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EFFECT OF CULTIVARS, GROWTH REGULATOR AND SUB-CULTURE ON THE PLANTLET SHOOTS FORMATION AND RAPD ANALYSIS OF STRAWBERRY CULTURES *IN VITRO*

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ABSTRACT

This experiment amid to study the effect of cultivars, growth regulator and number of sub-cultures on the shoots formation of strawberry plantlets during multiplication stage and Random amplified polymorphic DNA (RAPD) analysis of strawberry cultures *in vitro* during the fourth sub-culture. This experiment included 40 treatments, which were the combination between two strawberry cultivars (Festival and Sweet Charlie), five treatments of growth regulator (BA and GA_3) and four number of sub-cultures during shoots formation (multiplication stage). The obtained results showed that, the maximum increment of growth measurement of strawberry plantlets were recorded by Sweet Charlie cultivar .In addition , using ½ MS-medium without supplemented with any growth regulators (BA and GA_3) being the superior treatment for increasing both number of leaves per shoot and shoot length. On the other hand, generally, the fourth sub-culture being the most effective treatment on the growth measurement of strawberry plantlets during multiplication stage. Furthermore, Random amplified polymorphic DNA (RAPD) analysis varied according to the two tested cultivars and the type of for production of disease resistant plants and in plant breeding and crop improvement programs (Mohamed, 2003).

Key words: *Strawberry - Tissue cultures – Multiplication - Growth regulators - RAPD analysis*

INTRODUCTION

Strawberry (*Fragaria X ananassa* Duch.) is a natural hybrid of *Fragaria chilonsis* and *Fragaria virginiana* is a perennial herb belonging to the Rosaceae Family. The strawberry fruits is delicate in flavor, texture, shape, and rich in some vitamins particularly A, B1, B2, B6, C, E, and some minerals such as calcium, potassium, copper and iron (Glampieri *et al*., 2015). In addition, fruits are a good source of phytochemical compounds, mainly ellagic acids which have a wide range of biological activity.

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Tissue culture technique has been successful on the large scale multiplication of strawberry plants in many countries. This technique can produced millions of plants can be produced in short time from a few mother plants. Beside propagation, tissue culture technique have been used Mohamed (2003) on strawberry plants; Souza *et al*. (2008) and EL- Hosary and EL- Akkad (2015) on maize plants, working on molecular markers.

In general, the multiplication stage (shoots formation) of strawberry in the *in vitro* plant tissue

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and organs depends on some factors such as cultivars (Passey *et al*.,(2003), Sutan *et al*.,2010; Mozafari and Gerdakanch, 2012), growth regulators (such as BA and GA_3) and their concentration (Sachs *et al*.,1959; Haber and luippold, 1960; Gamborg *et al*.1976; Lal *et al*., 2003, Negi *et al*.,2008; Harugade *et al*.,2014); number of sub-cultures (Nowere *et al*., 2011;EL-Zeiny *et al*.,2013).

Moreover, genetic stability during micro propagation is controlled by various type including genotype, presence of chimera tissue, origin and explant type, medium components, type and concentration of growth regulators. In this connection, EL-Tarras *et al*. (2001) came to similar conclusion.

There for, the aim of this work was to study the effect of cultivars, growth regulators and number of sub-cultures on the formation of plantlet shoots, as well as Random amplified polymorphic DNA (RAPD) analysis of strawberry plantlets cultures *in vitro* during forth sub-culture.

MATERIALS AND METHODS

This experiment included 40 treatments, which were the interaction between two strawberry cultivars, five growth regulators and four number of sub-cultures, as follows:

A) Strawberry cultivars

1) Festival.
2) Sweet Charlie.

B) Growth regulators

1) ½ MS-medium (Half salts strength) without applications of antioxidant (the control treatment).
2) ½ MS-medium + 0.01mg/l BA.
3) ½ MS-medium + 0.5 mg/l BA.
4) ½ MS-medium + 0.01 mg/l BA + 0.01 mg/l GA_3.
5) ½ MS-medium + 0.5 mg/l BA + 0.3 mg/l GA_3.

C) Number of sub-cultures

1) First sub-culture.
2) Second sub-culture.
3) Third sub-culture.
4) Fourth sub-culture.

These treatments were arranged in a split-split plots design with four replicates. Each replicate contained of five glass jars (12.0 × 6.0 cm contained of 50 ml of the culture medium), and each one contained of four explants. The cultivars were arranged in the main plots, while growth regulator treatments were assigned randomly in the sub-plots and number sub-cultures were arranged randomly in the sub sub-plots.

