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Aloe vera leaf gel, a new approach to enhance plant tissue culture

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Abstract - This study investigates the nutritive properties of *Aloe vera* leaf gel (AvG) used in *in vitro* propagation from shoot tip explants of *A. vera* plant. Therefore, induced shoots of *A. vera* were prolifired on MS medium (Murashige and Skoog, 1962) supplemented with various concentrations of AvG as organic supplement. The highest shoot multiplication was found on M3 containing MS media + 0.2 mg/L IBA (β-indole butyric acid) + 3 mg/L BA(benzylamino-purine) + 50 g/L AvG (13.27 ±5.11). This demonstrated that adding 50 g/L AvG to the culture media has increased the number of shoots compared to the control treatment M1 (5.00 ±2.27). Moreover, the longest shoot (2.50 ±0.89 cm) was found on M2 medium (MS + 0.2 mg/L IBA + 3 mg/L BA + 25 g/L AvG) followed by M3 (50 g/L AvG) and M1 (control) respectively. For microshoots rooting, the effect of the substitution of MS medium by AvG was assessed. The AvG was used as a substitute source of nutrients to the culture media.
The maximum number of roots (5.93 ±1.39; 5.73 ±1.75) and the longest root (6.20 ±1.32; 5.90 ± 1.43 cm) were recorded on control medium (Full MS) and 1/2 strength MS medium supplemented with 10% AvG. All of the rooted explants (100%) survived during and after the acclimatization in the pots in the culture room. The regenerated plants looked healthy and they were morphologically similar to the mother plants.

Keywords: *in vitro* propagation, shoot tip explants, *Aloe vera* leaf gel, substitution.

1. Introduction

Aloe vera L. is an important commercial plant, which has enormous applications in pharmaceutical, cosmetic and food industries. Therefore, the demand for quality planting material of *A. vera* is increasing worldwide. Micropropagation is the biotechnological technique that can be used to meet the industrial demand for *A. vera*.
According to Devi (2009), each leaf is made up of three layers: (i) the outer layer, a thick cuticle with a protective function and giving rigidity to the plant; (ii) the middle layer of latex (the chlorophyll parenchyma), from which a bitter yellow sap flows spontaneously after cutting (the juice) and (iii) an inner layer consisting of a thick, mucilaginous liquid (the gel). A major feature of *A. vera* is its high-water content, in the range of 99–99.5%, with the 0.5–1.0% of remaining solids containing more than 200 different potentially active compounds (Rodríguez et al. 2010; Boudreau et al. 2013). Of these compounds, 55% are polysaccharides, 17% are carbohydrates, 16% are minerals and trace elements, 7% are proteins, 4% are lipids and 1% are phenolic compounds (Atherton 1997). Aloes, especially *A. vera*, have been used in traditional medicine for centuries and are found in many pharmacopoeias (Park and Jo 2006). Aloe gel contains many components known for their healing and anti-microbial, -inflammatory, -diabetic, -cancer and -viral properties, and their antioxidant effects are attracting interest (Lobine et al. 2015).
The aim of the present study was to develop an efficient protocol of high-frequency true-to-type plantlet regeneration from shoot tip explants of *A. vera*. The *A. vera* leaf gel was used to investigate its role as an organic supplement to the culture media.

2. Materials and methods

2.1. Plant material and explant disinfection

The *A. vera* plants (3 years old) used in this study were from the greenhouse of the Horticultural Science Laboratory of the National Agronomic Institute of Tunisia. Plants were thoroughly cleaned with water to eliminate soil residue. The tissue culture hood, its walls, and its air filters were cleaned with 70% ethanol. The shoot tip explants (0,5 cm height) were disinfected by soaking in pure alcohol for 30 s followed by 10% sodium hypochlorite (15 min) and finally a solution containing 0.1% mercury chloride (10 min). After these treatments, the remaining trace of the disinfectant was removed by washing explants three times using autoclaved distilled water.

2.2. Culture conditions and culture establishment

The basal medium used for culture induction was MS medium with 3% sucrose, 1 mg/L of (IBA), 0.1% rosemary essential oil and solidified by the addition of 0.6% agar. The pH was adjusted to 5.8. The culture media and the used material were sterilized for 20 min in an autoclave at a pressure of 1 bar. The tissue cultures were placed in an incubator under a 16/8 h of light/dark (36 µmole/m^2/s of light intensity). Explants were placed in test tubes (diameter 16 mm and length 100 mm) each containing 20 mL of medium. The cultures were maintained at a temperature of 22 ±2°C. The parameters measured after four weeks of culture were explant survival rate, intensity of browning, infection rate, number of leaves per explants, and average leaf length. All the experiments were implemented into ten replicates.

