SINAI Journal of Applied Sciences (ISSN: 2314-6079) Vol. (7) Is. (2), Aug. 2018 89-98

SINAI Journal of Applied Sciences

ARU-EGYPT SJAS Trust Science | It Creates

EFFECT OF LIGHT, EXPLANT TYPE, GROWTH REGULATORS AND MANNITOL ON (*Capparis spinosa* L.) CALLUS INDUCTION AND ACTIVE INGREDIENTS PRODUCTION

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ABSTRACT

This study was carried out at Tissue Culture Laboratory, Fac. Environ. Agric. Sci., Arish University, North Sinai, Egypt, during the period from 2016 to 2018. The main objective of this study was to study the effect of explants type, growth regulators and light on callus induction and growth as well as effect of mannitol as osmotic stress agent on production of active ingredients from *Capparis spinosa* L. callus culture. Callus cultures were initiated from *in vitro* leaves and stems which isolated from 6-weeks-old *in vitro* shoots and cultured on MS solid medium supplemented with various concentrations of (2, 4-D) combined with Kinetin (Kin) or 6-Benzyladenine(BA) . Cultures were maintained under darkness or continuous light conditions at 25 ± 2°C. After 4 weeks from subculture of callus on free MS medium, calli were individually weighed and about l g of callus was placed on MS medium fortified with mannitol (0, 2, 4 or 8 gl^{-1}). All cultures were maintained under dark condition at 24°C for 6 weeks. Results indicated that either control treatment or cytokinin treatments couldn't induce callus on both explant types. However, 2, 4-D either alone or combined with different cytokinin treatments induced callus on both explant types without significant differences among these treatments in most cases. Callus dry weight was reached to the maxinaum values when leaf explants were cultured on MS medium supplemented with 1.0 mgl^{-1} 2, 4-D + 2 mgl^{-1} Kin or BA and when nodal segments were cultured on MS medium fortified with 2,4-D and kin at 2.0mgl^{-1} for both of them under darkness. Also, the obtained results revealed that, addition of 4 gl^{-1} mannitol gave the highest values for each of callus fresh and dry weights (g) while, addition of 8 gl^{-1} mannitol gave the highest content of proline compared with control treatment. Moreover, the GC–MS analysis revealed to identify of thirty two components some of these compounds were higher with mannitol at 4gl^{-1} compared with control.

Keywords: *Capparis spinosa*, Callus, BA, Kin, active substances, GC–MS analysis.

INTRODUCTION

The caper bush (*Capparis spinosa* L.), belongs to family Capparidaceae, is a perennial winter deciduous species that bears rounded fleshy leaves and big white to pinkish-white flowers **(Watson and Dallwitz, 1992)**. Caper bush is present in almost all the circum-Mediterranean countries. The economic importance of caper plant (young flower buds, known as capers) are greatly favoured for seasoning and different parts of the plant are used in the manufacture of medicines and cosmetics. Caper root bark and leaves may have some anticarcinognic activity. Abiotic stress such as draught led to fluctuate ratio active substances in plant. Thus, micropropagation important to evaluate the performance of improves *Capparis spinosa* L. active substances under osmotic stress.

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Wang *et al.* (2007) studied the effect of different hormones on *Capparis spinosa* L. They reported that the high percentage of callus were obtained on MS medium supplemented with 0.5 mg/l 2, 4-D + 1.0 mg/l 6-BA and 1.0 mg/l 2, 4-D + 1.5 mg/l 6-BA, respectively. Also, **Al-Safadi and Elias (2009)** evaluated the influence of plant growth regulators on the formation of Caper (*Capparis spinosa* L.) callus using leaves and stem parts. They found that the best media was that contained 1 mg/l BA + 0.1 mg/l NAA or that contained 2 mg/l 2, 4-D + 1 mg/l NAA + GA_3 0.1 mg/l using leaves parts.

