

## **Effect of Salicylic acid, Ascorbic acid, and Proline to reduce the negative effect of salt stress on Pomelo seedlings**

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### **Abstract**

Antioxidants are used to reduce the impact of environmental stresses on plant growth, especially salt stress because they have an effect on reducing the oxidative stress resulting from effective oxygen radicals inside the plant in response to environmental stresses on the plant. Therefore, the study was conducted in Al-Hilla district, Al-Hakania region for the period from (1\9\2020 - 1\5\2021) to investigate the effect of salt stress on the vegetative and root traits of Pomelo seedlings treated with saline water (0, 2, 4, 6 dSm. m<sup>-1</sup>) using sodium chloride salt, The second factor is spraying the seedlings (salicylic, ascorbic, proline) at a concentration of 300 mg.L<sup>-1</sup> in addition to the (control ) treatment at the end of the experiment, the vegetative traits were measured (total chlorophyll pigment in leaves, dry weight of the shoot, number of branches) ) and root traits (dry weight of the root system, number of secondary roots). The results showed the treatment with salicylic acid at a concentration of 300 mg.l<sup>-1</sup> excelled in all the studied traits, and proline acid at a concentration of 300 mg.L<sup>-1</sup> recorded a significantly excelled in the root traits. The study was designed as a factorial experiment according to The Randomized Complete Block Design (RCBD) and with three replications with three seedlings for the experimental unit and with two factors (4 \* 4), the number of the experiment's treatments was (16) treatments distributed randomly to (144) seedlings of one and a half years of age, The results were analyzed using the analysis of variance table (ANOVA table) according to the EXCEL program, and the statistical differences between the treatments were tested using the least significant difference L.S.D at the probability level (0.05), and the most important results can be summarized as follows:

1. Increasing the salinity levels of irrigation water to the level of 6 ds.m<sup>-1</sup> (S3) led to a decrease in all vegetative and root growth traits (number of branches, vegetative dry weight, chlorophyll pigment, root dry weight, number of secondary roots), reaching (4.7 branches.plant<sup>-1</sup>, 29.2 g. seedling<sup>-1</sup>, 59.1 mg g<sup>-1</sup> fresh weight, 16.4 g seedling<sup>-1</sup>, 21.3 root) for the traits, respectively.

2. Treatment with salicylic acid, ascorbic or proline, resulted in a concentration of 300 mg. L<sup>-1</sup> improves vegetative and root growth indicators by reducing the effect of salinity of irrigation water in varying percentage, Salicylic acid (A3) achieved greater effectiveness in improving the traits of vegetative and root growth (number of branches, dry weight of vegetative, total chlorophyll, dry weight of the root system and number of secondary roots), reaching (7.1 branches. plant<sup>-1</sup>, 40.1 g. seedling<sup>-1</sup>, 72.9 mg g<sup>-1</sup> fresh weight, 21.6 g seedlings<sup>-1</sup>, 28.2 roots) for the traits, respectively.

