Effect of growth regulators, Salinity and Chitosan on vegetative traits for the shoots of Rootstocks of Citrus (Swingle citrumelo) multiplied in Vitro

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Abstract

The two factorial experiments were conducted in the tissue culture laboratory of the Plant Production Department / Al-Mussaib Technical College during the period 2018-2019 with the aim of proliferating the rootstocks of the citrus Citrumelo in vitro, where the first experiment was to study the effect of different concentrations of benzyl adenine BA (1, 2, 3 and 4 mg.L^{-1}) in the studied vegetative growth traits. As for the second experiment, it was to study the effect of three concentrations of NaCl salt (0, 50 and 100 mmol) by adding several concentrations of chitosan to the agricultural medium (0, 5, 15 and 25 mg. L^{-1}) and study the effect of the interaction between the factors in vegetative growth for the resulting plants. The plants were multiplied by using the single node as an Explant plant, as the Node were taken 1.5-2 cm long and then immersed in an antioxidant solution (100 mg.L^{-1} ascorbic acid + 150 mg. L^{-1} citric acid) ,Then the explant was sterilized with ethanol solution (70% concentration) for 10 seconds, after which they were immersed in 1% sodium hypochlorate solution for 15 minutes. Then the explant was immersed in distilled water three times for 3 - 5 minutes each time to ensure the removal of the harmful effect of alcohol. The explant was culture 1 cm long containing single nodes in MS culture medium. The seedlings were incubated at a temperature of 25 ± 2 and a light period of 16 hours. The results showed that the addition of chitosan reduced the harmful saline effect of cultures, where the combination (25 mg.L^{-1} chitosan + 50 and 100 mmol NaCl) significantly increased the average length of shoots, the weight of the fresh and dry plant, the average water content and the chlorophyll content of the Cultures reached (2.55 cm, 305 mg, 48.4 mg, 285 mg and 1,301 mg. fresh weight), compared to other interaction factors. In the second experiment, the concentration of 2 mg. L^{-1} BA excelled in the percentage of multiplication, the number of shoots and the length of the shoots (90%, 4.2% and 2.9 cm) and the concentration 3 mg.L^{-1} in the average of fresh and dry weights of the shoots (263 and 43 mg).

Key words: Swingle Citrumelo, salinity, Chitosan , micropropagation, water content.

** **Research from Ph.D thesis of the First author**

تأثير منظمات النمو و الملوحة و الكايتوسان في معدل الصفات الخضرية لافرع أصل الحمضيات السيونكل سترومیلو المتضاعفة خارج الجسم الحي

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الخلاصة

نفذت تجربتان عامليتان في مختبر زراعة الأنسجة التابع لقسم الإنتاج النباتي/الكلية التقنية المسيب خلال الفترة 2018 - 2019 بهدف إكثار أصل الحمضيات السترومیلو خارج الجسم الحي , حيث كانت التجربه الاولى لدراسة تأثير تراكيز مختلفة من

