both in terms of meeting the society's need for its amazing compounds and commercially. According to the obtained results, the best combination for induction of callus in leaf explants was in the presence of 2.5 mg/l of Naphthalene acetic Acid (NAA) as an auxin and 0.5 mg/l of benzyl adenine (BA) as a cytokinin hormone. According to these results, the use of the combination of these two growth hormones in callus induction are much more effective than using them separately, and also the greater amount of auxin hormone compared to the other hormone will have a greater effect on the samples.

Keywords: Aloe Vera, Tissue Culture, Callus Formation, Micropropagation, Climate-Resilient Crops

1. Introduction

Aloe vera L, a member of the liliacceae family, is a perennial plant with succulent green leaves with high medicinal values and is a native of southern Africa [1]. Studies revealed that among over 300 Aloe species, a few of them are commercially important. This plant has different uses such as pharmaceutical, healthcare, cosmetic products and food products for a very long time [2].

Investigation showed that this plant plays an important role in the treatment of some diseases including arthritis, high cholesterol, hypertension, chronic pelvic pain, immune system disorder and diabetes [3]. It also reported as an antibacterial and antifungal agent [4].

The plant Leaves contain many compounds such as vitamins, minerals, amino acids, carbohydrates, anthraquinones, saponins and lignin [5]. These significant compounds are used as astringent, antihelminthic, antiulcer, antiseptic, and also effective in treating stomach ailments, gastrointestinal problems and in treatment of skin diseases [6,7]. In addition, there are more than 160 secondary metabolites in Aloe vera leaves, among which anthraquinones such as barbaloin and homonataloin are very important [8]. The amount of these secondary metabolites in the plant changes under the influence of environmental factors. For example, the amount of barbaloin decreases by about 5% due to seasonal changes [9]. For this reason, in recent years, much attention has been focused on the use of tissue culture techniques to help produce secondary metabolites. Tissue culturing offers a promising approach to enhancing soil health by propagating plants that are resistant to abiotic stress, ensuring sustainable agricultural productivity in changing environmental conditions. On the other hand, the traditional propagation of Aloe vera plant, with such nutritional and medicinal value is done very slowly through grafting and there are many restrictions in its planting as a result in vitro cultivation of this fascinating plant is a crucial demand [10]. Tissue culturing complements areas utilizing non-chemical fertilizers by enabling the propagation of robust, stress-tolerant plants that thrive in nutrient-rich, organic systems, promoting sustainable agriculture

and improved soil health [11,12]. The micropropagation technique helps to produce higher quality plants in a shorter time. Investigations proved that in vitro cultivation of Aloe species faces serious difficulties because the release of phenolic compounds from the explants makes their initial establishment difficult. In this study we achieved callus formation of leaf explants by different concentration of 2 kind of growth regulators including Naphthalene acetic Acid (NAA) and benzyl adenine (BA).

2. Materials and Methods

2.1 Explants Preparation

Aloe vera plants were obtained from the local nursery in North of Tehran, Iran with an average of 8 leaves in August 2020 (Fig 1a). All the tools used, including petri dish, forceps, scalpel, culture bottles, were sterilized in the oven at a temperature of 200oC. The leaves separated from the potted plant were first washed with distilled water to remove the dust on them and then, in order to disinfect the samples, they were placed in 15% brine for 15 minutes. All the surfaces of the samples were completely exposed to the disinfectant. Then the samples were completely immersed in 70% alcohol for one minute. Finally, the samples were washed three times with sterile distilled water. All these steps were performed in the laminar air flow device of the sterile cabinet (Besta).

2.2 Callus Induction

The leaf explants with a size of 1.0 cm2 were placed in petri dishes containing solid MS medium with different combinations of 2 growth hormones including NAA (Naphthalene acetic Acid), and BA (benzyl adenine) with 10 replication each treatment and the surrounding area was closed with parafilm (3.0 pieces of explants per dish).The samples were kept at a temperature of 22 ± 2 0C with 16 hour continued photoperiod and after 15 days callus induction was initiated.Samples moved to new culture medium every two weeks .Finally, the average fresh weight of the calli obtained from different mediums (with three repetitions) was measured. PVP (polyvinyl pyrrolidine) was used to reduce the secretion of phenolic compounds from explants.

3. Results and Discussion

The results related to callus induction from leaf explants of Aloe Vera are given in Table 1. Our results indicated that the samples cultivated on MS media with 2.5 mg/l NAA (Naphthalene acetic) and 0.5 mg/l BA (benzyl adenine) inducted a higher percentage of callus induction, while MS medium contained 1 mg/l NAA and 1 mg/l BA reached the second place (Fig 1.b.c). Moreover, our results showed that the use of two types of growth hormones (NAA and BA) compared to one type was more effective to obtain more callus from leaf explants.

