Iraqi Journal of Science

ISSN: 0067-2904

In Vitro Regeneration of *Pimpinella Anisum* L. Using Different Plant Growth Regulators

Ansam Ghazi Abdulhalem*, Fatma H. Ghani, Noora M. Mohsin
Department of Biology, College of Science, Baghdad University, Baghdad, Iraq

Abstract

Plant regeneration protocols were developed for medicinally important anise (*Pimpinella anisum* L.) that successfully achieved from seeds. Seeds were sterilized and inoculated on Murashige and Skoog (MS) medium with and without gibberellins (GA_3) until full germination. The highest percentage of germination (100%) was recorded on MS medium treated with 2.0 mg/L GA_3 after 7 days. For shoot proliferation, different concentrations of 6- benzyl adenine BA (1, 1.5, 2 mg/L) were used. To enhance shoot induction, 0.1 mg/L of naphthalene acetic acid (NAA) and 0.01 mg/L of thidiazuron (TDZ) were tested along with BA. Direct regenerated shoots were obtained on MS medium supplemented with BA alone (2mg/L) which gave (7shoot/explant), while the presence of NAA and TDZ at low concentration encourage of somatic embryogenesis. A single stem explant was able to produce 25 micro shoots after (24) days of culture. Rooting was experimented at different levels of NAA (0, 0.5, 1, 1.5 mg/L). The medium that devoided from growth regulator and the media with the lowest level of NAA were the most effective in inducing root. Finally, it was concluded that cytokinins are essential for inducing the direct regeneration and the addition of auxin along with there is necessary for the induction of somatic embryogenesis.

Keywords: *in vitro* regeneration, callus, BA, NAA, TDZ, medicinal plants, *Pimpinella anisum*

إكثار نبات *Pimpinella anisum* L. خارج الجسم الحي باستعمال منظمات نمو نباتية مختلفة

أنسام غازي عبد الحليم *، فاطمة حيدر غني، نورا محمد محسن
قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة

تمّ تطوير بروتوكولات تنمية لنبات الينسون *Pimpinella anisum* L ذو الاهمية الطبية بصورة ناجحة خارج الجسم الحي. عقمت البذور و وضعت على الوسط الزرعي موراشيغ و سكوغ (MS) مرة بوجود الجبرلين$_3$ (GA_3) و بدونه مرة اخرى لحين الانبات الكامل للبذور. اعلى نسبة انبات تم الحصول عيها (100%) في وسط MS المعامل بـ (2.0 ملغم/لتر) GA_3 بعد سبعة ايام من الزراعة. لتكوين النبيتات, استخدمت عدة تراكيز من بنزل ادنين BA (1, 1.5, 2 ملغم/لتر) و لتحسين نشوء الافرع , اختبرت مع BA, 0.1 ملغم/لتر من نفثالين حامض الخليك (NAA) و 0.01 ملغم/لتر من الثيدازورون (TDZ). تم الحصول على تكوين مباشر للأفرع الخضرية على الأجزاء النباتية في الوسط MS مزود فقط بالبنزل ادنين (2 ملغم/لتر) الذي اعطى (7 افرع / جزء نباتي), بينما شجع وجود حمض النفثالين و الثيدازورون بتراكيز قليلة

*Email: ansamghazi@yahoo.com

على تكوين الاجنة الخضرية. قطعة ساق واحدة كان لها القدرة على انتاج 25 فرع صغير بعد مرور (24) يوم من الزراعة. اجريت عملية التجذير باستعمال عدة تراكيز حمض النفثالين (0, 0.5, 1, 1.5 ملغم/لتر) كان الوسط الغذائي الخالي من منظمات النمو و الحاوي على اوطئ تركيز من حمض النفثالين الأفضل في تكوين الجذور. اخيرا, يستنتج من ذلك ,ان السايتوكاينينات تعد العامل الرئيسي في التكوين المباشر للافرع الخضرية على الاجزاء النباتية وان إضافة الأوكسين معها ضروري لتحفيزها في تكوين الاجنة الجسمية.

