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PROPAGATION PROTOCOL OF THE MEDICINAL PLANT - *ALOE VERA* USING TISSUE CULTURE

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SUMMARY

Aloe vera is one of the most popular cactus-type plants in the global market due to its widespread uses in pharmaceutical, cosmetic, food, and decorative purposes. The present study derived callus cultures from the *Aloe vera* plant leaves, then reproduced on agar-solidified MS medium from June to December 2021 at the University of Mosul, Iraq. Results revealed that the MS medium + 3.0 mg L^{-1} benzyl adenine (BA) proved suitable for induction of leaf callus up to 85%, while the MS medium supplement with 1.0 and 2.0 mg L^{-1} BA reached 70%. The MS medium with 1.0 mg L^{-1} BA showed the best results for growing apical shoots of *A. vera* plants and producing vegetative branches. The formation of roots emerged within two weeks after placing them on the rooting medium. The shoots regenerated from the growing apices and were rooted easily in agar-solidified MS medium. The obtained plants attained successful acclimatization in terms of their growth and length, afterward, transferred to the peat-moss mixture.

Keywords: *Aloe vera*, callus cultures, propagation, leaves, stems, apical shoot

Key finding: The study aimed to identify the behavior of *Aloe vera* plants in the culture medium represented by the formation of callus cultures and their differentiation.

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INTRODUCTION

Aloe, a popular indoor house plant, has a long history as a multipurpose folk remedy, commonly known as *Aloe vera*, native to Africa and Spain (Sajjad and Sajjad, 2014). *Aloe Vera* is a member of the family Liliaceae and has cactus-like characteristics. The plant has several therapeutic and pharmacological properties, as well as, possesses counteractions for oxidation (Rajeshwari and Andallu, 2011). *Aloe vera* is a shrubby plant with many medicinal applications and substantial uses in cosmetics and health care (Eshun and He, 2004). The plant stem snaps off easily for immediate placing on cuts and burns for relief.

Aloe vera is a clump-forming succulent whose fleshy gray-green leaves form in a rosette shape atop a very short stem. The leaves have small grayish teeth on the margins. The central rosette gets up to about two feet high, and the plant continuously produces little offset rosettes. In winter and spring, medicinal aloe bears small tubular yellow flowers on inflorescence up to three feet tall. The original *Aloe vera* has yellow flowers, but most available varieties have orange flowers. The human body requires 22 amino acids for good health, of which eight consist

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"essential amino acids" because the body cannot fabricate them. *Aloe vera* contains over 20 minerals that are essential to the human body. *Aloe Vera* also contains eight essential amino acids and 11 of the 14 "secondary amino acids." *Aloe vera* also has vitamins A, B_1, B_2, B_6, B_{12}, C, and E (Surjushe *et al*., 2008).

Conventional plant breeding methods can improve both agronomic and medicinal traits. *In vitro* propagation or tissue culture of plants holds tremendous potential for producing high-quality plant-based medicines. Different methods can achieve this, including micropropagation (Yushkova *et al*., 1998; Jathunarachchi *et al*., 2021; Almukhtar, 2022). Plant tissue culture is one of the significant approaches for producing healthy plants free from diseases, especially viral ones. This technology has provided broad horizons for improving plant species of economic and medical importance (Cardoza, 2008). The use of tissue culture technique has obtained numerous plants speedily to conserve the genetic characteristics of various plant resources (Purohit, 2007).

Several studies indicated the possibility of propagating *A. vera* through tissue culture to produce callus cultures from leaves on agar-solidified MS medium, supplemented with the addition of 0.5 mg L^{-1} of each 2,4-D and Kin, with shoot regeneration by the addition of 1.0 mg L^{-1} BAP and 0.5 mg L^{-1} NAA (Murashige and Skoog, 1962; Choudhary *et al*., 2011). Another recent study indicated that *Aloe vera* responded best *in vitro* growth in leaf explants cultivated on MS medium supplemented with 1.0 mg L^{-1} BAP and 1.0 mg L^{-1} NAA (Wahab *et al*., 2020).

Enhancing the production with the maximum number of shoots of *A. vera* plants resulted from agar-solidified MS medium provided with a mixture of 1.0 mg L^{-1} IAA and 4.0 mg L^{-1} BAP. The same MS medium supplemented with 0.2 mg L^{-1} IAA and 0.8 mg L^{-1} BAP achieved the rooting of regenerated shoots in the same medium composition (Molsaghi *et al*., 2014). Reports also stated the high shoot production at 2.90, and the multiplication factor approached 2.87 on MS medium supplemented with 0.2 mg L^{-1} IBA and 1.0 mg L^{-1} BAP, respectively (Surafel *et al*., 2018).

