Annals of Agric. Sci., Moshtohor **ISSN 1110-0419**
Vol. 60(4) (2022), 1199 – 1206 **https://assjm.journals.ekb.eg**

Effect of Some Plant Growth Regulators on The Mulltiplication Stage of Strawberry *In Vitro*

Lawand, L. S. H., M.M.ELNagar., M. H. Mohamed., M. E. Ragab and L.A. Badr
Horticulture Dept., Faculty of Agriculture, Benha University, Egypt
Horticulture Dept., Faculty of Agriculture, Ain Shams University, Egypt
Corresponding Author: mahran.elnagar@fagr.bu.edu.eg

Abstract

The present study was conducted at the tissue culture lab. Center of Genetic Engineering and Biotechnology, Faculty of Agriculture, Ain Shams University, Egypt during the period from 2020 to 2022 to study the effect of plant growth regulators and their concentration on the multiplication stage of a strawberry (*Fragaria x ananassa Duch*) cultivars, i.e. Fortuna, through tissue culture technique. Data showed that Fortuna cv. The medium amended with kinetin at concentrations of (5 mg/l) was the best for giving the highest number of shoots/explant, while the highest length of shoots/explant was observed when BA at concentrations of (0.5 mg/l) and IBA at concentrations of (1 mg/l) during the multiplication stage.

Keyword: *in vitro*, tissue culture, shoot multiplication, strawberry, BA, Kinetin, IBA, NAA, IAA

Introduction

Due to its flavor, aroma, and nutritional value, the strawberry (*Fragaria x ananassa Duch*.) is a significant and well-liked vegetable grown in temperate and subtropical climates. It is the berry fruit that is most commonly consumed around the world (**Sultana et al., 2011**). Strawberry is a member of the rose family (Rosaceae) (**Nellist et al., 2019)** Additionally, it contains a lot of vitamins and minerals (**Naing et al., 2019**). Egypt is regarded as one of the top producers and exporters of strawberries in the world, trailing only China and the US with a production value of 597,029 tones and a planted area of roughly 15,345 hectares (**FAO, 2020**).

The strawberry (*Fragaria x ananassa Duch*.) is a well-known vegetable that may be eaten either raw or cooked, and is commonly used in puddings, jams, pies, ice cream, shakes, and fruit juices. Egypt is one of 15 nations that export strawberries, with a value of 2.8% of all fresh strawberry exports valued at about 78.5 million dollars and 12.8% of all frozen strawberry exports valued at about 151.5 million dollars (**worldstopexports, 2020**).

Fungal diseases negatively affect strawberry production in Egypt and cause severe economic losses each year. A technique of plant tissue culture *in vitro* appears as an applicable and efficient method to propagate strawberry plants exposed to many pathogens and insects, which allows for rapid multiplication and preservation of the genetics of virus-free plants under strictly controlled conditions. The advantages of tissue culture beyond preserving genetics are the opportunity to produce many more daughter plants in a short time than conventionally propagated plants.

Additionally, compared to conventionally propagated plants, micro-propagated strawberry plants are thought to have comparative advantages in terms of characteristics including crown size, runner count, blooming period, and strawberry output (**Karhu et al., 2000**). However, problems with different types, especially a hyper-flowering traits, have been reported **(Boxus et al., 1999)**. Limiting the number of subcultures and reducing hormones in the media could treat hyper-flowering problems **(Boxus et al., 1999)**.

Plant tissue culture is the science or art of extracting plant cells, tissues, or organs from the mother plant to grow them on artificial media. Through direct or indirect morphogenesis and somatic embryogenesis, entire new plants can be created from various explants utilizing plant tissue culture techniques.

Meristem cultures, shoot cultures, fetal cultures, and sequestered root cultures are the primary organ culture types utilized in micro-propagation.

The nutritional content varies based on the kinds of cells, tissues, organs, protoplasts, and plant species, even though the basic nutritional requirements of *in vitro* cultivated plant cells are quite similar to those used by plants. Additionally, nutritional needs vary amongst genotypes or cultivars of the same species.

Mineral salts, a carbon supply, vitamins, plant growth regulators, and other organic additions make up a nutrient medium, which is what it is called. Unless otherwise stated, a given medium is recognized by the salt content.

Due to the potential commercial value of strawberries, it is very desirable to create speedy, effective, affordable, and large-scale tissue culture multiplication techniques.

