RAPID PROTOCOL OF *Aloe vera* In Vitro propagation

G. H. Danial[1] D. A. Ibrahim[1] A. N. Yousef[2] S. B. Elyas[1]
Assist. Prof. Assist. Prof. Lecturer Biologist
[1] Scientific Research Center / Coll. Sci. / University Duhok. Email: diaa.ibrahim@uod.ac
2Dept. Biology / Science Coll. Sci. / University Duhok Email: atheel.yousef@uod.ac

ABSTRACT

The current research was carried out to enhance *Aloe vera* propagation via tissue culture technique. BA at 0, 1, 2, 3, and 4 mg/l alone or combined with 0.1 mg/l NAA were used for shoot proliferation. The best result was recorded by using 2mgl^{-1} BAP (5.33 shoots/ explant). Meanwhile, combination between 2 mg/l BAP with (0.2, 0.4 and 0.6) mg/l kinetin, NAA or IBA were tested and best protocol was shown by using 2 mg1^{-1} BAP + 0.6 NAA which recorded 4.89 shoots/ explant. For rooting, MS medium at half and full strength salts were used supplemented with 0, 1, 2, 3, 4mgl^{-1} of NAA. The result revealed that 3 mg1^{-1} NAA at half strength of MS medium regenerate developed roots (8.67 roots / shoot) within 4 weeks. The well successful healthy plantlets were transferred into a potting mix composed of sand and peat moss which shows 100% survival ratio.

Keywords: Aloe vera; shoot tip; Micropropagation; BAP; kinetin; cultural technique.

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الاكثار السريع لنبات الالوفيرا *Aloe vera* خارج الجسم الحي

غربية هرمز دانيال ضياء ايوب ابراهيم اثيل نجيب يوسف سازان بركات الياس
استاذ مساعد استاذ مساعد مدرس بايولوجي
[1] جامعة دهوك / كلية العلوم / مركز الابحاث العلمية
[2] جامعة دهوك / كلية العلوم / قسم علوم الحياة
diaa.ibrahim@uod.ac ; atheel.yousef@uod.ac

المستخلص

اجريت الدراسة الحالية لتحسين اكثار نبات الالوفيرا بوساطة تقنية الزراعة النسيجية. استخدم البنزايل ادنين لوحده بتراكيز مختلفة (0 ، 1،2، 3 و 4 ملغم / لتر) ومتداخلاً مع نفثالين حامض الخليك بتركيز 0.1 ملغم / لتر لغرض اكثار المجاميع الخضرية . وقد سجلت افضل النتائج عند التركيز 2 ملغم / لتر (5.33 فرع خضري / قطعة نباتية) ، وفي الوقت عينة فقد استعمل تداخل 2 ملغم / لتر من البنزايل ادنين مع (0.2 ، 0.4 و 0.6 ملغم / لتر) كاينتين و نفثالين حامض الخليك واندول حامض البيوتاريك لاختبار اكثار المجاميع الخضرية والذي اظهرت بان افضلها (4.89 فرع خضري / قطعة نباتية) باستخدام التداخل 2 ملغم / لتر بنزايل ادنين مع 0.6 ملغم / لتر نفثالين حامض الخليك . ولغرض التجذير، فقد استخدم وسط MS بنصف وكامل قوته الملحية مدعما باضافة تراكيز مختلفة (0 ، 1 ، 2 ، 3 و 4 ملغم / لتر) نفثالين حامض الخليك وقد اشارت النتائج الى ان استخدام نصف القوة التركيبة لاملاح هذا الوسط بوجود 3ملغم / لتر قد شجع تكوين الجذور (8.67 جذر / فرع خضري خلال اربعة اسابيع . نقلت النباتات السليمة وذات النمو الجيد الى اصص صغيرة حاوية خليط من التربة الرملية والبتموس والتي اظهرت معدل نجاح وبقاء بلغ 100% .

الكلمات المفتاحية: نفثالين، حامض الخليك، بنزين ادنين.

