CHIA SEED AS A SOURCE OF *IN VITRO* ESTABLISHMENT OF *Salvia hispanica* L. PLANTS

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ABSTRACT

Technique of tissue culture for Chia (*Salvia hispanica*) micropropagation was achieved, this study investigated the impact of various concentrations of plant growth regulators on shoot multiplication and root induction with the Chia's mature seed as a source explant. The highest percentage of shoot formation (80%), shoots number per explant(3.20) and shoot length(3.26 cm), were recorded on MS medium enriched with BAP(1.0 mgl^{-1}) after eight weeks of seed culture. The optimal medium for the rhizogenesis was achieved on half strength MS medium fortified with 1.0 mgl^{-1} IBA after four weeks of culture, which had the highest rooting percentage (100%) with highest mean of roots number (5.6 roots per shoot) with (3.40 cm root length). The rooted plants were successfully adapted *ex vitro* with a survival rate of 85%.

Keywords: micropropagation, plant growth regulators, MS , IBA , BAP, Shoot

مجلة العلوم الزراعية العراقية -2020 :51 (3):976-981 **الدباغ وصالح**

الإكثار السريع لنبات الشيا *Salvia hispanica* خارج الجسم الحي

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المستخلص

أجريت هذه الدراسة بهدف إيجاد برنامج متكامل لإكثار نبات الشيا خارج الجسم الحي باستعمال تقانة زراعة الأنسجة النباتية. درِسَ تأثير منظمات النمو النباتية بتراكيز مختلفة في مراحل النشوء, التضاعف والتجذير مع تحديد الظروف الملائمة لإنجاح نقل النباتات النسيجية الى تربة الحقل. أمكن التوصل الى تحفيز التضاعف الخضري وتكوين فروع عدة من أصل قمة نامية بزراعتها في وسط غذائي مجهز بـ 1 ملغم/لتر BAP, إذ بلغت نسبة نشوء الفروع 80%, معدل عدد الفروع 3.20 فرع/قمة نامية بمعدل طول 3.26 سم. أما عملية التجذير, فقد تمت بزراعة الفروع المكثرة على وسط MS بنصف قوة أملاحه مضافاً له 1 ملغم/لتر IBA وأعطت أعلى نسبة للتجذير ومعدل عدد وطول للجذور بلغت 100%, 5.60 جذر/فرع و 3.40 سم بالتتابع. أشارت نتائج أقلمة النباتات المجذرة الى نسبة بقاء بلغت 85%.

الكلمات المفتاحية: الأكثار الدقيق, منظمات النمو النباتية, MS , BAP , IBA , الأفرع

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INTRODUCTION

Salvia hispanica L., popularly known as Chia, is an annual oil seed crop, a flowering plant in the mint family (Lamiaceae), native to northern Guatemala and Southern Mexico (2 , 5). The *Salvia* genus consists of more than 900 perennial or annual species that are used for therapeutical, nutritional or decorative purposes (4,17). In Mexico, since ancient times, Chia seed have been planted but it has been increased in all worldwide (Southeast Asia, Caribbean, Australia…etc.) because is a good source of unsaturated fatty acids, proteins and high quantities of natural antioxidants like bioactive peptides and phenolic compounds (3, 12, 13, 22). The clean and dry Chia seed can be kept for several years because it has antioxidant compounds that prevent deterioration of integral oils, when these seeds placed in water, they exude a mucilaginous polysaccharides compounds which have interesting properties for care, food and pharmaceutical industries (8, 14, 24). Pharmacological and high nutritional value of Chia seeds create interest to be investigated opportunities the species to be micropropagated by tissue culture technique (10,15). Therefore, the aim of current study is to establish an efficient and simple micropropagation method by evaluating the response of different growth regulator concentrations.

MATERIALS AND METHODS

This study was implemented from May 2018 to March 2019 in the laboratory of plant tissue culture, Department of Plant Genetic Resources, Ministry of Agriculture, Baghdad**.** *Salvia hispanica* L. seeds were collected from the plants growing at Baghdad gardens. Authentically was confirmed by the Iraqi National Herbarium.