البنزل أدنين BA (1, 2, 3 و4 ملغم.لتر$^{-1}$) في صفات النمو الخضري المدروسة. اما التجربه الثانية فكانت لدراسة تأثير ثلاث تراكيز من الملح NaCl (0, 50 و100 ملي مول) من خلال إضافة تراكيز عدّة من الكايتوسان إلى الوسط الزراعي (0, 5, 15 و25 ملغم.لتر$^{-1}$) ودراسة تأثير التداخل بين العاملين في النمو الخضري للنبيتات الناتجة. كثرت النبيتات باستخدام العقدة المفردة كجزء نباتي Explant إذ أخذت العقدة بطول 1.5 – 2 سم ثم غمرت في محلول مضاد للأكسدة (100 ملغم.لتر$^{-1}$ حامض الاسكوربيك + 150 ملغم.لتر$^{-1}$ حامض الستريك) ثم عقمت الأجزاء النباتية بمحلول الأيثانول (تركيز 70%) لمدة 10 ثوان بعدها غطست في محلول هايبوكلورات الصوديوم بتركيز 1% لمدة 15 دقيقة. بعدها غطست الأجزاء النباتية في الماء المقطر لثلاث مرات لمدة 3 – 5 دقيقة لكل مرة لضمان إزالة التأثير الضار للكحول, زرعت الاجزاء النباتية بطول 1سم تحتوي على عقد مفردة في وسط غذائي MS. حضنت الزروعات على درجة حرارة 25±2 وفترة إضاءة 16 ساعة. أظهرت النتائج إن إضافة الكايتوسان قللت من الأثر الملحي الضار للزروعات إذ أحدثت التوليفة (25 ملغم.لتر$^{-1}$ شيتوسان + 50 و100 ملي مول NaCl) زيادة معنوية في معدل طول الأفرع, وزن النبات الرطب والجاف, معدل المحتوى المائي ومحتوى الزروعات من الكلوروفيل بلغت (2.55سم, 305ملغم , 48.4ملغم, 285ملغم و1.301ملغم.غم وزن طري), قياساً مع باقي معاملات التداخل. أما في التجربة الثانية فقد تفوق التركيز 2 ملغم.لتر$^{-1}$ BA في النسبة المئوية للتضاعف, عدد الأفرع المتضاعفة وطول الأفرع (90%, 4.2% و2.9سم) والتركيز 3 ملغم.لتر$^{-1}$ في معدل الوزنين الطري والجاف للأفرع (263 و43 ملغم).

الكلمات المفتاحية : السيونكل سترومیلو , الملوحة , الكايتوسان , الاكثار الدقيق , المحتوى المائي .

- **البحث مستل من أطروحة الباحث الاول**

Introduction

Swingle Citrumelo (Citrus paradisi Macf × Poncirus trifoliata (L.) Raft) is an important rootstock of citrus , a hybrid resultant from cross Citrus paradise with Poncirus trifoliata whose seedlings are identical in growth and have a large root group, And the yield of grafted trees on it is abundant, the fruits are larger and the characteristics are good, and it resists extreme coldness, rapid deterioration disease, gumosis and nematode infection. It is not recommended for use in wet soil and Non-good drainage, It is well compatible in grafting with Eureka lemon and grapefruit cultivars and is incompatible with some orange and Mandarin cultivars (Lacey et al., 2006). Clonal micropropagation of plants, including citrus fruits, is one of the most widespread applications, where vegetative propagation in large numbers in a short time period by controlling apical dominance and stimulating lateral shoots or encouraging callus cells to formation organs or Somatic embryogenesis that detect vegetative growth (George et al., 2008). The results of previous studies have proven that citrus cultivars can be multiplied by various plant tissue culture techniques successfully by encouraging shoots multiplication (Mukhtar et al., 2005 Siwach et al., 2012) or organ formation via callus(Ali and Mirza, 2006), or by using the formation method Somatic embryogenesis (Sajera et al., 2008; Salim et al., 2015). And the safest pathway for the accurate multiplication of citrus fruits without the possible genetic differences of plants resulting from the parent plant is by stimulating the formation of lateral shoots by planting peripheral and axillary buds compared to other pathways. Salinity is considered one of the most important problems facing the cultivation of many plants, including citrus fruits, when plants are exposed to high concentrations of salinity that lead to an imbalance in the different physiological functions within the plant that indicate that the plant has been exposed to salt stress (Taiz and Zeiger, 2002). In a study conducted by Singh et al. (2004) on the physiology and mechanism of Salinity tolerance at the cellular level of citrus rootstock Rough lemon, Rangpur lime, and trifoliate oranges, there was a reduction in the dry and fresh weights of callus grown on the nutrient medium with concentrations ranging from 0 to 200 ml. A mole of sodium chloride salt 6 weeks after transplantation. Pérez-Tornero et al. (2009) confirmed that the vegetative growth traits of the proliferating branches in vitro were negatively affected under the influence of salinity, especially at

