Effect of cytokinins and nano-iron on some vegetative and chemical growth indicators for citrus rootstock citrumelo *in vitro*.

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Abstract

The experiment was conducted in the Department of Horticulture and Landscape Engineering, Plant Tissue Culture Laboratory - College of Agriculture - Al-Qasim Green University during the period from November 2021 to May 2022. The aim of the research was to study the effect of adding two types of cytokines: (BA 2.0 mg.L-1 , TDZ 0.2 mg.L-1) and two concentrations of nano-iron (2.5 , 5 mg.L-1) and the addition of nano-iron at two concentrations (2.5 mg.L^{-1} and 5 mg.L^{-1}) to the medium of propagation of in vitro of Citromelo rootstock .The results showed that the addition of benzyl adenine at a concentration of 2.0 mg. L^{-1} to MS medium showed significant differences in the rate of shoots height and number of leaves, given (2.40 cm, 10.33 leaves/shoot) respectively. As for the chemical indicators, the same concentration gave the highest average concentration of phosphorous, which amounted to (0.2777%), and carbohydrates at an average of (21.92%). As for the concentration of 0.2 mg.L^{-1} thidiazoron excelled and gave the average content of shoot of chlorophyll (6.07 mg.100 gm^{-1} fresh weight).As for the concentration of 2.5 mg.L^{-1} nano-iron, it gave the highest average for shoot lengths (2.40 cm), number of leaves (9.66 leaves/shoot) and carbohydrates (21.61%). While the concentration of 5 mg. L^{-1} excelled in chlorophyll content (6.24 mg. 100 gm^{-1} fresh weight) and phosphorous (0.2760%) in the interaction treatment 2.0 mg. L^{-1}BAP + 2.5 mg. L^{-1} nano iron.Significantly excelled in shoot length (3.20 cm), number of leaves (13 leaves/shoot), and carbohydrates (23.24%) as for the interaction between BAP 2.0 mg. L^{-1} +5 mg.L^{-1} Nano iron excelled in the content of phosphorous shoots (0.4720%) Also, the interaction treatment between 0.2 mg . L^{-1} TDZ + 2.5 mg. L^{-1} of nano iron significantly excelled in its chlorophyll content (7.85 mg. 100 gm^{-1}).

Keywords: Nano iron. Benzyl adenine. TDZ

Introduction:

Genus Citrus belongs to the order Geraniales, Rutaceae family , which includes about 160 genera and 1650 species consisting of trees, and evergreen shrubs, Citrus is one of the most important groups of plants Where it grows and bears fruit in the tropics and subtropics extending from the southeast, from which it spread towards Europe and Africa (5). The assets used in the propagation of citrus plays an important role in production because they affect the qualities of the grafted cultivated in terms of their impact on the quality and quantity of the crop, as well as on the shape and size of the tree growing on it, and thus there is a guarantee of the quality of the fruits and the abundance of production (4).The rootstock of citrus Swingle Citrumelo is one of the rootstocks of citrus that is widely used in the world due to its traits and its positive effects on the grafts growing on it. It is a rootstock that resulted from cross-breeding between grapefruit and three-leafed orange and trees grafted on it have good productivity, strong growth, medium to abundant yield. In addition to its resistance to many diseases, including the rapid deterioration of Tristeza and root rot. Xyloporosis, as well as nematodes, as well as its tolerance to low temperatures and drought-resistant conditions, because it has a large root system deep in the soil, but it is sensitive to iron and manganese deficiency (1). Micropropagation technology is one of the most important modern propagation methods used in the production of disease-free plants, the development of new cultivars, the production of seedless fruits and the preservation of genetic assets, including

citrus. Plant tissue culture technology plays an important role in plant biotechnology due to its huge potential to produce improved crop cultivars and high yields of important secondary compounds. Several efforts have been made to improve the efficacy and yield of plant tissue culture, using biotic and abiotic factors. Nowadays, the addition of nanoparticles, for example, has gained worldwide attention due to its success in producing secondary compounds (6).Cytokinins are the main catalyst and regulator of cell division in tissue culture in the presence of auxin, noting that stimulating and regulating cell division is a centre of growth and discovery in plants (2).Iron has an auxiliary role for many enzymes that catalyze biochemical reactions, and iron deficiency is a widespread problem in many plants, including citrus (7).

