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RESEARCH ARTICLE
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In Vitro Propagation of Lemon Citrus lemon local using WPM media.

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ABSTRACT

This research was conducted in the Plant Tissue Culture Laboratory in the Department of Horticulture and Landscape Design/College of Agriculture/University of Kirkuk-IRAQ for period from March (2023) to April (2024) to propagate local lemon (Citrus lemon local.) In vitro. During the sterilization stage, the effect of NaOCl was tested at concentrations (2 - 4 - 6) % with duration (2 - 4 - 6) minutes. The proliferation and multiplication stage used different concentrations of BA (0.0, 0.5, 1.0, 1.5, 2.0) mg L-1 and Kin (0.0, 2.0, 4.0, 6.0, 8.0) mg L-1 for WPM media. in the multiplication stage after eight weeks, the highest average number of branches was 4.60 branches. Plant part-1 and the highest average number of leaves was 17.10 leaves. Plant part-1 on. The percentage of chlorophyll in the leaves of plants grown in WPM media supplemented with BA was measured, where the highest wavelength at the chlorophyll level was (0.1971) nm in the media supplemented with 1.5 mg. L-1, while the highest wavelength at the level of chlorophyll b was (1.810) nm in a media supplemented with 0.5 mg. L-1, while the highest total wavelengths were (1.970) nm in the media supported with 0.5 mg. L-1, while in the media supported by Kin, where the highest wavelength was at the level a (4.490) nm in the media supplied with 8.0 mg. L-1, while the highest wavelength at the b level (19.74) nm in the center of the supply was 6.0 mg. L-1, where the highest total wavelength (21.80) nm at the center of the supply was 6.0 mg. L-1, where the leaves collected the cotyledons in the multiplication stage 8 weeks after planting. The resulting vegetative growths were used in the multiplication stage in the process of in vitro micrografting on bitter orange seed stock.

***Keywords*:** In Vitro, WPM, BA, Kin, Citrus lemon local..

INTRODUCTION

Lemon trees, whose scientific name is *Citrus Limon*, belong to the citrus genus *Citrus*, which belongs to the family Rutaceae. The regions of southwestern China and northeastern India are considered the original home of these genera. The local lemon variety, *Citrus Lemon* local, is very desirable in Iraq because its fruits are excellent and of good quality. The fruits are very juicy and small in size, the skin is thin, and the fruits are less acidic than other international varieties, so they are preferred. The fruits are early ripening, as the harvest of lemon fruits begins in the months of September and October, and the harvest season continues until the spring months while the fruits are still green in color,[1]. *Citrus lemon* fruits are used in the manufacture of juices and fresh consumption, in addition to being used as flavorings for many foods, in addition to their important role in treating many diseases [2]. Lemons are a source of vitamin C and good amounts of vitamins [3]. (B2, B1, A)

• Plant Tissue Culture:

Plant tissue culture means isolating a cell, tissue, or organ for plants under sterile conditions free of pathogens, sterilizing it and cultivating it in sterile artificial nutritional media as well, and then developing the cultivated plant part under controlled conditions of humidity, temperature, and light [4]. Plant tissue culture, or what is called *In vitro* culture, by removing or isolating a cell, tissue, organ, or part of an organ, sterilizing it, cultivating it on artificial nutrient media, and then incubating the cultivated plant part in controlled conditions in terms of temperature and light [5]. [6].

• Materials and methods:

• Explants:

In this experiment, different explants of Shoot tips and nodal stems were used. These were divided into small parts so that each part contained one bud for propagation using tissue culture technology. These parts must be free of any pathogens, insects, or growth abnormalities. Therefore, identifying explants must be done with precision and perfection, especially since different plant tissues are not considered to have the same ability to grow and branch when grown in a nutrient media [7].

• Sterilize the Explants:

Explants were sterilized with a sodium hypochlorite solution obtained from a commercial minor solution containing 6% sodium hypochlorite, and all tools used in transplantation, including tweezers, blades, and the transplant cabinet, were

sterilized with ethyl alcohol at a concentration of 70% immediately before transplantation. The explants were placed in NaOCl at concentrations (2,4,6) % and then complete the volume to 100 ml with sterile distilled water for a period of (2, 4, 6) minutes, after which the parts were washed with distilled water three times in a row to remove any trace of the sterile solution to avoid damage to the plant tissue. In an experiment conducted by [8], This method was effective in reducing pollution and not causing damage to the treated plant parts.