concentrations greater than 60 mmol of C. macrophylla implants. and in a study by Ghaleb et al. (2010), they used culture medium provided with different concentrations of two salts of sodium chloride and calcium chloride at concentrations of 0, 50, 100, 150, 200 and 300 mmol to grow side shoots of two types of citrus rootstock, namely C. aurantium and volcamariana. (C. volkameriana Ten. & Pasq.) in vitro , Where they found that the high concentrations of these salts in the nutritional medium caused a reduction in the growth of the cultures in terms of fresh and dry weight, length of shoots and number of leaves, and led to increased damage to the plants after a period and for both rootstock. Chatzissavvidis et al (2014) when using a culture medium equipped with different concentrations of NaCl (0, 50, 100, 150, 200 and 300 mmol) found that most of the salt concentrations showed a negative effect on the growth indicators of tissue cultures represented by the number of shoots, lengths and weights when culture the apical tips of the trifoliate orange (P. trifoliata L.) in vitro. It is also characterized by its non-toxicity and biodegradation and has no local or general effects on living tissues and is a compound with bio functions (Hossain and Iqbal, 2014). Therefore, it attracted the attention of researchers in the past few years for its commercial uses in the food, medical, chemical and pharmaceutical industries. Chitosan has the ability to inhibit the growth of fungi because it stimulates the enzyme Kinetine, which is one of the defensive enzymes, and it was observed that reduced respiration and ethylene production from the fruits treated with Kinetine, As well as its ability to encourage plants to withstand environmental stresses (Krupa-Malkiewicz and Fornal, 2018). The aim of this study is to investigate the effect of different concentrations of Chitosan to reduce salinity damage on the Swingle Citrumelo in vitro.

Materials and methods

Preparing and sterilizing the explant

Young shoots of modern growths of 5-10 cm length were taken from the seedlings of the Swingle Citrumelo citrus rootstock used for study and growing in plastic bags in the Lath house Certified citrus nursery located in Karbala province belonging to the General Company for Horticulture and Forestry / Ministry of Agriculture, Where all thorns and leaves were removed from the plant parts taken, then washed with liquid soap and placed under running water for 30 minutes, after which they were immersed in an antioxidant solution consisting of 100 $mg.L^{-1}$ of ascorbic acid and 150 $mg.L^{-1}$ citric acid, This was followed by the transfer of the explant to the Laminar Air Flow Cabinet to perform the surface sterilization process, as the explant were sterilized inside the airflow cabin by submerging them with ethyl alcohol at a concentration of 70% for a period of ten seconds, and then they were submerged in NaOCl solution with a concentration 1% (commercial minor, Clorax, 5.25% chlorine concentration) for 15 minutes with continuous shaking to remove air bubbles formed on explant, after which the explant were washed three times with sterile distilled water and for 3-5 minutes each time, to remove the harmful effect of sterile matter and to maintain

Preparing nutrition media

The explants were placed after sterilization was done in sterile Petri dishes where the two ends of explants were cutting in order to get rid of the damaged parts by the sterilization material and to get 1cm-long explants containing a single nod. These nodes were cultured vertically so that the lower part was immersed in the prepared nutrition media (MS Murashing and Skoog, 1962) manufactured by Media plant company. As 4.8 g of ready-media powder was added, according to the manufacturer's instructions, to a glass flask containing 900 ml of sterile distilled water and Vitamins (pyridoxine, thymine and nicotinic acid) were all added at a concentration of 1 $ml.L^{-1}$ each, as well as adding 100 mg. $L^{-1}$of inositol. A growth regulator BA was added to the 2 mg concentrations. L^{-1} with 0.1 mg. L^{-1} NAA with 1.0 mg. L^{-1} GA_3 Then the mean acidity (pH) was set to the limits of 5.7 using 1

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standard NaOH or HCl, then 7 g was added. 1 liter of agar to harden the medium and complete the volume to 1000 ml and put it on a mixer with a hot plate in order to melt the medium and homogeneity, then pour the medium in a glass container of 10 ml for each container and sterilized in the Autoclave sterilizer under pressure 1.4 bar and a temperature of 121 C° for 20 Minute. After the sterilization is complete, it is kept in a sterile place until culturing. The cultures were incubated in the growth chamber at a temperature of 25 ± 2م m and a brightness of 1000 lux for 16 hours followed by 8 hours of darkness. The explants resulting from the Initiation stage were re-cultivated before starting the multiplication stage in order to obtain homogeneous plants for use in the multiplication stage.

