

#### **Plant Production Science**

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## EFFECT OF CULTIVARS, AUXINS AND ACTIVATED CHARCOAL ON IN VITRO ROOTS FORMATION OF STRAWBERRY PLANTLETS

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**ABSTRACT:** This experiment amid to investigate the effect of two strawberry cultivars (Festival and Sweet Charlie), two auxins, i.e. Indole-3-butyric acid (IBA) and 1-Naphthalene acetic acid (NAA) and activated charcoal on the in vitro roots formation (rooting stage) of strawberry plantlets. The obtained results showed that, Sweet Charlie cultivar being the superior one and recorded the maximum values of both root and shoot length, number of roots and leaves, the fresh and dry weight of roots and leaves of plantlet as compared with Festival cultivar. It is evident that, using ½ MS-medium supplemented with 1.0 mg/l IBA+0.5 g/l activated charcoal being the most effective and superior treatment for increasing both root and shoot length. While, rooting medium contained ½ MS-medium + 1 .0 mg/l IBA or 0.5 mg/l IBA +0.5 mg/l NAA recorded the highest values of number of both roots and leaves per plantlet .In addition ,using ½ MS-medium supplemented with 1.0 mg /l NAA recorded the maximum values of the fresh and dry weight of roots per plantlet. Furthermore, the highest value for each of fresh weight of crown and leaves per plantlet, was obtained by using ½ MS-medium supplemented with 0.5 mg/l IBA+0.5 mg/l NAA. On the other hand, using ½ MS-medium + 0.5 mg /l ÎBA + 0.5 mg /l NAA + 0.5g /l activated charcoal being the superior treatment and recorded the maximum values of nitrogen, phosphorus, potassium, total carbohydrate and total protein content (%). Transfer shoots of Sweet Charlie cultivar to the rooting medium contained ½ MS- medium +1.0 mg/l IBA +0.5 g/l activated charcoal recorded the maximum values of both root and shoot length, number of leaves per plantlet, the fresh and dry weight of leaves, as well as the dry weight of crown per plantlet. While, the highest number of roots per plantlet was more achieve via using ½ MS-medium + 1.0 mg/l IBA. Moreover, cultured shoots of Festival cultivar on ½ MS-medium supplemented with 0.5 mg /l IBA+0.5 mg /l NAA being the most effective and favorable treatment for increasing the fresh weight of roots per plantlet. On the other hand, the highest increase of the fresh weight of crown per plantlet were more distinct via the interaction treatment between such cultivar and using ½ MSmedium supplemented with 1.0 mg/l NAA. Furthermore, using the rooting culture medium contained ½ MS- medium+0.5 mg/l IBA+0.5 g /l activated charcoal recorded the highest value for each of nitrogen and total protein content (%) in the plantlets of Festival cultivar, while cultured shoots of Sweet Charlie cultivar on the same rooting medium, registered the maximum increase of phosphorus, potassium and total carbohydrates content (%).

Key words: Strawberry, Indole-3-butyric acid ,1-Naphthalene acetic acid, activated charcoal, in vitro, roots formation

#### INTRODUCTION

Strawberry (Fragaria X ananassa Duch.) belongs to family Rosaceae. It is a natural hybrid of Fragaria chiloensis Mill., and

\* Corresponding author: Tel.: +201002143287 E-mail address: boba100100@yahoo.com Fragaria virginiana Duch. The Strawberry fruits is delicated in flavor ,texture ,shape, and rich in some vitamins particularly A, B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, C, E and some minerals such as calcium, potassium, copper and iron (Giampieri *et al.*,

**2015).** In addition, fruits are a good source of phylochemical compounds, mainly ellagic acid which have a wide range of biological activity. Strawberry is grown in many regions of Egypt mainly at Beheira, Ismailia, Sharkia and Qalyubia Governorates. The cultivated area of strawberry in Egypt were about 15614.4 faddans with production valued 283471 tons **(FAO, 2014)**. This area of strawberry is in increasing over the years.

Tissue culture technique has been successful on the large scale multiplication of strawberry plants in many countries. This technique can produce several millions of plants in short time from a few mother plants. Beside propagation, tissue culture technique have been used for production of disease resistant plants, and in plant breeding and crop improvement programs (Mohamed, 2007).

Among the pathways for improvement the performance of strawberry plants in tissue cultures, cultivars (Ara et al., 2013; Murti and Yeoung, 2013), some auxins, such as IBA and NAA (Moradi et al., 2011; Badr-ELden, 2013; Harugade et al., 2014). as well as activated charcoal (Erenoglu et al., 1995; Adak et al., 2001; Kaushal et al., 2006; Adak et al., 2009) play a major role in this respect.

The main purposes of this work were to study the effect of two strawberry cultivars, two auxins (IBA and NAA) and activated charcoal on the roots formation of plantlets which cultured *in vitro*.

#### MATERIALS AND METHODS

This work was carried out during the period from 2012 to 2017 in the Tissue Culture Laboratory and Greenhouse of Improvement of the Main Vegetable Crops and Hybrids Production Project (I.M.V.C.H.P.), Horticulture Research Institute (HRI), Agriculture Research Center (ARC), Dokki, Giza Governorate, Egypt, to study the effect of two strawberry cultivars (Festival and Sweet Charlie),two auxins, *i.e.* Indole-3-butyric acid (IBA) and1-Naphthalene acetic acid (NAA) and activated charcoal on the *in vitro* roots formation.

This experiment included 12 treatments which were the combination between two strawberry cultivars and two auxins and activated charcoal treatments, as follows.

#### **Strawberry Cultivars**

- 1. Festival.
- 2. Sweet Charlie.