The selected shoots are shown in Fig. (1) were used as a plant material in this stage and sub-cultured on the half salts strength of basal nutrient (½ MS-medium) which supplemented with the previously growth regulator combinations of BA and GA_3.

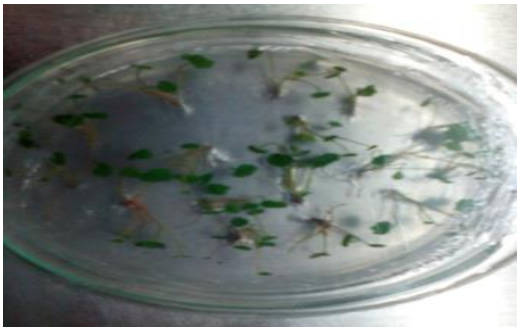

Fig. (1): The selected shoots were used as a plant material in multiplication experiment.

Data recorded

Data were recorded after 21 days between sub-cultures from culture in vitro, as follows:

A) Growth measurements of plantlets

1) Number of shoots per plantlet.
2) Shoot length (cm).
3) Number of leaves per plantlet.

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4) Fresh weight of shoots per plantlet.
5) Dry weight of shoots per plantlet.

B) Polymerase chain reaction (PCR) analysis (Molecular analysis)

Deoxyribo nucleic acid (DNA) was extracted from *in vitro* plantlets after the fourth subculture of both cultivars (Festival and Sweet Charlie) and mother plants, ex vitro. Briefly, new and fresh leave samples were collected separately from each cultivar, and the Deoxyribo nucleic acid (DNA) extraction was performed using DNeasy plant Mini Kit (QIAGEN).The method of Williams et al. (1990) used in this connection.

RAPD –PCR reactions were conducted using six primers for two strawberry cultivars i.e. Festival and Sweet Charli to determine the polymorphism that could be associated with differences among five treatments Table (1).

Table (1): List of the primer names and their nucleotide sequences used in the study for RAPD procedure.

No.	Primer	Sequence
1	OP-B09	5` TGGGGGACTC 3`
2	OP-C09	5` CTCACCGTCC 3`
3	OP-K01	5` CATTCGAGCC 3`
4	OP-K03	5` CCAGCTTAGG 3`
5	OP-O05	5` CCCAGTCACT 3`
6	OP-O10	5` TCAGAGCGCC 3`

Statistical analysis

All collected data were subjected to proper statistical analysis using Co-Stat software program version 3. The least significant difference (**L.S.D.**) 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 DISCCUSION

A) Growth measurements of plantlets

1) Effect of cultivars

The effect of the two tested strawberry cultivars (Festival and Sweet Charlie) on the shoots formation of plantlets during multiplication stage *in vitro* are illustrated in Table (2). It is quite clear from such table that, Sweet Charlie being the superior one in number of both shoots formation per explant and leaves per shoot, as well as shoot length as compared with Festival cultivar.

On the other hand, it is also evident from such results that, no obvious differences were detected between the two cultivars on the fresh and dry weight of shoots formation, which were mostly similar in this connection. In this regard, **Passey *et al*. (2003)** tested the regeneration ability of seven commercial strawberry cultivars using a range of the explants, they mentioned that, two genotypes showed a limited ability to regenerated shoots in all explants tested. In addition, **Sutan *et al*. (2010)** reported that, genotypes was proven to be a critical factor for indirect differences in callus formation ability and shoots regeneration frequency between the two investigated strawberry cultivars**. Mozafari and Gerdakanch (2012)** found that, shoots multiplication occurred in strawberry cultivars, i.e. Kucrdislun and Merck. The highest number of shoots/culture (3.44) were recorded from Kurdistan cultivar.

2) Effect of growth regulators

Concerning the effect of some growth regulators (BA and GA_3) on explant shoots formation of strawberry cultures *in vitro*, the obtained results in Table (3) and Fig. (2) revealed that, half salts strength of MS-medium (½ MS-medium) which supplemented with 0.5 mg/l BA + 0.3 mg/l GA_3 recorded the highest number of shoots formation per plantlet. Half salts strength of MS-medium (½ MS-medium) without application of any growth regulators (the control

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treatment) being the superior one in respect of number of leaves formation per shoot and shoot length with no significant differences between this treatment and the treatment of ½ MS-medium + 0.01 mg / l BA + 0.01 mg / l GA_3 for shoot length only. In addition using ½ MS-medium which supplemented with 0.5 mg/l BA + 0.3 mg/l GA_3 or 0.01mg/l BA increased the fresh weight of shoots formation, while the treatments of growth regulators (BA and GA_3) did not reflect any significant effect on the dry weight of the obtained shoots of strawberry cultures *in vitro*.

