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# Callus Induction of Kiwifruit Plant *Actinidia deliciosa In Vitro*

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## ABSTRACT

The study has been conducted in the tissue and plant cells culture laboratory of the college of Agriculture and Forestry at the university of Mosul in 2013-2014 to test the effects of both of Kinetin (Kin) with concentrations of (0, 0.5, 1.0, and 2.0) mg.L$^{-1}$, and 2,4-dichlorophenoxy with concentrations of (0 , 0.5) mg.L$^{-1}$, in addition to various concentrations of sucrose (25, 30, 60, , 80) g.L$^{-1}$ and phenylalanine (0 , 10) mg.L$^{-1}$, on the growth and development of Kiwifruit plant (cultivar : Bruno) callus after five weeks of culture . the results have shown Significant variations between the studied factors of both fresh and dry weight of the callus. The addition of Kin at the concentrations (0.5 , 1.0 , 2.0)mg.L$^{-1}$ gave the highest Significant difference in fresh weight that reached (59.44, 69.01, 37.79)mg respectively, compared to the control treatment. whereas treatment with 2,4-D at concentration of 0.5 mg.L$^{-1}$ gave highest results that reached 61.15mg of fresh weight compared to the control treatment that. the treatment by adding Kin and 2,4-D in a concentration of 0.5mg/L$^{-1}$ also surpassed by increasing the dry weight of the callus that reached the values (25.117, 20.009)mg respectively, compared to other concentrations. As for the interaction between Kin and 2,4-D at a concentration of 0.5mg.L$^{-1}$ , it gave the highest values in both fresh and dry weight that reached (98.21, 39.360)mg respectively. The results also showed that the addition of sucrose the medium in a concentration of 30g.L$^{-1}$ gave a maximum fresh weight that reached 179.49mg. whereas the addition of sucrose in concentrations of (30, 60, 80) g.L$^{-1}$ gave the highest values in the dry weight that reached (43.268, 37.352, 33.765)mg respectively, compared control treatment. While the addition of phenylalanine led to Significant variations when added in a concentration of 10mg.L$^{-1}$, causing increment in both fresh and dry weight that reached (163.98 , 40.741)mg respectively, compared to the Comparative treatment that gave less values of (86.75, 22.939)mg respectively. Whereas the interaction between these factors led to significant results. in which, the interaction treatment using sucrose in a concentration of 30g.L$^{-1}$ and phenylalanine in a concentration of 10mg.L$^{-1}$ led to the highest results in both fresh and dry weight which reached (237.04, 52.877)mg respectively, compared to other treatments.

# استحداث كالس نبات الكيوي *Actinidia deliciosa* خارج الجسم الحي

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## الخلاصة

أجريت هذه الدراسة في مختبر زراعة الأنسجة والخلايا النباتية التابع لكلية الزراعة والغابات جامعة الموصل 2013–2014 لاختبار كل من الكاينتين Kinetin بتركيز (0 و 0.5 و 1.0 و 2.0) ملغ.لتر$^{-1}$ و 2,4-D بتركيز (0 و 0.5) ملغم.لتر$^{-1}$ وتراكيز مختلفة من السكروز (25 و 30 و 60 و 80) غم.لتر$^{-1}$ وفنيل الانين (0 و 10) ملغم.لتر$^{-1}$ في نمو واستحداث كالس نبات الكيوي صنف Bruno بعد خمسة اسابيع من الزراعة . بينت النتائج وجود فروق معنوية بين المعاملات المدروسة في الوزن الطري والجاف للكالس .ان اضافة Kin بتركيز (2.0 , 1.0 , 0.5) ملغم.لتر$^{-1}$ قد اعطت اعلى فرق معنوي في الوزن الطري بلغ ( 59.44 37.79 , 69.01) ملغم على التوالي مقارنة مع معاملة المقارنة ، في حين اعطت المعاملة بـ 2,4-D بتركيز