### تأثير المعاملة بحامض الساليسيليك والاسكوربيك والبرولين في تقليل الأثر السلبي للإجهاد الملحي على نمو شتلات السندي

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#### الخلاصة

تستخدم مضادات الاكسدة في تقليل اثر الاجهادات البيئية على نمو النبات وخاصة الاجهاد الملحي لما لها تأثير في تقليل الجهد التأكسدي الناتج من الجذور الاوكسجينية الفعالة داخل النبات استجابة للإجهادات البيئية على النبات لذا أجريت الدراسة في قضاء الحلة منطقة الحكاية للفترة من (2020\9\1 – 2021\5\1) لبحث تأثير الإجهاد الملحي في الصفات الخضرية والجذرية لشتلات السندي المعاملة بمضادات الملوحة وتضمنت دراسة عاملين ، العامل الاول هو سقي الشتلات بربع مستويات من المياه المالحة وهي ( 0, 2, 4, 6 ديسيسمنز. م<sup>-1</sup>) باستخدام ملح كلوريد الصوديوم، اما العامل الثاني هو رش الشتلات ( الساليسيليك , الاسكوربيك , برولين) بتركيز 300 ملغم.لتر<sup>-1</sup> بالإضافة الى معاملة (المقارنة)، في نهاية التجربة، تم اخذ قياس الصفات الخضرية (صبغة الكلوروفيل الكلي في الأوراق , الوزن الجاف للمجموع الخضري , عدد الافرع ) و الصفات الجذرية (الوزن الجاف للمجموع الجذري, عدد الجذور الثانوية ), أظهرت النتائج تفوق المعاملة بحامض الساليسيليك بتركيز 300 ملغم.لتر<sup>-1</sup> في جميع الصفات المدروسة كما ان حامض البرولين بتركيز 300 ملغم.لتر<sup>-1</sup> قد سجل تفوق معنوي في الصفات الجذرية وتم تصميم الدراسة كتجربة عاملية وفق تصميم القطاعات العشوائية الكاملة R.C.B. D وبثلاث مكررات بواقع ثلاث شتلات للوحدة التجريبية وبعاملين ( 4\*4 ), بلغ عدد معاملات التجربة (16) معاملة وزعت عشوائيا على (144) شتلة بعمر سنة ونصف، وتم تحليل النتائج باستخدام جدول تحليل التباين (ANOVA table) وفقاً لبرنامج EXCEL وتم اختبار الفروق الإحصائية بين المعاملات باستخدام أقل فرق معنوي L.S.D عند مستوى احتمال (0,05) ويمكن تلخيص اهم النتائج كما يلي:

1. إن زيادة مستويات ملوحة مياه الري الى مستوى 6 ديسيسمنز. م<sup>-1</sup> (S) أدت إلى حدوث انخفاض في جميع صفات النمو الخضري والجذري (، عدد الافرع، الوزن الجاف الخضري، صبغة الكلوروفيل، الوزن الجاف الجذري ، عدد الجذور الثانوية)، إذ بلغت (4.7 فرع. نبات<sup>-1</sup>، 29.2 غم. شتلة<sup>-1</sup>، 59.1 ملغم. غم<sup>-1</sup> وزن طري، 16.4 غم. شتلة<sup>-1</sup>، 21.3 جذر) للصفات على التوالي.
2. ادت المعاملة بحامض (الساليسيليك أو الاسكوربيك أو البرولين) بتركيز 300 ملغم. لتر<sup>-1</sup> إلى تحسين مؤشرات النمو الخضري والجذري من خلال تقليل تأثير ملوحة مياه الري وينسب متفاوتة إذ حقق حامض الساليسيليك (3A) فعالية أكبر في تحسين صفات النمو الخضري والجذري (عدد الافرع والوزن الجاف للمجموع الخضري والكلوروفيل الكلي والوزن الجاف للمجموع الجذري وعدد الجذور الثانوية)، إذ بلغت (7.1 فرع. نبات<sup>-1</sup>، 40.1 غم. شتلة<sup>-1</sup>، 72.9 ملغم. غم<sup>-1</sup> وزن طري، 21.6 غم. شتلة<sup>-1</sup>، 28.2 جذر) للصفات على التوالي.

## **1. Introduction**

Citrus is one of the evergreen fruit trees and belongs to the Rutaceae family, which includes a number of genera, the most important of which is the genus Citrus.