Materials and methods:

The experiment was conducted in the plant tissue culture laboratory in the Department of Horticulture and Landscaping Engineering - College of Agriculture - Al-Qasim Green University during the period from (9/11/2021) to (13/5/2022). To demonstrate the effect of cytokinin and nano iron on the growth indicators of in vitro propagation of the rootstock Citromelo, the explant of the fresh shoots of 10-5 cm length were used from one-year-age seedlings grown in plastic bags in lathhouse of the certified citrus nursery of the General Company for Horticulture and Forests In Holy Karbala - Iraqi Ministry of Agriculture. Leaves and thorns were removed, then washed with liquid soap and water for 5 minutes and sterilized by dipping it in 70% ethyl alcohol for 10 seconds.Then it was immersed in a solution of sodium hypochlorite NaOCl at a concentration of 1% commercial minor chlorine concentration of 5.25% for 15 minutes and then the plant parts were washed 4 times. 2.5-2cm each part contains one Node .The explants were planted in tubes containing 10 sterile medium prepared from the nutritional medium approved by (23) known as MS weight was approved 4.43 g/L^{-1} according to the recommended cultivation instructions, 3% sucrose and 100 mg/L myo-inositol, 0.2 mg/L BA, and 0.1 mg/L NAA (3) were added, and then agar was added at a concentration of 7 g/L. After that, the acidity of the medium (pH) was adjusted in the range of 5.7 ± 1 using 1 N of NaOH or HCl. The base solutions of cytokinin and nano iron were prepared and added to the multiplication medium containing 0.1 mg / L NAA and 30 mg / L adenine sulfate in addition to the addition of sucrose and inositol with the same Concentrations used in the upbringing stage. The minute cuttings were planted after cutting them into two-node cuttings and the plants were incubated in the growth room at a temperature of 25±2°C and a light intensity of 1000 lux for 16 hours, followed by 8 hours of darkness.

Results and discussion

shoot height (cm)

The results in Table(1) showed that there were significant differences for the addition of cytokinin, where the concentration of 2 mg / L^{-1} of BAP gave the highest average shoot lengths of 2.40 cm .As for the lowest rate of shoot length at a concentration of 0.2 mg/L^{-1} TDZ .The results of the same table showed a significant difference for the addition of nano-iron, as the concentration of 2.5 mg/L^{-1} nano-iron at the average of shoot lengths reached 2.40 cm, excelled to the concentration of 5 mg/L^{-1} with the lowest rate of 1.70 cm.As for the bi-interaction of the results of the same table, significant differences were shown between the treatments, where the treatment of 2.5 mg/L^{-1} nano-iron + 2 mg/L^{-1} BAP excelled with a shoot length of 3.20 cm, followed by 2.5 mg/L^{-1} nano-iron + cytokinin-free medium with an average of 2.50 cm The minimum length was for the treatment of 5 mg/L^{-1} nano iron + 0.2 mg/L-1 TDZ 1.40 cm

Table 1 Effect of nano iron and the type of cytokinin TDZ and BAP and the interaction between them on the average shoot length of in vitro explant of Citromelo rootstock

nano iron average	Cytokinin type (mg.L-1)			Nano iron concentrations (mg.L-1)
	TDZ 0.2	BA 2.0	0	
2.06	2.00	2.00	2.20	**0**
2.40	1.50	3.20	2.50	**2.5**
1.70	1.40	2.00	1.70	**5**
	1.63	2.40	2.13	**Cytokinin average**
Cytokinin = 0.2099 nano iron = 0.2099 interaction = 0.3636				**LSD (0.05)**

Average number of leaves \ shoot^{-1}

It is evident from the results presented in Table (2) that there were significant differences in the number of leaves when adding cytokinin, where the concentration of 2 mg/L^{-1} BAP exceeded at a rate of 10.33 leaves/shoot, followed by a concentration of 0.2 mg/L^{-1} TDZ with 7.33 leaves/shoot compared to the control treatment. Which amounted to 5.67 leaves/shoot. As for the addition of nano iron, it had a significant effect at a concentration of 2.5 mg/L^{-1}, excelled to the concentration of 5 mg/L^{-1} at a average of 9.66 leaves/shoot. The interaction between iron and the type of cytokinin had a significant effect on the average number of leaves that exceeded 2 mg/L^{-1} BAP +2.5 mg/L^{-1} Nano iron by recording the highest average in the number of 13 leaves/shoot followed by a concentration of 0.2 mg/L^{-1} TDZ +2.5 mg /L^{-1} nano iron 10 leaves/shoot, while the comparison treatment recorded the lowest rate of 4 leaves/shoot

Table 2 Effect of nano iron and the type of cytokinin TDZ and BAP and the interaction between them on the average number of leaves of plant shoots grown in vitro of Citromelo rootstock

nano iron average	Cytokinin type (mg.L-1)			Nano iron concentrations (mg.L-1)
	TDZ 0.2	BA 2.0	0	
7.00	7.00	10.00	4.00	**0**
9.66	10.00	13.00	6.00	**2.5**
6.67	5.00	8.00	7.00	**5**
	7.33	10.33	5.67	**Cytokinin average**
Cytokinin = 0.814 nano iron = 0.814 interaction = 1.410				LSD **(0.05)**