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0.5 ملغم.لتر$^{-1}$ اعلى النتائج بلغ 61.15 ملغم وزن طري مقارنة مع معاملة المقارنة .كما تفوقت معاملة اضافة Kin و 2,4-D بتركيز 0.5 ملغم.لتر$^{-1}$ لكل منها في الوزن الجاف للكالس بلغ (25.117 و 20.009) ملغم على التوالي على باقي التراكيز .اما التداخل بين Kin و 2,4-D بتركيز 0.5 ملغم.لتر$^{-1}$ لكل منهم فقد اعطت اعلى القيم في الوزن الطري والجاف بلغ (98.21 و 39.360) ملغم على التوالي . كذلك بينت النتائج ان اضافة السكروز الى الوسط الغذائي بتركيز 30 غم.لتر$^{-1}$ اعطت اعلى وزن طري بلغ 179.49 ملغم في حين اعطت اضافة السكروز بتركيز ( 80 , 60 , 30) غم.لتر$^{-1}$ اعلى القيم من الوزن الجاف بلغ ( , 43.268 33.765 , 37.352) ملغم وزن جاف على التوالي مقارنة مع معاملة المقارنة . في حين ادت اضافة فنيل الانين الى حدوث فروق معنوية عند اضافتة بتركيز 10 ملغم.لتر$^{-1}$ في الوزن الطري والجاف بلغ (163.98 و 40.741) ملغم على التوالي مقارنة مع معاملة المقارنة والتي اعطت اقل القيم بلغ (86.75 , 22.939) ملغم على التوالي. في حين كان التداخل معنوياً حيث اعطت معاملة تداخل اضافة السكروز بتركيز 30 غم.لتر$^{-1}$ وفنيل الانين بتركيز 10ملغم.لتر$^{-1}$ اعلى القيم في الوزن الطري والجاف بلغ (237.04 , 52.877) ملغم على التوالي مقارنة مع بقية المعاملات.

**Introduction :**

China is the original home of kiwifruit *Actinidia deliciosa*, which belongs to the family "Actinidaceae" and to the genus "*Acitinidia*" . there is also other species of this plant that grows in India, Japan, and in the north at south-east of Siberia (AL-diri, 2003). Italy is considered the leading producer of kiwifruit in the world (FAO, 2007), while Iraq is still falling behind in about cultivating this plant or it's not even being cultivated, despite the fact that it has been cultivated in some of the neighboring countries and despite of its environmental conditions of growth which is similar to the conditions of a lot of fruit trees being cultivated in this country (Obaid and AL-hayani, 2012). The kiwifruit is oval in shape and has a brown skin and usually green flesh with black seeds. Kiwi plant is a Dioecious, bisexual, woody, climbing shrub with deciduous leaves. Kiwi has a Great economic returnbecause of the Gradual maturity of its fruits that gives it the ability to be stored up to six months. Kiwifruit is rich with vitamin C. that is, one kiwifruit contains an amount of vitamin C that equals the content of 10 lemon fruits (AL-diri, 2003). Kiwi also contains various minerals many copper, zinc, manganese, and magnesium (Rugini and Gutierrez-pesce, 2003). It also contains proteins, salts, phosphorus, calcium, iron, and potassium with a concentration equal to the one of the banana fruit (Ibrahim, 1996). Kiwi leaves and branches can also being benefited from by boiling it in water to be used as a treatment for Scabies (Nasib *et al*., 2008). A study carried out by (Akbas *et al*., 2007) showed that the best way to growth kiwi Seedlings is by culturing seeds on a food medium supplied with a concentration of 0.5mg.L$^{-1}$ of Kin or Benzyl adenine. While (Ibrahim et al., 2012) referred that adding Kin a concentration of 0.1mg.L$^{-1}$ to the food medium led to the highest branching rate in the grape plant of a cultivar called "kamali" that reached 2,2 branch.plant. Whereas (Jawad et al., 2014) demonstrated that the addition of phenylalanine in a concentration of (5 ,10)mg.L$^{-1}$ gave the highest increment in the fresh weight of Lemon Balm plant that reached 3688mg and 3578mg, respectively. A study committed by on water hyssop plant showed that the addition of 2,4-D in a concentration of 3mg.L$^{-1}$ led to the highest percentage grow rate of the callus reached 85% .

**Materials and Methods:**

The study was conducted in the Plant Tissue Culture laboratory in the Department of Horticulture and Landscape Design, College of Agriculture and Foresty at the University of Mosul in 2013-2014. The kiwifruit seeds cultivar Bruno.