#### **Auxins and Activated Charcoal**

- 1. Half salts strength of MS (½ MS-medium) + 1.0 mg/l IBA
- 2.  $\frac{1}{2}$  MS-medium + 1.0 mg/l NAA
- 3.  $\frac{1}{2}$  MS-medium + 0.5 mg/l IBA + 0.5 mg/l NAA
- 4. ½ MS-medium + 1 mg/l IBA + 0.5 g/l activated charcoal.
- 5. ½ MS-medium + 1 mg/l NAA +0.5 g/l activated charcoal.
- 6. ½ MS-medium + 0.5 mg/l NAA + 0.5 mg/l IBA + 0.5 g/l activated charcoal.

These treatments were arranged in split-plot design with six replicates. Each replicate contained two glass jars, and each one contained four explants. The strawberry cultivars were arranged in the main plots, while auxins and activated charcoal treatments were assigned randomly in the sub-plots.

Murashing and Skoog (1962) medium (MS) was used. All used shoots in this experiments were obtained from cultured meristems on half salt strength of basal MS-medium (½MS-medium) supplemented with the previouse auxins concentrations (IBA and NAA) and activated charcoal.

#### **Data Recorded**

Data were recorded after 6 weeks of culture *in vitro*, as follows:

#### **Growth Measurements of Plantlets**

- 1. Root length (cm).
- 2. Shoot length (cm).
- 3. Number of roots per plantlet.
- 4. Number of leaves per plantlet.
- 5. Fresh weight of roots per plantlet (g).
- 6. Fresh weight of crown per plantlet (g).

- 7. Fresh weight of roots, crown and leaves/ plantlet were oven dried at 70°C till constant weight according to **Dogras** *et al.*(1991) and the following data were recorded:
- 8. Fresh weight of leaves per plantlet (g).
- 9. Dry weight of roots per plantlet (g).
- 10. Dry weight of crown per plantlet (g).
- 11. Dry weight of leaves per plantlet (g).

#### **Chemical Composition of Plantlets**

Fresh samples of shoots per plantlets were oven dried at 70°C till constant weight according to **Dogras** *et al.* (1991). The dry matter of shoots was finely ground and wet digested with sulfuric acid and perchloric acid (3:1). The following data were determined and recorded:

#### Nitrogen (N) content (%)

It was determined in shoot dry weight according to the methods described by **Bremner** and Mulvaney (1982).

#### Phosphorus (P) content (%)

It was determined according to the methods described by **Olsen and Sommers** (1982).

Potassium (K) content (%): was determined according to the methods described by **Jackson** (1970).

#### **Total carbohydrates content (%)**

Was determined according to the methods described by **James** (1995).

#### **Total protein content (%)**

Was calculated by multiplying total nitrogen x 6.25, as described by **Pregle (1945).** 

#### **Statistical Analysis**

All collected data were subjected to proper statistical analysis using Costat Soft Ware Program. The least significant difference (LSD) test at 0.05 level of probability was used to determine statistically the significance of differences among the compared means of various treatments according to **Snedecor and Cochran (1980)**.

#### RESULTS AND DISCUSSION

#### **Growth Measurements of Plantlets**

#### Effect of cultivars

The presented results in Table 1 show the effect of strawberry cultivars, i.e. Festival and Sweet Charlie on the growth measurements of complete plantlets during rooting stage in vitro. In this regard, Sweet Charlie cultivar being the superior one for increasing length of both root and shoot, number of roots and leaves, fresh and dry weight of roots and leaves per plantlet as compared with Festival cultivar. On the other hand, it was quite clear from such results that, there are no obvious significant differences detected between the two cultivars in their fresh and dry weight of plantlet crown formation. In this connection, Ara et al. (2013) came to similar results on some strawberry genotypes. They concluded that, number of roots/shoots and frequency of roots induction ranged from 78 to 95% according to the genotypes. In addition, Murit and Yeoung (2013) reported that, Red Pearl strawberry cultivar recorded the highest root length as compared with Camarosa cultivar, the superior effect of Red Pearl cultivars, might be due to absorbed more water and reduced transpiration than Camarosa cultivar.

From the forgoing results and discussion, it could be suggested that, strawberry cultivars varied greatly from their response of roots formation per plantlet.

#### Effect of auxins and activated charcoal

As for the effect of the two auxins (IBA and NAA) and activated charcoal on in vitro strawberry plantlet development during rooting stage, the obtained results in Table 2 show that, all treatments had a significant effect on the growth measurements of plantlets, except the dry weight of plantlet crown which did not reflect any significant effect. Using Half salts strength MS-medium  $(\frac{1}{2})$ MS-medium) supplemented with 1 mg/l IBA+0.5 g/l activated charcoal being the superior treatment in increasing root and shoot length of plantlet .In addition, using rooting medium contained ½ MS-medium and supplemented with 1.0 mg/ l IBA recorded the maximum increase in roots and leaves number per complete plantlet, followed by using rooting medium contained ½ MS-medium supplemented with 0.5 mg/l IBA + 0.5 mg / 1 NAA.