Chemical indicators: included

chlorophyll content in the plant (mg. 100gm^{-1} fresh weight):-

It is noted from the results presented in Table (3) that there is a significant effect between the type of cytokinin added to the MS medium on the chlorophyll content of shoots and leaves, where TDZ at a concentration of 0.2 mg/L^{-1} was excelled on the highest content of chlorophyll 6.07 mg. 100gm^{-1} fresh weight While the cultures grown in BAP medium at a

concentration of 2.0 mg/L-1 gave the lowest chlorophyll content of 5.05 gm^{-1} fresh weight, the results of the same table showed significant differences in the chlorophyll content of plant parts when adding nano-iron, as the concentration of 5 mg/L^{-1} was excelled on the highest content. It was 6.24 mg. $100gm^{-1}$ fresh weight .This the control treatment excelled giving the lowest rate of 4.21 mg. 100 g^{-1} fresh weight, but it did not differ significantly from the concentration of 2.5 mg/L^{-1}, which was 6.20 mg. 100 gm^{-1} fresh weight. As for the interaction between the type of cytokinin and nano iron. It is noticed from the results in the same table that there are significant differences between the interactions, where the concentration of 0.2 mg/L^{-1} TDZ +2.5 mg/L^{-1} nano-iron gave the highest chlorophyll content of 7.85 mg. 100 g^{-1} Followed by medium without cytokinin +5 mg/L^{-1} nano iron with a content of 7.14 mg. 100 gm^{-1} The lowest content was when BAP 2.0 mg/L^{-1} + nano iron present in the medium 3.55 mg. 100 gm^{-1}.

Table 3 Effect of nano iron , type and concentration of cytokinins BAP and TDZ and the interaction between them on content Relative chlorophyll in explant cultured in vitro to rootstock of citrumelo

nano iron average	Cytokinin type (mg.L-1)			Nano iron concentrations (mg.L-1)
	TDZ	BA	0	
4.21	5.23	3.55	3.87	**0**
6.20	7.85	5.18	5.57	**2.5**
6.24	5.13	6.44	7.14	**5**
	6.07	5.05	5.53	**Cytokinin average**
Cytokinin = 0.565 nano iron = 0.565 interaction = 0.979				LSD **(0.05)**

Percentage of phosphorous in shoots(%):-

The results presented in Table (4) indicated that there were significant differences between the type of cytokinin in the percentage of phosphorous concentration Whereas, BAP at a concentration of 2.0 mg/L^{-1} was excelled 0.27%, while the concentration of TDZ 0.2 mg/L^{-1} gave a rate of 0.17%.The results of the same table showed that there were significant differences between the concentrations of nano iron , where the addition of nano iron at a concentration of 5 mg/L^{-1} led to obtaining the highest phosphorous percentage in shoots 0.27%, while the concentration of 2.5 mg/L^{-1} recorded the lowest percentage of 0.14%As for the interaction between nano iron and cytokinin for the same table, BAP treatment gave 2.0 mg/L^{-1} + 5 mg/L^{-1} nano iron the highest percentage.

It recorded 0.47%, followed by TDZ treatment 0.2 mg/L^{-1} +5 mg/L^{-1} nano iron with a rate of 0.21%, while the lowest rate was recorded when treating TDZ 0.2 mg/L^{-1} +2.5 mg/L^{-1} nano iron by 0.11%

Table 4 Effect of nano iron , type and concentration of cytokinins TDZ and BAP and the interaction between them on the phosphorous content of explants grown in vitro of Citromelo rootstock

nano iron average	**Cytokinin type (mg.L-1)**			**Nano iron concentrations** (mg.L-1)
	TDZ	**BA**	**0**	
0.19	0.19	0.19	0.17	**0**
0.14	0.11	0.16	0.16	**2.5**
0.27	0.21	0.47	0.14	**5**
	0.17	0.27	0.16	**Cytokinin average**
Cytokinin = 0.02250 nano iron = 0.02250 interaction = 0.03897				LSD **(0.05)**

The percentage of carbohydrates in shoots: (%)