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INTRODUCTION

Aloe vera is a perennial herbal succulent plant from Liliaceae family and is an important medicinal plant. It has been used for pharmaceutical, food, and it demand for cosmetic industries. (1) mentioned that this genus contain over 200 bioactive constituents, such as anti- oxidant (Vitamins) and immune regulator (glucomannans), anti-inflammatory (steroids and salicylic acid), antitumoral (mucopolysaccharides) and antiseptic (saponins and anthraquinones). It was revealed that the extracted gel from its leaves makes an excellent treatment for burns, wounds, a protective coat above infected area, reducing the time of healing and the risk of infection. Today, it is also believed to possess anti-cancer agents (2). Vegetative propagation is the common method of *Aloe vera* using lateral buds. Moreover, two major barriers reduce the vegetative propagation rate. First only three to four lateral shoots will initiated in a year via single plant. Second, is the presence of male sterility (3). Thus, *in vitro* propagation introduced the ability to solve such problems. Many reports (4,5,6,7,8, 9,10, 11, 12, 13,14,16,17 and 18) studied the rapid and efficient *A.vera* micropropagation and some other crops using different protocols of plant growth regulators.. The goal of this study was to grow new protocols for quick and high shoot proliferation of *Aloe vera* and standardized new combinations of plant growth regulators for fast and proficient micropropagation using shoot tip explants.

MATERIALS AND METHODES

Aloe vera L. off shoot-derived plants from the commercial greenhouse was the source of shoot tip explants which were free of disease symptoms and pests. Each shoot was cut along with shoot apex of about 1.5-2.0 cm in length. Commercial detergent solution containing 5% Sodium hypochlorite (NaOCl) was used at concentration of 3.75% for 15 min under gentle vacuum for explants surface sterilization. [19] basal medium augmented with 0.5 mg/l nicotinic acid + 0.5 mg/l pyridoxine HCl + 0.4 mg/l thiamine HCl + 0.2 mg/l BA+ 0.2 mg/l IBA+ 30 g/l sucrose + 0.7% w/v agar and 100 mg/l inositol was used for the initiation stage. The pH was adjusted to 5.7 ± 0.1 utilizing 0.1 N HCl and /or 0.1 N NaOH earlier autoclaving at 121°C temperature and 15 lb pressure for twenty minutes. Every single aseptic culture inoculated with three explants was kept up at 16 h photoperiod at 25± 2°C temperature. At shoot multiplication stage, various levels of BA was tested (0.0, 1.0, 2.0, 3.0 and 4.0 mg/l) alone or combined with 0.1 mg/l NAA to encourage shoots proliferation from the successful established explants, after six weeks best concentration from BA (2.0) mgl^{-1} was selected and tested with (0.2, 0.4 and 0.6 mgl^{-1}) from NAA, IBA and Kinetin for multiplication. For rooting stage, NAA was used at different concentrations (0.0, 1.0, 2.0, 3.0 and 4.0mgl^{-1}) to test their ability for root induction in half and full concentration of MS salts. After six weeks in culture, data of both multiplication and rooting stages were recorded. Complete Randomized Design (CRD) was used with three replicates for each treatment and Duncan's multiple range test ($P \leq 0.05$) were carried out to compare the means via computerized program of SAS [20]. Successfully rooted plantlets were removed from culture vessels after removing the agar and washing them with distilled water and immersed in Benlate fungicide 0.1% for 10 minutes for acclimatization stage, then transferred to plastic pots containing a steam sterilized mixed soil (peatmoss, loam and Styrofoam (1:1:0.5, v:v:v) in open greenhouse condition.

RESULTS AND DISCUSSION

The response of *Aloe vera* explants for mass proliferation utilizing shoot tips started obviously during second weeks of the cultured in MS media supplemented with various concentrations and blends of cytokinins and auxin. However, the amount of new shoots assorted due to BAP concentrations (Table1). Average issue of shoots increased significantly with the company of BAP. The maximum value of multiplication rate was reached in the medium containing 2.0 mg/l of BAP. Whereas, the number of shoots increased significantly among all treatments especially at the control (1.0) to more than (5.33) shoots at 2.0 mgl^{-1}.