Table 1. Effect of strawberry cultivars on the growth measurements of plantlets cultured *in vitro* at 6 weeks of culture

Cultivar	Length		Number per		Fı	esh weiş	ght	Dry weight			
	(cm)		Plantlet			<b>(g)</b>		<b>(g)</b>			
	Root	Shoot	Roots Leaves		Roots	Crown	Leaves	Roots	Crown	Leaves	
Festival	1.19	5.35	6.46	9.53	0.29	0.022	0.30	0.041	0.004	0.02	
Sweet Charlie	3.19	8.40	12.28	12.42	0.41	0.029	0.52	0.071	0.003	0.06	
LSD at 0.05 level	0.41	0.6	1.81	0.95	0.08	NS	0.16	0.01	NS	0.04	

NS: Not significant at 0.05 level of probability

Table 2. Effect of two auxins (IBA + NAA) and activated charcoal on the growth measurements of strawberry plantlets cultured *in vitro* at 6 weeks of culture

Treatments of auxins and activated charcoal		ngth	Numb	er per	Fresh weight			Dry weight			
		(cm)		plantlet		<b>(g)</b>			<b>(g)</b>		
	Root	Shoot	Roots	Leaves	Roots	Crown	Leaves	Roots	Crown	Leaves	
½ MS-medium+ 1.0 mg/l IBA.	1.92	6.54	15.08	11.83	0.16	0.03	0.05	0.02	0.003	0.05	
½ MS-medium + 1.0 mg/l NAA.	1.42	6.54	9.17	11.58	1.14	0.04	0.06	0.13	0.003	0.05	
½ MS-medium + 0.5 mg/l IBA + 0.5 mg/l NAA.	2.13	7.54	12.5	11.75	1.06	0.04	0.08	0.05	0.005	0.08	
1/2 MS-medium + 1 mg/l IBA + 0.5 g/l activated charcoal.	2.78	8.04	8.08	11.42	0.05	0.02	0.06	0.01	0.004	0.07	
1/2 MS-medium + 1 mg/l NAA + 0.5 g/l activated charcoal.	2.41	6.63	5.27	9.92	0.02	0.02	0.04	0.01	0.003	0.04	
½ MS-medium + 0.5 mg/l IBA + 0.5 mg/l NAA+ 0.5 g/l activated charcoal.		5.96	6.10	9.33	0.02	0.01	0.04	0.01	0.003	0.04	
LSD at 0.05 level	0.72	1.05	3.14	1.64	0.28	0.02	0.02	0.06	NS	0.02	

NS: Not significant at 0.05 level probability.

On the other hand, using ½ MS-medium supplemented with 1.0 mg/l NAA being the most effective treatment and recorded the maximum increase of fresh and dry weight of roots per plantlet. Furthermore, the fresh weight of crown and leaves, as well as the dry weight of leaves per complete plantlet were obtained *via* using ½ MS-medium supplemented with 0.5 mg/l IBA + 0.5 mg/l NAA.

As for the role of auxins such as naphthalene acetic acid (NAA) and indol butyric acid (IBA), **Krishnamoorthy (1981)** reported that the stimulatory effect of auxins addition to the culture medium, both roots and shoots formation may be attributed to cell division by mitoses which added new cells and cell elongation of already existing cell by enlargement of the vacuole. In addition, **Torres (1989)** stated that, auxin are required for cell elongation, stimulation of callus induction, cell growth, as well as intiation of shoots and particularly roots.

The obtained results are in harmony with those reported by Moradi et al. (2011) they found that, the highest number for each of roots and root length were obtained from ½ MSmedium contained 0.2-0.5 mg/l IBA. Moreover, Zobayer et al. (2011) revealed that the maximum frequency of rooting and the highest number of roots were obtained on MS-medium contained 1.0 mg/l IBA. Ashrafuzzaman et al. (2013) found that, application of IBA at a concentration of 0.5 mg/l to ½ MS-medium being the best performance of strawberry roots, as well as the highest number of roots of per culture (6) and the longest root (3.05 cm), while half strength MS-medium (1/2 MS-medium) without IBA did not reflect any significant response on the roots induction. In addition, Bader-Elden (2013) reported that, the shoots were transferred to MS-medium supplemented with five concentrations of both IBA or NAA (0.0, 0.5, 1.0 and 1.5 and 2.0 mg/l) individually, showed that IBA at 0.5 mg/l proved to be the most suitable treatment for roots indication (8,4 roots per shoot and the average root length was 7.21cm). Harugade et al. (2014) concluded that, the different concentration of IBA (0.1 and 1.5 mg/l) and 1.0 mg / l IBA being the most superior and suitable treatment for roots induction (5 roots per explant, and the average root length was 3.68 cm).

Furthermore, Sutter et al. (1997), Bhatt and Dhar (2000), Hemant et al. (2001), Elmana et al. (2003), Kaushal et al. (2006), Sakila et al. (2007), Adak et al. (2009), Sutan et al. (2009), Haddadi and Abd El-Aziz (2010) and Hasan et al. (2010) came to similar findings.

Finally, from the previously mentioned results and discussion, it could be suggested that, the depressive or the stimulating effect on the roots formation was differed greatly according to the type of auxin and its concentration in the cultured medium of rooting stage.

As for the effect of activated charcoal, it is quite clear from Table 2 that, addition of activated charcoal in the rooting medium improved the potenial for adventitious rooting, not only in terms of rooting rates, but also in enhancement of the number and length of the roots, as well as roots score per plantlet. This stimulating effect of activated charcoal on roots ability was particularly associated with mature explants. Activated charcoal may affect the activity and/or stability of plant growth regulators by reducing or excluding light in in vitro culture. In addition, activated charcoal can be attributed to removal the inhibitory substance from the medium, or released by the tissue itself (Fridborg et al., 1978). Moreover, activated charcoal may be able to absorb toxic brown or black pigments (phenol like compounds and melanin), as well as other unknown colourless toxic compounds. Also, the obtained results are in agreement with those reported by Lopez-Aranda et al. (1994) who found that addition of 500 mg/l activated charcoal, enhanced roots formation of strawberry. Erenoglu et al. (1995) reported that, the highest number of roots per plantlet was obtained with 0.5 g/l activated charcoal or 1.0 mg/l IBA. Furthermore, Adak et al. (2001) showed that, using activated charcoal was more successful on rooting formation than IBA and NAA. In addition, application of 5.0g/l, activated charcoal had more rooting success than application 1.0 and 2.5g/l activated charcoal. Gautam et al. (2001) found that, the highest roots induction frequency (95.23%) were obtained with ½ MS- mediam +1.0mg/l IBA and charcoal at a rate of 200 mg/l In addition, Mahajan et al. (2001), Kaur et al. (2005), Kaushal et al. (2006) and Adak and