**The media preparation:** MS salts (Murashige and Skoog, 1962), vitamins (1.0 mg.L$^{-1}$), plant growth regulators are using in the medium of callus induction. The pH of medium is adjusted to 5.7 by sodium hydroxide and hydrochloric acid.

**Table(1)The chemical material composition additives to MS medium used for callus induction.**

| Seq. | Chemical material | Quantity (mg l-1) |
|---|---|---|
| 1 | Salt | full strength |
| 2 | Pyrodoxine –Hcl | 0.5 |
| 3 | Glycine | 2.0 |
| 4 | Nicotine acid | 0.5 |
| 5 | Thiamine–Hcl | 0.1 |
| 6 | Myo-inositol | 0.1 |
| 7 | Agar | 7000 |
| 8 | Sucrose | 25000,30000,60000,80000 |
| 9 | Kinetin | (0, 0.5, 1.0, and 2.0) |
| 10 | 2,4-dichlorophenoxy | (0 , 0.5) |
| 11 | phenylalanine | (0 , 10) |

**Explants sterilization**, kiwifruit seeds of current study equipped by the American company for seed production. This seeds were isolated and washed thoroughly under tap water to remove dust on the seed coat. Then the seeds were sterilized with 4.5 % sodium hypochlorite solution with 3 drops of tween20 for 20 minutes and washed 3 times with distilled water inside the laminar air-flow cabinet. The sterilized seeds cultured on MS medium without hormones. They placed in a growth room under controlled conditions (temperature 25±2°C, 16/8 h photoperiod). Cotyledons were excised from cultures after 4 weeks from seedling culture. kinetin(kin) added to MS medium in different concentrations (0.0, 0.5, 1.0 and 2.0) $mg.L^{-1}$. 2,4-D added at two concentrations (0.0 and 0.5) $mg.L^{-1}$. The different concentration of kin and 2,4-D were used to determine the optimal concentration for callus induction.

**Callus induction:**

1. The cotyledons cultured in MS medium (10ml) supplemented with 0.0 or 0.5 $mg.L^{-1}$ 2,4-D and 0.0, 0.5, 1.0 or 2.0 $mg.L^{-1}$ kin . Each treatment represented ten replications.
2. Callus was indution through the cultivation of the best medium MS salts + 0. 5 $mg.L^{-1}$ 2,4-D + 0.5 $mg.L^{-1}$ kin.

**Effect of 2,4-D and kin on callus induction:**

Has been taking the weight of 100 mg of callus was grown on MS medium containing: 0.0 or 0.5 $mg.L^{-1}$ 2,4-D + 0.0, 0.5, 1.0 or 2.0 $mg.L^{-1}$ kin. Each treatment represented ten replications. They placed in a growth room under controlled conditions (temperature 25±2°C and darkness). The fresh and dry weights of callus were calculated after 5 weeks from culture.

**Effect of sucrose and phenylalanine on callus induction:**

Has been taking the weight of 150 mg of callus was grown on MS medium containing: 0.5 $mg.L^{-1}$ 2,4-D + 0.5 $mg.L^{-1}$ kin + 25, 30, 60 or 80 $g.L^{-1}$ sucrose + 0.0, 10 $mg.L^{-1}$phenylalanine. Each treatment represented ten replications. They placed in a growth room under controlled conditions (temperature 25±2°C and darkness). The fresh and dry weights of callus were calculated after 5 weeks from culture.

**Statistical analysis:**

The factorial experiments were carried out using Completely Randomized Design (CRD). The data were analyzed using SAS (2002). The means of treatments were measured by Duncan Multiple Range Test under the 5% probability level. Each treatment included 10 replicates, each containing one explant (Al-Sahuki and Wahib, 1990).

**Results and discussion :**

**1-The effect of kinetin and 2,4-D and the interaction on the induction of kiwi plant callus from the Cotyledons leaves *in vitro*.**

**Ftesh weight :**

After five weeks of culture, the results shown in table (2) have demonstrated significant variations in the fresh weight of the callus, in which, the mediums supplied with (2.0, 1.0, 0.5) $mg.L^{-1}$ of Kin gave the highest results (59.44, 69.01 and 37,79)mg of fresh weight, respectively. compared to the control treatment which did not give any value in fresh weight.