Abu Khalaf and Arafeh (2010) on *Capparis spinosa* determined the effect of media supplemented with different levels of 2, 4-D. Results indicated that callus was successfully inducted on MS media + 0.5 mg/l 2, 4-D and subcultured on MS medium with 1.0 mg/l 2, 4-D and 1.5mg/l BA. Drought is an important stress factor limiting the plant growth, reproductive, development and finally survival.

Drought stress tolerance is seen in all plants, but its extent varies from speices to species. Plants which are exposed to drought stress frequently were affected the synthesis and accumulation of secondary metabolite contents **(Al-Gabbiesh *et al.*, 2015)**.

Hadi *et al.* (2014) tested the response of *Ruta graveolens* callus to different concentrations of mannitol as an induced water stress agent on callus growth and proline accumulation. A quantity of 100 mg callus weighting was grown on Murashige and Skoog (MS) medium supplemented with 1 mg/l 2, 4-Dichlorophenoxyacetic acid (2, 4-D) and 0.5 mg/l kinetin (Kin) then mannitol at (0, 60, 120, 180, 240 or 300 g/l) was added. Results revealed that increasing water stress generated by increasing mannitol concentration caused a progressive reduction in callus fresh weight with a significant increase in response to 240 and 300 g/l mannitol after twelve weeks and a significant increase in proline accumulation in callus was recorded compared to control.

Hussein and Aqlan (2011) cut hypocotyls of fenugreek (*Trigonella foenum graecum* L.) into 5mm long segments and used it as explants. For obtaining callus under various water and salt stress levels, MS medium supplemented with 2.0 mg/l BA and 1.0 mg/l NAA was prepared; then divided into nine portions, the first was kept as control while the other portions mannitol (0.1 and 0.3% W/V) was added separately and interacted. Callus formation was allowed to take place for a period of six weeks in the dark at 25 ^{o}C. Results of this study have shown that the lower concentration of mannitol reduced growth while the higher concentration enhanced total phenolics and total flavonoid contents in comparison to the control.

This study aimed to investigate the effect of growth regulators on callus induction and growth as well as mannitol as osmotic stress agents on production of active ingredients from *Capparis spinosa* L. callus culture.

MATERIALS AND METHODS

This study was carried out at Tissue Culture Laboratory, Faculty of Environmental Agricultural Sciences, Arish University, North Sinai, Egypt, during the period from 2016 to 2018.

Plant material

Plant materials were collected from mature plants (*Capparis spinosa* L.) grown naturally in El-Hasana region, North Sinai.

Explants sterilization

Explants (nodal segments and shoot tips) were taken directly from their native plants and rinsed in water with a few drops of liquid soap for 5 minutes then rinsed again under running tap water for 60 minutes. Finally the explants were soaked for 5 minutes in 30% Clorox (containing 5.25% sodium hypochlorite) then washed again with sterilized distilled water for 3-5 times.

Culture Media

Explants were cultured aseptically on free MS-medium **(Murashige and Skoog 1962)** supplemented with 100 mgl^{-1}myo-inositol and 3% sucrose. All media were adjusted to pH 5.7 – 5.8 before gelling with 7.00 gl^{-1} agar and the medium was cooked on the hot plate. Medium was poured in 375 ml glass jars. Each jar contained 50 ml of medium.

Callus Induction

Callus cultures were initiated from *in vitro* leaves and nodal segments which isolated from 6-weeks-old shoots. The leaves and stems were cut into small cuts nearly uniform in size. Explants were cultured on MS solid medium supplemented with various concentrations of 2, 4-D (0.0, 0.5, 1.00, 1.5, 2.00 mgl^{-1}) combined with Kin or BA (0.0, 1.0, 2.0 mgl^{-1}). Cultures were maintained under continuous light at intensity of 2000 -3000 Lux from white cool light of florescent lamps (Phillips, Egypt) or continuous darkness.

All cultures were incubated at 25 ± 2^{o}C for 60 days. Eeach treatment consisted of three replicates and each replicate consisted of three jars each one containing three explants. After 60 days of culture the following parameters were recorded, callus formation percentage (%), callus fresh weight (g /jar) and callus dry weight (g /jar).