The first experiment: the effect of BA in the presence of NAA and GA3 on shoots multiplication

The experiment was conducted by adding BA at concentrations of (1.0, 2.0, 3.0, 4.0 mg.L^{-1}) with NAA at a concentration of 0.1 mg. L^{-1} and also in the presence of GA3 at a concentration of 1.0 mg. L^{-1} with 10 replicates per treatment in order to better identify A combination that gives the best effect on the growth of the vegetative shoots by taking the following measurements four weeks after culture. The data were taken and included the percentage of multiplication, the number of shoots , the length of the shoots, and the fresh and dry weight of the multiple vegetative shoots.

The effect of NaCl and Chitosan on multiplication the shoots

Through the results obtained from the multiplication stage, the best combination for multiplication of Cultures was determined (2.0 mg.L^{-1} BA, 1.0 mg.L^{-1} GA3, 0.1 mg.L^{-1} NAA) and then this combination was used in addition to different concentrations of NaCl (0.0, 50, 100 mmol) in combination with the concentrations of Chitosan (0.0, 5.0, 15, 25 mg. L^{-1}) The shoots resulting from the previous experiment were cultured with 10 replicates per concentration. After four weeks of cultivation, the readings took the experiment. The following measurements were taken (the shoot content of nitrogen, phosphorus, potassium, sodium, chlorine, carbohydrates, proline, ascorbic acid, chlorophyll).

Experimental design and statistical analysis:

The research was conducted as factorial experiments according to the completely randomized design (CRD), and the differences were compared according to the least significant difference test (LSD) to show the statistical differences between the treatments and the probability level 0.05 (Alrawi and Khalaf Allah, 2000).

Results and discussion:

The results in Table (1) showed that the concentration of benzyl adenine had a significant effect on the percentage of shoot multiplication resulting during the multiplication stage, where the concentration 2.0 mg. L^{-1} significantly excelled on the treatment of the concentration of 3.0 mg. L^{-1} of benzyl adenine only by giving the highest average for this trait. It reached 90% while it did not differ significantly with the rest of the experiment treatments. Where, the treatment of concentration, 3.0 mg. L^{-1}, recorded the lowest average for this trait, which was 30%. The reason for the excelled may be due to the effectiveness of benzyl adenine in inducing replication due to its ability to break the apical dominance through which it results in stimulating the growth of lateral shoots (Sharma et al., 2009),In addition to the role of cytokinin as a catalyst for the processes of division and differentiation, and its establishment of sinks attraction centers in the lateral shoots, through which it stimulates the speed of transporting nutrients that result in stimulating the growth process (Taiz and Zeiger, 2006). This result did not agree with Hamad and Al-Jubouri (2012), as the concentration of 1.0 mg. L^{-1} caused a significant increase in the multiple percentages