In general, the establishment, proliferation, germination, rooting, growth, and survival of the *in vitro* cultured plant tissues and organs are influenced by a variety of factors, including the energy source (sucrose), the composition of the mineral elements, the strength of the total salt, and the growth regulators auxins and cytokinins (GA_3, IAA, IBA, and BA). Acclimatized plants' ability to grow well depends on the substrate's composition and quality.

Therefore, this study was conducted to investigate the effect of some plant growth regulators on multiplication stage of strawberry.

Materials and Methods

Establishment stage.

Strawberry plants from the variety Fortuna were quickly cultured in jars with (50 ml) of solidified **Murashig and Skoog (MS) 1962**, which contained BA at a concentration of (0.5 mg/l) either singly or in combination with different concentrations of IBA at (1, 2, and 3 mg/l), and BA at concentrations of (1 mg/l) either singly or in combination with different concentrations of IBA at (1, 2, and 3 mg/l), and using three concentration of kinetin at (1, 2.5, and 5 mg/l) as plant growth regulators. After three subcultures of cultivation, the produced shoots of the cultivar (cv. Fortuna) were used in this experiment (figure 1).

Figure 1: Strawberry materials of tissue culture from fortuna strawberry plants *in vitro*.

Culture Media

Murashig and Skoog (MS) (1962) Medium was used in the first experiments. The media were supplemented with minerals, organic substances, and other additions according to the aim of the study in each experiment. 30 g/l of sucrose and 8 g/l of agar were added to all culture media as supplements, and the pH of the MS medium was adjusted to 5.7–5.8 and autoclaved at 121°C for 20 min. (1.06kg/cm2).

Conditions of the incubation.

For each experiment, white fluorescent lamps were used to provide 2000 Lux of light intensity during the establishment, multiplication, and rooting stages, and about 3000 Lux during the acclimatization stage for each experiment's culture, which were kept at a constant temperature of 25°C under a 16/8-hour (light/dark) photoperiod.

Data Recorded

After 3 subculture from incubation the following trails were recorded:

1. Number of shoots/explants.
2. Shoots length (cm).

3.7. Statistical analysis and experimental design

Five replications of each treatment were used in the one-factorial, totally randomized design experiment. Data were statistically examined using the analysis of variance (ANOVA) approach, and Duncan's multiple range test (DMRT) at the 5% level of confidence was used to compare differences between treatment means. **M. L. Tiku (1971)**.

Results and Discussion

This study has been done to determine the best hormone for the multiplication stage for cv. Fortuna with different concentrations of (BA , IBA , and Kin).

1-Effect of (BA) on multiplication stage.

Data presented in table (1) and figures (2 & 3) showed the effect of benzyl adenine (BA) at different concentrations (0.5, 1.0 mg/l) on the multiplication stage of cv. Fortuna strawberry cultivar when grown on MS Medium **Murashing and skoog (1962)**.

Data illustrated that BA at concentrations of (0.5 mg/l) had a high mean of shoots/explant (8.31),

with a high mean length/explant (1.34 cm) (figure 1), and took (11-13) days for shoot formation.

While BA at a concentration of (1.0 mg/l) had the highest mean number of shoots/explant (9.16), with a mean length/explant (1.22 cm) (figure 2), and it took (12-14) days for shoot formation.

In general, when BA was used at the concentration (1.0 mg/l), it induced a higher number of shoots/explants. Meanwhile, the average shoot length was higher when BA was used at concentrations of (0.5 mg/l).

The result was disproved with the result of **Rattanpal et al. (2011), Ashrafuzzaman et al. (2013), and Danial et al. (2016).**

Table 1. Effect of BA on multiplication stage of strawberry.

Growth regulator Concentrations (mg/l)	No. of days to shoot Formation	No. of Shoots/explants	Average Shoot length (cm)
0.5 BA	11-13	8.31±0.2	1.34
1 BA	12-14	9.16±2.1	1.22

Figure 2: Effect of (BA) at (0.5 mg/l) on multiplication of fortuna strawberry cultivar.
A: Before multiplication. B: After multiplication.