Several investigations have been performed to evaluate induction of callus by different kind of explants in order to explore optimum condition for micropropagation of Aloe. Investigations proved that source of explant has a vital effect on in vitro propagation of this species. There is a report of using plant seeds which resulted to callus initiation, but failed to have any positive achievement for regeneration of *Aloe pretoriensis plant* [14]. Later on, performed reproduction of this plant through stem meristems. According to previous experiments a variety of explants including different parts of stems, leaves, shoots and buds were selected for in vitro production of this Aloe plants [15-19]. According to their results, apical parts, as explants could play a crucial role in tissue culture and consequently, have a proper differentiation path which leads to suitable micropropagation [20].

On the other hand, combination of different type of growth regulators is another determinant factor in samples response to in vitro production. Several experiments have been performed to evaluate and recognize optimum protocol for successful in vitro cultivation of Aloe species by using different combinations of growth regulators, both in type and amount, added to culture medium. Researchers added various kind of auxin such as α-naphthalene acetic acid (NAA), Indole-3-acetic acid (IAA), 2,4 dichlorophenoxy acetic acid (2,4-D), Indole butyric Acid (IBA) and cytokinin such as Kinetin (KIN), N6-benzylaminopurine (BAP) in MS culture medium to obtain more callus from explants [21-25].

Investigated the effect of auxin and different cytokinin on callus induction from vegetative meristem explants and reported that the use of 0.02 mg/l 2,4-D and 0.5 mg/l BA was the best combination for callus induction [26]. Similar results were also obtained by regarding the induction of callus in culture medium containing 1 mg/l 2,4-D and 0.2 mg/l kinetin [27]. They induced callus formation in plant underground stems, and reported rapid propagation by shoot formation from calli. Generally, according to achieved results it seems that presence of these two growth regulators simultaneously, have a greater effect on callogenesis of explants, rather than one and also a higher concentration of auxin is more helpful for this process.

Investigated the induction of callus in *Aloe barbadensis* in the presence of different combinations of NAA, IAA, 2,4-D and kinetin hormones. The results of this study showed that the highest callus fresh weight was obtained after 60 days in the presence of 3 mg/l 2,4-D with 1 mg/l kinetin and the lowest fresh weight of callus was obtained in culture medium with 1 to 3 mg/l IAA along with kinetin. Our findings are consistent with the results of which showed an increase in callus induction in MS medium by using 2 times more auxin than cytokinin [27].

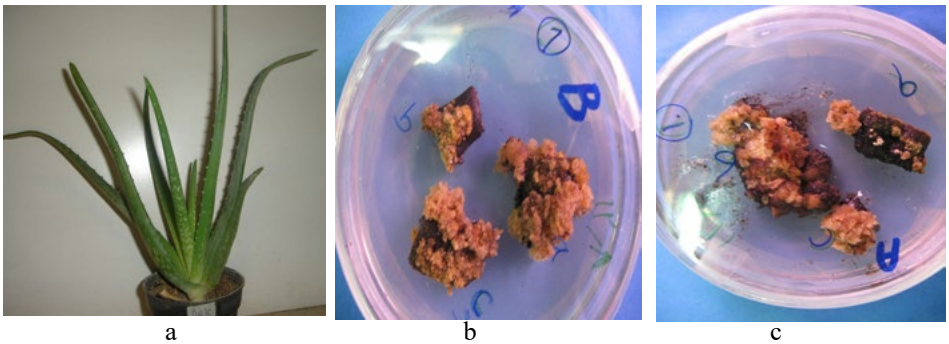
4. Conclusion

Identify the most proper instruction for in vitro propagation of *Aloe vera*, as a unique instance for medicinal plants, could play an enormous role in preserving this unique and multifunctional species which is a vigorous step for responding society demands [28]. Micropropagation of Aloe can produce healthier plant in a shorter period of time without encountering environmental challenges, which is a crucial path to better future.

MS+ Growth Hormones(mg/l)	% Response of Explant
MS+ 1 NAA+1BA	62
MS+ 2.5 NAA+ 0.5 BA	77
MS+ 0.5 NAA+ 0 BA	33
MS+ 0 NAA+ 1 BA	45
MS+ 1 NAA+ 0 BA	36

*20 Samples Per Treatment, NAA- Naphthalene Acetic, BA Benzyl Adenine

Table 1: Callus Formation of Aloe Vera Explants Under Different Concentration of Growth Hormones



a b c

Figure 1: a. In Vivo Aloe Vera Plant Used as Explants Source. b. Callus Induced from Explant on Medium Culture with NAA+BA (2.5+0.5) mg/L and. c with NAA+ BA (0.5+0) mg/l.

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