From the previously mentioned results, it could be suggested that, the promotion effect of BA and GA_3 on the formation of both number of shoots per plantlet and leaves per shoot, as well as shoot length and the fresh weight of shoots per plantlet is due to its simulative effect on both cell division and cell enlargement (**Sachs *et al*. 1959** and **Haber and Luippold, 1960**). Moreover, **Gamborg *et al.* (1976)** mentioned that, cell division are stimulated by application of cytokinin (such as BA) to the culture medium.

In this connection, **Malodobry *et al*. (1997)** found that, the greatest number of strawberry shoots (5.2) were obtained when the explants cultured on MS-medium which supplemented with 0.5 mg / l BA +0.1 mg / l IBA. Moreover, **Wei *et al*. (2001)** found that the most suitable regeneration medium for strawberry was MS medium + 0.5-1.0 mg / l BA+ 0.05-0.1mg /l IBA. **Lal *et al*. (2003)** reported that, the maximum shoots regeneration (100%) of strawberry after 7 weeks of incubation and the maximum number of shoots per explant was observed via using MS-medium which supplemented with BA at a concentration of 4.0 mg / l. Furthermore, **Negi *et al*. (2008)** found that, **MS-**medium which supplemented with BA (0.5mg / l), IBA (0.5 mg / l) and GA_3 (1.0 mg/ l) recorded the maximum value of shoot length (10.50 cm). **Nankali and Azghandi (2009)** reported that, the greatest shoots proliferation was more achieved in full strength of MS-medium which contained of BA at concentration of 0.5 mg / l. On the other hand, **Ashrafuzzaman *et al.* (2013)** found that, the maximum values of both shoots number (7) shoot length (3.34 cm) and number of leaves per explant (5) were more distinct via using MS-medium which contained of 0.5 mg / l BA. **Waliur Rahman *et al.* (2015)** reported that, the maximum percentage of shoots regeneration (93.33%) and number of shoots (15) per leaf disc were found to be induced by using MS-medium which supplemented with 3.0 mg / l BA+ 0.5 mg/l GA_3. In addition, **Kaur *et al*. (2005), Bhat *et al*. (2012), Harugade *et al*. (2014)** came to similar conclusion.

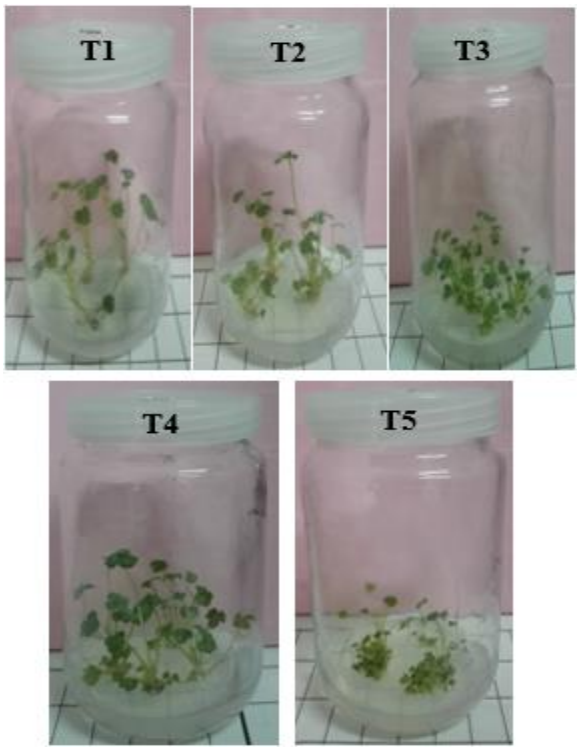

Fig. (2): Effect of growth regulator (BA and GA_3) on the plantlet shoots formation of strawberry cultures *in vitro*.