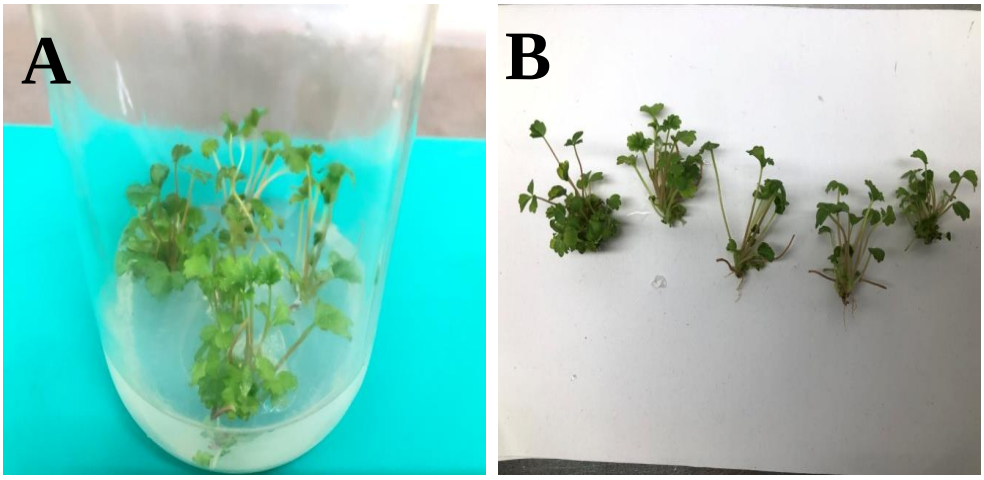

Figure 3: Effect of (BA) at (1mg/l) on multiplication of fortuna strawberry cultivar.
A: Before multiplication. B: After multiplication.

2- Effect of BA combined with different concentrations of IBA on multiplication stage.

Data presented in table (3) and figures (4, 5, and 6) show the effect of benzyladinin (BA) at concentration of (0.5, and 1 mg/l) combined with IBA at three concentrations (0.1, 0.2, and 0.3 mg/l) on the number of days to shoot formation, the number of shoots/explant, and the average shoot length (cm) of cv. Fortuna strawberry cultivar grown on MS Medium.

Data clarified that BA at concentrations of (1 mg/l) combined with IBA at concentrations of (0.1 mg/l) gave the highest mean of shoots/explant (9.9), with a mean length/explant of 1.31 cm), and it took (9-11) days for shoot formation (figure 4). While BA at the concentration (0.5 mg/l) combined with IBA at concentrations of (0.3 mg/l) gave the lowest mean number of shoots per explant (2.8) and had a mean high length/explant (1.55 cm), and it took (11-13) days for shoot formation (figure 5).

The highest shoot length was observed when BA was at a concentration of (1 mg/l) combined with IBA at a concentration of (3 mg/l) which was (1.71cm) per explant (figure 6).

These result were confirmed with the result of **Dogan et al. (2021)** and on the other hand the result are not agree with **Rattanpal et al. (2011).**

Table 2. Effect of (BA) combined with different concentrations of (IBA) on multiplication stage of strawberry**.**

Growth regulator Concentrations (mg/l)	No. of fays to Shoot Formation	No. of Shoots/explants	Average Shoot length (cm)
BA+IBA			
0.5+0.1	10-12	3.78±0.4	1.56
0.5+0.2	11-14	5.65±0.7	1.5
0.5+0.3	11-13	2.80±0.1	1.55
1+0.1	9-11	9.96±1.9	1.31
1+0.2	12-15	5.02±0.5	1.35
1+0.3	9-11	3.17±0.4	1.71

