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In vitro Propagation of pomegranate (*Punica granatum* L).

Hadeer Hassan Ali[1] Jassim Mohammed Khalaf[2] Ali Mohammed Noori[2]
[1] *Kirkuk Agriculture Directorate, Ministry of Agriculture, Kirkuk, IRAQ.*
[2] *Department of Horticulture and Landscape, College of Agriculture, University of Kirkuk, Kirkuk, IRAQ.*
*Corresponding Author: Hasanhadeer521@gmail.com.

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ABSTRACT

This study was conducted in the Plant Cell and Tissue Culture Laboratory of the Department of Horticulture and Landscape Engineering/College of Agriculture/University of Kirkuk, IRAQ to demonstrate the study of the effect of adding benzyl adenine 0.0, 0.5, 1.0, 1.5, and 2.0 mg L^{-1} and kinetin 0.0, 2.0, 4.0, 6.0, and 8.0 mg L^{-1} on the growth of pomegranate plants in Woody Plant Medium (WPM) According to the results, the medium containing 1.0 mg L^{-1} (BA) had the highest average number of branches at 3.3 branches. $part^{-1}$ in contrast, the medium containing 1.5 mg L^{-1} had the longest branches, on average, measuring 1.5 cm, while the medium containing 2.0 mg L-1 (BA) produced a highest average of 20 leaves. The results of adding kin to agricultural media showed that the highest average number of branches was 2.5 branches. Part-1 when treated with two concentrations (8.0 and 4.0) mg L^{-1}, the length of the longest branch is 2.13 cm, and the highest average number of leaves is 17.6 leaves. Plant part when grown in medium supplemented with 2.0 mg L^{-1}.

***Keywords*:** *Punica granatum* L, Micropropagation, WPM, Kin, BA..

INTRODUCTION

Punica granatum L., the pomegranate, is in the punicaceae family, which means "apples with many seeds." Pomegranates are one of the oldest fruits that can be eaten. Both Jews and Christians have written about them in their holy books. The pomegranate was talked about three times in the Quran [1, 2]. There are carbs in pomegranate seeds, mainly sugars, as the percentage of sugars in their juice reaches 0.16%. The juice also contains 0.5% protein and 1.1% citric acid, 0.3% fatty substances, and mineral elements such as calcium, phosphorus, iron, and potassium. The fruit's peel also contains 28% tannin, an astringent. Therefore, it is used to treat diarrhea and combat leukemia by reducing the effectiveness of leukemia cells and their spread [3]. The Salimi variety is one of the most valuable pomegranate types in Iraq. It is also the most common, grown and harvested on farms in the central and northern regions. [4] [5] [6]. This variety is characterized by the fact that its fruits are large, round, with a thick peel, and the color of the peel is green with a red tint. Then, a dark red color covers all the fruits at the end of the season (when fully ripe). The red seeds are juicy and have a bitter taste. As the fruits mature, their sweetness increases and their acidity decreases [6], [7]. Different sciences are progressing and thriving, which depends on the technologies available to them that can be adopted to expand the scope of experiments, including plant tissue culture technology. The concept of plant tissue culture is expressed by culturing various plant cells or tissues in glass or plastic vessels containing artificial nutrient environments consisting of food materials under controlled conditions and are completely sterilized [8].At present, with the development of science, development in the cultivation of plant cells and tissues has reached such an extent that it has become possible to grow different plant parts on nutrient media and obtain complete plants from them, as well as use them in research and application. Including its fields of plant breeding and improvement, the production of medicines and medicinal drugs, and the production of seedlings. From medical injuries [9].