T1) ½ MS-medium without growth regulators (Control)
T2) ½ MS-medium + 0.01mg/l BA
T3) ½ MS-medium + 0.5 mg/l BA
T4) ½ MS-medium + 0.01 mg/l BA + 0.01 mg/l GA_3
T5) ½ MS-medium + 0.5 mg/l BA + 0.3 mg/l GA_3

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3) Effect of number of sub-cultures

It is evident from the obtained results in Table (4) and Fig. (3) that, number of sub-cultures exerted a marked and significant effect on the shoots formation per plantlets. In addition, the fourth sub-culture recorded the highest number of shoots per plantlet and number of leaves per shoot, as well as shoot length, followed by third sub-culture, respectively. In this connection, **Nower *et al.* (2011)** found that, the third sub-culture significantly recorded the highest response in increasing both number of shoots (14.0 shoots per explant) and number of leaves (8.30 per explant) as compared to the first and the second sub-cultures. While, the number of sub-cultures did not reflect any differences in shoot length. Moreover, **El-Zeiny *et al.* (2013)** reported that there are relationship between number of sub-cultures and rates of shoots production of global artichoke plantlets. Increasing the number of sub-cultures till fifth times increased gradually the number of shoots production and decreased shoot length.

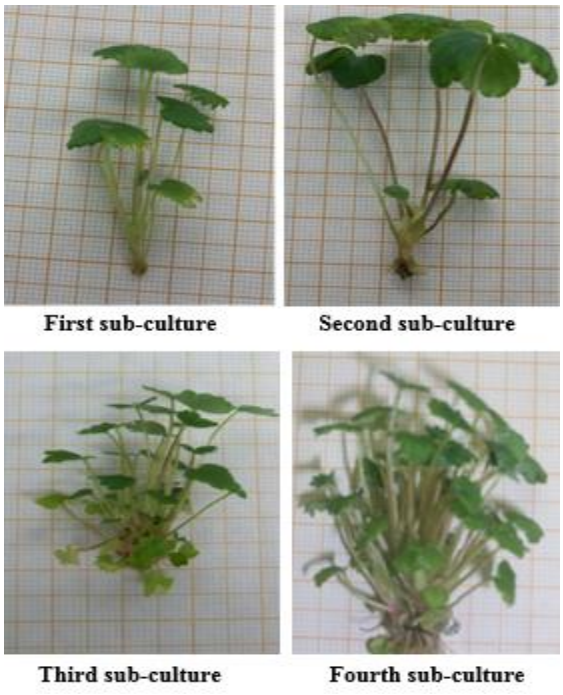

Fig. (3): Effect of number of sub-cultures on the plantlet shoots formation of strawberry cultures *in vitro*.

4) Effect of the interaction between cultivars and growth regulators

The obtained results in Table (5) showed clearly that, the maximum increase in number of shoots per plantlet were more achieved via the interaction treatment between Sweet Charlie cultivar and using ½MS-medium which supplemented with 0.5 mg/l BA + 0.3 mg/l GA_3. Moreover, the highest values of both number of leaves per shoot and shoot length were recorded by the interaction treatment between such cultivar and using ½ MS-medium without application of any growth regulators (the control treatment), followed by the interaction between the same cultivar and using ½ MS-medium which contained of 0.01 mg/l BA + 0.01 mg/l GA_3. On the other hand, the maximum increase in the fresh weight of soot were more distinct by the interaction treatment between Sweet Charlie cultivar and using ½ MS-medium+0.01 mg/l BA.

On the contrary, all interaction treatments did not reflect any significant effect on the dry weight of shoots per plantlet. In this regard, **Zebrowska and Hortynski (2002)** studied the effect of various concentrations of BA (0, 1.6, 3.2 and 6.4 mg /l) in MS-medium in clone B-302 and Kama strawberry cultivar. They found that, leaf explants regenerated only at a concentrations of 3.2 mg and 6.4 mg/l BA in the medium. The higher shoots formation was found to be in clone B-302 than in Kama cultivar and obtained at 3.2 mg/l of BA level (average 6 and 8 shoots /explant). However, at the same concentration of BA in MS-medium did not formation any shoots in Kama cultivar, which regenerated only at concentration of 6.4 mg/l BA. Moreover, **Tanziman *et al*. (2013)** and **Murti and Yeoung (2013)** came to similar findings in some strawberry cultivars.

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5) Effect of the interaction between cultivars and number of sub-cultures

The obtained results in Table (6) indicated that, the interaction treatment between Sweet Charlie cultivar and the fourth sub-culture being the most effective treatment and recorded the maximum values of number of both shoots per plantlets and leaves per shoots, as well as shoot length. In addition, the interaction treatment between Festival cultivar and the fourth sub-culture came in the second rank in this respect, for except number of leaves per shoot.

On the other hand, the interaction treatment between Festival cultivar and the first sub-culture being the inferior one and recorded the lowest values of all parameters which were studied.

6) Effect of the interaction between growth regulators and number of sub-cultures

It is quite clear from the obtained results in Table (7) that, the fourth sub-culture treatment being the most effective as compared with the other sub-culture treatments.