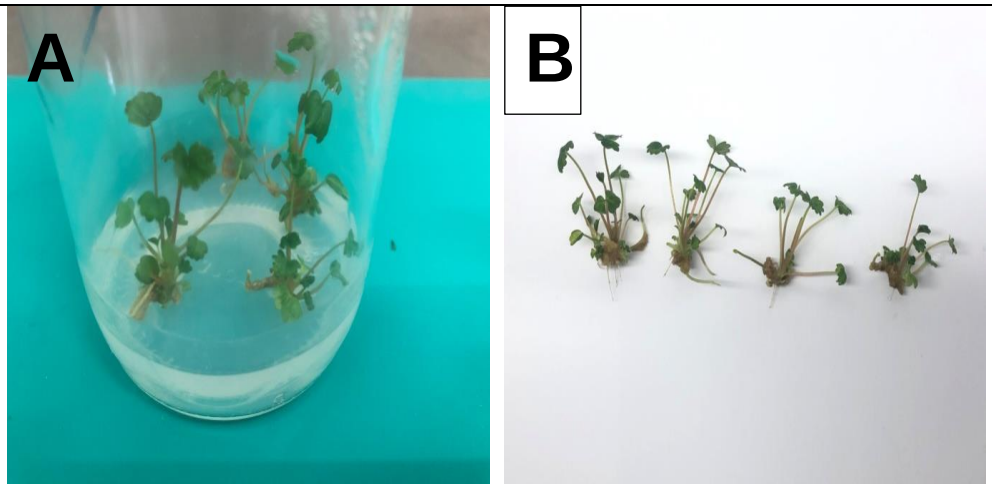

Figure 4: Effect of (1 mg/l BA + 0.3 mg/l IBA) on multiplication of fortuna strawberry cultivar. A: Before multiplication. B: After multiplication.

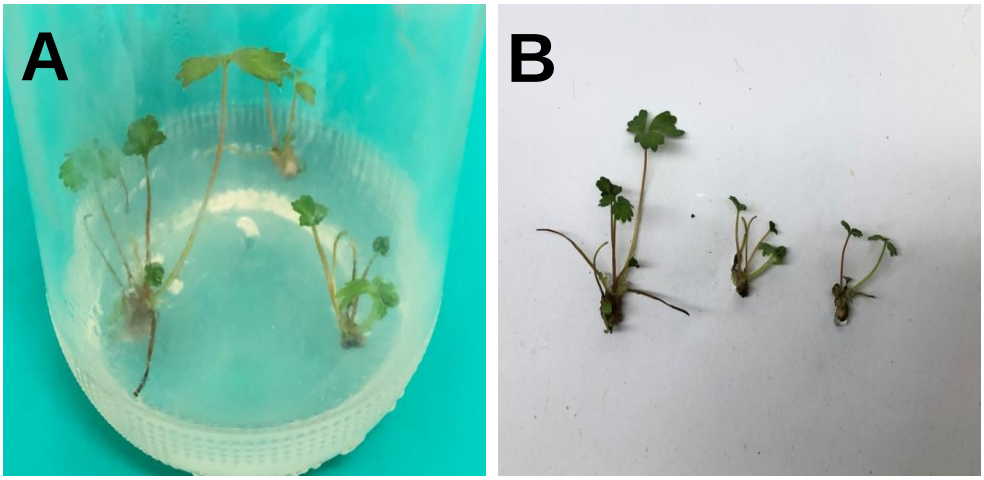

Figure 5: Effect of (0.5 mg/l BA + 0.3 mg/l IBA) on multiplication of fortuna strawberry cultivar. A: Before multiplication. B: After multiplication.

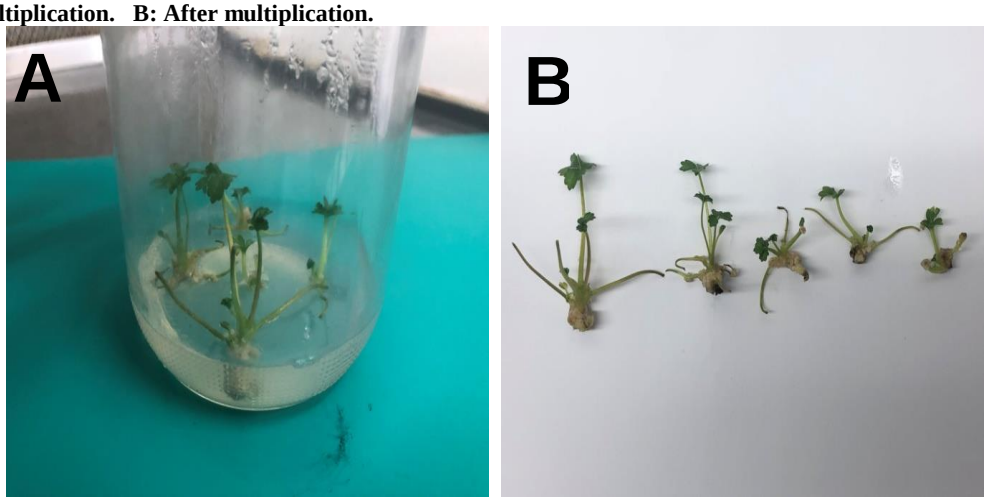

Figure 6: Effect of (0.5 mg/l BA + 0.1 mg/l IBA) on multiplication of fortuna strawberry cultivar. A: Before multiplication. B: After multiplication.