The effect of 2,4-D was also significant, and it can be noticed in the same table above. Treatment with $0.5mg.L^{-1}$ of 2,4-D gave a maximum fresh weight value that reached 61.15mg , compared to the control treatment which did not give any value in fresh weight.

As for the effect of interaction between Kin and 2,4-D , as it can be noticed in the same table, the maximum fresh weight of the callus was achieved by treatment with (0.1 and 0.5) $mg.L^{-1}$ of kin interacted with $(0.5)mg.L^{-1}$ of 2,4-D . it gave the highest increment in the fresh weight of callus that reached 98.21mg and 89.45mg, exceeding the control treatment which did not give any value in fresh weight.

**dry weight :**

It has been demonstrated in table (3) that after five weeks of cultivation, there were significant variationsin the dry weight of the callus, in which the treatment of adding $0.5mg.L^{-1}$ concentration of kin to the medium gave the highest value 25.117 mg of the dry weight, compared to the control treatment that gave 0.00 mg increment in dry weight.

The effect of 2,4-D on the dry weight of the callus can be noticed in the same table. The maximum dry weight increment of the callus was achieved by cultivation on medium supplied with a $0.5mg.L^{-1}$ concentration of 2,4-D . the resulted value reached 20.009mg, compared to the control treatment that gave a minimal dry weight that reached 5.546mg.

The effect of interaction between kin and 2,4-D was significant. Treatment with $0.5mg.L^{-1}$ of kin and $0.5mg.L^{-1}$ of 2,4-D gave the highest dry weight value of callus that reached 39.360mg, compared to the control treatment which did not give any value in dry weight. This may be due to the physiological balance between auxin and cytokinin. The addition of both growth regulators to the medium of culture is necessary for the induction of callus. Cytokinin works with auxin as a key to initiating cell division. Adenine, the cytokinin molecule, may be the optimal balance. The difference between explants may be due to the anatomical structure and its physiological development. (Mineo, 1990).

**2-The effect of sucrose and phenylalanine and the interaction on the induction of kiwi plant callus from the Cotyledons leaves *in vitro*.**

**fresh weight :**

It has been demonstrated in table (4) that the addition of sucrose to the medium had a significant effect on the fresh weight of the callus. In which, the treatment supplied with sucrose at a concentration of $30g.L^{-1}$ gave the highest increment in the fresh weight of the callus that reached 179.49mg, compared to the comparative treatment that has been supplied with a $25g.L^{-1}$ concentration of sucrose and gave a minimal value that reached 38.66mg.

The addition of phenylalanine to the medium led to significant variations in the fresh weight of the callus. That is, treatment supplied with a 10mg.$L^{-1}$ concentration of phenylalanine led to a maximum fresh weight value of the callus that reached 163.89mg. compared to the control treatment which gave the minimal value 86.75 mg of fresh weight.

On the other hand, the interaction of sucrose and phenylalanine has a significant effect on the fresh weight of the callus, where in the interacting treatment of sucrose at concentrations of 30g.$L^{-1}$ and 60g.$L^{-1}$ with phenylalanine at a concentration of 10mg.$L^{-1}$ gave the highest values of fresh weight that reached 237.04mg and 202.80mg respectively, compared to the interaction treatment of sucrose 25g.$L^{-1}$ and 0.00mg.$L^{-1}$ concentration of phenylalaninethat did not give any value in fresh weight.

**dry weight :**

As it shown in table (5) the addition of sucrose to the medium led to the occurrence of significant variations in weight, wherein treatment by adding sucrose to the medium at concentrations of (30, 60, 80)g.$L^{-1}$ resulted in a maximum dry weight of the callus that reached (33.765, 37.352, 43.268)mg respectively, compared to the addition of sucrose at a concentration of 25g.$L^{-1}$ which gave the minimal value 21.976mg of the dry weight of callus.

The addition of phenylalanine to the medium, as it shown in the previous table, in a concentration of 10mg.$L^{-1}$ gave the highest increment in dry weight compared to the control treatment that gave a minimal value that reached 22.939mg.