As it is known, *the Aloe vera* plant reproduces naturally by offshoots, with a relatively slow way of multiplication to meet the growing demand. Each plant produces 2–3 offshoots per year, showing insufficient for commercial cultivation (Saggoo and Kaur, 2010). Therefore, this research is a vital step in plant biotechnology to preserve the essential genotypes of medicinal plants, providing great potential for producing high-quality plant medicines. The current study aimed to identify the response of *Aloe vera* plants in the culture medium represented by the formation of callus cultures and their differentiation.

MATERIALS AND METHODS

Explant source

One-year-old *Aloe vera* plants obtained from private nurseries in Mosul City, Iraq, underwent thorough washing with tap water and detergent (liquid soap) for 10 min. Carefully separating explants ranged with 3–6 leaves and apical shoot, their surfaces gained sterilization with 3% NaOCl (sodium hypochlorite) for 5 min, afterward, washed 3–4 times with sterile autoclaved distilled water to remove NaOCl (Chukwujekwu *et al*., 2002). Distributing 20 ml agar-solidified MS medium proceeded into flasks with 250 ml capacity. Upon approval, the current research work took place from June to December 2021 following the instructions of the Central Scientific Research Ethics Committee at the University of Mosul, Iraq.

Callus induction

The growth regulators, such as 2,4-D and BA, were added and tested in concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg L^{-1} to the MS medium. *Aloe vera* leaf explants cut into portions of 1.0 cm^2 received each treatment, including 10 flasks with 2.0 pieces of leaf explants per flask. The samples placed at 25 °C temperature underwent alternate light conditions of 16 h of light / 8 h of darkness.

Culture of apical shoot

Sterilized apical shoot and stems (length 2.0 cm) of *Aloe vera* followed cultivation by implanting their bases upright in 25 ml of MS medium supplemented each with 1.0, 1.5, 2.0, and 2.5 mg L^{-1} BA (Chukwujekwu *et al*., 2002), planting their bases in the center with one tip per flask. Samples remained in the culture room in the same condition previously mentioned. Cutting out *Aloe vera* vegetative branches obtained from cultivation of the apical shoot and stems used a sharp scalpel, with their bases planted upright in 25 ml of

agar-solidified MS medium supplemented with 1.0 mg L^{-1} NAA (Hailu *et al*., 2020).

Regenerated parts of *Aloe vera* removed from the culture medium, and roots proceeded water-rinsing to remove the medium. Planting a group of these plants in peat moss took place, with another group planted in a mixture of soil and peat moss in plastic pots, then covered with transparent and perforated nylon bags for seven days. Later, removing the bags allowed the plants to continue growing in the cultivation room under the earlier mentioned conditions.

RESULTS

The results indicated that, in general, difficulties also occurred during the development of calli from *Aloe vera* leaves, and the MS medium provided with the addition of 3.0 mg L^{-1} benzyl adenine (BA) demonstrated superior for leaf callus development, with the recorded development rate at 85% (Table 1). However, observations showed that the *Aloe vera* samples planted on other media did not encourage development significantly.

Results further indicated that leaf explants involved 12–20 days to start the callus development. The results showed that few explants emerged with enhanced induction of callus from the edges of the explant; however, it also stopped soon after a short period. The callus texture was compact and yellowish-green in color grown in the MS medium containing 2,4-D (Figure 1-A), while the callus appeared white in the MS medium containing BA (Figure 1-B).

The results specified that apical shoot cultivation produced intact plants readily, later cultivated in various culture media. The investigations also showed a significant decrease in the number of shoots obtained with growing apical shoots with increased BA concentration in the MS medium (Table 2). However, it does not encourage the formation of shoots from stem explant in said MS medium.

It also seemed that apical shoots showed to regenerate many shoots on agar-solidified MS medium (Figure 1-C). Meanwhile, the agar-solidified MS medium containing 1.5 mg L^{-1} BA regenerated several vegetative shoots ranging between 3–5 branches (Figure 1-D). The results also exhibited that the MS medium with 1.0 mg L^{-1}BA provided the best medium for producing vegetative twigs from stem explants (Figure 1-E). However, all the treatments required three to eight weeks to make the vegetative branches in good condition (Table 2).

The vegetative shoots produced from the apical shoot rooted fast, with their bases cultivated in agar-solidified MS medium supplemented with 1.0 mg L^{-1} NAA. The formation of roots appeared within two weeks of placing them on the rooting medium (Figure 1-F). The results also displayed vegetative shoots that showed success after transferring to the peat moss, then acclimatized in terms of

Table 1. Callus induction from leave explant of *Aloe vera* plant.