Pekmezci (2011) came to similar conclusion. On the other hand, Nower et al. (2011) reported that, MS-medium supplemented with 0.5 mg/l IBA with activated charcoal being the supperior treatment and recorded the highest increase of number of roots, root length and the fresh weight of plantlet, as compared with the other treatments of unxins (IAA or NAA).

Accordingly, from the above mentioned results and discussion, it may be suggested that, the two factors of study, *i.e.* auxins (IBA and NAA) and activated charcoal exerted a marked and significant effect on roots formation of strawberry plantlets during rooting stage *in vitro*.

# Effect of the interaction between strawberry cultivars, auxins (IBA and NAA) and activated charcoal

The presented results in Table 3 and illustrated in Fig. 1 indicated that, the effect of interaction treatments between strawberry cultivars, different concentrations of the two auxins (IBA and NAA) and activated charcoal caused a significant effect on length of both root and shoot, number of roots and leaves, as well as the fresh and dry weight of roots, crown and leaves per plantlet which cultured in vitro during rooting stage. Transfer shoots of Sweet Charlie cultivar to the rooting medium contained of ½ MS-medium + 1 mg/l IBA + 0.5 g/l activated charcoal recorded the highest value for each of root and shoot length, number of leaves per plantlet, the fresh and the dry weight of leaves, as well as the dry weight of crown. While, the highest number of roots was recorded by culturing shoots of the same cultivar on ½ MS-medium supplemented with 1.0 mg/l IBA, also cultured shoots of the same cultivar on ½ MS-medium + 1.0 mg/l NAA gave the maximum values of roots dry weight. Moreover, the highest fresh weight of each of roots and crown per plantlet was obtained from cultured shoots of Festival cultivar on ½ MS-medium supplemented with 0.5 mg/l IBA + 0.5 mg/l NAA or 1.0 mg/l NAA, respectively. On the other hand, the highest increase of crown fresh weight per plantlet was more distinct via the interaction treatment between such cultivar and using 1/2 MS-medium supplemented with 1.0 mg/l NAA.

Finally, from the previously mentioned results and discussion, it could be concluded that, the depressive or the stimulating effect on the roots formation of strawberry plantlets was differed greatly according to the cultivars, the type of auxin, and its concentration, as well as to the dose of activated charcoal in the culture medium.

#### **Chemical Composition of Plantlets**

#### Effect of cultivars

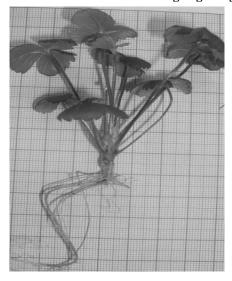
It is quite clear from the illustrated results in Table 4 that, there is no significant differences between the two tested strawberry cultivars on the content of nitrogen, phosphorus, potassium, total carbohydrates and total protein (%) in strawberry plantlets which cultured *in vitro* during rooting stage.

#### Effect of auxins and activated charcoal

Concerning the effect of two auxins (IBA and NAA) and activated charcoal on the chemical composition of the plantlets, the obtained results in Table 5 show that supplementation of ½ MS-medium by different concentrations of two auxins (IBA and NAA) and activated charcoal caused a marked effect on the content of N, P, K, total carbohydrates and total protein (%) of formed plantlets *in vitro*. In addition, using ½ MS-medium supplemented with 0.5 mg/l IBA+ 0.5 mg/l NNA + 0.5 g/l activated charcoal being the superior one and came in the first rank in this respect, followed by using ½ MS-medium supplemented with 0.5 mg/l IBA+ 0.5 mg/l NNA.

### Effect of the interaction between cultivars, auxins and activated charcoal

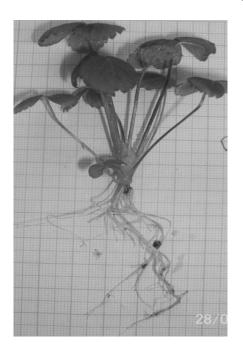
The presented results in Table 6 show that, N, P, K, total carbohydrates and total protein content(%) in produced strawberry plantlets were significantly affected by the interaction between cultivars and different concentrations of the two auxins (IBA and NAA) and activated charcoal added to the rooting medium of strawberry mass propagation *in vitro*. Horeover, sub-cultured shoots of Festival cultivar *in vitro* on rooting medium contained of ½ MS-medium + 0.5 mg/l NAA+0.5 mg/l IBA+ 0.5 gm/l activated charcoal produced the highest value for each of nitrogen and total protein content (%) in the formed plantlets. While, sub-cultured

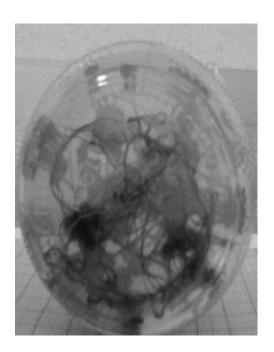




The highest roots number ( $\frac{1}{2}$  MS-medium+ 1.0 mg/l IBA).

Festival cultivar





The highest roots number (½ MS-medium + 1.0 mg/l IBA).