Osmotic Stress Experiment

After 4 weeks of subculture of callus on free MS medium, calli were individually weighed at 1g placed on MS medium fortified with mannitol at 0, 2, 4 or 8 gl^{-1}. All cultures were maintained under darkness condition at 25°C± 2. After 6 weeks, callus fresh and dry weights (g /jar), Proline content (according to **Bates *et al*., 1973)** and Callus water content (CWC %) were recorded. CWC % was calculates according to **Nawaz *et al*. (2013)** since CWC% = callus fresh weight (CFW) - callus dry weight (CDW) / CFW x CDW**.**

In order to identify active ingredients in callus, about 15 g of callus was collected from control and $4gl^{-1}$ mannitol treatments and active ingredients extracted by immersed each sample in absolute ethyl alcohol at 1: 100 (W/V). The mixture was placed in water bath at 99 ºCfor 30 min. In order to replace the evaporated alcohol, the same volume of ethylalcohol was boiled in water bath until reaching to 78ºC (the boiling point of ethylalcohol is 78.4ºC) and then were added to the flask with mixing. When the flask is cold, it was closed by parafilm and covered with aluminum foil and then kept on magnetic stirrer overnight. In the next day, the mixture was centrifuged at 5000 rpm for 15 min and the supernatant was taken and placed in falcon tubes and kept opened in the hood to evaporate the solvent. The dried extract was stored at -20ºC after ethyl alcohol was completely evaporated **(Abu Khalaf, 2011)**. The solvent was evaporated under reduced pressure and the oily residues were weighed and stored in dark at 4°C. The chromatographic separation for the identification of Silicate components was performed on an Agilent Technologies 6850 coupled with a Mass Selective Detector 5973 and a 7683B Series Injector auto sampler.

Statistical Analysis

The statistical layout of all above mentioned experiments was simple completely randomized design. Data were

statistical analyzed with analysis of variance (ANOVA) procedure using MSTAT-C Statistical software Package **(Michigan state University, 1983)**. Differences between means were compared by using Duncan's multiple ranged test **(Duncan, 1955)**.

RESULTS AND DISCUSSION

Effect of Growth Regulators and Light on Callus Induction and Growth

Callus Formation (%)

From results illustrated in Table 1 it could be observed that either MS free medium or MS fortified with any applied concentration of Kin or BA alone did not induce callus on both explant types either under light or dark conditions. On the other side, there were slight significant differences among different combinations between light, explant type, 2, 4-D and cytokinins (Kin and BA) levels concerning callus formation percentage.

These results are in the same way with those found by **Jahan *et al.* (2009)** since they showed that the high frequency of calli was obtained from leaf and spadix segments of *Anthurium andraeanum* L. when cultured on medium containing 2.5 mg/l BAP and 0.2 mg/l 2, 4-D in dark condition. Also, **Al-Hussaini *et al.* (2015)** studied the effect of different media on callus induction in potato. The results showed that the good callus formation was obtained on medium containing 2 mg/l 2, 4-D + 2 mg/l BA

Callus Fresh Weight

Results observed in Table 2 show that there were significant differences among different applied treatments on *Capparis spinosa* callus fresh weight. Results showd that culturing nodal explant under dark condition on MS medium supplemented with 2.0 mgl^{-1} 2, 4-D and Kin at 2.0 mg l^{-1} recorded the highest callus fresh weight (6.13g). This treatment did not significantly comparing with the two other treatments which the leaf explant grown under dark conditions on MS basal medium supplemented with Kin or BA at 2.0 mg l^{-1} with 1.0 mg l^{-1} 2, 4-D since they produced 5.73 and 5.66 g fresh callus, respectively.

The obtained results are in agreement with those found by **Abu Bakar *et al.* (2014)** on *Celosia argentea* where they reported that both stem and leaf explants cultured on medium contained 2, 4-D at 0.1 mg l^{-1} combined with kin at 1.0 mg l^{-1} showed the highest callus biomass. Also, **Roy *et al.* (2008)** on *Gymnema sylvestris* stated that the highest efficiency of callus formation was observed in the medium containing different concentrations of 2, 4-D and kinetin.