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compared to a concentration of 2 mg. L^{-1} on the Citrumelo plant. these results agree with Hamad et al (2012), the concentration of 2 mg. L^{-1} gave the highest percentage of response to the increase of the citrus rootstock of the Swingle Citrumelo in vitro.It also agreed with Salman and Khairy (2016), as the concentration of 2.0 mg. L^{-1} produced a significant excelled in the response percentage, reaching about 93.33%, compared to the rest of the concentrations used from benzyladenine in the propagation of the citrus rootstock of the Swingle Citrumelo and **Troyer** citrange in vitro.The results of the same table also show that significant differences occurred in the average of the number of multiple vegetative shoots where a result of its treatment with BA growth regulator, where the concentration treatment 2.0 mg. L^{-1} recorded a significantly excelled in the above traits of all other treatments used and gave the highest average of 4.20 vegetable shoots compared to the concentration treatment. 3 mg. L^{-1} that achieved the lowest average amounted to of 1.40 vegetable shoot without significantly differing with all other treatment. These results were not in agree with Hamad and Al-Jabouri (2012), as the concentration of 1.0 mg.L^{-1} a significant difference in the number of shoots formed, compared to the rest of the concentrations used when the propagation of the citrus rootstock of Troyer citrange in vitro.While the results were in agreement with Kiran and Singh (2012), Hamad et al. (2012), Salman and Khairy (2016), who found that the prepared nutrient medium with a concentration of 2 mg. L^{-1}BA contributes to a significant increase in the average number of shoot formed by the citrus rootstocks, the Citrumelo and the Troyer citrange compared to with the rest of the other concentrations. The reason for the excelled in the number of shoots formed as a result of its treatment with BA may explain its role in inducing tissue cells to divide and differentiate, which results in the differentiation of tissues transplanted in vitro vegetative shoot (Miah et al., 2008).From observing the results of the same table, it is evident that there are differences between the treatments used in the study without raising the level of significance in the traits of the length of the formed vegetative shoots, where the BA concentrations did not have a significant effect on this traits, so it was investigated. The reason for the decrease in the average length of the vegetative shoots formed on the vegetative shoot, with the increase in the concentration of cytokinin used, may be due to the increased competition of the vegetative shoot for the nutrients and hormonal substances present in the nutrient medium, which affects their growth and in return works to encourage the growth of buds by the action of cytokinin, increasing their number and increasing the number, leading to a decrease Average multiple shoot lengths (Neumann et al. 2009). These results were not identical with the results of Hamad and Al-Jubouri (2012), Hamad and Al-Jabouri (2014) and Salman and Khairy (2016), as they obtained significant differences in the length of the vegetative shoots of the citrus rootstocks, the Truer Strang and the Citrumelo, where a result of treating them with several concentrations of BA when propagated in vitro. It is evident from the traits mentioned and their results shown in Tables (1) that the concentration of 2 mg.L^{-1} liter of benzyl adenine was the best in stimulating and encouraging growth according to the cultivar conditions in the research experiment, while the high concentrations negatively affected all the studied traits. It was observed through the data of the tables that raising the concentration of benzyl adenine from the concentration of 2.0 mg.L^{-1} caused a significant decrease, and this may mean that the increase in its concentration had a negative effect in achieving any significant increase in all characteristics due to its effect on internal hormone levels or its ability to stimulate the emergence of callus. At the same time, it may inhibit the growth of vegetative shoots (Al-Rifai and Al-Shobaki, 2007 and Al-Khalifa, 2011). or the concentration of 2.0 mg. L^{-1} of BA may be the appropriate concentration in giving the optimal results and the required response for growth and development due to its induction of hormonal balance in the tissue of the explant plant that is the culture in vitro. Al-Hushaibi (2004) showed