2.3. Shoot multiplication

Shoots multiplication trials have been conducted to determine the effect of the *A. vera* leaf gel (AvG) on the regeneration of axillary shoots. For *A. vera* leaf gel preparations, mature fresh leaves of *A. vera* were collected from the greenhouse and kept for an hour to remove aloine exudate, then washed thoroughly in running water. The leaf skin was removed, the mucilaginous leaf gel was peeled off, homogenized in mixture-grinder and then the filtered. The culture media used consist of the basic medium MS supplemented with 0.2 mg/L IBA and 3 mg/L BA with different concentrations (0; 25; 50; 100 g/L) of *A. vera* leaf gel (AvG). The measured traits recorded after one month of culture were the average number of axillary shoots and the average height of the axillary shoots.

2.4. Rooting of microshoots

For rooting tests, the axillary shoots measuring 2 -3 cm obtained during the multiplication phase are placed on media with different strength of MS (Full MS, ½ MS, ¼ MS) and *A. vera* leaf gel (AvG) (0; 10; 20; 30 %) supplemented with 1 mg/L IBA. The observations made, after four weeks of culture, were the rooting rate, the number of roots, and the average length of the roots.

2.5. Acclimatation and soil transfer

Rooted plantlets were thoroughly washed and planted in plastic pots containing soilrite and covered with transparent polythene bags to maintain humidity and were kept in 25 -30°C temperature for 30 days. The acclimatized plants were transferred to soil mixture of soil, sand and manure (1:1:1, v/v/v) kept in the greenhouse for growth and watered every two days interval. Finally, the plants were transferred to the field under full sunlight.

2.6. Statistical analysis

All the experiments were implemented into ten replicates. Statistical analyses were performed with STATISTICA software 10. The one-way analysis of variance (ANOVA) was performed to identify the effect of each treatment. The Fisher's least significant difference test at the 5% threshold was used to compare means.

3. Results and discussion

The explants began to show the signs of shoot induction after 1 weak of culturing. Nineteen per cent of explants gave aseptic culture and were free from fungal as well as bacterial contamination. All the induced shoots showed no signs of browning from the oxidation of phenolic compounds. The average number of leaves per explant was 3.71 with an average length of 2.94 cm. Scientists obtained various results applying different formulations of plant growth regulators for *in vitro* propagation of *A. vera*. The hormonal requirement for *in vitro* differentiation differs for different genotypes (Molsaghi et al. 2014). Biswas and colleagues (2013) found that when explants are treated with benzyaladenin (BA), the best outcome of shoot induction percentage and average number of shoots per explant was respectively

90% and 2.70 for 1.5 mg/L hormone concentration; whereas for kinetin (Kn) it was 100% and 4.20 for 2 mg/L hormone concentration. However, the percentage of success for the initiation phase reached 93.35% with 0.25 mg/L of α-naphthalene acetic acid (NAA) and 1.5 mg/L of benzylaminopurine (BAP), the average number of shoots per explant observed was 4.67 (Gantait et al. 2011).

After successful initiation of the culture (30 days after culturing), newly formed shoots were excised individually from the induced explants and further cultured on MS supplemented with 0.2 mg/L IBA and 3 mg/L BA with different concentrations of *A. vera* leaf gel (AvG) to determine its effect on the regeneration of axillary shoots (Table 1). The highest shoot multiplication was found on M3 containing MS media + 0.2 mg/L IBA + 3 mg/L BA + 50 g/L AvG (13.27 ±5.11) followed by M4 : MS + 0.2 mg/L IBA + 3 mg/L BA + 100 g/L AvG (9.73 ±5.06). The M1 media (MS + 0.2 mg/L IBA + 3 mg/L BA) and M2 (MS + 0.2 mg/L IBA + 3 mg/L BA + 25 g/L AvG) produced the lowest average axillary shoots (5.00 ±2.27 and 6.40 ±3.46 shoots respectively). These results are close to those obtained in the study of Haque and Ghosh (2013a), on MS medium supplemented with 2.5 mg/L of BAP and 5% *A. vera* gel, the number of formed shoots per explant (≥ 2 cm) was in the order of 15.9 shoots. Shoot elongation (2.50 ±0.89 cm) was found the longest on M2 medium (MS + 0.2 mg/L IBA + 3 mg/L BA + 25 g/L AvG) and the least shoot height was found in M4 (1.82 ±0.45). The control media M1 (MS + 0.2 mg/L IBA + 3 mg/L BA) was placed in a group with M3 (MS + 0.2 mg/L IBA + 3 mg/L BA + 50 g/L AvG) with an estimated average height of 2.27 ±0.94 and 2.21 ±0.84 respectively. Haque and Ghosh (2013b) noted that AvG was used as a supplementary source of organic and inorganic ingredients for better growth and production of healthy *in vitro* plants of *Bacopa*. In addition to the different carbohydrates, AvG contains 75 potentially active constituents including vitamins, enzymes, minerals, lignin, saponins, salicylic acids, amino acids and different inorganic salts (Hamman 2008), which may enhance the multiplication rate of *A. vera*. The total solid content of *A. vera* gel is 0.66% and soluble solids are 0.56%. Aloe gel contain 55% of polysaccharides, 17% of sugars, 16% of minerals, 7% of proteins, 4% of lipids and 1% of phenolic compounds. The *A. vera* gel contains many vitamins including the important antioxidant vitamins A, C and E. Vitamin B1 , niacin, Vitamin B2 , choline and folic acid (Muthukumaran et al. 2018).