Callus Dry Weight

The illustrated results in Table 3 present the effect of interaction between light, explant type, 2, 4-D, BA and Kin levels on callus dry weight. It is clear that either MS free medium or MS medium fortified with any applied concentration of Kin or BA alone failed to induce callus on both explant types either under light or dark conditions. While, most of applied combination treatments did not differ from each other significantly concerning callus dry weight.

These results are in a harmony with those found by **Abu Bakar *et al.* (2014)** on *Celosia argentea*. They stated that both stem and leaf explants cultured on MS fortified with 2, 4-D at 0.1 mg l^{-1} combiened with 1.0 mgl^{-1} kin showed the highest callus biomass.

Table 1: Effect of light, explant type and growth regulators on callus formation (%) of *Capparis spinosa*

Treatment			Control	Kinetin (mg l^{-1})		BA (mg l^{-1})	
Explant type		2,4-D (mg l^{-1})	(0.0 mg l^{-1})	1.0	2.0	1.0	2.0
Light	Leaves	0.0	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f
		0.5	100.00 a	85.00 ab	50.00 cde	100.00 a	100.00 a
		1.0	100.00 a	100.00 a	100.00 a	100.00 a	86.66 ab
		1.5	100.00 a	46.66 de	100.00 a	100.00 a	100.00 a
		2.0	100.00 a	25.00 ef	83.33 ab	100.00 a	100.00 a
	Nodal	0.0	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f
		0.5	100.00 a	100.00 a	100.00 a	66.66 b-d	100.00 a
		1.0	100.00 a	100.00 a	86.66 ab	86.66 ab	90.00 ab
		1.5	100.00 a	100.00 a	100.00 a	100.00 a	86.66 ab
		2.0	100.00 a	100.00 a	86.66 ab	100.00 a	76.66 ab
Dark	Leaves	0.0	0.00 f	100.00 a	0.00 f	0.00 f	0.00 f
		0.5	100.00 a	100.00 a	91.66 ab	96.66 a	25.00 ef
		1.0	100.00 a	100.00 a	100.00 a	96.66 a	95.00 a
		1.5	100.00 a	100.00 a	100.00 a	88.33 ab	93.33 a
		2.0	100.00 a	100.00 a	100.00 a	100.00 a	88.33 ab
	Nodal	0.0	0.00 f	100.00 a	0.00 f	0.00 f	0.00 f
		0.5	100.00 a	100.00 a	100.00 a	100.00 a	66.66 b-d
		1.0	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a
		1.5	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a
		2.0	100.00 a	100.00 a	100.00 a	95.00 a	100.00 a

- Means followed by the same letters within the same column are not significantly different according to Duncan's multiple range test($P \leq 0.05$)

Table 2: Effect of light, explant type and growth regulators on callus fresh weight (g/jar) of *Capparis spinosa*

Treatment			Control	Kinetin (mg l^{-1})		BA (mg l^{-1})	
Explant type		2,4-D (mg l^{-1})	(0.0 mg l^{-1})	1.0	2.0		
Light	Leaves	0.0	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k
		0.5	3.28 c	2.10 d	2.83 c	2.93 c	4.13 b
		1.0	2.91 c	1.70 e	2.20c	2.73 c	3.36 c
		1.5	1.31 j	1.58 g	2.05 d	2.06 c	4.20 f
		2.0	1.61 c-g	1.43 i	3.43 k-o	1.81 d	3.00 c
	Nodal	0.0	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k
		0.5	1.24 g-j	1.10 ij	2.33 d	3.28 c	3.13 c
		1.0	0.97 j	1.42 i	1.51 h	2.41 d	3.13 c
		1.5	1.46 i	1.42 i	1.37 i	2.06 d	2.91 c
		2.0	1.43 i	1.33 j	1.50 h	1.81 d	3.37 c
Dark	Leaves	0.0	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k
		0.5	1.24 j	1.33 j	3.69 b	3.34 c	4.03 b
		1.0	1.94 d	0.00 k	5.73 a	1.20 h-j	5.66 a
		1.5	0.00 k	2.66 d	3.58 b	1.49 h	4.79 b
		2.0	0.00 k	2.53 d	2.07 d	1.64 f	3.68 b
	Nodal	0.0	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k
		0.5	3.80 b	2.73 c	3.26 c	1.44 i	4.58 b
		1.0	5.24 b	4.30 b	4.62 b	1.70 e	3.34 c
		1.5	2.38 d	3.48 c	3.81 b	2.10 d	1.63 f
		2.0	3.93 b	3.52 b	6.13 a	3.07 c	3.66 b