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this to the fact that low concentrations of benzyl adenine have higher catalytic action in inducing cells to divide and differentiate, which results in the differentiation of tissues cultivated in vitro into vegetative organs because of their ability to transport nutrients towards dormant buds, which leads to stimulation of their growth in vitro. The results in Table (1) showed significant differences in the fresh weight of the multiple vegetative shoots, where the treatment of concentration 2.0 mg. L^{-1} of BA growth regulator showed a significant effect on the average fresh weight of the resulting growths. It gave the highest average for these traits of 263.0 mg compared to the concentration treatment 1.0 mg. L^{-1}, while the treatment for concentration 4 mg. L^{-1} gave the lowest average of 159.0 mg without there being a significant difference with the concentration treatment 3.0 mg. It recorded an average of 163.0 mg. Also results in the same table showed that there were significant differences in the dry weight of the multiple vegetative shoots as a result of being affected by BA growth regulator, where the treatment achieved a concentration of 2 mg. L^{-1} BA was significantly excelled to the dry weight of the growths of 43.00 mg compared to the other treatments used in the experiment except for the concentration treatment 1 mg. L^{-1} (32.10 mg)Where, the concentration 3 mg. L^{-1} treatment gave the lowest average for the aforementioned trait of 24.20 mg without significantly differing with the concentration treatment of 4 mg. L^{-1}, which recorded an average of 24.50 mg. The above results agree with Al-Asadi found (2012)as the concentration of 2 mg. L^{-1} had a significant effect on both fresh and dry weights, compared to the rest of the concentrations used in the research experiment of the growths formed from the culture of Citrus medica seeds in vitro. Nayef (2013) found, the addition of benzyl adenine at a concentration of 2 mg. L^{-1} to the aqueous medium resulted in a significant increase in the dry and fresh weights of the growths of five citrus rootstocks, including the Citrumelo. It also agreed with Naji (2013) obtained, as it was found that the addition of benzyl adenine at a concentration of 2 mg. L^{-1} to the medium supplied with NaCl resulted in a significant increase in the dry and fresh weights of the growths arising from the two-node micropropagation of the rootstocks of some citrus fruits, including Stromello.While the results did not agree with the results of the Hamiri (2009) study in the propagation of Citrus sinensis L. Osbeck, where the lowest concentration of benzyl adenine was 0.5 mg. L^{-1} had a significant response in the lean weight of the resulting growths, while it gave the lowest results at a concentration of 2 mg.L^{-1}, Whereas Khazali (2016) indicated that the benzyl adenine concentrations used in the research experiment had no significant effect on the lean weight of the growths resulting from callus culture of the citrus rootstocks of volcamariana. The reason for the increase in weights of vegetative growth may be due to the role of the cytokinin growth regulator represented by benzyl adenine, which is important in stimulating plant cell division, where this regulator is known for its ability to stimulate and divide cells and thus increase and accelerate growth. Therefore, it was commonly used in phylogenetic experiments in plant tissue culture. As for high concentrations of BA, which caused a significant reduction in the aforementioned trait, it may have an inhibitory effect on growth and development processes (George et al. 2008, Al-Salehi and Al-Sumaida'i, 2013). This was confirmed by Shukri and Al-Moaikil (2013) that the use of low concentrations in the upbringing of growths gives higher effects to the part grown in-vitro, which contributes to encouraging vegetative growth, and this, in turn, increases plant efficiency in food processing and improves its growth and development.

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Table (1) The effect of AB concentrations on the percentage of multiple vegetative shoots, the average number and length of multiple shoots and the average dry and dry weight in the presence of 0.1 (mg. L^{-1}) NAA and 1 GA3 mg. L^{-1} in the Swingle Citrumelo plant invitro

Average dry weight of multiply shoots (mg)	Average fresh weight of multiply shoots (mg)	Average length of multiply shoots (cm)	Average number of multiply shoots(%)	multiple percentage (%)	BA concentration (mg.l-)
32.00	179.0	2.74	2.00	60	1
32.10	218.0	2.90	4.20	90	2
43.00	263.0	2.52	1.40	30	3
24.20	163.0	2.17	2.00	50	4
24.50	159.0	N.S	1.318	43.3	LSD 0.05

The effect of the interaction between Chitosan and salinity in:

1- Number of vegetative shoots

The results in Table (2) show that chitosan concentrations had a significant effect on the number of vegetative shoots formed, where the concentration treatment 15.00 mg. L^{-1} significantly excelled on the 5.00 mg.L^{-1} treatment only without differing significantly with the rest of the treatments and It recorded the highest average of 2.53 vegetative shoots. While the salinity levels did not have any significant response in the aforementioned trait, while there were no significant differences between the concentrations of chitosan in the traits of the length of the vegetative shoots. As for adding the salinity levels of NaCl, no significant differences were recorded between the treatments. Whereas, the interaction of the two study factors had a significant effect on this traits as the control treatment (not adding chitosan and NaCl) to the culture medium achieved a significantly excelled on all the interaction treatments used in the experiment except for the interaction treatment (the two higher concentrations of chitosan and salt (NaCl)).As there were no significant differences between them, they recorded the highest average for this trait, which was (4.20 and 3.40), respectively, while the interaction treatment recorded 5.00 mg. L^{-1} of chitosan without adding NaCl, the lowest average for these traits was 1.40 vegetative shoots.