3- Effect of Kinetin on multiplication stage.

Data presented in table (4) and figures (7, 8, and 9) show the effect of different concentrations of kinetin on the multiplication stage of the cv. Fortuna strawberry cultivar grown on MS Medium.

Result indicate that kinetin at concentrations of (5 mg/l) was showed the highest mean number of shoots per plant (12.2), had a mean high length/explants (1 cm), and took (9-11) days for shoot formation (figure 7).

While kinetin at concentrations of (2.5 mg/l) gave mean of shoots per explant (6.30), it gives the lowest mean of shoot length (0.92 cm), and it takes (9-12) days for shoot formation (figure 8). Kinetin at a concentration of (1 mg/l) gave the lowest mean of shoots per explant (3.69), while it gave the highest mean of shoot length (1.32 cm), and it took (11-13) days for shoot formation (figure 9).

In general, the results show that kinetin at a concentration of (5 mg/l) gives the highest number of shoots/plant (12.2) and that (1 mg/l) of kinetin gives the higher length of shoots/explant at (1.32).

In these experiments, we use kinetin at concentrations of 5 mg/l, such as **Sehrawat, et al. (2016)** tested in their experiment, and the results were diverse. The other researchers used kinetin at concentrations of (0.5, 1, 1.5, 2, 2.5, and 3 mg/l). Similar results were found by **Gantait et al. (2010), and Zobayer et al. (2011)**.

Table 4. Effect of kinetin on multiplication stage of strawberry.

Kinetin Concentrations (mg/l)	Days to shoot Formation	No. of Shoots/explants	Average Shoot length (cm)
1	11-13	3.69±0.6	1.32
2.5	9-12	6.30±1.0	0.92
5	9-11	12.2±3.6	1

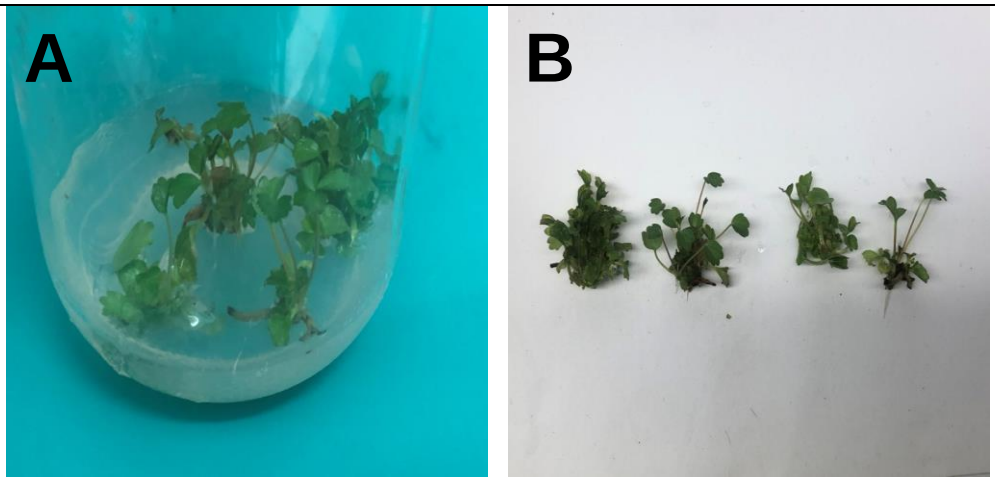

Figure 7: Effect of contain Kinetin at (5 mg/l) on multiplication of Fortuna strawberry. A: Before multiplication. B: After multiplication.