In this connection, the interaction treatment between using ½ MS-medium+ 0.5 mg /l BA + 0.3 mg/l GA_3 and the fourth sub-culture recorded maximum value of number of shoots per plantlet. In addition , the interaction treatments between the same sub-culture and using ½ MS-medium which supplemented with 0.01 mg/l BA + 0.01 mg/l GA_3 being the superior one and recorded the highest increase of number of leaves per shoot. On the other hand, the fourth sub-culture and using ½ MS-medium without application any growth regulators (the control treatment) recorded the maximum value of shoot length.

7) Effect of the interaction between cultivars, growth regulators and number of sub-cultures on the plantlet shoots formation of strawberry cultures in vitro

It is evident from the presented results in Table (8) that, the maximum increase in number of shoots per plantlet were recorded via the interaction treatment between Festival cultivar, using ½ MS-medium which supplemented with 0.5 mg/l BA + 0.3 mg/l GA_3 and the fourth sub-culture. Moreover, the interaction treatment between Sweet Charlis cultivar, the same medium and the fourth sub-culture came in the second rank in this respect .

Furthermore, the maximum, increase in number of leaves per shoot were more achieved via the interaction treatment between Sweet Charlie cultivar, using ½ MS-medium without application any growth regulators and the second sub-culture.

With regard to shoot length, it is quite clear from such results in Table (8) that, the maximum value in this respect was recorded by the interaction treatment between Sweet Charlie cultivar, using ½ MS-medium without application any growth regulators (the control treatment) and the fourth sub-culture.

From the for going results, it could be suggested that ,all parameters of the plantlets ,i.e. number of both shoots per plantlet and leaves per shoot, as well as shoot length varied greatly according to the tested cultivars, the contained of ½ MS-medium and number of sub-cultures.

Molecular Markers

Identification of six primers for two strawberry varieties, *i.e.*, Festival and Sweet Charlie which generated polymorphic markers were used to

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determine the polymorphism that could be associated with differences among five treatments:

B) RAPD Analysis:

DNA markers are proved to be powerful tools to evaluate the genetic diversity **(Selim *et al*., 2010, Hussein *et al*., 2013** and **Suprasanna and Jain 2017)**. Recently, RAPD markers are commonly used for identification and testing genetic

purity in several crops such strawberry **(EL-Tarras *et al*. 2001 and Mohamed, 2003)** and other crop plants **(Souza *et al.* 2008; EL-Hosary and EL-Akkad 2015).**

Identification of six primer for two strawberry varieties, *i.e*., Festival and Sweet Charlie which generated polymorphic markers were used to determine the polymorphism that could be associated with differences among five treatments.

1) Festival cultivar

Identification of six primers which generated polymorphic bands was used to determine the polymorphism that could be found among five treatments. RAPD analysis of Festival cv using six primers are illustrated in Table (9).

Primer **OP-B09** generated two monomorphic bands and the two polymorphism bands with 71.429% polymorphism. 283.196 to 1074.240 b.p.

Whereas, the highest number of bands (12) was generated from Primer **OP-C09**, and generated three monomorphic bands with 80% polymorphism. The size of bands ranged between 334.001 to 1118.946 b.p.

Primer **OP-K01** generated five monomorphic bands and the five polymorphism bands with 50% polymorphism. The size of bands ranged between 297.625 to 1186.595 b.p.

Primer **OP-K03** generated four monomorphic bands and three polymorphism bands with 42.857% polymorphism. The size of bands ranged between 204.064 to 629.310 b.p.

Primer **OP-O05** generated seven monomorphic bands and four polymorphism bands with 36.346% polymorphism. The size of bands ranged between 245.693 to 1154.764 b.p. Fig. (4) Showed the pattern of amplification product of primer **OP-O05**.

It is clear that Primer **OP-O10** generated five monomorphic bands and nine polymorphism bands with 64.286% polymorphism. The size of bands ranged between 233.292 to 989.581 b.p.

It could be concluded that, the six primers produced 64 bands among them 38 were found polymorphic with 59.375% polymorphism. The number of polymorphic bands per locus ranged from three (OP- K03) to 12 (OP-C09) with an average number of 6.0 bands per locus. DNA markers are proved powerful tools to evaluate the polymorphism (**Selim *et al*., 2010, Hussein *et al*., 2013** and **Suprasanna and Jain 2017).**

2) Sweet Charlie cultivar

Identification of six primers which generated polymorphic bands was used to determine the polymorphism that could be found among five treatments. RAPD analysis of Sweet Charlie cv. using six primers are illustrated in Table (10).