• Preparing the nutrient media:

In this experiment, Woody Plant Media (WPM) was imported from Caisson Labour Company (USA) with a weight of 2.58 g. L^{-1} Were used To prepare one liter of nutrient media, put distilled water in Beakers on a hot plate magnetic stirrer device, then add 7.5 grams of agar (Agar - Agar) to it until boiling, and after the agar has completely dissolved and the solution is homogeneous, add 2.58 grams of media to it. Then 30 grams of sucrose were added, then the volume was added to a liter, and then growth regulators were added (the type and concentration of the regulator according to the goal of the study), and the ingredients were mixed well, then the pH was adjusted at 5.7 ± 0.1 by adding sodium hydroxide (NaOH) or hydrochloric acid. HCl using a pH meter. Then pour the nutrient media directly into a 240 ml culture glass laboratory tube with 40 ml of nutrient media. The experiment was carried out using the (CRD) with ten repetitions, the findings were analyzed using a statistical program, and the means were compared using (LSD) 0.05. [9].

• Sterilization of the nutrient media and growing conditions for the crops:

After placing the nutrient media in a test tube designated for growing explants according to the quantity required for each experiment, it was sterilized at a temperature of 121 °C and a pressure of 1.04 kg/cm2 for 20 minutes using an Autoclave device. After the sterilization period, the test tubes were taken out and left in the development room until the media solidified. Then it is ready for planting. Plants during the proliferation, multiplication and rooting stage were incubated in a growth room at a temperature of (25 ± 2 °C) and a lighting intensity of (3000) lux, with a photoperiod of 16 hours followed by 8 hours of darkness daily, equipped with white fluorescent tubes.

• Initiation stage: Initiation stage

Explants (Shoot tips & Nodal stem) with a length of (0.5 and 1 cm) were sterilized with commercial trace element (NaOCl) at a concentration of (2, 4, 6) % ml v/v for a period of (2, 4, 6) minutes. These plant parts were grown on Woody Plant Media (WPM) supplemented with 0.5 mg. L^{-1} BA and data were taken 4 weeks after planting.

• Multiplication stage: Multiplication stage

This stage is one of the important stages in tissue culture, in which the growth of buds and the formation of new branches continues to be stimulated by controlling the phenomenon of apical dominance of the abundant branches in tissue culture when they are re-cultivated on nutrient media prepared with cytokines [10]. Two types of cytokines, BA and Kin, were used in this experiment. In WPM nutrient media, the developing nodes and shoots resulting from the proliferation stage were planted on WPM media supplied with BA at concentrations of (0.0, 0.5, 1.0, 1.5, 2.0) mg. L^{-1}. The growing nodes and shoot tips resulting from the proliferation stage were grown on WPM media supplied with Kinetin at concentrations of (0.0, 2.0, 4.0, 6.0, 8.0) mg. L-1. I used 10 replicates for each treatment and one plant part for each replicate, and then incubated in the Growth Room at a temperature of 23-25°C and a lighting intensity of 3000 lux for 16 hours of light and 8 hours of darkness. Day 1, and data were taken after (4, 8) weeks of transplantation.

• Results & Discussion:

• The effect of sodium hypochlorite NaOCl on the survival of shoot tips & Nodal stem on WPM media.

Among the results in Table (1), the sterilization period and the concentration of the sterilizing agent NaOCl have a significant impact on the highest survival rate of 100% when the sterilization duration is (2) minutes. It is also clear from the table that the use of sodium hypochlorite NaOCl at a concentration of (2) ml led to obtaining a percentage Survival is 100%. As for the interaction of duration and concentration of the sterile substance, we note that the greatest survival rate was 83% at a concentration of (2) ml and for a duration of (2) minutes.

Table (1) the effect of sodium hypochlorate NaOCl on the survival of shoot tips and nodal stems of the local lemon plant Citrus lemon local. Cultivated on WPM media.

T (min) C (ml) %	2	4	6	Effect Concentration (ml) %
2	100 a	100 a	90 ab	96 a
4	90 ab	80 ab	80 ab	83 ab
6	80 ab	70 ab	60 b	70 b

Effect time	90	83	76
(min)	a	ab	b

* Values with similar characters for each factor are not significantly different according to the Dunkin Multipliers test below the 5% probability level.