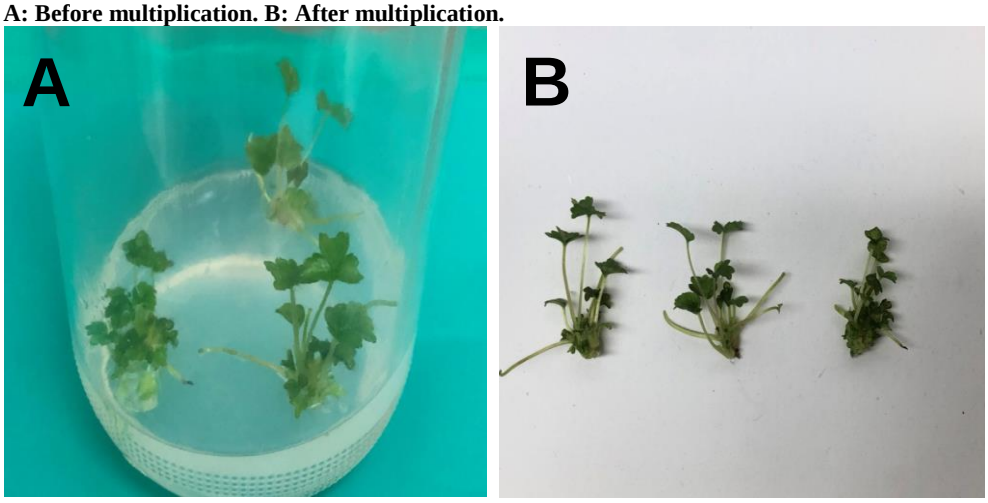

Figure 8: Effect of Kinetin at (2.5 mg/l) on multiplication of Fortuna strawberry cultivar. A: Before multiplication. B: After multiplication.

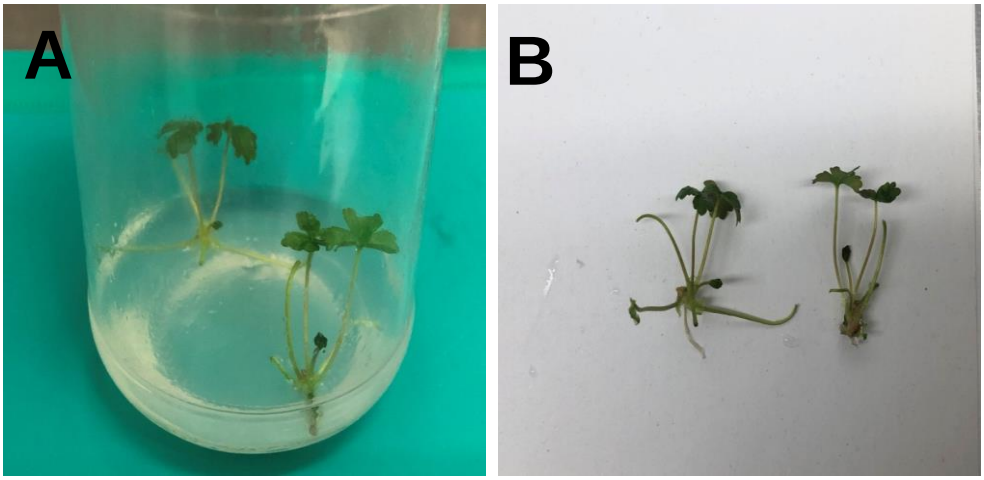

Figure 9: Effect of Kinetin at (1 mg/l) on multiplication of Fortuna strawberry cultivar. A: Before multiplication. B: After multiplication.

Conclusions

A method was developed for rapid shoots multiplication of one commercially important strawberry cultivars, 'Florida Fortuna', by using shoots tip explants. Using this technique, a large quantity of high-quality planting material can be obtained in a short time. With the help of this protocol, more than ten thousand plants can be produced from a single shoot tip within one year.

Among the growth regulators tested, IBA at a concentration of 1 mg/l added to half-strength MS was found to be more suitable and economical for the multiplication stage of strawberry cv. Fortuna.

References

Ashrafuzzaman, M., Faisal, S. M., Yadav, D., Khanam, D., and Raihan, F. (2013). Micropropagation of strawberry (*Fragaria* × *ananassa* Duch.) through runner culture. Bangladesh J.,Agric., Res., 38(3): 467-472.

Boxus, P. H., Jemmali, A., Terzi, J. M., & Arezki, O. (1999, August). Drift in genetic stability in micropropagation: The case of strawberry. In International Symposium on Methods and Markers for Quality Assurance in Micro-propagation. International Soc., for Hort., Sci., 530 (pp. 155-162).

Danial, G. H., Ibrahim, D. A., and Omer, M. S. (2016). Response of running shoot tips of strawberry (*Fragaria* × *ananassa* Duch.) for in vitro propagation in Kurdistan Region of Iraq. Interna., J., Environ., Agric., and Biotech., 1(2): 238516.