Introduction

Iraq, like any other country in the world, characterizes with two communities: the urban and the rural; Each of these communities depends to a great extent on the rich folk heritage of handling with medicinal plants for disease treatments, and therefore folk medicine is practiced largely by the people in these communities. Iraq has more or less than 363 medicinal plant species belonging to approximately 270 genera from about 98 families. Folk medicine in Iraq is back to the Sumerian and Babylonian era [1].

Over the past decade, Folk medicines have become a topic of global value. Now a day, a large ratio of the population depends largely on folk remedies and medicinal herbs in many developing countries to meet the needs of primary health treatment. Although modern medicine is available in these countries, herbal medicines have often maintained their popularity for many reasons: cultural and historical. Simultaneously, in developed countries, numerous people have begun to resort to medicinal herbs as alternative or complementary therapies [2]. Safety insurance, medicinal plant quality, and efficacy and herbal products have become a major issue in both industrialized and developing countries. Both professionals of health care and public consumers need to be updated about the safety and effectiveness of medicinal plants [3].

Now a day, tissue culture is one of the most important techniques used for rapid and large scale clonal propagation in short regeneration time and small space. It is important for the basic and applied areas of botany, such as nutrition, cytology, embryogenesis, morphogenesis, genetic manipulation, generation of pathogen-free plants that tolerate to herbicide resist to pests and diseases, production of edible vaccines or other new products and useful metabolites with quality improvement and production of plant transformation [4-6]. By germplasm conservation, rare plants and plants free of seeds or needed pollinators to form seeds are multiplied and such plants are saved. *In vitro* stock can be quickly proliferated as it is season independent and long term storage of valuable germplasm possible. Tissue culture is also used as a method to develop many species with tolerance capacities to stress [7].

Many studies were conducted to evaluate the *in vitro* production of medicinal and other plants *in vitro* [4, 8-9].

P. anisum (anise), belongs to Apiaceae family, is an annual grassy. It is about 30–70 cm in high, has very small white flowers, and small greyish-green to greyish-brown seeds, which distributes in the Eastern Mediterranean Region, Egypt, Spain, West Asia, the Middle East and Mexico [10]. This genus contains 23 species, 3 of which are endemic in Iraq [11]. It is the oldest and most important medicinal plants in the world. Its active compounds are utilized in varied pharmaceutical and food industries [12].

In anise, the time of sowing to seed ripening takes 4-5 months: Germination usually occurs from 17 to 25 days, the emergence of stems takes 35-40 days after germination (slow initial growth). Flowering usually starts after 65-75 days of germination, while the period of flowering and seed development is 20-25 days; the time varies from sowing to flowering, depending on the date of cultivation [13]. The plants that cultured in March needed 88-102 days to grow and needed 175-200 days when cultured in November, indicating that anise needs for flowering long photoperiodism. Seed ripening is uneven.

In folk medicine, anise has been used as an appetizer and stimulant for vital organs such as lungs, liver, heart, brain and as a used diuretic drug. It has many therapeutic effects on many conditions such as respiratory, digestive, gynecological and neurological disturbances [14]. Several properties like aromatic, carminative, antimicrobial [15], antifungal [10,16], antiviral, antioxidant [17], anticancer [18], anti-convulsive and analgesic effect, muscle relaxant, in addition to the variant effects on gastrointestinal duct have been recorded of anise. Also, it has useful effects on menopausal hot flashes

and dysmenorrhea in women and in reducing morphine dependence. In diabetics, aniseeds viewed hypoglycemic and hypolipidemic effect as well as depressed lipid peroxidation [19].

Thus, the aim of the present research is to plant an efficient micropropagation protocol suitable for large scale cloning of the anise plant by using seeds through direct regeneration or somatic embryogenesis.

Materials and methods

Preparation of culture medium

To prepare the culture medium, sucrose (30) g/L and the plant growth regulators (BA, NAA, and TDZ) at different concentrations were added to (4.9) g/L of MS medium. NaOH or HCl (1N) was added to adjust the pH to 5.8 and then (7) g/L of Agar-Agar was added. Components were dissolved in double distilled water at a required volume, put on a hotplate magnetic stirrer until boiling, and then 10 ml of the medium was poured into each vial. Culture medium was sterilized by using the autoclave at 121°C and 1.04 $kg.cm^2$ for 17 minutes. The vials were left to cool at room temperature and became prepared to culture the explants.