The results of this experiment showed that NaOCl hypochlorite is a sterilizing substance that plays a major role in eliminating microorganisms represented by fungi and bacteria when used to sterilize explants with the appropriate concentration and duration [6]. These results are consistent with [14], and [15].

• Effect of BA on proliferation and multiplication of growing shoot tips and nodes on WPM media

The results in Table (2) showed that benzyl adenine BA has a major role in the proliferation and multiplication of tissue cultures from (Shoot tips & Nodal stem) cultures, as all treatments responded by 100%, and this in turn outperformed the comparison treatment, in which the response rate was 60%, and the highest response rate was obtained. The average number of branches was 3.40 branches. Plant part^{-1} from planting (Shoot tips & Nodal stem) on the media supplemented with 1.0 mg. L^{-1}, while the highest average length of the longest branch was (1.40, 1.30) cm from planting on the media supplemented with (0.5, 1.5) mg. L^{-1}, respectively, and the highest average number of leaves was 10.10 leaves. Plant part^{-1} from the planting in the media supplied with 1.5 mg. L^{-1}, and it was significantly superior to the rest of the treatments and this comparison treatment for the proliferation stage.

From reviewing the data during the doubling phase, we notice an increase in the number of branches to 4.60 branches. Plant part $^{-1}$ when grown on WPM media prepared with BA at a concentration of 1.0 mg. L^{-1} and grown (Shoot tips & Nodal stem) on WPM media supplied with 0.5 mg. L^{-1} resulted in the highest average length of the longest branch being 1.65 cm, while the highest average number of leaves was 17.10 leaves. Vegetable part-1 was significantly superior to the rest of the treatments.

Table (2) Effect of BA on the proliferation and multiplication (Shoot tips & Nodal stem) of Citrus lemon local grown on WPM media after 4 and 8 weeks.

WPM-BA mg. L^{-1}	After 4 weeks				After 8 weeks		
	Initiation				Multiplication		
	Response %	Leaves no.	Shoots no.	Shoots Length /cm.	Leaves no.	Shoots no.	Shoots Length /cm.
0.0	0.60	0.80	0.30	0.11	1.40	0.80	0.17
		c	d	c	c	c	d
0.5	1.00	5.80	1.40	1.43	10.60	2.10	1.65
		b	c	a	b	bc	a
1.0	1.00	9.00	3.40	1.03	17.10	4.60	1.08
		ab	a	ab	a	a	bc
1.5	1.00	10.10	2.00	1.30	15.50	3.20	1.46
		a	bc	a	ab	ab	ab
2.0	1.00	8.20	2.70	0.69	13.70	3.90	0.84
		ab	ab	b	ab	a	c

* Values with similar characters for each factor are not significantly different according to the Dunkin Multipliers test below the 5% probability level.

• Effect of Kin on proliferation and multiplication (Shoot tips & Nodal stem) on WPM media

The results in Table (3) showed that the treatments Kinetin has a major role in the proliferation of tissue cultures from (Shoot tips & Nodal stem) cultures, as all treatments responded by 100%, and this, in turn, outperformed the comparison treatment, in which the response rate was 60%, and the highest rate of number Branches 2.40 branches. Plant part^{-1} of culture on media prepared with 4.0 mg. L^{1}. The highest average length of the longest branch was 1.54 cm when grown on media supplemented with 2.0 mg. L^{-1}, while the highest average number of sheets was 10.30 sheets. Plant part-1 of culture on media prepared with 4.0 mg. L^{-1} is significantly superior to the rest of the treatments and the comparison treatment for this stage.

The data during the doubling phase showed an increase in the number of leaves, reaching the highest rate of 18.10 leaves. Plant part^{-1} of cultivation on media supplemented with 4.0 mg. L-1, which exceeds the rates for other parameters. While the

highest average length of the longest branch was 1.74 cm when grown on the media supplemented with 2.0 mg. L-1 was significantly superior to the rest of the treatments where the cultivation of (Shoot tips & Nodal stem) on the media prepared with 4.0 mg. L-1 to obtain the highest average number of branches, 3.90 branches. Vegetable part-1 was significantly superior to the comparison treatment.

Table (3) Effect of Kin on the proliferation and multiplication of shoots and nodes of Citrus lemon local grown on WPM media after 4 and 8 weeks.