It is clear that Primer **OP-B09** generated three monomorphic bands and maximum number of polymorphism bands (12) with 80% polymorphism. The size of bands ranged between 186.954 to 1109.140 b.p.

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Furthermore, Primer **OP-K01** generated only one monomorphic bands and higher number of polymorphism bands (10) with 90% polymorphism. The size of bands ranged between 204.575 to 978.236 b.p.

Whereas, the lowest number of polymorphic bands (2) was generated from Primer **OP-O10** with 22.222% polymorphism. This primer gave seven monomorphic bands. The size of bands ranged between 279.770to 1077.644 b.p. Fig. (5) showed the pattern of amplification product of primer **OP-O10**.

Primer **OP-C09** generated three monomorphic bands seven number of polymorphism bands with 70% polymorphism. The size of bands ranged between 353.293 to 1093.959 b.p.

Primer **OP-K03** generated three monomorphic bands and only one polymorphism bands with 25% polymorphism. The size of bands ranged between 380.732 to 538.020 b.p.

Primer **OP-O05** generated six monomorphic bands and eleven polymorphism bands with 64.706% polymorphism. The size of bands ranged between 179.259 to 1449.114 b.p.

It could be concluded that, the six primers produced 66 bands among them 43 were found polymorphic with 59.375% polymorphism. The number of polymorphic bands per locus ranged from 1 **(OP-K03**) to 12 (**OP-B09**) with an average number of 7.0 bands per locus. In this respect, and **EL-Hosary and EL-Akkad 2015** demonstrated that primers produced reliable and reproducible banding pattern and that the number, size of amplified DNA fragments and polymorphic bands varied among primers.

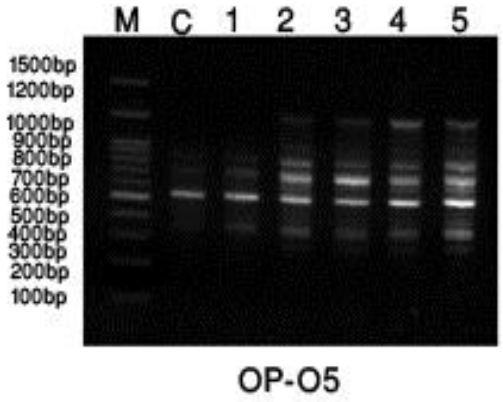

Fig. (4): RAPD pattern obtained by primer OP-O5 for Festival cultivar

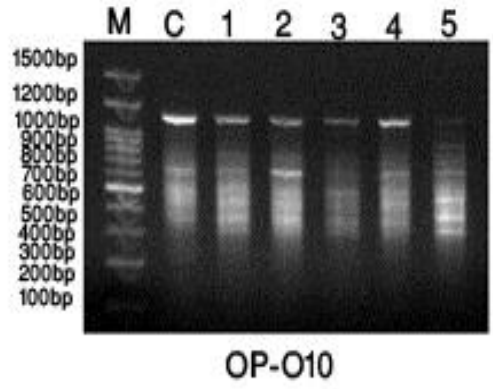

Fig. (5): RAPD pattern obtained by primer OP-O10 for Sweet Charlie cultivar

M = DNA Marker, C = Original cultivar, 1 = Control, 2 = 0.01 mg/l BA, 3 = 0.5 mg/l BA, 4 = 0.01 mg/l BA + 0.01 mg/l GA_3, 5 = 0.5 mg/l BA + 0.3 mg/l GA_3.

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Table 2. Effect of cultivars on the plantlets shoots formation of strawberry cultures *in vitro*.

Cultivars	Number		Shoot length (cm)	Weight of shoots (g)	
	Shoots/ explant	Leaves/ shoot		Fresh	Dry
Festival	1. 87	5.18	2.42	2.52	0.21
Sweet Charlie	2.59	5.64	2.99	2.44	0.26
L.S.D. at 0.05 Level	**0.22**	**0.35**	**0.28**	**N.S.**	**N.S.**

N.S.: Not significant at 0.05 level of probability

Table 3. Effect of growth regulators (BA and GA_3) on the plantlets shoots formation of strawberry cultures *in vitro*.