The results in Table (5) showed a significant effect of the cytokinin type on the percentage of BAP carbohydrates at a concentration of 2.0 mg/L^{-1}, which gave the highest percentage of 21.92%, while the lowest percentage was 17.79% when the control treatment .The results of the same table showed a significant effect of adding nano iron. As the concentration of 2.5 mg/L-1 gave the highest value, excelled on the rest of the concentrations, which amounted to 21.61%, while the concentration of 5 mg/L^{-1} gave 19.90%, and for the traditional iron in the middle the lowest percentage of 18.72%In the same table, a significant effect of the bi-interaction of the proportion of carbohydrates was observed. The treatment of 2.0 mg/L-1BAP + 2.5 mg/L^{-1} Nano iron gave the highest percentage of 23.24%. Followed by TDZ treatment 0.2 mg/L^{-1} +2.5 mg/L-1 nano-iron 22.34%, while the lowest percentage was when treating 5 mg/L-1 nano-iron +0 cytokinin 16.87%

Table 5 Effect of nano iron , type and concentration of cytokinins TDZ and BAP and the interaction between them on carbohydrate content of in vitro explants of Citromelo rootstock

nano iron average	**Cytokinin type (mg.L-1)**			**Nano iron concentrations** (mg.L-1)
	TDZ	**BA**	**0**	
18.72	18.65	19.70	17.80	0
21.61	22.34	23.24	19.24	2.5
19.90	21.89	20.95	16.87	5
	20.96	**21.29**	**17.97**	**Cytokinin average**
Cytokinin = 0.999 nano iron = 0.999 interaction = 1.731				LSD **(0.05)**

Discussion:

The reason for the elongation of shoots (Table 1) when adding BAP is due to several different factors, including the effect of cytokines in cell division and elongation, which in turn is reflected in the growth traits as well as its effect on the construction of nucleic acids (8)The results of adding BAP agreed with many researchers, (9) in pomelo tissue culture,and (10) in the medium of Carrizo multiplaction. As for the positive response in the average (Table 2) and (Table 1), it is due to the stimulatory action of BAP in urging cells to differentiate, and this results in the differentiation of the transplanted tissues and the density of the vegetative system (11) .The study of adding BAP agreed with what was found (12) (13) as the concentration BAP had a significant effect in increasing the number of leaves and the length of shoots of the origin of the citromelo relative to the rest of the concentrations. The reason for the increase (table 2.1) may be due to the fact that nano-iron increases the effectiveness of biochemical conversion processes in the cell membrane, which facilitates the penetration of nutrients into the plant, and this helps in increasing cell divisions and encouraging vegetative growth, and thus provides a continuous demand for Nutrients (14).The reason for increasing chlorophyll schedule 3 is due to the interaction of cytokinin with light, then it stimulates gene expression and increases the formation of plastid proteins. Addition of cytokinin increases chloroplast DNA, Which increases the construction of the protoplast, maintains the level of pigments and changes the permeability of the membranes, as well as stimulates the proliferation of plastids and the formation of the grana membrane and has a high effectiveness in retaining chlorophyll especially in high concentrations (15).These results agreed with the findings of (16) for palm multiplication, (17) for tissue-growing banana branches,. In the plant and increase the content of chlorophyll inside the plant shoots (18)The reason for the increase is also attributed to the role of nano iron in its impact on many vital activities in vitro, where nano iron affects the formation of chlorophyll in explants directly, and it is one of the important foundations in the process of photosynthesis in addition to a role in the formation of many compounds (ferdoxin and cytochromes) that have Importance in the process of photosynthesis (19). This result agreed with (20) when multiplying fulcamrina. As for the reason for the increase in the phosphorous content of the shoots, table 4 is due to the fact that cytokines act as a sink for nutrients, which leads to an increase in the proportion of elements within the explants and the increase in the percentage of sugars in the nutritional medium that is important in the processes of building tissues, which leads to the accumulation of sugars (table 5) in it, which is reflected in the increase in vegetative growth (table 2.1), and thus the increase in the accumulation of mineral elements in addition to its vital role, which increases the opening of the stomata, which may reach the width of the stomata by 50%, which It leads to an increase in transpiration and thus an increase in the process of absorbing elements from the medium (21).As for the increase in the phosphorous content of the branches by adding nano-iron, it is due to the fact that the nano-fertilizers provide a larger surface area for the various metabolic reactions, which increases the photosynthesis process and thus encourages the demand for nutrients and produces more dry matter represented by the increase in the content of the branches of elements Table 4 and carbohydrates Table 5.The addition of iron in the form of nanoparticles increases the efficiency of the enzyme H + ATPase in the plasma membrane in the wall of the guard cells and its accumulation leads to an increase in the opening of stomata 5 times its normal rate, which increases the process of entering CO2 and thus the efficiency of the food-making process, which provides a continuous demand for the nutrients that it provides plant nutritional medium (22)As for the increase in the carbohydrate content of the shoots, Table 5, these results can be explained on the basis of the increase in the amount of chlorophyll in

the explants (Table 3) and the increase in the vegetative total (Table 2 and 1)

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