WPM-Kin mg. L^{-1}	After 4 weeks Initiation				After 8 weeks Multiplication		
	Response %	Leaves no.	Shoots no.	Shoots Length /cm	Leaves no.	Shoots no.	Shoots Length /cm.
0.0	60	0.80 b	0.30 c	0.11 b	1.40 b	0.80 c	0.17 c
2.0	100	10.10 a	1.90 ab	1.54 a	15.80 a	2.30 b	1.74 a
4.0	100	10.30 a	2.40 a	1.23 a	18.10 a	3.90 a	1.41 ab
6.0	100	9.30 a	1.90 ab	0.80 ab	18.00 a	3.10 ab	0.92 b
8.0	100	9.10 a	1.40 b	1.29 a	14.10 a	2.20 b	1.41 ab

* Values with similar characters for each factor are not significantly different according to the Dunkin Multipliers test below the 5% probability level.

• Effect of BA on the chlorophyll wavelength at levels (b, a) of the local lemon plant Citrus lemon. Cultivated on WPM media.

The data is shown in Table (4). BA plays an important role in the wavelength of chlorophyll in plant leaves, as the highest wavelength at the chlorophyll level was (0.1971) nm in the media supplemented with 1.5 mg. L^{-1}, while the highest wavelength at the chlorophyll b level was (1.810) nm in a media supplemented with 0.5 mg. L^{-1}, while the highest total wavelength (1.970) nm was in the media supplemented with 0.5 mg. L^{-1}, where the leaves were collected in the doubling stage after 8 weeks of planting.

Table (4) Effect of BA on the chlorophyll percentage of Citrus lemon local. Cultivated on WPM media

	WPM - BA		
Concentrations (ml)	a	b	Total
0.0	0.000 e	0.000 e	0.000 e
0.5	0.1601 b	1.810 a	1.970 a
1.0	0.0406 c	0.678 b	0.719 d
1.5	0.1971 a	0.569 c	0.766 c

2.0	0.0199	0.020	1.275
	d	d	b

Values with similar characters for each factor are not significantly different according to the Dunkin * Multipliers test below the 5% probability level.

• The effect of Kin on the chlorophyll wavelength at levels (b, a) of the local lemon plant Citrus lemon. Cultivated on WPM media.

It is clear in Table (4) that Kin has a significant effect on the wavelength of chlorophyll in plant leaves, and the highest wavelength was at the chlorophyll a level (4.490) nm in the media supplied with 8.0 $mg.L^{-1}$, while the highest wavelength was at the chlorophyll b level (19.74 nm in the media supplemented with 6.0 $mg.L^{-1}$, while the highest total of the longest wavelength was (21.80) nm in the media supplemented with 6.0 $mg.L^{-1}$, where the leaves were collected in the doubling stage after 8 weeks of planting

Table (5) Effect of Kin on the chlorophyll percentage of Citrus lemon local. Cultivated on WPM media.

WPM - Kin			
Concentrations (ml)	a	b	Total
0.0	0.000	0.000	0.000
	e	e	e
2.0	1.754	10.53	12.29
	c	d	d
4.0	0.449	19.28	19.73
	d	b	c
6.0	2.062	19.74	21.80
	b	a	a
8.0	4.490	16.18	20.67
	a	c	b

* Values with similar characters for each factor are not significantly different according to the Dunkin Multipliers test below the 5% probability level.

The results obtained from NETs (4-5) in chlorophyll stocks in the operational phase can be explained by the role played by cytokines in this phase. Based on [11], in tissue culture, tissue culture in the absence of light and the absence of cytokinin becomes white plastids (i.e. Japanese in color) and plastid granules do not arise, but the signatures of cytokinin lead to multiple endplates without the formation of cataphiles and plastid granules in the dark. Because you are exposed to the allergy to the presence of cytokinin in the event of the formation of both plastid and cytoplasmic granules. Meanwhile, [12] notes that the vascular growth regulator helps heal the tiny blood vessels of various plants, which leads to an increase in chloroplasts and thus an increase in the vitality of plants. It turns out in this study that BA was more effective in achieving significant progress compared to its relatives, [13]. The reason for this may be because BA is considered one of the most effective cytokines compared to the rest of the cytokines, as its benefits are linked to a benzyl ring containing three Double bonds which makes it superior in its activities to cytokines, and hence the desired results with [14, 15, 16, 17].