Table 1. Effect of BAP on *Aloe vera* shoots multiplication on MS medium after 6 weeks in culture

BAP concentrations (mg/l)	number of shoots / explant	mean length of shoots (cm)	number of leaves / explant
0. 0	1. 00 e	2. 32a	4.17b
1.0	1.67 d	2.02 a	6.00 b
2.0	5.33 a	1.32 b	10.33 a
3.0	3.50 c	1.58 b	5.50 b
4.0	4.50 b	1.62 b	9.50 a

On the other hand, the shoot length was significantly reduced by increasing BAP concentration. Least shoot length was attained at the 2.0 mg/l BAP level, which reached 1.32 cm as compared to 2.32 cm in BAP- free medium. Meanwhile, the highest number of leaves / explants was recorded on 4.0 mg/l BAP treatment (9.50) which was diminished significantly to 4.17 leaves/ explant in the control treatment. From the preceding results, it is clear that BAP was powerful to instigate shoot multiplication in *Aloe vera*, this positive role of BAP on multiplication stage is may be due to on be due to cytokinins great effects on liberate the lateral buds from the domination of terminal buds without the need to evacuate the apical bud by encourage the formation of xylem and phloem (vascular tissues) of buds that ease water conduction and nutrients leading to lateral bud growth. In addition to the vital role of cytokinins in stimulate RNA synthesis, followed to raising the intracellular proteins and enzymes which improve bud growth [21]. The blend among BAP with NAA exhibit similar impact in shoot proliferation (Table 2) which revealed that the most elevated number of regenerated shoots (4.5 shoots /explants) in MS media containing 3.0 mg/l BAP + 0.1 mg/l NAA. (Fig.1c). All levels raise significantly the number of shoots / explants when compared to control treatment. Presence of BA and NAA caused significant decreased in shoot lengthen compared to the control (3.62cm), Whereas, the addition of 1.0 mg/l BAP + 0.1 mg/l NAA gave the most elevated number of leaves / explant (7.17 leaves/explants) and varies significantly when compared to the control treatment (3.17 leaves/explants). Many researchers mentioned that shoot proliferation generally require the presence of both auxins and cytokinins who revealed that cytokinins, particularly BA stimulate axillary bud improvement. However at high concentration, shoot elongation is smothered. Moreover, This outcomes are in accordance with many researchers who observed variables Impacts of cytokinins and auxin in shoot regeneration of many species (22).

Table 2. Effects of BAP blend with NAA on *Aloe vera* shoots multiplication after 6 weeks in culture

BAP+ NAA concentrations (mg/l)	number of shoots / explant	mean length of shoots (cm)	number of leaves / explant
0.0+ 0.0	1.16 c	3.62 a	3.17 c
1.0+ 0.1	3.00 b	1.49 b	7.17 a
2.0+ 0.1	3.17 b	1.52 b	6.83 ab
3.0+ 0.1	4.50 a	0.70 c	7.00 a
4.0+ 0.1	3.50 b	1.33 b	6.00 b

According to results in Table 1 which shows that the best concentration of BAP (2.0 mg/l) for *Aloe vera* shoots proliferation, so this protocol was selected and tested by combination with different concentration from NAA, IBA and Kinetin. The outcomes in