Growth regulator	Number		Shoot length (cm)	Weight of shoots (g)	
	Shoots/ explant	Leaves/ shoot		Fresh	Dry
½ MS-medium without growth regulators (Control)	1.02	7.49	3.72	1.63	0.25
½ MS-medium + 0.01mg/l BA	2.04	5.04	2.76	3.14	0.34
½ MS-medium + 0.5 mg/l BA	2.47	4.15	2.07	2.75	0.21
½ MS-medium + 0.01 mg/l BA + 0.01 mg/l GA_3	1.09	6.81	3.55	1.71	0.18
½ MS-medium + 0.5 mg/l BA + 0.3 mg/l GA_3	4.57	3.52	1.44	3.19	0.21
L.S.D. at 0.05 Level	**0.35**	**0.56**	**0.44**	**1.36**	**N.S.**

N.S.: Not significant at 0.05 level of probability

Table 4. Effect of number of sub-cultures on the plantlet shoots formation of strawberry cultures *in vitro*.

Number of sub-cultures	Number		Shoot length (cm)
	Shoots/ explant	Leaves/ shoot	
First	1.92	5.10	1.99
Second	1.88	5.66	2.56
Third	2.30	4.87	2.58
Fourth	2.84	5.97	3.69
L.S.D. at 0.05 Level	**0.31**	**0.50**	**0.39**

Table 5. Effect of the interaction between cultivars and growth regulators (BA and GA_3) on the plantlet shoots formation of strawberry cultures *in vitro*.

Treatments		Number		Shoot length (cm)	Weight of shoots (g)	
Cultivars	Growth regulator	Shoots/ explant	Leaves/ shoot		Fresh	Dry
Festival	**½ MS-medium without growth regulators (Control).**	1.01	6.07	3.08	1.86	0.35
	½ MS-medium + 0.01mg/l BA.	1.31	5.82	2.78	2.69	0.28
	½ MS-medium + 0.5 mg/l BA.	2.12	4.22	1.63	2.58	0.11
	½ MS-medium + 0.01 mg/l BA + 0.01 mg/l GA_3.	1.09	6.10	3.13	1.71	0.16
	½ MS-medium + 0.5 mg/l BA + 0.3 mg/l GA_3.	3.82	3.67	1.48	3.76	0.17
Sweet Charlie	**½ MS-medium without growth regulators (Control).**	1.02	8.90	4.35	1.39	0.15
	½ MS-medium + 0.01mg/l BA.	2.77	4.26	2.74	3.58	0.39
	½ MS-medium + 0.5 mg/l BA.	2.82	4.07	2.52	2.90	0.31
	½ MS-medium + 0.01 mg/l BA + 0.01 mg/l GA_3.	1.08	7.51	3.96	1.70	0.19
	½ MS-medium + 0.5 mg/l BA + 0.3 mg/l GA_3.	5.33	3.38	1.41	2.63	0.25
L.S.D. at 0.05 Level		**0.49**	**0.79**	**0.62**	**1.92**	**N.S.**

N.S.: Not significant at 0.05 level of probability

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Table 6. Effect of the interaction between cultivars and number of sub-cultures on the plantlet shoots formation of strawberry cultures *in vitro*.

Treatments		Number		Shoot length (cm)
Cultivars	Number sub-culture	Shoots/ plantlets	Leaves/ shoot	
Festival	First	1.23	4.89	1.80
	Second	1.36	5.27	2.24
	Third	2.10	4.73	2.47
	Fourth	2.79	5.81	3.17
Sweet Charlie	First	2.61	5.31	2.19
	Second	2.40	6.06	2.89
	Third	2.50	5.01	2.68
	Fourth	2.89	6.12	4.22
L.S.D. at 0.05 Level		**0.44**	**0.70**	**0.55**

Table 7. Effect of the interaction between growth regulators (BA and GA_3) and number of sub-cultures on the plantlet shoots formation of strawberry cultures *in vitro*.

Treatments		Number		Shoot length (cm)
Growth regulators	Number of sub-culture	Shoots/ plantlet	Leaves/ shoot	
½ MS-medium without growth regulators (Control).	First	0.99	6.32	2.72
	Second	2.44	4.12	3.48
	Third	1.06	6.48	3.42
	Fourth	1.00	9.39	5.25
½ MS-medium + 0.01 mg/l BA.	First	2.44	4.12	1.61
	Second	2.20	4.46	2.36
	Third	1.15	5.48	2.72
	Fourth	1.89	6.09	4.35
½ MS-medium + 0.5 mg/l BA.	First	1.99	4.98	1.64
	Second	2.18	4.68	2.11
	Third	2.99	3.28	1.49
	Fourth	2.73	3.66	3.05
½ MS-medium + 0.01 mg/l BA + 0.01 mg/l GA_3.	First	2.99	5.88	2.32
	Second	1.00	7.63	3.27
	Third	1.13	6.03	3.96
	Fourth	1.06	7.69	4.64
½ MS-medium + 0.5 mg/l BA + 0.3 mg/l GA_3.	First	3.04	4.22	1.59
	Second	3.00	3.78	3.05
	Third	4.71	3.09	1.29
	Fourth	7.54	3.01	1.18
L.S.D. at 0.05 Level		**0.69**	**1.11**	**0.87**

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Table 8. Effect of the interaction between cultivars, growth regulators (BA and GA_3) and number of sub-cultures on the plantlet shoots formation of strawberry cultures *in vitro*.