The interaction effect of adding both sucrose and phenylalanine to the medium was also significant. That is, the interaction treatment of adding a 30g.$L^{-1}$ concentration of sucrose and a 10mg.$L^{-1}$ concentration of phenylalanine had led to a maximum increment value of 52.877mg in the dry weight, which differs significantly from the comparative treatment which did not give any value in dry weight. These results may be explained by the fact that Phenylalnine is one of the amino acids involved in building proteins that work on enzymes that play a role in most bio-processes It is also considered to stimulate cell division , This is consistent with (Jawad et al., 2014). This may be due to the importance of adding sugar to the center from the fact that the process of photosynthesis carried out by the planted plant part is insufficient to grow as it depends on growth Sugar added.

**Table (2): Effect of Kin and 2,4-D on fresh weight of callus (mg) induced from cotyledonary leaf of the kiwifruit (*Actinidia deliciosa* ) by *In vitro*.**

| 2,4-D **concentration (mg.l$^{-1}$)** | Kin **concentration (mg.l$^{-1}$)** | | | | **Mean of 2,4-d** |
|---|---|---|---|---|---|
| | **0.0** | **0.5** | **1.0** | **2.0** | |
| **0.0** | **0.000 C** | **20.68 BC** | **51.58 ABC** | **15.64 BC** | **21.97 B** |
| **0. 5** | **0.000 C** | **98.21 A** | **86.45 A** | **59.95 AB** | **61.15 A** |
| **Mean of kin** | **0.000 B** | **59.44 A** | **69.01 A** | **37.79 A** | |

**Table (3): Effect of Kin and 2,4-D on dry weight of callus (mg) induced from cotyledonary leaf of the kiwifruit (*Actinidia deliciosa* )by *In vitro*.**

| 2,4-D **concentration (mg.l$^{-1}$)** | Kin **concentration (mg.l$^{-1}$)** | | | | **Mean of 2,4-D** |
|---|---|---|---|---|---|
| | **0.0** | **0.5** | **1.0** | **2.0** | |
| **0.0** | **0.000 D** | **10.874 CD** | **6.633 CD** | **5.074 CD** | **5.645 B** |
| **0. 5** | **0.000 D** | **39.360 A** | **24.477 B** | **16.199 BC** | **20.009 A** |
| **Mean of** Kin | **0.000 C** | **25.117 A** | **15.555 B** | **10.637 B** | |

**Table (4): Effect of sucrose and phenylalanine on fresh weight of callus (mg) induced from cotyledonary leaf of the kiwifruit (*Actinidia deliciosa* )by *In vitro*.**

| phenylalanine **concentration ($mg.l^{-1}$)** | sucrose **concentration ($mg.l^{-1}$)** | | | | Mean of phenylalanine |
|---|---|---|---|---|---|
| | **25** | **30** | **60** | **80** | |
| **0.0** | **0.00 D** | **121.93 BC** | **100.05 BC** | **107.67 BC** | **86.75 B** |
| **10** | **69.59 BC** | **237.04 A** | **202.80 A** | **146.12 B** | **163.89 A** |
| Mean of sucrose | **38.66 C** | **179.49 A** | **151.43 AB** | **126.90 AB** | |

**Table (5): Effect of sucrose and phenylalanine on dry weight of callus (mg) induced from cotyledonary leaf of the kiwifruit (*Actinidia deliciosa* )by *In vitro*.**

| phenylalanine **concentration ($mg.l^{-1}$)** | sucrose **concentration ($mg.l^{-1}$)** | | | | Mean of phenylalanine |
|---|---|---|---|---|---|
| | **25** | **30** | **60** | **80** | |
| **0.0** | **0.000 D** | **33.660 BC** | **26.000 C** | **32.079 BC** | **22.939 B** |
| **10** | **25.951C** | **52.877 A** | **48.703AB** | **35.433BC** | **40.741 A** |
| Mean of sucrose | **12.976 B** | **43.268 A** | **37.352 A** | **33.765 A** | |

**Conclusions**:

We are concluded from the present study that cotyledon leaf of kiwifruit plants have ability of growth and induction of indirect callus when they are cultured in the right medium and concentration of 2,4-D, kin, sucrose and phenylalanine, according to the nature of growth**.**

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