Table 3 indicate that the high number of shoots (4.89 shoots) was achieved in 2.0 mg/l BAP with 0.6 mg/l NAA (fig. c) and differs significantly from the medium contained2.0 mg/l BAP combined with 0.2 mg/lNAA. Presence of 2.0 mg/lBAP combination with different concentration from NAA did not demonstrate significant impacts in shoot length .Whereas, the addition of 2.0mg/l BAP + 0.4 mg/l NAA record the most noteworthy number of leaves per explants (8.00) and this increment was significant when compared to the lower concentration from NAA (5.11). The combination results between 2.0mg/l of BAP with different concentration from IBA clarified that there is no significant differences among the treatment on the shoots number per explants and mean shoots length, while the leaves number per explants was significantly increased in the medium supplemented with BAP combine with 0.4 mg/l IBA. Meanwhile, the impact of various levels of kinetin combined with 2.0 mg/l BAP on *Aloe vera* plant shoots multiplication reviled that the 2.0 mg/l BAP combined with 0.2mg/l kinetin records the most elevated number of branches / explant (4.0) and highest number of leaves/explants (7.78), while the highest average shoots length (1.28) was appear in the combination between BAP and 0.4 mg /l kinetin. These results emphasized the necessity of using both cytokinin and auxin for shoot proliferation, this result corresponded with many reports (23) in addition of the role of cytokinins via breaking the apical dominance in buds and induce subsidiary meristem grown into shoots

Table 3. Effect of BAP combination with kinetin or auxin on *Aloe vera* shoots multiplication after 8 weeks in culture

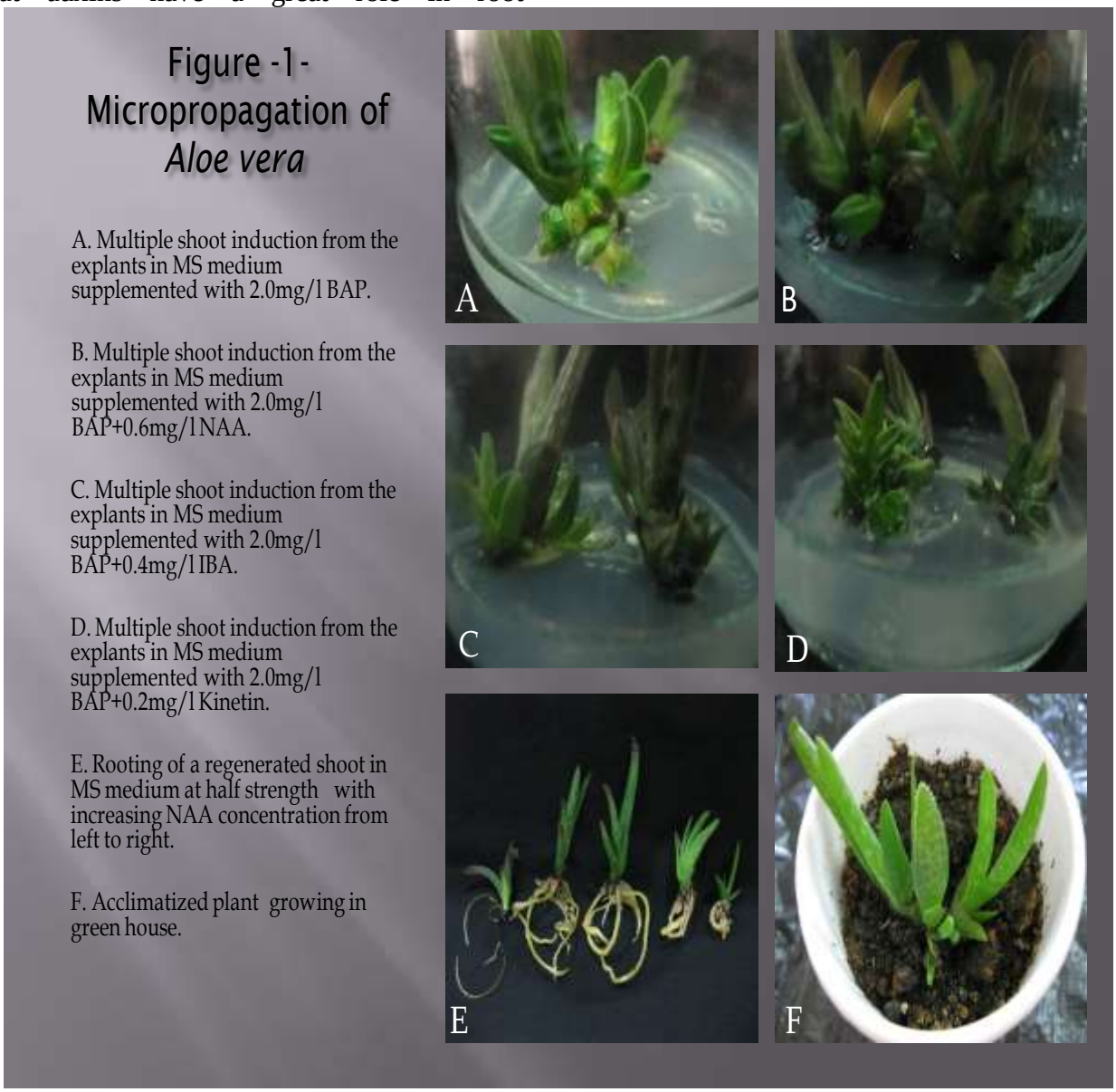
BAP + NAA concentrations (mg/l)	number of shoots / explant	mean length of shoots (cm)	number of leaves/ explant
2.0 +0.2	**1.56 b**	**1.74 a**	**5.11 b**
2.0 +0.4	**4.11 a**	**1.29 a**	**8.00 a**
2.0+0.6	**4.89 a**	**1.28 a**	**7.78 a**
BAP+ IBA (mgl^{-1})			
2.0+ 0.2	**3.56 a**	**1.83 a**	**2.84 c**
2.0+ 0.4	**3.78 a**	**1.33 a**	**9.56 a**
2.0+ 0.6	**3.11 a**	**1.94 a**	**6.55 b**
BA+ Kinetin (mgl^{-1})			
2.0+ 0.2	**4.00 a**	**0.85 b**	**7.78 a**
2.0+ 0.4	**1.44 b**	**1.28 a**	**4.44 b**
2.0+ 0.6	**1.22 b**	**0.92 b**	**4.11 b**