Treatments		Number			Shoot length (cm)
Cultivars	Growth regulator treatments	Number of sub-culture	Shoots/plantlet	Leaves/ shoot	
Festival	½ MS-medium without growth regulators (Control)	First	0.96	4.76	2.04
		Second	1.11	5.07	2.63
		Third	1.59	5.68	1.29
		Fourth	1.07	8.78	3.22
	½ MS-medium + 0.01 mg/l BA	First	1.11	5.11	1.927
		Second	1.33	5.24	2.26
		Third	1.51	5.24	2.68
		Fourth	1.28	7.19	4.27
	½ MS-medium + 0.5 mg/l BA	First	1.59	4.57	1.29
		Second	1.33	5.47	2.12
		Third	2.75	3.38	1.57
		Fourth	2.82	3.47	2.68
	½ MS-medium + 0.01 mg/l BA + 0.01 mg/l GA_3	First	2.75	5.18	2.04
		Second	1.16	6.56	2.76
		Third	4.02	5.83	1.55
		Fourth	1.00	6.83	4.17
	½ MS-medium + 0.5 mg/l BA + 0.3 mg/l GA_3	First	1.40	4.84	1.70
		Second	2.08	3.98	1.43
		Third	4.02	3.06	1.24
		Fourth	7.75	2.80	1.42
Sweet Charlie	½ MS-medium without growth regulators (Control)	First	1.00	7.89	3.39
		Second	3.78	10.45	4.33
		Third	2.39	7.27	3.61
		Fourth	1.22	10.00	6.05
	½ MS-medium + 0.01 mg/l BA	First	4.67	3.13	1.30
		Second	3.07	3.67	2.47
		Third	1.72	5.24	2.75
		Fourth	2.50	4.98	4.43
	½ MS-medium + 0.5 mg/l BA	First	2.39	5.37	1.99
		Second	3.02	3.89	2.10
		Third	3.22	3.17	1.41
		Fourth	2.64	3.84	4.56
	½ MS-medium + 0.01 mg/l BA + 0.01 mg/l GA_3	First	1.22	3.59	2.59
		Second	1.11	3.58	3.78
		Third	5.39	6.22	4.38
		Fourth	1.00	8.56	5.11
	½ MS-medium + 0.5 mg/l BA + 0.3 mg/l GA_3	First	4.67	4.98	1.71
		Second	3.92	3.84	1.76
		Third	5.39	3.11	5.11
		Fourth	7.33	3.23	0.94
L.S.D. at 0.05 Level			**0.97**	**1.57**	**1.24**

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Table 9: RAPD analysis of strawberry mutant genotypes using six primers for Festival cv genotype.

Primer	Size of bands (b.p.)	Monomorphic bands	Polymorphic bands	Total number of bands	Polymorphism (%)
OP-B09	283.196-1074.240	2	5	7	71.42
OP-C09	334.001-1118.946	3	12	15	80.00
OP-K01	297.625-1186.595	5	5	10	50.00
OP- K03	204.064-629.310	4	3	7	42.85
OP-O05	245.693-1154.764	7	4	11	36.36
OP-O10	233.292-989.581	5	9	14	64.28
Total		**26**	**38**	**64**	**59.37**

Table 10: RAPD analysis of strawberry mutant genotypes using six primers for Sweet Charlie genotype.

Primer	Size of bands (b.p.)	Monomorphic bands	Polymorphic bands	Total number of bands	Polymorphism (%)
OP-B09	186.954-1109.140	3	12	15	80.00
OP-C09	353.293-1093.959	3	7	10	70.00
OP-K01	204.575-978.236	1	10	11	90.90
OP- K03	380.732-538.020	3	1	4	25.00
OP-O05	179.259-1449.114	6	11	17	64.70
OP-O10	279.770-1077.644	7	2	9	22.22
Total		**23**	**43**	**66**	**65.15**