Plant material and culture conditions

Anise seeds were obtained from the local herbalist in Baghdad. They were raised with tap water for 30 min and then transferred to the air flow cabinet, sterilized with 70% ethanol for 30 sec, submerged in 2% sodium hypochlorite (NaClO) for 20 min [20] and then were washed three times with sterile distilled water for removing all the residues of chloride. Seeds were inoculated on culture medium under sterile conditions and incubated in a growth incubator at 25 ± 2°C in dark until full germination and then transferred to light conditions under 16 h. illumination (2000 Lux, daylight fluorescent tubes).

In vitro seeds germination

Firstly the viability of seeds was examined. For this purpose, petri-dishes were used with the addition of distal water under laboratory conditions.

Sterilized seeds were cultured on MS medium the half strength solid. Hormone – free medium and MS medium provided with 2.0 mg/L GA_3 were used for seed germination. Seeds were kept under dark at 21±2 °C until full germination. Treated seeds were cultured separately on the surface of MS medium for 14 days in the incubator under dark conditions. Finally, the date and percentage of germination in each treatment were recorded. 20 replicates for each treatment were used.

Shoot formation and plant regeneration

For establishment massive mother stocks, micro shoots (1.5-2 mm) of anise were grown on MS medium provided with 6-furfuryl-aminopurine (kinetin; Kin) at 0.5 mg/L, and 0.1 mg/L NAA [21] under aseptic conditions. Mother stock cultures were kept in growth incubator for 6 weeks, overall performance of the cultures and growth was monitored and then used in the following stages of culture. 20 replicates were used.

For the shoot regeneration experiment, two explants were used: leaves and stem. The leaves had no responses in all treatments. About 1.0 cm stem segments were cultured horizontally on MS medium containing BA at different concentrations (1.5, 2 mg/L) alone and in combination with 0.05 mg/L TDZ and 0.1 mg/L NAA as shown in the Table-1. 10 replicates for each treatment were used.

Table 1-Components of five medium protocols used for shoot formation.

protocol	Shoot regeneration (Protocol components) MS medium with growth regulators (mg/L)		
	BA	NAA	TDZ
A	1.5	-	-
B	2.0	-	-
C	1.0	0.1	0.05
D	1.5	0.1	0.05
E	2.0	0.1	0.05

Root initiation

For rooting experiments, shoots were planted on MS medium devoid from hormone for one week to remove any excess effect of any hormone that might adversely affect rooting. To motivate rooting, individual shots (2 cm long) were separately transferred to MS rooting medium contained different concentrations (0.5, 1.0 and 1.5 mg/L) of NAA. One set of cultures were cultured on basal hormone-free MS media as a control. Cultures were assessed for rooting (number and length of roots) per explants after six weeks. 10 replicate for each treatment were used. Plants were transferred to pods containing 1:1 soil and PittMoss for acclimatization and growth under greenhouse conditions.

Results and discussion

Seeds germination

Seeds viability was tested. Result viewed that was viable, and the percentage of viability recorded for the seeds was 85% after 14 days. *In vitro* seeds germination was carried out on MS medium with and without GA_3. The optimal medium was in the present of GA_3. With the addition of 0.5 mg/L of GA_3 on MS medium, marked an increase in the germination percentage was obtained that reached %100 after **7** days as shown in Figure-1, whereas in MS free medium the maximum ratio was recorded % 65 after 15 days of culture. In addition, Figure-2 showed that there was an exponential increased in the length of the aerial part of the plantlets. The results are in accordance with Bae [22] who reported that the increase of GA_3 in culture medium led to a raise germination percentage. Gibberellins induce the synthesis and production of hydrolysis enzyme, chiefly amylase through prompting a range of genes, which are essential for the production α-amylase, proteases and glucanases, resulting in seeds germination [23, 24].