Sweet Charlie cultivar

Fig. 1. Effect of the interaction between strawberry cultivars, auxins (IBA and NAA) and activated charcoal on roots number of strawberry plantlets cultured *in vitro* during rooting stage

Table 3. Effect of the interaction treatments between strawberry cultivars, auxins (IBA and NAA) and activated charcoal on the growth measurements of strawberry plantlets cultured *in vitro* at 6 weeks of culture

Treatment  Cultivar Auxins and activated charcoal		Length (cm)		Number per plantlet		Fresh weight (g)			Dry weight (g)		
Cultival	Adams and activated chareout	Root	Shoot	Roots	Leaves	Roots	Crown	Leaves	Roots	Crown	Leaves
Festival	½ MS-medium + 1.0 mg/l IBA.	6.000	2.0800	11.830	10.830	0.090	0.030	0.250	0.011	0.003	0.039
	$\frac{1}{2}$ MS-medium + 1.0 mg/l NAA.	6.170	1.1700	7.670	10.500	0.050	0.046	0.460	0.047	0.004	0.057
	$\frac{1}{2}$ MS-medium + 0.5 mg/l IBA + 0.5 mg/l NAA.	2.000	2.000	10.330	10.830	1.490	0.039	0.580	0.042	0.005	0.078
	$\frac{1}{2}$ MS-medium + 1 mg/l IBA + 0.5 g/l activated charcoal.	4.420	0.390	2.330	8.000	0.012	0.004	0.097	0.003	0.002	0.022
	$\frac{1}{2}$ MS-medium + 1 mg/l NAA + 0.5 g/l activated charcoal.	4.580	0.740	2.200	8.330	0.006	0.004	0.230	0.003	0.002	0.023
	$1\!\!/_{\!2}$ MS-medium + 0.5 mg/l NAA + 0.5 mg/l IBA + 0.5 g/l activated charcoal.	3.580	0.780	4.370	8.670	0.013	0.008	0.117	0.005	0.003	0.025
Sweet	1/2 MS-medium + 1.0 mg/l IBA.	7.080	1.750	18.330	12.830	0.230	0.026	0.410	0.028	0.003	0.061
Charlie	½ MS-medium + 1.0 mg/l NAA.	6.920	1.670	10.670	12.670	0.770	0.028	0.440	0.216	0.003	0.071
	$\frac{1}{2}$ MS-medium + 0.5 mg/l IBA + 0.5 mg/l NAA.	7.330	2.250	14.670	12.670	0.630	0.039	0.427	0.062	0.005	0.073
	$\frac{1}{2}$ MS-medium + 1 mg/l IBA + 0.5 g/l activated charcoal.	11.670	5.170	13.830	14.830	0.090	0.035	0.672	0.016	0.006	0.115
	$\frac{1}{2}$ MS-medium + 1 mg/l NAA + 0.5 g/l activated charcoal.	8.670	4.080	8.330	11.500	0.042	0.026	0.300	0.007	0.004	0.054
	$1\!\!/_{\!2}$ MS-medium + 0.5 mg/l NAA + 0.5 mg/l IBA + 0.5 g/l activated charcoal.	8.330	4.250	7.830	10.000	0.032	0.021	0.232	0.006	0.004	0.054
LSD at 0.05 level		1.49	1.01	4.44	2.32	0.402	0.025	0.194	0.088	0.002	0.026

Table 4. Effect of cultivars on the chemical composition of strawberry plantlets cultured *in vitro* at 6 weeks of culture

Cultivar	Chemical composition of plantlets (%)									
	N	P	K	Total carbohydrate	s Total protein					
Festival	0.41	0.13	0.63	17.36	2.55					
<b>Sweet Charlie</b>	0.42	0.14	0.64	17.35	2.59					
LSD at 0.05 level	NS	NS	NS	NS	NS					

NS: Not significant at 0.05 level of probability.

Table 5. Effect of auxins (IBA and NAA) and activated charcoal on the chemical composition of strawberry plantlets cultured *in vitro* at 6 weeks of culture

Treatments of auxins and activated charcoal	Chemical composition of plantlets (%)								
	N	P	K	Total	Total				
				carbohydrates	protein				
½ MS-medium + 1.0 mg/l IBA.	0.39	0.12	0.59	17.17	2.44				
½ MS-medium + 1.0 mg/l NAA.	0.37	0.11	0.59	16.54	2.32				
$\frac{1}{2}$ MS-medium + 0.5 mg/l IBA + 0.5 mg/l NAA.	0.43	0.15	0.66	17.74	2.71				
½ MS-medium + 1 mg/l IBA + 0.5 g/l activated charcoal.	0.42	0.14	0.63	17.47	2.62				
1/2 MS-medium + 1 mg/l NAA + 0.5 g/l activated charcoal.	0.41	0.13	0.62	17.32	2.56				
½ MS-medium + 0.5 mg/l IBA + 0.5 mg/l NAA + 0.5 g/l activated charcoal.	0.45	0.16	0.69	17.89	2.78				
LSD at 0.05 level	0.02	0.01	0.01	0.45	0.11				

Table 6. Effect of the interaction between cultivars, auxins (IBA and NAA) and activated charcoal on the chemical composition of strawberry plantlets which cultured *in vitro* at 6 weeks of culture