Materials and methods:

Experiments were conducted in the Plant Tissue Culture Laboratory – Department of Horticulture and Landscape Design-University of Kirkuk-IRAQ, this study used plant parts (explants) taken from vegetative branches of pomegranate (*Punica granatum* L.) seedlings obtained from a nursery in Kirkuk- IRAQ. The plant parts were prepared by cutting the plant branches into parts of equal length and placing them inside a glass cup in preparation for washing them under running water for 5 minutes to remove any remaining dust and dirt. Add regular washing powder (Brite) and let sit for a few minutes. After that, place in a strainer and soak for 12 minutes under running water. The explant was passed on to a 250 ml glass container and an air chamber with laminar flow, then immersed in a commercial sodium hypochlorite (NaClO) solution at three concentrations (2, 4 and 6%) for (2, 4 and 6) minutes. Until the explant are submerged. Completely to ensure complete sterilization. After the sterilization ended, to eradicate any potential hazardous effects of the sterile chemical, the plant portions were washed three times consecutively for about 5 minutes with distilled water. The plant parts were cut into lengths (1 cm)

to be ready for transplantation in Woody Plant Medium (WPM) containing Benzyl Adenine (BA) at concentrations of (0.0, 0.5, 1.0, 1.5 and 2.0) mg L^{-1} and Kinetin (Kin) at concentrations of (0.0, 2.0, 4.0, 6.0 and 8.0) mg L^{-1}, which was previously prepared by placing 400 ml of twice-distilled water in a 1000 ml glass beaker, then adding sucrose in an amount of 30 gl^{-1}, then adding agar in an amount. 7.5 gL^{-1} (agar), then (WPM) is added to it according to the recommended amount, and the volume is added to 1000 ml of sterile distilled water, then heated on a device (Magnetic stirrer hot plant), then the pH is adjusted. Measurement. Accordingly, between (5.7) use (HCl and NaOH), and after the solution is completely homogeneous, it is poured into tubes designated for cultivation until used. The coefficients were compared using Duncan's multinomial test at a probability level of 5%. [10].

Results and discussion:

The results in Table 1 indicate that there is a significant effect of sterilization concentrations and sterilization periods on the contamination rate. The 2% concentration significantly outperformed the rest of the concentrations, as it is noted that the percentage of pollution reached the lowest possible level at this concentration, in which the plant parts were not exposed to any damage or pollution. This may be due to the percentage of the active ingredient in the sterilization (NaOCL). This may be suitable for sterilizing the plant part without harming its components [11]. While the highest percentage of contamination of plant parts was at a concentration of 6% for 2 minutes

The results of Table 2 note that (BA) showed a significant effect on the opening of buds of the parts that were grown on media supplemented with different concentrations of WPM media, as the concentration of 1.0 mgL^{-1} was superior to the rest

Table 1: The effect of sodium hypochlorite (NaClO) and the duration of sterilization on the percentage of contamination of pomegranate plant parts *Punica granatum* L.

Duration (min) Concentration (mL)	2	4	6	Concentration effect (mL)
2	100 a	100 a	100 a	100 a
4	50 b	40 b	70 b	51.72 b
6	70 b	10 b	60 b	76.67 b
Duration effect (minute)	73 a	80 a	76 a	

Similar letters do not differ in terms of statistical significance at 5%.

of the concentrations. The comparison treatment gave an average number of branches of 3.3. While the concentration of 1.5 mgL^{-1} Ba led to an increase in the length of the branches to reach 1.57 cm, the highest average number of leaves reached 20.4 at a concentration of 2.0 mgL^{-1} (BA).

The effects of (Kin) treatment on plant parts growing in (WPM) medium were observed in Table 3. The maximum average

Table 2: The effect of)BA(on Initiation Stag of pomegranate *Punica granatum* L.

(BA) mg L^{-1}	Number of branches	Length of longest branch (cm)	number of leaves
0.0	0.7 b	0.58 b	3.7 c
0.5	2.9 a	1.25 a	14.5 b
1.0	3.3 a	1.45 a	18.7 ab
1.5	2.6 a	1.57 a	16.6 ab
2.0	2.7 a	1.50 a	20.4 a

Similar letters do not differ in terms of statistical significance at 5%

number of branches reached 2.5 branches when treated with two concentrations (4.0 and 8.0) mg L^{-1}. (Kin), as there were no significant differences between the two concentrations, and they outperformed the comparison treatment and the rest of the treatments. The highest average number of longest branches reached 2.13 cm when treated with
a concentration of 2.0 mg L^{-1} (Kin), and the highest number of leaves reached 17.6 leaves when treated with a concentration of (2.0) mg L^{-1} (Kin).