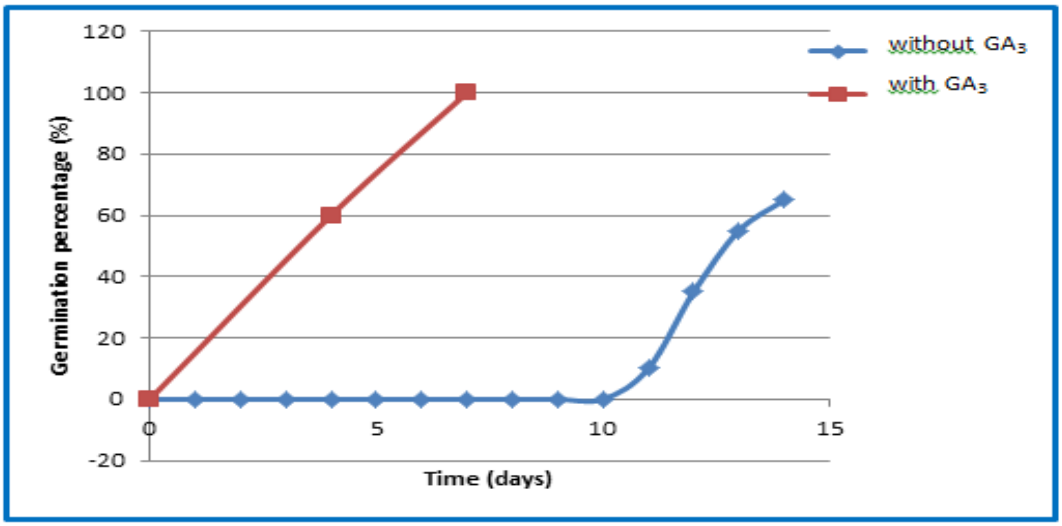

Figure 1-The germination percentage of *P. anisum* seeds on MS medium with and without GA_3

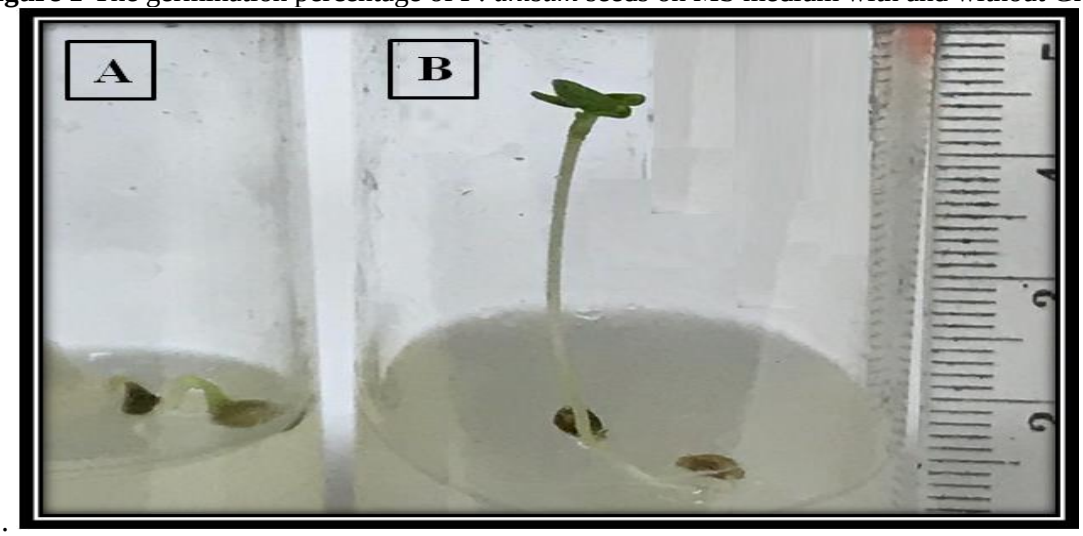

Figure 2-Seeds germination of *P. anisum*: (A) on free MS medium, (B) with 0.5 mg/l of GA_3.