* Means followed by the same letters within the same column are not significantly different according to Duncan's multiple range test($P \leq 0.05$)

Table 3: Effect of light, explant type and growth regulators on callus dry weight (g/jar) of *Capparis spinos*a

Treatment			Control	Kinetin (mg l^{-1})		BA (mg l^{-1})	
Explant type		2,4-D (mg l^{-1})	(0.0 mg l^{-1})	1.0	2.0		
Light	Leaves	**0.0**	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
		0.5	0.64 a	0.53 a	0.68 a	0.43 a	0.33 ab
		1.0	0.46 a	0.48 a	0.37 ab	0.37 ab	0.49 a
		1.5	0.51 a	0.41 a	0.36 ab	0.36 ab	0.41 a
		2.0	0.44 a	0.34 ab	0.33 ab	0.33 ab	0.34 ab
	Nodal	**0.0**	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
		0.5	0.48 a	0.53 a	0.44 a	0.34 ab	0.50 a
		1.0	0.44 a	0.45 a	0.39 a	0.41 a	0.33 ab
		1.5	0.47 a	0.43 a	0.34 ab	0.38 a	0.42 a
		2.0	0.49 a	0.40 a	0.42 a	0.33 ab	0.39 a
Dark	Leaves	**0.0**	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
		0.5	0.42 a	0.50 a	0.45 a	0.34 ab	0.37 ab
		1.0	0.65 a	0.35 ab	0.63 a	0.00 b	0.33 ab
		1.5	0.47 a	0.43 a	0.40 a	0.38 a	0.00 b
		2.0	0.38 a	0.34 ab	0.31 ab	0.33 ab	0.00 b
	Nodal	**0.0**	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
		0.5	0.48 a	0.37 ab	0.49 a	0.50 a	0.57 a
		1.0	0.51 a	0.37 ab	0.45 a	0.47 a	0.47 a
		1.5	0.48 a	0.33 ab	0.45 a	0.47 a	0.49 a
		2.0	0.43 a	0.40 a	0.55 a	0.49 a	0.46 a

*Means followed by the same letters within the same column are not significantly different according to Duncan's multiple range test ($P \leq 0.05$)

Effect of Osmotic Stress

Results in Table 4 show significant differences in callus growth parameters as affected by mannitol concentration after 6 weeks. It's clear that addition of mannitol at4 gl^{-1} gave the heights value for each of fresh and dry weights (g) (5.66g and 3.31g, respectively). While, the lowest values were recorded by control treatment (2.33g and 1.10g, respectively). On the other hand, the greatest water content (%) was found with control and the lowest concentration of mannitol without significant difference between both treatments. Moreover, addition of 8 gl^{-1} mannitol to the medium gave the highest concentration of proline, while the lowest value was recorded with control.

These results are in agreement with those found by **Somayeh and Vahid (2011)** since they studied the comparative effects of drought on activity of antioxidant enzymes and carotenoids in callus of *Salicornia persica* and *Salicornia europaea*. They cultured the callus on Murashige and Skoog medium containing mannitol at (200, 500, and 1000 mM). Results indicated that proline content increased in callus of both species under mannitol induced stresses.