Disinfection and establishment

After transferring to laminar air flow chamber, seeds of Chia (Figure 1A) were cleaned by removing all the damaged and impurities seeds, then surface disinfected by immersion for 10 minutes in a solution of Clorox (4%active chloride) plus 1 drop of tween-20, followed by 3 rinses with sterile double-distilled water (each 3 minutes). The sterilized seed were inoculated in test tubes (25×100 mm) on Murashige and Skoog (16) medium supplemented with 3% sucrose. The inoculated seeds were maintained in the culture room at a temperature of 24±1ºc and with 8/16 hours dark/light cycles supplied by cool-white fluorescent lamps.

In vitro shoot multiplication

In order to optimize an efficient method for shoot multiplication, an experiment was designed to investigate the influence of different concentrations as well as presence of two cytokinins involving BAP and Kinetin. For this purpose, shoot tips from 4 weeks old seedlings were aseptically cut off and cultivated on solid medium of MS supplemented with BAP and Kinetin by adding them independently at various concentrations (0.5, 1.0, 1.5 mgl^{-1}). In the primary culture and after 2 successive subcultures (4 weekly intervals), we evaluated the multiplication potential of shoot tips by measuring the following parameters:

1- The percentage of shoot tips producing shoots.

2- The multiplication rate which refers to the mean number of shoots per shoot tip at the end of the culture period.

3- The average length of shoots after two successive subcultures (four weeks each).

In vitro shoot rooting

The second experiment was carried out to evaluate the performance of two auxins including IBA and NAA with their varied concentrations for shoot rooting. For root induction, the elongated shoots (>4.0 cm in length) were placed on 0.5 strength MS medium fortified with 3% sucrose, 0.7% agar and IBA (0.5, 1.0, 1.5 mgl^{-1}) or NAA (0.5, 1.0, 1.5 mgl^{-1}).

Data were recorded on:

1- Rooting percentage

2- Mean number of roots per plant

3- Root length (cm) after four weeks of culture

For all mentioned experiments, the pH of the media was adjusted to 5.7 and then autoclaved at 121℃ for 15 min at 15 psi. The culture were maintained in culture room conditions (16/8 hours of photoperiod, temperature was sustained at 24±2℃ and 60% relative humidity). Notes were recorded every week and analyzed statistically.

***Ex vitro* acclimatization:** Regenerated plantlets with well-developed roots were

carefully removed from the culture jars and their roots were rinsed gently with tap water. Then they were transferred to plastic pots having a mixture: organic peat moss and soil (ratio 2:1, v/v.). The plants were transplanted into green house and enclosed with transparent polythene membrane to avoid rapid dehydration and ensure high humidity levels (90%). Potted plants were watered with ½ strength MS solution tow times weekly and after 3 weeks, the polythene membrane were opened. After 8 weeks of adaption, calculated the survival rate of acclimatized plants.

Statistical analysis

Both the shoot multiplication and shoot rooting experiments were laid out in the Completely Randomized Design (CRD). All the treatments were replicated twice, per replication, ten culture test tubes were used. The data were analyzed using SPSS 16 software, and differences among means of treatments were compared by using Fisher's Least Significant Differences (LSD) test as significant at $p \leq 0.05$ (20).

RESULTS AND DISCUSSION

Seed surface sterilization and *in vitro* germination

In this study, an axenic cultures of Chia with low levels of bacterial and fungal contamination (about 3%) was established by using:

1- Seed initial surface sterilization (NaOCl 4%, for 10 minutes).

2- Three rinses each 3 minutes in sterile double-distilled water due to the sticky gel that coated Chia seeds which requires reducing the wash duration time.

The sterilized seeds were germinated after 4 weeks of culture, shoot tips were cut off from the *in vitro* seedling and used as a source of explants.