Induction media (mg L^{-1})	Total number of induced explants*	Induction (%)
MS (control)	0	0
MS + 0.5 BA	16	80
MS + 1.0 BA	14	70
MS + 1.5 BA	15	75
MS + 2.0 BA	14	70
MS + 2.5 BA	16	80
MS + 3.0 BA	17	85
MS + 0.5 2,4-D	0	0.0
MS + 1.0 2,4-D	2	10
MS + 1.5 2,4-D	0	0.0
MS + 2.0 2,4-D	4	20
MS + 2.5 2,4-D	0	0.0
MS + 3.0 2,4-D	0	0.0
MS + 0.5 2,4-D + 1.0 BA	10	50
MS + 1.0 2,4-D + 1.0 BA	8	40
MS + 1.5 2,4-D + 1.0 BA	2	10
MS + 2.0 2,4-D + 1.0 BA	11	55
MS + 2.5 2,4-D + 1.0 BA	13	65
MS + 3.0 2,4-D + 1.0 BA	11	55

*20 samples per treatment

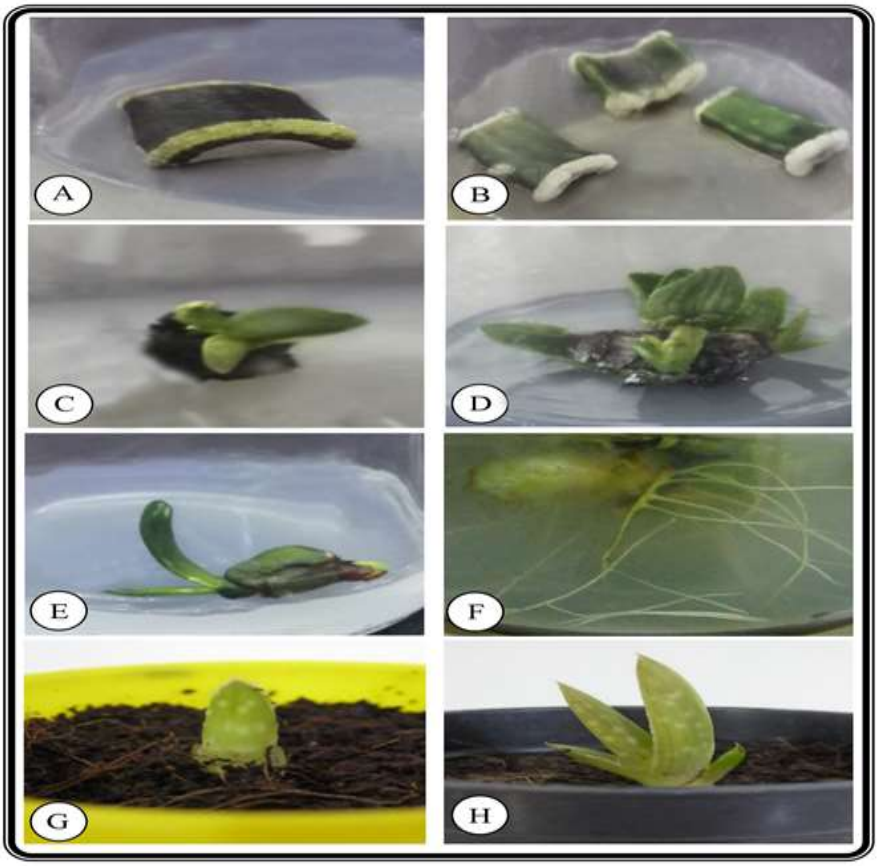

Figure 1. Plant propagation and callus formation from the stem and apical shoot explant of *Aloe vera* L.
(A) Leaf callus on MS medium + 2.0 mg L^{-1} 2,4-D (15 days age). (B) Leaf callus on MS medium + 3.0 mg L^{-1} BA (18 days age). (C) Formation of shoots after three weeks from the apical shoot on MSO medium. (D) Formation of shoots after four weeks from the apical shoot on MS medium + 1.5 mg L^{-1} BA; note the number of branches (arrows). (E) Formation of shoots after four weeks from stems on MS medium + 1.0 mg L^{-1} BA. (F) Rooting of shoots resulting from cultivation of apical shoot on MS medium + 1.0 mg L^{-1} NAA. (G) Acclimatization of plantlet produced from the cultivation of apical shoots in peat moss. (H) Increasing growth of plants and formation of additional leaves after three weeks of acclimatization.