2 - Length of vegetative shoots (cm)

The results presented in Table (2) show that there were no significant differences between the concentrations of chitosan in the traits of the length of the vegetative shoots. As for the addition of salt levels of NaCl, no significant differences were recorded between the treatments. Where, the interaction of the two study factors, where the interaction treatment achieved 15.00 mg.L^{-1} of chitosan without adding NaCl, had the highest average for this trait of 2.70 cm, which significantly excelled on the two interaction treatments without adding chitosan and NaCl and the concentration was 5.00 mg. L^{-1} of chitosan with the level. Only 100 mmol of NaCl had the lowest average for this trait of 1.82 and 1.55 cm, respectively.

3- Average wet weight (mg)

The results presented in Table (2) indicated that there were significant differences in the fresh weight due to the effect of adding different concentrations of chitosan, so the treatment of concentration 25.00 mg. L^{-1} recorded the highest average for this trait of 300.0 mg. As for the effect of different levels of NaCl, the control treatment was given (without adding of NaCl to the culture medium) the highest fresh weight average of 283.0 mg. As for the interaction of both study factors, the interaction treatment of 15.00 mg. L^{-1} of chitosan + 0.0 mmol of NaCl achieved a

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significantly excelled on the interaction treatments, without adding chitosan with concentrations of 50 and 100 mmol and 5.00 mg. L^{-1} for chitosan + 100 mmol NaCl, while it was not There, are significant differences with the other interaction factors, It achieved the highest average of fresh weight which was 331.0 mg, while the interaction gave 5.00 mg. L^{-1} for chitosan + 100 mmol NaCl, the lowest average of fresh weight of 144.0 mg.

4 - Average dry weight (mg)

The results in Table (2) show that there are significant differences at high concentrations of chitosan, where the concentration treatment 25.00 mg. L^{-1} gave the highest dry weight average for multiple vegetative shoots was 46.5 mg. As for the effect of salinity levels, the control treatment gave the highest average dry weight. It reached 44.8 mg, which was significantly excelled to the concentration treatment of 100 mmol NaCl without significantly differing with the concentration of 50 mmol. Where, the bi-interaction had a significant effect, where the interaction treatment achieved 15.00 mg. 1 liter of chitosan + 0.0 mmol NaCl (the highest average was 53.7 mg dry weight, where it significantly excelled the interaction factors (0.0 mg chitosan + 50 mm NaCl and 0). .0 mg. L^{-1} of chitosan + 100 mmol NaCl and 5.0 mg. Liter-1 of chitosan + 50 mmol of NaCl, while it did not differ significantly with the other interaction factors used in the experiment, while the interaction treatment gave 5.00 mg.L^{-1} liter of chitosan + 100 mmol NaCl, the lowest dry weight for the multiple vegetative shoots was 21.2 mg.

5- Average water content (mg)

The results in Table (2) show that there are significant differences in the water content of the vegetative shoots, where the treatment of concentration of 25.00 mg. L^{-1} of chitosan significantly excelled on the control treatment and the treatment of concentration 5.00 mg. due to the effect of NaCl salt levels added to the culture medium, the same table shows that the control treatment plants significantly excelled on the 100 mmol salt concentration treatment plants without significantly differing with the 50 mmol salt concentration treatment plants, which gave the highest average for the mentioned recipe (269 mg). Where, the interaction of the study factors had a significant effect on the water content of the shoots, where the treatment achieved 15.00 mg. L^{-1} of chitosan + 0.0 mmol NaCl significantly excelled to the interaction factors 0.0 mg. L^{-1} of chitosan + 100 mm NaCl and 5.00 mg. L^{-1} of chitosan + 100 mmol and 0.0 mg. L^{-1} of chitosan + 50 mmol of NaCl without there being significant differences with the other treatments, where it achieved the highest average of the water content of 310 mg, while the interaction treatment recorded 5.00 mg. L^{-1} Of chitosan + 100 mmol NaCl the lowest was 140 mg.