GC/MS Analysis of Produced Callus Under Osmotic Stress Conditions

GC–MS analysis leads to identify thirty compounds (Table 5) in callus of selected treatments (control and 4 gl^{-1} mannitol). Results revealed that 4 gl^{-1} mannitol treatments increased callus content of (1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl ester) from 42.38 mg/g for control to 48.11 mg/g for mannitol at $4gl^{-1}$. In the second order, Oleic acid (%) in *Capparis spinosa* callus was increased from 0.28 mg/g for control to 1.19 mg/g for mannitol at $4gl^{-1}$. Also, the same trend was obtained for 9-Hexadecenoic acid,

Table 4: Effect of mannitol concentration on growth and proline content (mg/g) of *Capparis spinosa* after 6 weeks

Mannitol ($g\ l^{-1}$)	Callus fresh weight (g)	Calus dry weight (g)	Callus water content (%)	Proline content (mg/g)
0.0	2.33 c	1.10 c	52.22 a	0.06 d
2.0	3.66 b	2.00 b	41.38 ab	0.08 c
4.0	5.66 a	3.31 a	45.00 b	0.12 b
8.0	3.33 bc	1.63 b	31.11 c	0.16 a

- Means followed by the same letters within the same column are not significantly different according to Duncan's multiple range test($P \leq 0.05$)

Table 5: GC/MS analysis of produced callus of *Capparis spinosa* under osmotic stress conditions

No.	Compound name	content (mg/g)	
		Control	Mannitol at 4 gl^{-1}
1	Muramic acid	0.01	0.00
2	Pterin-6-carboxylic acid	0.01	0.06
3	Imidazole-4-carboxylic acid	0.01	0.00
4	E-9-Tetradecenoic acid	0.28	0.00
5	9-Hexadecenoic acid	0.28	1.19
6	Oleic Acid	0.28	1.19
7	Dodecanoic acid, 3-hydroxy	0.01	0.04
8	Dodecanoic acid, 3-hydroxy	0.05	0.05
9	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	0.01	0.00
10	Pterin-6-carboxylic acid	0.03	0.03
11	Tetradecanoic acid,2-hydroxy	0.03	0.00
12	9-Hexadecenoic acid	0.02	1.19
13	Oxiraneoctanoic acid, 3-octyl-	0.02	0.32
14	Phenol	0.92	0.55
15	Phenyl-á	0.03	0.07
16	à-d-Gulofuranoside, phenyl	0.03	0.05
17	Phenyl-á-D-glucoside	0.03	0.03
18	Phenyl-d-mannoheptuloside	0.03	0.00
19	9-Octadecenoic acid,(2-phenyl-1,3-dioxolan-4-yl)methylester,cis-	0.00	0.12
20	Phen-1,4-diol,2,3-dimethyl-5-trifluoromethyl-	0.00	0.00
21	1, 2-Benzenedicarboxylic acid, mono(2-ethylhexyl)ester	42.38	48.11
22	Di-n-octyl phthalate	0.10	2.11
23	Dibutyl phthalate	1.00	2.00
24	Phthalic acid, butyl 4-octyl ester	5.00	0.74
25	Phthalic acid, butyl tetradecyl ester	0.00	0.74
26	Phthalic acid, isobutyloctadecyl ester	0.00	0.74
27	E,E,Z-1,3,12-Nonadecatrie ne-5,14-diol	0.22	0.00
28	Oleic acid,3-(octadecyloxy) propylester	0.03	0.08
29	1, 2-Benzenedicarboxylic acid, diisooctyl ester	42.38	36.25
30	Hexadecanoic acid, ethyl ester	0.13	0.00

Oxiraneoctanoic acid, 3-octyl-, Di-n-octyl phthalate, Dibutyl phthalate and Oleic acid,3-(octadecyloxy) propylester. On the other hand, addition of mannitol at $4gl^{-1}$ led to decrease in 1, 2-Benzenedicarboxylic acid, diisooctyl ester from 42.38 mg/g for control to 36.25 mg/g for mannitol at $4gl^{-1}$.