Table 2. Formation of vegetative shoots of *Aloe vera* from the apical shoot and stem explant grown on differentiation media.

Explant	Differentiation media (mgl^{-1})	Total number of explants		Induction (%)	Time (week)
		Tested	Induced		
Apical shoots	MS + 0.0 BA	10	7	70	4
	MS + 1.0 BA	10	5	50	3
	MS + 1.5 BA	10	3	30	7
	MS + 2.0 BA	10	2	20	4
	MS + 2.5 BA	10	0	0.0	0
Stems	MS + 0.0 BA	10	0	0.0	0
	MS + 1.0 BA	10	4	40	8
	MS + 1.5 BA	10	0	0.0	0
	MS + 2.0 BA	10	0	0.0	0
	MS + 2.5 BA	10	2	20	6

enhanced growth and length reaching 5–7 cm (Figure 1-G). However, seven of the total plants observed have continuous development, producing additional leaves (Figure 1-H).

DISCUSSION

Several past studies on the *Aloe vera* plant concentrated on propagation using stems and shoot tips, rooted easily with the formation of whole plants (Hailu *et al*., 2020). It seems likely that certain obstacles faced by callus formation than the complexities challenged its growth histologically, despite providing the required media and supporting it with reasonable growth regulator types and concentrations. It could refer to the *Aloe vera* plant containing a group of chemical compounds that may sometimes hinder the differentiation processes (Surjushe *et al*., 2008).

Growth regulators could play a vital role and manifest themselves in different ways in rebellious plant species. Studies indicate that BAP and IBA had an effective and significant role in plant organogenesis by direct differentiation of shoots from cotyledon leaf explants in *Catharanthus roseus* (Abdul-Jaleel *et al*., 2009). In the *Tagetes erecta* plant*,* multiple shoots resulted with the MS medium containing BAP, with the plant growth regulators (PGRs) added to the media acting as stimulators of metabolic pathways for phytochemicals. Therefore, the regenerated plants appeared metabolically similar or even superior to the parental plant (Misra and Datta, 2001). The type of plant part used in micropropagation proved to have an effective role in the formation of vegetative branches, indicating more success and efficiency in the axillary buds in the propagation of *A. vera* (Molsaghi *et al*., 2014). Other researchers also recommend using the tip of the plant and the apical tissue for micropropagation of *A. vera* (Ahmed *et al*., 2007).

Several studies described the process of callus formation and differentiation in *Aloe vera* as a multipurpose plant with medicinal values (Nayanakantha *et al*., 2010). Cultivation of shoot tips and stem explants of *Aloe vera* plants in the culture medium demonstrated the superiority of BA over 2,4-D, with cytokinins found most efficient in the sustainable development and elongation of shoots on the medium supplemented with 1.0 mg L^{-1} IAA + 4.0 mg L^{-1} BAP and 0.2 mg L^{-1} IAA + 0.8 mg L^{-1} BAP (Molsaghi *et al*., 2014). Additionally, the success of shoots rooting in MS medium could attribute to the fact that the level of endogenous auxins was high enough to promote the said process in the species *Aloe polyphylla* (Chukwujekwu *et al*., 2002).

Several reports have noted rapid *in vitro* propagation of *A. vera* (Aggarwal and Barna, 2004; Hosseini and Parsa, 2007). Scientists obtained different results applying a different formulation of plant growth regulators for *in vitro* propagation of *A. vera*. The hormonal requirement for *in vitro* differentiation differs for different genotypes. The ability of plants to stimulate callus and produce vegetative branches depends on the internal hormonal level, and growth regulators added to the nutrient media until they reach the equilibrium stage (Acharjee *et al*., 2012). Several researchers have reported different responses to plant production with *A. vera*. Daneshvar *et al*. (2013) noted that the highest plant yield obtained used 0.15/2.5 mg/l BAP/NAA. Zakia *et al*. (2013) also recorded that the tallest vegetative shoot formation emerged using 0.5/0.5 mg L^{-1} BAP/NAA. Moreover, other researchers observed 14 to 16 shoots per plant with 4.0/0.2 mg L^{-1} of BAP/NAA in *A. vera* (Nayanakantha *et al.*, 2010; Khanam and Sharma, 2014). This difference could attribute to the type of plant and genetic variation that often affect the response of tissue culture (Lobine *et al*., 2015).

CONCLUSIONS

Establishing a successful protocol for creating *Aloe vera* plants from stems and apical shoot constitutes an imperative path in producing that crucial medicinal plant by tissue culture.

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