***In vitro* shoot multiplication**

The micropropagation technology is considered to be the most efficient technique to get large number of plants in a continuous process under controlled conditions with irrespective of weather. The type and concentration of used plant growth regulators influence the efficiency of propagation, so and in order to optimization of medium for shoot multiplication, shoot tips were laid onto multiplication medium fortified with different plant growth regulators such as BAP and Kin (Tables 1,2). All shoot tips produced shoots on all concentrations of both cytokinins used (BAP, 0.5: 1.0: 1.5 mgl^{-1}) (Kin, 0.5: 1.0: 1.5 mgl^{-1}). Shoots were grown directly from shoot tips, the highest percentage of shoot formation(80%), shoots number per explant (3.20) and shoot length (3.26 cm) were obtained when the shoot tips were cultured on MS medium supplemented with BAP(1.0 mgl^{-1}) (table1) (figure 1B). Shoot formation was also obtained of media supplemented with Kin(1.0 mgl^{-1}) (Table 2) were maximum of 40% which was lower than those on BAP, also the shoots number per explant (1.00) and shoot length (1.86 cm) were lower if compared to the same BAP concentration (1.0 mgl^{-1}).

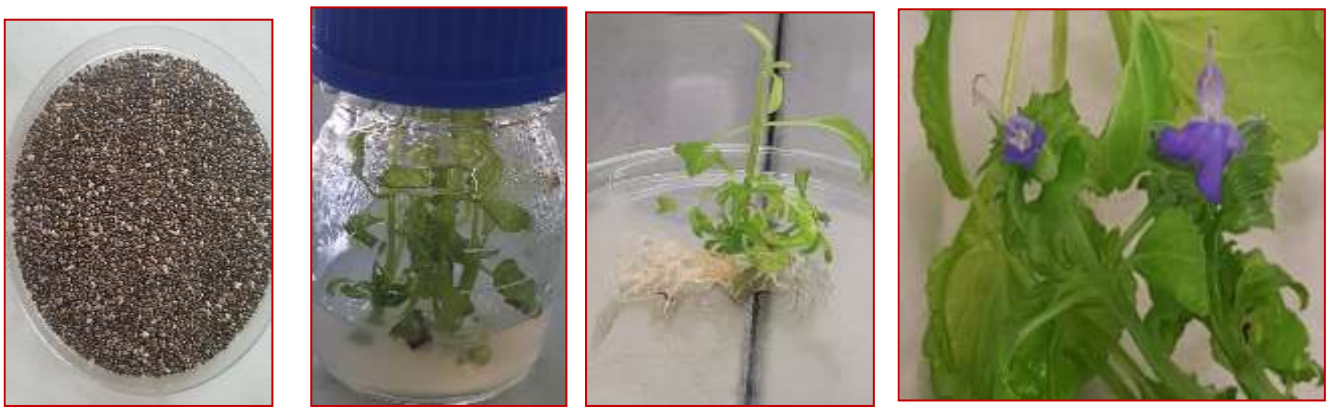

Figure 1. Micropropagation of *Salvia hispanica*

A: Chia seed, B: *in vitro* shoot multiplication on MS medium supplemented with 1.0 mgl^{-1} BAP

C: *in vitro* rooted shoot on ½ MS medium supplemented with 1.0 mgl^{-1} IBA, D: acclimatized plant with flowers

Table 1. Effect of various concentrations of BAP on shoot multiplication from shoot tips in *Salvia hispanica*

Concentrations (mgl^{-1})	Shoot Formation%	Number of shoots per explant	Shoot height (cm)
0.00	0.00 C	0.00 B	0.00 B
0.50	40.00 B	0.80 B	0.70 B
1.00	80.00 A	3.20 A	3.26 A
1.50	60.00 AB	0.80 B	2.30 A

Various letters indicate significant differences evaluated by the Fisher LSD test p≤0.05

Table 2.Effect of various concentrations of Kin on shoot multiplication from shoot tips in *Salvia hispanica*

Concentrations (mgl^{-1})	Shoot Formation%	Number of shoots per explant	Shoot height (cm)
0.00	0.00 B	0.00 B	0.00 B
0.50	20.00 AB	0.40 AB	0.78 AB
1.00	40.00 A	1.00 A	1.86 A
1.50	20.00 AB	0.60 AB	0.74 AB

Various letters indicate significant differences evaluated by the Fisher LSD test p≤0.05