Treatm	ent	Chemical composition of plantlets (%)							
Cultiva	r Auxins and activated charcoal								
		N	P	K	Total	Total			
					carbohydrates	protein			
Festiva	l ½ MS-medium + 1.0 mg/l IBA.	0.39	0.12	0.59	17.08	2.41			
	½ MS-medium + 1.0 mg/l NAA .	0.37	0.11	0.58	16.87	2.29			
	½ MS-medium + 0.5 mg/l IBA + 0.5 mg/l NAA.	0.43	0.15	0.65	17.66	2.68			
	$\frac{1}{2}$ MS-medium + 1 mg/l IBA + 0.5 g/l activated charcoal.	0.41	0.14	0.63	17.45	2.57			
	½ MS-medium + 1 mg/l NAA + 0.5 g/l activated charcoal.	0.40	0.13	0.61	17.27	2.53			
	½ MS-medium + 0.5 mg/l NAA + 0.5 mg/l IBA + 0.5 g/l activated charcoal.	0.45	0.16	0.69	17.85	2.84			
Sweet	1/2 MS-medium + 1.0 mg/l IBA.		0.13		17.26	2.47			
Charlie	e ½ MS-medium + 1.0 mg/l NAA.	0.38	0.12	0.59	16.19	2.35			
	½ MS-medium + 0.5 mg/l IBA + 0.5 mg/l NAA.	0.44	0.15	0.67	17.83	2.74			
	½ MS-medium + 1 mg/l IBA + 0.5 g/l activated charcoal.	0.43	0.15	0.63	17.50	2.66			
	½ MS-medium + 1 mg/l NAA + 0.5 g/l activated charcoal.	0.42	0.14	0.62	17.37	2.59			
	½ MS-medium + 0.5 mg/l ANA + 0.5 mg/l IBA + 0.5 g/l activated charcoal.	0.44	0.17	0.69	17.95	2.73			
LSD at	0.05 level	0.03	0.01	0.01	0.63	0.16			

shoots of Sweet Charlie cultivar on the same rooting medium recorded the highest value for each of phosphorus, potassium and total carbohydrates content (%) of the plantlet cultured *in vitro*.

On the other hand, there is no significant differences of potassium content (%) between the plantlets of the two tested cultivars under the same rooting medium ½ MS-medium + 0.5 mg/l NAA+0.5 mg/l IBA+0.5 gm/l activated charcoal.

Finally, from the previously mentioned results, it could be suggested that, the depressive or the stimulative effect on the chemical composition of strawberry plantlets cultured *in vitro* during rooting stage were differed significantly according to the cultivars, the type of auxin and its concentration, as well as the dose of activated charcoal in culture medium.

#### REFERENCES

- Adak, N. and M. Pekmezci (2011). The effect of different auxin types and active charcoal levels on plant growth and development at the acclimization stage in different strawberry cultivars. Anadolu Tarm Bilimleri Dergisi, 26 (2): 91-100.
- Adak, N., M. Pekmezci and H. Gubbuk (2001). Investigations on propagation of different strawberry cultivars by meristem culture. Ziraat Fakultesi Dergisi, Akdeniz Univ., 14 (1): 119-126.
- Adak, N., L. Kaynak, M. Pekmezci and H. Gubbuk (2009). The effect of various hormone types on *in vitro* propagation of strawberry. Acta Hort., 829: 305-308.
- Ara, T., M.R. Karim, M.A. Aziz, R. Karim, R. Islam and M. Hossain (2013). Micropropagation and field evaluation of seven strawberry genotypes suitable for agroclimatic condition of Bangladesh. Afr. J. Agric. Res., 8:1194-1199.
- Ashrafuzzaman, M., S.M. Faisal, D. Yadav, D. Khanam and F. Raihan (2013). Micropropagation of strawberry (*Fragaria X ananassa* Duch) through runners. Bangladesh J. Agric Res., 38 (3): 467 472.

- Badr-Elden, A.M (2013). An effective protocol for *in vitro* storage and *ex vitro* re-growth of strawberry capsules. Atlas J. Chem. and Biochem., 1: 30 38.
- Bhatt, I.D. and U. Dhar (2000). Micropropagation of Indian wild strawberry. Plant Cell, Tissue and Organ Culture, 60:83 88.
- Bremner, J.M. and C.S. Mulvaney (1982). Total nitrogen. In: Page, A.L.; R.H. Miller and D.R. Keeney (Ed.). Methods of Soil Analysis. Part 2, Ame. Soc. Agron. Madison. WI. USA, 595 624.
- Dogras, C., A.S. Iomos and C.P. Asomakelis. (1991). Suger and dry matter changes in potatos stored in clamp in mountainous region of northern Dreece. Potato Res., 34: 211-214.
- Elmana, H.E.M., A.A. El Hassan and M.M.A. Elballa (2003). *In vitro* propagation of strawberry (*Fragaria X ananassa* Duch) using shoot tip explants. UKJ. Agric. Sci., 11 (3): 349 358.
- Erenoglu, B., M. Burak and G. Gorur (1995). Study on the effects of etiolation, IBA concentration and activated charcoal on the rooting of micropropagated Aliso and Tioga strawberry plants. Bahce, 24: 1/2, 3-12.
- FAO (2014). Searchable on line statistical database from Food and Agriculture Division of the United Nations http://www.fao.org.
- Fridborg, G.; M. Pedersen; L. Landstrom, and T. Eriksson (1978). The effect of activated charcoal on tissue cultures: adsorption of metabolites inhibiting morphogenesis. Physiol. Plant., 43: 104 106.
- Gautam, H., R. Kaur, D.R. Shama and T. Neetu (2001). A comparative study on *in vitro* and *ex vitro* rooting of micropropagated shoots of strawberry (*Fragaria X ananassa* Duch.). Plant Cell Biotech. and Molec. Biol., 2: 149-152.
- Giampieri, F., J.M. Alvarez-Suarez and M. Battino (2015). Strawberry and human health: Effects beyond antioxidant activity. J. Agric. and Food Chemis., 62: 3867-3876.