6 - Average chlorophyll (mg / g fresh weight)

The results presented in Table (2) indicate that the chitosan compound had a significant effect on the content of multiple vegetative shoots of chlorophyll, where the control treatment significantly outperformed all other treatments and recorded the highest average of 1.304 mg. Of the salt of NaCl, the results of the same table showed significant differences between the treatments, as the control treatment significantly excelled on the treatment of 100 mmol NaCl only, as it recorded the highest average for this trait of 1.312 mg.Where, the interaction of the study factors had a significant effect on the aforementioned trait, as the interaction treatment recorded 25.00 mg. L^{-1} of chitosan + 0.0 mmol of NaCl significantly excelled to all other interaction factors, except for 15.00 mg. NaCl, as well as the two coefficients of non-addition and the concentration of 5.00 mg. L^{-1} of chitosan + 50 mmol of NaCl, as there were no significant differences between them, achieved the highest average for this trait of 1.380 (mg. g^{-1} fresh weight), respectively. Whereas, the interaction treatment gave 25.00 mg. L^{-1} of chitosan + 100 mmol of NaCl, the lowest average for

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chlorophyll content was 1.086 (mg. g^{-1} fresh weight).

We note from the results in tables (2) that the concentrations of chitosan treated by the nutritional media of citrus plants, the Rootstocks of the Citrumelo has worked to end the damage caused by adding NaCl salt to the nutrient medium, which means reducing or stopping the damage of salt tension affecting growth and the course of bioactivities of the explant plant in vitro. This improvement was due to an increase in the concentrations of chitosan added to the growth medium, and this may be due to Amin (2013) mentioned that chitosan has the ability to bring about positive changes in the plant metabolism process that lead to improving its growth indicators and increasing the effectiveness of the physiological processes shown by the plant during the period Its development, in addition to the fact that the plant is more resistant to withstanding conditions caused by various biological and abiotic stresses or diseases that cause bio infections. This is confirmed by Ali et al. (2013) mentioned that the chitosan compound has properties that make it anti-bacterial, fungal and bio infections, and at the same time it limits the spread of the pathogen, which allows the plant to have a higher capacity for growth and development. Weak growth as a result of treatment with different levels of NaCl can be due to its negative role in reducing the average of cell division and elongation as a result of its disruption of physiological activities and its direct effect on the nutritional and hormonal balance of growth (Wani and Hossain, 2016).

Table (2) The effect of Chitosan concentrations (mg. Liter-1) and sodium chloride (mmol) in the presence of 2 mg. Liter-1 BA and 0.1 mg. L^{-1} NAA and 1.0 mg. L^{-1} GA3 on the average vegetative growth traits for Swingle Citrumelo plant.

Average chlorophyll (mg / g fresh weight).	Average water content (mg)	Average dry weight (mg)	Average fresh weight (mg)	Average length of shoots(cm)	Average number of shoots	Treatments
1.323	246	42.3	263	1.82	4.2	CH 0
1.206	229	39.2	245	2.11	1.4	CH 5.0
1.34	310	53.7	331	2.7	1.8	CH 15.0
1.38	289	44.1	293	2.15	1.6	CH 25.0
0.031	64.4	9.41	68.9	N.S	0.81	LSD 0.05 CH
1.354	169	26.8	181	2.36	1.6	NACL50
1.234	180	30.8	192	2.09	1.7	NACL 100
0.027	55.8	8.18	59.7	N.S	N.S	LSD 0.05 NACL
1.359	230	36.8	247	2.28	1.6	5+50 CH+NaCl
1.209	140	21.2	144	1.55	1.9	5+100 CH+NaCl
1.206	264	41.1	284	2.2	3.8	15+50 CH+NaCl
1.197	212	39.2	219	2.25	2	15+100 CH+NaCl
1.301	284	46.9	304	2.55	2.2	25+50 CH+NaCl
1.086	285	48.4	305	2.43	3.4	25+100 CH+NaCl
0.054	111.6	16.31	119.3	0.73	1.41	LSD 0.05 CH+NACL

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