Table 1. Shoot multiplication

Culture media additives				Average number of axillary shoots	Average height of axillary shoots (cm)
Name of the	IBA[a] (mg/L)	BA[b] (mg/L)	AvG[c] (g/L)		
M1	0.2	3	0	5.00 ±2.27[a]	2.27 ±0.94[ab]
M2	0.2	3	25	6.40 ±3.46[a]	2.50 ±0.89[b]
M3	0.2	3	50	13.27 ±5.11[c]	2.21 ±0.84[ab]
M4	0.2	3	100	9.73 ±5.06[b]	1.82 ±0.45[a]

[a] *IBA: β-indole butyric acid*
[b] BA : Benzyl amino-purine
[c] AvG*: Aloe vera gel*
Different letters indicate significant differences (Fisher's test at p ≤ 0.05)

All three strength of MS medium (1, 1/2, ¼) with 0; 10; 20; and 30% of AvG resulted in cent percent root induction (Table 2). The maximum number of roots (5.93 ±1.39; 5.73 ±1.75) and the longest root (6.20 ±1.32; 5.90 ± 1.43 cm) were recorded on control medium (Full MS) and 1/2 strength MS medium supplemented with 10% AvG. A higher number of roots per cultured shoot were obtained with AvG (10%) treatment when compared with 1/2, 1/4 strength of MS medium with 20 and 30% of AvG respectively. The lowest number and length of roots (3.73 ±1.62; 4.64 ±0.91cm and 3.87 ±1.6; 3.77 ±0.59 cm) were obtained with 30% AvG treatment when supplemented with 1/2 and 1/4 strength of MS medium respectively. The findings of present study are in line with those of Haque and Ghosh (2013a) which reported that the addition of AvG to the medium not only increased the percentage of response and number of root per shoot but also the growth of the plantlets was improved.

Table 2. Effect of the substitution of MS medium by AvG on the rooting of microshoots

Name of the media	Culture media			Rooting rate (%)	Average number of roots	Average root length (cm)
	IBA[a] (mg/L)	MS strength	AvG[b] (%)			
M1	1	Full MS	0	100.00 [a]	5.93 ±1.39[c]	6.20 ±1.32[c]
M2	1	½ MS	10	100.00 [a]	5.73 ±1.75[c]	5.90 ± 1.43[c]
M3	1	½ MS	20	100.00 [a]	4.87 ±1.81[abc]	5.53 ±1.59[bc]
M4	1	½ MS	30	100.00 [a]	3.73 ±1.62[a]	4.64 ±0.91[ab]
M5	1	¼ MS	10	100.00 [a]	5.27 ±2.05[bc]	4.67 ±2.36[ab]
M6	1	¼ MS	20	100.00 [a]	4.33 ±2.19[ab]	4.63 ±2.08[ab]
M7	1	¼ MS	30	100.00 [a]	3.87 ±1.60[a]	3.77 ±0.59[a]

[a] *IBA: β-indole butyric acid*
[b] AvG*: Aloe vera gel*
Different letters indicate significant differences (Fisher's test at $p \leq 0.05$)

The plantlets with actively growing roots were transferred from *in vitro* conditions to plastic pots and containing garden soil, manure, and sand in the proportion of 1:1:1, respectively placed under net to keep the environment wet and shade. Survival of plantlets was observed after one month. All of the explants (100%) survived during and after the acclimatization in the pots in the culture room. The regenerated plants looked healthy and they were morphologically similar to that of mother plants.

4. Conclusion

Using the protocol described in this study, it is possible to improve tissue culture protocol for *in vitro* propagation of *A. vera* through addition of *A. vera* leaf gel in the culture medium as an alternative plant source of organic nutrients. In this context, the present findings are innovative and unique.

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