- Haddadi, F. and M. Abd El-Aziz (2010). Micropropagation of strawberry cv. Camarosa: Prolific shoot regeneration from *in vitro* shoot tips using Thidiazuron with N6-benzylamino- purine. Hort. Sci., 45: 453 456.
- Harugade, S., R.H. Tabe and S. Chaphalkar. (2014). Micropropagation of Strawberry (*Fragaria X ananassa* Duch.). Int. J. Curr. Microbiol. Appl. Sci., 3: 344 347.
- Hasan, M.N., S. Nigar, M.A.K. Rabbi, S.B.
  Mizan and M.S. Rahman (2010).
  Micropropagation of Strawberry (*Fragaria X ananassa* Duch.). Int. J. Sustain. Crop Prod., 5 (4): 36-41.
- Hemant, G., R. Kaur, D.R. Sharma, T. Neetu, H. Gautam and N. Thakur (2001). Acomparative study on *in vitro* and *ex vitro* rooting of micropropagated shoots of strawberry (*Fragaria X ananassa* Duch.). Plant Cell Biotech. and Molecular Biol., 2: 149 152.
- Jackson, M.L. (1970). Soil Chemical Analysis. Prentice Hall. Englewood Gliffs, N.J.
- James, C.S. (1995): Analytical Chemistry of Foods. Blokie Acad. And Professional, London, England.
- Kaur, R., H. Gautam and D.R. Sharma (2005). A low cost strategy for micropropagation of strawberry (*Fragaria X ananassa* Duch.) ev. Chandler. Acta Hort., 696:129-133.
- Kaushal, K., A.K. Nath and D.R. Sharma (2006). Establishment of callus cultures and plant regeneration in strawberry (*Fragaria X ananassa* Duch.) cv. Chandler. Indian J. Plant Physiol., 11 (2): 136-144.
- Krishnamoorthy, H.N. (1981). Plant Growth Substances. TATA. Mc Graw-Hill Pub. Comp. Limt., New York (USA), New Delhi, (India).
- Lopez-Aranda, J.M., F. Pliego-Alfaro, I. Lopez-Navidad and M. Barcelo-Munoz (1994). Micropropagation of strawberry (*Fragaria X ananassa* Duch.). Effect of mineral salts, benzyladenine levels and number of subcultures on the *in vitro* and field behaviour of the obtained microplants and the fruiting capacity of their progeny. J. Hort. Sci., 69 (4): 625-637.

- Mahajan, R., R. Kaur, A. Sharma and D.R. Sharma (2001). Micropropagation of strawberry cultivars Chandler and Fern. Crop Improve., 28 (1): 19-25.
- Mohamed, A.E. (2007). Somaclonal variation in micropropagated strawberry detected at the molecular level. Int. J. Agric. Biol., 9 (5): 721-725.
- Moradi, K., M. Otroshy and M.R. Azimi (2011). Micropropagation of strawberry by multiple shoots regeneration tissue cultures. J. Agric. Tech., 7: 1755 1763.
- Murashige, T. and F. Skoog (1962). Arevised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15: 473 497.
- Murti, R.H. and Y.R. Yeoung (2013). Effects of BA and IBA concentrations and subculture frequent on meristem culture of strawberry. ARPN J. Agric. and Biol. Sci., 8(5): 405 410.
- Nower, A.A., A.I. Ibrahim, H.A. Emara, S.Y. Mohamed and M.S. Atfi (2011). Preliminary study on propagation and genetic stability of strawberry (*Fragaria X ananassa* Duch.) *in vitro*. Proced. 3<sup>rd</sup> Int. Conf. Genet. Eng. and its Appl., 203-225.
- Olsen, S.R. and L.E. Sommers (1982). Phosphorus. In: AL; R.H. Miller and D.R. Keeney (Ed.). Methods of Soil Analysis. Part 2, Ame. Soc. Agron. Madison, WI. USA, 403 340.
- Pregle, E. (1945). *Quantitative organic micro-analysis*. 4<sup>th</sup> Ed., J. Chundri, London, 385 387.
- Sakila, S., M.B. Ahmed, U.K. Roy, M.K. Biswas, R. Karim, M.A. Razvy, M. Hossain, R. Islam and A. Hoque (2007).
  Micropropagation of Strawberry (*Fragaria X ananassa* Duch.). A Newly Introduced Crop in Bangladesh. Ame.-Euras. J. Sci. Res., 2 (2): 151-154.
- Snedecor, G.W. and W.G Cochran (1980). Statistical Methods, 7<sup>th</sup> Ed., Iowa State Univ. Press, Iowa, USA.
- Suţan, A.N., A. Popescu and V. Isac (2009). The effects of culture medium composition on *in vitro* rooting of two intergeneric hybrids

*fragaria X potentilla*. Analele Universitatii din Oradea, Fascicula Biol., 16: 150 –154.

Sutter, E.G., H. Ahmadi, J.M. Labavitch, A. Altman and M. Ziv (1997). Direct regeneration of strawberry (*Fragaria X ananassa* Duch.) from leaf disks. Acta. Hort., 447: 243–245.

Torres, K.C. (1989). Tissue Culture Techniques for Horticultural Crops. An AVI Book, New York (USA).

Zobayer, N.; S.H. Prodhan; S.U. Sikdar; F. Azim and M. Ashrafuzzaman (2011). Study of shoot multiplication of strawberry (*Fragaria X ananassa* Duch.). int. J. Agric. Res. Innov. and Tech., 1 (1-2): 69 - 72.