Shoot multiplication and *in vitro* regeneration

Shoot proliferation was successfully obtained when about (2 cm) stem segments were cultured on MS supplemented with Kin at 0.5 mg/L and 0.1 mg/L NAA. The maximum shoot number 6 shoots/explant was obtained that used in the following stages of culture as mother stock culture (see Figure-3).

Growth regulators in different types at different concentrations and different explants (leaves and stem) were used to determine optimal culture conditions to regenerate shoots of *P.anisum in vitro*. Table-2 and Figure-3 showed the growth response of the explants grown on MS medium contained BA, TDZ in addition to NAA. Stem explants were found to be an extra prolific over the leaves. Low shoot regeneration and late response were observed on MS medium provided with BA alone at 1.5 mg/L that may be attributed to the low level of cytokinin that is used, while the increase of BA concentration to (2mg/L) induced the direct regeneration shoot (7 shoots /explants). BA was the best cytokinin for *in vitro* multiplication in most bud cultivation and shoot proliferation in the different type of plant [25-28]. The synergetic effect of auxin and cytokinin has been evaluated in various medicinal plants such as *Santolina canescens* [29], *Bupleurum fruticosum* [30] and *Rotula aquatic* [31]. In addition, auxin at low concentrations has substantially effect on micropropagation [32]. The presence of BA along with NAA and TDZ at (1.0, 0.1 and 0.05 mg/L respectively) or at (1.5, 0.1 and 0.05 mg/L) encouraged of callus proliferation that is developed to somatic embryo after a short period and then gave (24±2 microshoots). Additionally, the combination of TDZ, NAA and BA at concentrations (0.05, 0.1, 2.0 mg/L respectively) initiated callus and shoot (4 shoots /explants). The minimum concentration of TDZ was able to induce all growth parameters to the max when added to the media [21]. The combination of cytokinins and auxins is an essential requirement to stimulate shoot formation from callus because cytokinins can significantly alter the gene expression. These genes are usually regulated by additional stimuli, like auxin and the products of regulated genes have a key role in diverse biological processes such as cell elongation, cell division, protein synthesis and chloroplast development [33]. The endogenous level of growth regulators greatly affects the demand of exogenous growth regulators in the plant system [34] and plays a main role in organ growth and patterning [35].

Table 2-Effect of various types of cytokinins (BA and TDZ) and NAA on shoot formation after 24 days of culture

protocol	Shoot regeneration (Protocol components) MS medium with growth regulators (mg/L)			Response
	BA	NAA	TDZ	
A	1.5	-	-	Late response and low shoot regeneration
B	2.0	-	-	Direct shoot (7shoot/explants)
C	1.0	0.1	0.05	Somatic embryo that gave (24±2 microshoots).
D	1.5	0.1	0.05	Callus andshoot (4shoot/explants)
E	2.0	0.1	0.05	Somatic embryo that gave (20±2 microshoots).

Figure 3-*In vitro* shoot proliferation of *P. anisun* grown on MS medium supplement with **A-** 0.5 mg/L of Kin. **B-** 2mg/L of BA . **C-** 1.5 mg/L of BA.**D-** 1, 0.1 and 0.05 mg/L of BA, NAA and TDZ . **E-**1.5, 0.1 and 0.05 mg/L of BA ,NAA and TDZ . **F-** 2 ,0.1 and 0.05 mg/L of BA, NAA and TDZ.

Root initiation

Rooting of regenerated plantlets is essential to achieve successfully *in vitro* culture. Shoots were transferred onto MS medium contained 0.0, 0.5, 1.0 and 1.5 mg/l NAA for rooting. Results, as shown in Table-3, revealed that optimal root formation was noted on the control medium (free MS medium) where 100% of shoots formed roots after 6 weeks of culture. The mean of roots number 20/explant and root length 15 cm were obtained, while no response occurred in the medium contained NAA. But after 10 weeks, shoots at low concentration of NAA were responded for rooting (results are not shown). It was concluded that the best rooting medium was hormone free MS medium (Figure-4). The results are in accordance with Kozak [35] who recorded the optimal root formation on the control medium and reviewed the same results in other Agavaceae species. Rooted plants transferred to pods under greenhouse conditions showed high survival ratio (80%).