The results of this analysis were in agreement with those previously reported by **Al-Gabbiesh *et al*. (2015)** since they stated that drought is an important stress factor limiting the plant growth, reproductive, development and finally survival. It is known that accumulation of secondary metabolites often occurs in plants subjected to stresses including various elicitors or signal molecules **(Ramakrishna and Ravishanka, 2011).**

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الملخص العربي

تأثير الإضاءة ونوع المنفصل النباتي وبعض منظمات النمو والمانيتول على تكوين الكالس وإنتاج المواد الفعالة لنبات اللصف

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أجريت هذه الدراسة بمعمل زراعة الأنسجة النباتية- كلية العلوم الزراعية البيئية، جامعة العريش، شمال سيناء- مصر خلال الفترة من ٢٠١٦ حتى ٢٠١٨ والهدف الرئيسي لهذه الدراسة هو دراسة تأثير الإضاءة ونوع المنفصل النباتي وبعض منظمات النمو على تكوين الكالس ونموه بالإضافة إلى إختبار تأثير المانيتول كعامل إجهاد إسموزي على إنتاج المكونات الفعالة بمزارع كالس نبات اللصف، بدأ إنتاج الكالس من أوراق وعقد ساقية تم عزلها معمليا من زراعات معملية عمرها ٦ أشهر على بيئة موراشيج وسكوج الصلبة مضاف إليها تركيزات مختلفة من داي كلوروفينوكسي حامض الخليك مع الكينيتين والبنزيل أدينين ، وتركت تحت ظروف الإضاءة أو الإظلام الدائم على درجة حرارة ٢٥ ± ٢°م. بعد ٤ أسابيع من الزراعة لإنتاج الكالس على بيئة موراشيج وسكوج، تم وزن قطع من الكالس وزن كل منها جرام ثم نقلت إلى بيئة موراشيج وسكوج تحتوي علي المانيتول بتركيزات (صفر، ٢ ، ٤ أو ٨ جم/اللتر). وقد تم حفظها تحت ظروف الإظلام على درجة حرارة ٢٥ ± ٢°م لمدة ٦ أسابيع. وقد أظهرت النتائج أن كل من معاملة الكنترول وكذا معاملات السيتوكينينات قد فشل في تشجيع تكوين كالس علي كلا نوعي المنفصلات النباتية ولكن إستخدام كل من الداي كلوروفينوكسي حامض منفردا" أومشتركا" مع معاملات السيتوكينينات قد أدي لتكوين كالس عل كلا نوعي المنفصلات النباتية بدون فروق معنوية بين تلك المعاملات في معظم الحالات . وقد أمكن الحصول علي أعلي وزن جاف للكالس عن زراعة المفصلات الورقية علي بيئة موراشيج وسكوج المزودة بالداي كلوروفينوكسي حامض الخليك بتركيز ١مللجم/لتر+٢مللجم / لتر إلي بنزيل أدنين أو كينتين وكذلك عند زراعة منفصلات ساقية عقدية علي بيئة تحتوي علي الداي كلوروفينوكسي حامض الخليك والكينتين بتركيز ٢مللجم / لتر لكل منها تحت ظروف الإظلام .وعلاوة علي ذلك فقد أشارت النتائج المتحصل عليها أن إضافة ٤جم/لتر مانيتول أعطي اعلي القيم بالنسبة للوزن الطازج والوزن الجاف للكالس (جم). بينما، إضافة ٨,٠ جم/لتر مانيتول أعطى اعلي القيم لتركيز البر ولين، بينما أقل القيم سجلت مع معاملة المقارنة (الكنترول). كما أن نتائج تحليل كروماتوجرافيا الغاز الكمي تشير إلى إمكانية فصل ٣٠ مركب وبعض هذه المركبات كان تركيزه أعلي عند إستخدام معاملة ٤جم/لتر مانيتول مقارنة بمعاملة الكنترول .

الكلمات الاسترشادية :اللصف، الكالس، الكينيتين، البنزيل أدينين، المواد الفعالة، تحليل كروماتوجرافيا الغاز الكمي.

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