Dogan, S., Sahin, M., and Kaya, O. (2021). Researches on the Reproduction of "Ottoman Strawberry" with Tissue Culture. Alinteri J.,Agric., Sci., 36(1): 27-32.

FAO. (2020) Food and agriculture organization of the united nations, report on world-wide crop production, Rome, Italy. https://www.fao.org/faostat/en/?fbclid=IwAR37wQIkBufhOdCJK_dQ7ALG4Iv29tU955C3niTeVi5PiBwvgCly4osgekM#data/QCL

Gantait, S., Mandal, N., Bhattacharyya, S., and Das, P. K. (2010). Sustainable *in vitro* propagation and clonal fidelity in strawberry. Int., J., Plant Dev., Biol., 4, 19-25.

Karhu, S., and Hakala, K. (2000). Micropropagated strawberries on the field. In IV International Strawberry Symposium 567, 321-324).

Naing, A. H., Kim, S. H., Chung, M. Y., Park, S. K., and Kim, C. K. (2019). *In vitro* propagation method for production of morphologically and genetically stable plants of different strawberry cultivars. Plant methods, 15(1): 1-10.

Nellist, C. F., Vickerstaff, R. J., Sobczyk, M. K., Marina-Montes, C., Wilson, F. M., Simpson, D. W. and Harrison, R. J. (2019). Quantitative trait loci controlling Phytophthora cactorum resistance in the cultivated octoploid strawberry (*Fragaria* × *ananassa* Duch.). Hortic., res., Vol 6(2019) 60.

Rattanpal, H. S., Gill, M. I. S., and Sangwan, A. K. (2011). Micropropagation of strawberry through meristem culture. Acta horti., 890, 149-153.

Sehrawat, S. K., Poonia, A. K., Kajla, S., and Bhat, S. (2016). Production of strawberry plant by *in vitro* propagation. Research on Crops, 17(3): 545-549.

Sultana, H., HOSSAIN, M., Zakaria, M., and Bhuiyan, K. A. (2011). Study of the field performance of different planting materials of strawberry (*Fragaria* × *ananassa* Duch.). Ann. Bangladesh Agric. 15(1&2): 101-110

Tiku, M. L. (1971). Power function of the F-test under non-normal situations. *Journal of the American Statistical Association*, *66*(336), 913-916.

Worlds top export. (2020) Top strawberry exported by country. https://www.worldstopexports.com/top-strawberries-exporters-by-country/?fbclid=IwAR2P8rseK0HkR7hfHG4WxIgW3_rNi2fYecqSTPUq4BimBrF3g_2CgQsS_7o

Zobayer, N., Prodham, S. H., Sikdar, S. U., Azim, F., and Ashrafuzzaman, M. (2011). Study of shoot multiplication of strawberry (*Fragaria* × *ananassa* Duch.). Interna., J., Agric., Res., Innovation and Techno., Vol (1: 69-72.

تأثير بعض منظمات النمو في مراحل الاكثار الدقيق لنبات الفراولة

لاوند سعيد حاج علي – مهران مختار النجار – مصطفى حمزة محمد –محمد امام رجب– لطفى عبدالفتاح بدر

قسم البساتين – كلية الزراعة – جامعة بنها

أجريت الدراسة الحالية في معمل زراعة الأنسجة. مركز الهندسة الوراثية والتكنولوجيا الحيوية ، كلية الزراعية ، جامعة عين شمس ، مصر خلال الفترة ما بين (٢٠٢٠–٢٠٢٢) لمعرفة تأثير التركيزات المختلفة من منظمات النمو على الاكثار الدقيق و تكوين الجذور لنبات الفراولة بالإضافة إلى مرحلة الاقلمة التي تم إجراؤها لدراسة افضل طريقة لنقل نبتات الفراولة من المعمل إلى الحقل المفتوح و ذلك على صنف فورتونا. من خلال تقنية زراعة الأنسجة. أوضحت اللنتائج أن الكينتين بتركيز (٥ مجم / لتر) أعطت أعلى متوسط لتكوين الافرع لكل نبات و أن البنزيل ادنين بتركيز (٠،٥مجم / لتر) عند خلطه مع اندول بيورتيك اسيد بتركيز (١مجم / لتر) أ عطت أعلى متوسط متوسط طول لكل نبات.