Table 3-Effect of different concentrations of NAA on root regeneration of *P. anisum* after 6 weeks of culture

Concentration of NAA (mg/L)	Root length (cm)	roots number
0	8	20
0.5	0	0
1	0	0
1.5	0	0

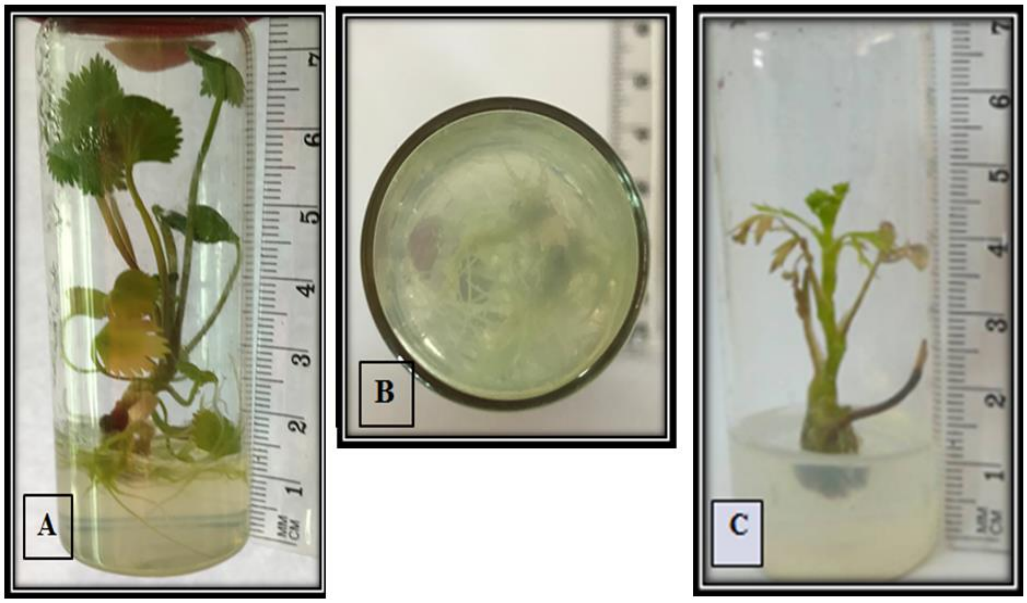

Figure 4-Root induction of regenerated shoots in MS supplicated with NAA after six weeks of *in vitro* culture: A, B at MS free medium; C treated with NAA.

Conclusions

This study showed that the addition of Gibberellins to the culture medium accelerates and encourages seeds germination of anise by increasing the germination percentage and the length of aerial part of plantlets. A comparison of regenerative capabilities of leaves and stem segments of shoots showed that many more shoots and roots regenerated from stems than from leaves. In addition, cytokinins are the main factor in stimulating direct shoot regeneration of shoots. *In vitro* multiplication offers a hopeful method for large scale production of anise. The medium devoid of growth regulators was the most effective in inducing roots.