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إكثار الليمون المحلي (*Citrus lemon local*.) خارج الجسم الحي بأستخدام وسط WPM.

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الخلاصة

أجريت هذا بحث في مختبر زراعة الأنسجة النباتية *Plant tissue culture* في قسم البستنة وهندسة الحدائق/ كلية الزراعة / جامعة كركوك للمدة من أذار (2023) الى نيسان (2024) في إكثار الليمون محلي (*Citrus lemon local*.) خارج الجسم الحي. خلال مرحلة التعقيم تم تجربة تأثير *NaOCl* بتراكيز (2 – 4 –6) % ولفترة (2 – 4 –6) دقائق، وكانت أعلى نسبة النجاة 100% عنده تركيز 2 مل في 2 دقائق أما تداخل الفترة والتركيز مادة المعقمة في وسط *Woody Plant Medium (WPM)* كانت أكبر نسبة البقاء 83 % عنده التركيز 2 مل ولفترة 2 دقائق. اما خلال مرحلة النشوء والتضاعف تم تجربة تأثير إضافة تراكيز مختلفة من *BA* (0.0، 0.5، 1.0، 1.5، 2.0) ملغم. لتر−1 و*Kin* (0.0، 2.0، 4.0، 6.0، 8.0) ملغم. لتر−1 لوسط الغذائي *WPM*. أظهرَ النتائج بعد مرور أربعة أسابيع من مرحلة النشوء بأن نسبة الاستجابة جميع المعاملات بنسبة 100% *WPM*− *BA* وهذه تفوقت على معاملة المقارنة التي كانت نسبة الاستجابة فيها 60% بينما كانت نسبة الاستجابة 100% *WPM* – *Kin* وهذه بدورها تفوقت على معاملة المقارنة التي كانت نسبة الاستجابة فيها 60 % ، أما في مرحلة التضاعف بعد مرور ثمانية أسابيع كانت أعلى معدل لعدد الأفرع 4.60 فرع.جزءالنباتي−1 وأعلى معدل لعدد الأوراق 17.10 ورقة.جزءالنباتي−1 على الوسط المدعوم بـ *BA*عند التركيز 1.0 ملغم.لتر−1 في حين التركيز 0.5 ملغم.لتر−1 أعطى أطول طول الفرع 1.65 سم . في حين كانت التركيز 4.0 ملغم. لتر−1 من *WPM*−*Kin* حصلة على أعلى معدل لعدد الأفرع 3.90 فرع. جزءالنباتي−1 وأعلى معدل لعدد الأوراق 18.10 ورقة. جزءالنباتي−1 بينما معاملة 2.0 ملغم. لتر−1 حصلة على أعلى معدل أطول طول الفرع 1.74 سم. تم قياس نسبة كلوروفيل في الأوراق النبيتات المزروعة في وسط *WPM* المزود بـ *BA* حيث كان أعلى طول الموجة عند المستوى كلوروفيل *a* هي (0.1971) *nm* عند وسط المدعوم بـ 1.5 ملغم. لتر−1 في حين كانت أعلى طول الموجة عند مستوى الكلوروفيل *b* هي (1.810) *nm* عند وسط المدعوم بـ 0.5 ملغم. لتر−1 بينما كانت أعلى مجموع أطوال الموجة (1.970) *nm* عند وسط المدعوم بـ 0.5 ملغم. لتر−1، بينما في وسط المدعوم بـ *Kin* حيث كانت أعلى طول الموجة عند المستوى *nm (4.490) a* في وسط المزود 8.0 ملغم. لتر−1 بينما كانت أعلى طول الموجة عند المستوى *nm (19.74) b* في وسط المزود 6.0 ملغم. لتر−1 حيث كانت أعلى مجموع أطول الموجة (21.80) *nm* عند وسط المزود 6.0 ملغم. لتر−1، حيث جمعت الأوراق النبيتات في مرحلة التضاعف بعد 8 أسابيع من الزراعة. تم استعمال نموات الخضرية الناتجة في مرحلة التضاعف في عملية التركيب الدقيق في المختبر *In vitro Micrografting* على أصول النارنج بذرية *Citrus aurantium*.

الكلمات المفتاحية : الزراعة النسيجية ،*wpm*، بنزل ادينين، كاينتين، *Citrus lemon local*.