Table 4. Effects of the NAA on *Aloe vera* rooting stage after 6weeks in half and full strength MS salts medium

MS salts	NAA concentration Mg/l	number of roots / explant	mean length of roots (cm)
Full strength	**0.0**	**0.67 d**	**3.50 b**
	1.0	**4.22 b**	**5.61 a**
	2.0	**6.89 a**	**3.83 b**
	3.0	**2.89 c**	**1.16 c**
	4.0	**2.72 c**	**0.71 d**
Half strength	**0.0**	**5.43 c**	**7.93 a**
	1.0	**7.33 b**	**7.63 a**
	2.0	**6.78 b**	**4.95 b**
	3.0	**8.67 a**	**3.39 b**
	4.0	**5.56 c**	**1.43 c**

After six weeks of culture on MS medium at full or half salts strength and different concentrations of NAA, establishing results showed that the nearness of NAA seemed positive effects on *in vitro* rhizogenesis of *Aloe vera*. Adding 2.0 mg/l of NAA increase

significantly the roots number in full strength salts medium(5.89) while the highest number of roots (8.67) showed by increasing the level of NAA to 3.0 mg/l by reducing the amount Ms medium salts into half (Table 4). On the other hand, roots length reached its maximum value at 1.0 mg/l NAA level in both full (5.61cm) and half (7.63 cm) respectively (Figure1E). From previous results it is obvious that NAA is more effective in half strength medium. Moreover, increasing the concentration of NAA reduce the roots length significantly. These outcomes demonstrated that auxins have a great role in root establishing process since they advance adventitious roots formation due to their effects via increasing the cell division and elongation (22).Then again (24) detailed that the moderately low salt concentrations in the medium are known to upgrade root establishing of small shoots. Moreover, when the NO_3/NH_4 proportion expanded from 0.1 to 3.0 increase the quantity of roots/explants and decline both KNO_3 and NH_4NO_3 concentrations is conceders the definitive factor for enhancing the root establishing rate (25).



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