References

1. Al -Douri, N.A. and Al-Essa, L. Y. **2010**. A Survey of plants used in Iraqi traditional medicine. *Jordan Journal of Pharmaceutical Sciences*, **3**(2): 100-108.
2. Al-Douri, N. A. **2000**. Survey of medicinal plants and their traditional uses in Iraq. *Pharmaceutical Biol*ogy, **38**(1): 74-79.
3. Tyler, V. E., Schultz, V. and Hansel, R. **1998**. *Rational Phytotherapy*. 3rd ed. Berlin. Springer.
4. CHaudhry, Z., Afroz, A. and Rashid, H. **2007**. Effect of variety and plant growth regulators on callus proliferation and regeneration response of three tomato cultivars *Lycopersicon esculentum*. *Pakistan Journal Bot*ony, **39**(3): 857-869.
5. Gaikawd, A.V., Singh, S. K. and Gilhotra, R. **2017**. Plant tissue culture- A review. *SGVU Journal of Pharmaceutical Research & Education*. **2**(1): 217-220.
6. Kim, D. H., Judy Gopal, J. and Iyyakkannu Sivanesan, I. **2017**. Nanomaterials in plant tissue culture: the disclosed and undisclosed. *RSC Advances*, **7**(58): 36492-36505.
7. Satyanarayana, U. **2010**. *Biotechnology*. 6th Ed. Vilayawada, India, Books and Allied Ltd. PP: 497-640.
8. Yusnita, Wahyuningsih, T., Sulistiana, P. and Hapsoro, D. D. **2013**. *In vitro* propagation of *Sansevieria trifasciata* 'Lorentii': shoot regeneration, rooting, and plantlet acclimatization. *Journal of Agronomic Indonesia*, **41**(1): 70-76.
9. Mohapatra, P. P. and Batra, V. K. **2017**. Tissue Culture of Potato (*Solanum tuberosum* L.): A Review. *International Journal of Current Microbiology Application Science*, **6**(4): 489-495.
10. Mohamed, H. S. A. A., Abdelgadir, W. S. and Aisha Z. I. Almagboul, A. Z. I. **2015**. *In vitro* antimicrobial activity of Anise seed (*Pimpinella anisum* L.). *International Journal of Advanced Research*, **3**(1): 359-367.