In this experiment, BAP proved to be most affective cytokinin than Kin for *in vitro* shoot multiplication and it has been reported to be the most efficient plant hormone on some species of *Salvia* genus. In 2008, (6) mentioned the necessity of BAP in *Salvia officinalis* culture media for shoot proliferation and multiplication and showed that the best level to be supplemented with MS medium depends on the endogenous concentrations of cytokinin. Also, Huang *et al* (9) demonstrated that the presence of 1 mgl^{-1} BAP in *Salvia chamelaegnea* multiplication media enhanced the shoot multiplication rate. Similar results have been recorded on *Salvia santolinifolia* by (23), they found out that the shoots multiplicity on different concentrations of BAP were higher as compared to the once multiplicated on Kin medium.

***In vitro* shoot rooting**

Regenerated plantlets of *Salvia hispanica* more than 4cm in length were cultured on half strength MS fortified with auxins IBA, NAA for induction of roots (Tables 3, 4). In the current study, no rooting response was achieved on half strength MS media devoid of auxins. Observed the initial root formation 21 days after transferring to the rooting medium, 0.5 MS medium supplemented with 1mgl^{-1} IBA induced the Best rhizogenesis, where 100% of plantlets produced roots with highest number of healthy roots(average 5.6 roots per plantlet) with (3.40 cm root length)(Table3) (Figure 1C). Treatment with IBA has been previously recorded in *S. hispanica* by (25), who mentioned that the highest percentage of plant rooting was achieved on 0.5 MS medium supplemented with 0.1 mgl^{-1} IBA after 4 weeks of culture.

Table 3. Effect of various concentrations of IBA on shoot rooting of *Salvia hispanica* micropropagated plants

Concentrations (mgl^{-1})	Rooting %	Number of roots per shoot	Root length (cm)
0.00	0.00C	0.00C	0.00C
0.50	60.00 B	2.20B	1.64B
1.00	100.00 A	5.60A	3.40A
1.50	80.00 AB	3.40 B	2.14B

Various letters indicate significant differences evaluated by the Fisher LSD test p≤0.05

Table4. Effect of various concentrations of NAA on shoot rooting of *Salvia hispanica* micropropagated plants

Concentrations (mgl^{-1})	Rooting %	Number of roots per shoot	Root length (cm)
0.00	0.00B	0.00B	0.00B
0.50	40.00 AB	1.00AB	0.84AB
1.00	60.00 A	1.60A	2.02A
1.50	40.00 AB	1.20AB	1.12AB

Various letters indicate significant differences evaluated by the Fisher LS D test $p \leq 0.05$

Furthermore, it is very clear from Tables 3 and 4 that the regenerated shoots of *Salvia hispanica* which laid onto medium fortified with IBA (1 mgl^{-1}) exhibited the maximum rooting percentage (100%) compared to those cultured with same concentration of NAA (60%) and average number of roots (1.6) with (2,02 cm) (Table 4). Present findings of this experiment is strengthened by the work of Arikat *et al* (1)who observed a high rooting percentage in *S. fruticosa* with 2.7 µM of IBA compared to those cultured with NAA. A number of internal and external factors are affected the process of the formation of adventitious root, among the internal factors, the auxins group have the main and most important role in rooting initiation (7). In plants, auxins control growth and development, involving the root initiation and response of root gravity (11). Chhun *et al* (21) have shown that the increased initiation of lateral roots is resulted by the exogenous application of auxins and the development of lateral roots be dependent on auxin transport.

***Ex vitro* acclimatization**

Salvia hispanica rooted plants were successfully acclimatized (Figure 1D) with 85% survival. The most important factors for the *ex vitro* acclimatization are the well-developed root system and the humidity reduction, which enhance the plants to synthesize more epicuticular wax to support the survival success during *ex vitro* acclimatization (19). Furthermore, reduction the micronutrients by testing ½ strength MS media during *in vitro* root induction, utilizes and avoids the salt complex accumulation by guard cells in the stomata. Hence, the transferred plantlets show high levels of water in the leaves due to minimal water loss by stomata (18).

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