The increase in the percentage of live, uncontaminated plant parts resulting from immersion in sodium hypochlorite solution may be due to the effectiveness of this substance when using it to sterilize plant components. The results can be explained by considering chlorine as a sterilizing chemical. When applied to plant parts at the right concentration and for the right amount of time, it kills microorganisms like fungi and bacteria. This substance is characterized by its ease of removal from the explant

by repeated washing, as this substance decomposes into a less toxic substance and thus is easier to remove from the plant part. [12] The results of Tables 2 and 3 can be interpreted to indicate the major role that cytokinins play in stimulating cell divisions and the formation of adventitious branches on plant organs, as well as encouraging the growth of buds. The benefits are after eliminating the phenomenon of apical dominance [13]. It was shown from calculations that BA had better results than Kin. This is attributed to the composition of BA, which is linked to its chain by several pieces of evidence. These three double bonds make it superior in its activities to other organizations. The bonds of BA are personally effective and compound active, which makes them more influential in terms of cell divisions and their expansion in size and differentiation, that is, more influential in the development of growth and development. This makes it one of the most prominent cytokinins in propagating many plant species [12] [14]. These results are in line with [15], [16], [17], [18] and [19].

Table 3: Effect of (Kin) on Initiation Stage of pomegranate *Punica granatum* L.

(Kin) mg L^{-1}	Number of branches	Length of longest branch (cm)	number of leaves
0.0	0.7 b	0.58 c	3.8 b
2.0	2.4 a	2.13 a	17.6 a
4.0	2.5 a	1.8 ab	16.4 a
6.0	1.8 a	1.55 b	15.2 a
8.0	2.5 a	0.92 c	15.4 a
Similar letters do not differ in terms of statistical significance at 5%			

Conclusions:

It was found that WPM medium was more effective than MS, as it led to an increase in the number of branches, their length, and the number of leaves of the plant parts growing in it.

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صنف سليمي خارج الجسم الحي إكثار الرمان *Punica granatum* L .

هدير حسن علي[1] جاسم محمد خلف[2] علي محمد نوري[2]

[1].مديرية زراعة كركوك، وزارة الزراعة، كركوك، العراق.

[2] قسم البستنة وهندسة الحدائق، كلية الزراعة، جامعة كركوك، كركوك، العراق.

الخلاصة

أجريت هذه الدراسة في مختبر زراعة الخلايا والأنسجة النباتية التابع لقسم البستنة وهندسة الحدائق , كلية الزراعة جامعة كركوك ، لبيان تأثير إضافة (0.0,0.5,1.0,1.5,2.0) ملغم.لتر$^{-1}$ BA و(0.0,2.0,4.0,6.0,8.0) ملغم.لتر$^{-1}$ Kin على وسط (WPM) لنبات الرمان *Punica granatum* L. أظهرت النتائج تسجيل أعلى معدل لعدد الأفرع 3.3 فرع.جزء نباتي$^{-1}$ في الوسط المزود ب1.0 ملغم .لتر$^{-1}$ BA في حين كان أعلى معدل لطول أطول فرع 1.5 سم في الوسط المزود ب1.5 ملغم/لتر وأعلى معدل لعدد الأوراق 20 ورقة.جزء نباتي$^{-1}$عند الزراعة بالوسط المزود ب2.0 ملغم.لتر$^{-1}$ BA . وبينت نتائج إضافة kin إلى الأوساط الزراعية تسجيل أعلى معدل لعدد الافرع2.5فرع. جزء نباتي$^{-1}$ عند المعاملة بتركيزي(4.0 و 8.0) ملغم/لتر$^{-1}$ وطول أطول فرع 2.13 سم وأعلى معدل لعدد الأوراق 17.6 ورقة .جزء نباتي$^{-1}$عند الزراعة بالوسط المزود ب 2.0 ملغم .لتر$^{-1}$.

الكلمات المفتاحية : الرمان,Kin,Ba,اوساط زراعة ,الاكثار الدقيق.