11. Al -Douri, N.A. **2014**. Some important medicinal plants in Iraq. *ASJ International Journal of Advances in Herbal and Alternative Medicine (IJAHAM)* **2**(1): 10 - 20.
12. Nabizadeh, E., Habibi, H. and Hosainpour, M. **2012**. The effect of fertilizers and biological nitrogen and planting density on yield quality and quantity *Pimpinella anisum* L. *European Journal of Experimental Biology*, **2**:1326-1336.
13. Nimet, K. A. R. A. **2015**. Yield, quality, and growing degree days of anise (*Pimpinella anisum* L.) under different agronomic practices. *Turkish Journal of Agriculture and Forestry*, **39**: 1-9.
14. Ullah, H. **2012**. *Fruit Yield and Quality of Anise (Pimpinella anisum L.) in Relation to Agronomic and Environmental Factors*. Ph. D. thesis, Faculty of Agricultural and Nutritional Sciences and Environmental Management, Justus Liebig University Giessen, Germany. pp: 149.
15. Akhtar, A., Deshmukh, A. A., Bhonsle, A. V. Kshirsagar, P. M. and Kolekar, M. A. **2008**. *In vitro* Antibacterial activity of *Pimpinella anisum* fruit extracts against some pathogenic bacteria. *Veterinary World*, **1**(9): 272-274.
1. 16. Obaid, A. J., Al-Janabi, J. K. A., Taj-Aldin, W. R. 2017. Antifungal activity of Anise Essential Oil against Growth and Morphological Characteristics of *Trichophyton Rubrum*. *Journal of Global Pharma Technology* , **7**(9): 53-68.
16. Jamshidzadeh, A., Heidari, R., Razmjou, M., Karimi, F., Moein, M. R., Farshad, O., Akbarizadeh, A. R. and Houshangi Shayesteh M. R. **2015**. An *in vivo* and *in vitro* investigation on hepatoprotective effects of *Pimpinella anisum* seed essential oil and extracts against carbon tetrachloride-induced toxicity. *Iran Journal of Basiccal Medicinal Science*, **18**: 205-211.
17. Kadan, S. **2013**. Anticancer Activity of Anise (*Pimpinella anisum* L.) Seed Extract. *Nutraceuticals Journal* , **6**(1):1-5.
18. Shojaii, A. and Fard, M. A. **2012**. Review of Pharmacological Properties and Chemical Constituents of *Pimpinella anisum*. *International Scholarly Research Network ISRN Pharmaceutics*, Volume **2012**, Article ID 510795, 8 pages doi:10.5402/2012/510795
19. Tambosi, G. and Rogge-Renner, G. D. **2012**. Evaluation of asepsis, agar concentration and culture medium composition methods for *in vitro* propagation of *Pimpinella anisum* (Linn.) –Apiaceae. *Semina*, **31**(2): 189-194 (abstract).
20. Shibli, R. A., Duwayri, N., Hadidi, N. and AL-Qudah, T. S. **2016**. *In vitro* propagation and cryopreservation by v-cryoplate technique of *Foeniculum vulgare* Mill. *Jordan Journal Aar.Science*, **12**(4):1099-1061.
21. Bae, K. H. **2015**. Improvenment of seed germinaton and *in vitro* propagation of *Bupleurum latissimum* Naxai through embryogenesis. *Turkish Journal of Biology*, **39**: 732-737.
22. Appleford, N. E. J., Lenton, J. R. **1997**. Hormonal regulation of a-amylase gene expression in germinating wheat (*Triticum aestivum*) grains. *Physiological Plantarum* **100**: 534–542.
23. Hopkins, W. G. and Hűner, N. P. A. **2010**. *Introduction to Plant Physiology*. 4th edition, U.S.A., John Wiley & Sons, Inc. E-book.
24. Silveira, D. G., Souza, F. V. D., Pelacani, C. R., Souza, A. d. S., Ledo, C. A. S., Santana, J. R. F. **2009**, Micropropagation and *in vitro* Conservation of *Neoglaziovia variegata* (Arr. Cam.) Mez, a Fiber Producing Bromeliad from Brazil. *Brazil Arch. of Biology and Technology Curitiba*, **52**(4): 923-932.
25. Shibli , R., Duwayri, M., Sawwan, J., Shatnawi, A., and Al-Qudah, T., **2012**. Regeneration via somatic embryogenesis of the endangered wild arum (*Arum palaestinum*). *In Vitro Cellular and Developmental Biology – Plant*, **48**:335-340.
26. Jamous, F. and Abu-Qaoud, H. **2015**. *In vitro* regeneration of tomato (*Lycopersicon esculentum* Mill). *Plant Cell Biotechnology and Molecular Biology*, **16**(3&4): 181-190.
27. Younis, L.; Shibli, R. and Al -Qudah, T. **2015**. *In vitro* propagation and acclimatization of of *Achillea fragrantissima* Forssk Sch. Bip. *Jordan Journal of Agricultural Sciences*. **15**: 339-351.
28. Cascado J. P., Navarro, M. C., Utrilla, M. P., Martinez, A. and Jimenez, J. **2002**. Micropropagation of *Santolina canescens* Lagasca and *in vitro* volatiles production by shoot explants. *Plant Cell Tissue Organ Culture*, **69**:147-153.
29. Fraternale, D., Giamperi, L., Ricci, D. and Rocchi, M. B. L. **2002**. Micropropagation of *Bupleurum fruticosum:* The effect of triacontanol. *Plant Cell Tissue Organ Culture*, **69**: 135-140.
30. Martin, K. P. **2003**. Rapid *in vitro* multiplication and *ex vitro* rooting of *Rotula aquatica* Lour., a rare rhoeophytic woody medicinal plant. *Plant cell rep*., **21**: 415-420.

31. Dakah, A., Zaid, S., Suleiman, M., Abbas, S. and Wink, M. **2014**. *In vitro* propagation of the medicinal plant *Ziziphora tenuior* L. and evaluation of its antioxidant activity. *Saudi journal of Biological Sciences*, **21**: 317-323.

32. AL-Hussaini, Z. A., Yousif, S. H. A. and AL-Ajeely, S. A. **2015**. Effect of Different medium on callus induction and regeneration in potato cultivars. *International Journal of Current Microbiology and Application Sciences*, **4**(5): 856-865.

33. Khan, M. C. P. I. **2013**. *Current trends in Coleus aromaticus: An Important Medical Plant.* Bookango, Blomington. pp58.

34. Zhao, y. **2008**. The role of local biosynthesis of auxin and cytokinin in plant development. *Current Opinion plant Biology*, **11**(1): 16-22.

35. Kozak, D. **2010**. The effect of 6-benzylaminopurine, thidiazuron and the type of explants on *in vitro* propagation of *Yucca elephantipes* Regel . *Acta Science Pol. Hortorum Cultus*, **9**(3): 211-219.