#### تأثير أصناف الفراولة، والأوكسينات، والفحم النباتي النشط على تكوين الجذور لنبيتات الفراولة معمليا

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تم إجراء هذه الدراسة خلال الفترة من عام ٢٠١٢ حتى ٢٠١٧ في معمل زراعة الانسجة والبيوت المحمية لتحسين إنتاج المحاصيل الرئيسية والهجن بمعهد بحوث البساتين - مركز البحوث الزراعية بالدقى – الجيزة – مصر، وذلك لدراسة بعض العوامل المؤثرة على تحسين أداء بناتات الفراولة في مزارع الانسجة من خلال تقنية زراعة الخلايا والانسجة النباتية، الهدف من هذه التجربة هو دراسة تأثير صنفين من الفراولة (فيستفال، وسويت شارلي) واثنين من الأوكسينات (إندول حامض البيوتريك، ونفثالين حامض الخليك)، والفحم النباتي النشط على تكوين الجذور (مرحلة التجذير) لنبيتات الفراولة معملياً، أوضحت النتائج المتحصل عليها أن الصنف سويت شارلي كان الأكثر تفوقاً وسجل أقصي القيم لطول كل من الجذر والفرع، وعدد الجذور والأوراق، والوزن الطازج والجاف للجذور والأوراق لكل نبيتة، بالمقارنة مع الصنف فيستفال، من النتائج المتحصل عليها وجد أن استخدام نصف قوة تركيز أملاح بيئة موراشيج وسكوج المضاف لها ١,٠ مجم/لتر إندول حامض البيوتريك +٥,٠ جم/لتر فحم نباتي نشط كانت أكثر المعاملات تأثيراً وتفوقاً في زيادة طول كل من الجذروالفرع ، بينما البيئة المحتوية على نصف قوة تركيز أملاح بيئة موراشيج وسكوج + ١,٠٠ مجم/لتر إندول حامض البيوتريك أو ٠,٥ مجم/لتر إندول حامض البيوتريك +٥,٠ مجم/لتر نفثالين حامض الخليك سجلت أعلى القيم لعدد الجذور والأوراق لكل نبيتة، وبالإضافة الى ذلك، فإن استخدام نصف قوة تركيز أملاح بيئة موراشيج وسكوج المضاف لها ١,٠ مجم نفثالين حامض الخليك قد سجلت أقصى القيم للوزن الطازج والجاف لجذور كل نبيتة، وعلاوة على ذلك ،فإن أقصى القيم للوزن الطازج للتاج والأوراق لكل نبيتة كانت ناتجة باستخدام نصف قوة تركيز بيئة أملاح موراشيج وسكوج المضاف لها ٠,٠ مجم/ لتر إندول حامض البيوتريك + ٠,٥ مجم/لتر نفثالين حامض الخليك، ومن ناحية أخرى، فإن استخدام نصف قوة تركيز أملاح بيئة مور اشيج وسكوج +٥,٥ مجم/لتر إندول حامض البيوتريك+ ٥,٥ جم/لتر نفثالين حامض الخليك + ٥,٥ جم/لتر فحم نباتي نشط كانت أكثر المعاملات تفوقاً وسجلت أقصِي القيم لمحتوى النيتروجين، والفوسفور، والبوتاسيوم والكربوهيدرات الكلية والبروتين الكلى (%) في نبيتات الفر اولة، بخصوص تأثير معاملات التفاعل، فقد أوضحت النتائج المتحصل عليها أن نقل النموات الخضرية لصنف سويت شارلي الى بيئة التجذير المحتوية علي نصف قوة تركيز أملاح بيئة موراشيج وسكوج المضاف لها ١,٠ مجم/لتر إندول حامض البيوتريك +٥,٥ جم/لتر فحم نباتي نشط سجلت أقصى القيم لكلّ من طول الجّدر والساّق، وعدد الأوراق، والوزن الطازج والجاف للأوراق، وكذلك الوزن الجاف للتاج لكل نبيتة، بينما كان أعلى عدد للجذور لكل نبيتة أكثر وضوحاً من خلال استخدام نصف قوة تركيز أملاح بيئة موراشيج وسكوج ٠٠,١ مجم/لترإندول حامض البيوتريك، وعلاوة على ذلك، فإن زراعة النموات الخضرية لصنف فيستفال على نصف قوة تركيز أملاح بيئة موراشيج وسكوج المضاف لها ٠,٥ مجم/لتر إندول حامض البيوتريك +٥,٠ مجم/لتر نَقْالينَ حامض الخليك كانتَ أكثر المعاملات تأثيراً وفاعليةٍ في زيادة الوزن الطازج للجذور لكل نبيتة، ومن ناحية أخرى، فإن أقصى زيادة في الوزن الطازج لتاج كل نبيتة كان أكثر وضوحاً من خلال معاملة التفاعل بين نفس الصنف مع استخدام نصف قوة تركيز أملاح بيئة موراشيج وسكوج المضاف لها ١,٠ مجم/لتر نفثالين حامض الخليك. وفوق ذلك، فإن استخدام بيئة التجذير المحتوية على نصف قوة تركيز أملاح بيئة موراشيج وسكوج +٠,٥ مجم/لتر إندول حامض البيوتريك +٠,٥ جم/لتر فحم نباتي نشط سجلت أعلى القيم لمحتوى النيتروجين، والبروتين الكلي (%) في نبيتات الصنف فيستفال، بينما زراعة النموات الخضرية لصنف سويت شارلي علي نفس بيئة التجذير سجلت أقصى زيادة لمحتوى الفوسفور، والبوتاسيوم، والكربوهيدرات الكلية (%) في نبيتات الفراولة المزروعة

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