It grows in the tropics and subtropics, and the regions of Southeast Asia are its origin country. In Iraq, most citrus fruits are cultivated in the central and southern regions [3] and [11]. Pomelo fruit (*Citrus grandis*) is one of the species belonging to the genus (*Citrus*), where its fruits contain high amounts of carbohydrates, about 17.5%. It also contains mineral salts, organic acids, fiber, vitamins, and a little protein [7] and [34]. Salinity is one of the most important problems facing agriculture on a global scale, specifically in arid and semi-arid regions [27] and [28] and it is considered the most biotic stress factor affecting plant growth and the outcome. The negative impact of salinity is due to direct effects due to the high osmotic pressure of the soil solution, which reflects on Water availability and other indirect ones by affecting the chemical and physical properties of the soil, which affect the balance of nutrients inside the plant [9] and [10]. Therefore, it is necessary to use some alternative technologies at the present time that work to increase the tolerance of sandy trees to salinity in order to reduce its damage, including

antioxidants, hormones, and some bio-stimulants. Such as external treatment with salicylic acid, and studies have shown that there are alternative means other than salicylic to improve the salt tolerance of plants, including external treatment with the amino acid proline as well as anti-oxidants such as ascorbic acid [33]. Due to the lack of studies on the use of these compounds and their effect in reducing salt stress on Pomelo seedlings and thus on plant growth, this study was carried out with the aim of: -

- 1- Reducing the effect of using saltwater to irrigate Pomelo seedlings through the use of some antioxidants and osmotic preservatives.
- 2- Determining the best of the used acids (salicylic acids, ascorbic, proline) in improving the growth of Pomelo seedlings under salt stress conditions.

## **2. Materials and methods**

The study was conducted in Al-Hilla district, Al-Hakania region for the period from (1\9\2020 - 1\5\2021) to study the effect of salt stress on the vegetative and root traits of Pomelo seedlings treated with antioxidants. Pomelo seedlings were obtained from the certified citrus production nursery belonging to the Iraqi Ministry of Agriculture / General Directorate of Horticulture and Forests in the Holy Karbala

Governorate / Hindiya District. The seedlings were selected as homogeneous as possible, planted in pot of 8 kg capacity. The service operations were conducted on it, which included irrigation, removing the bush manually, adding Humic Asad and Dixon by irrigation method, and Rifas fungicide using the foliar spray method in a homogeneous manner throughout the duration of the experiment. The experiment included (144) seedlings of Pomelo grafted on the rootstock of *Citrus aurantium*, and the experiment of its two factors was followed according to the Completely Randomized Design, with two factors ( $4 \times 4$ ) and three replications. The experiment included 16 experimental units with three seedlings for each experimental unit in one replicate. The experiment was conducted with two factors, the first factor is irrigation of the seedlings with four levels of saline water (0, 2, 4, 6 dSm.  $m^{-1}$ ) using sodium chloride salt, and the concentrations were determined by the EC device. The second factor is spraying seedlings with antioxidants (without spraying, proline acid, ascorbic acid, salicylic acid) at a concentration of 300  $mg.L^{-1}$ . These concentrations are within the recommended limits, where the seedlings were sprayed with saline water at 10 sprays for the spring and Autumn season, with a period of 10 days between one spraying and another. The

experiment was terminated a month after the last spray date and samples were taken for analysis and results were recorded, then the analysis of variance was conducted using the EXCEL program. The differences between the means were compared according to the least significant difference (L.S.D) test at the probability level of 0.05 [4]. Indicators of vegetative and root growth were studied as follows: -

#### **vegetative traits:**

Leaves content of total chlorophyll pigment ( $mg. g^{-1}$  fresh weight), dry weight of the shoot ( $g. seedling^{-1}$ ), Number of branches ( $branch. plant^{-1}$ .)

#### **Root traits:**

The dry weight of the root system ( $gm. seedlings^{-1}$ ), Number of secondary roots ( $root$ ):

### **3. Results and discussion**

#### **1.3. Effect of salt stress on vegetative traits**

The results in tables (1-3) indicate that the salinity of irrigation water has a significant negative effect on all vegetative traits (chlorophyll, vegetative dry weight, number of branches). The high concentrations caused a decrease in the rate of chlorophyll pigment in leaves, vegetative dry weight, and the number of branches, and it reached the lowest average when irrigated at the level of salinity (6) ds.  $M^{-1}$

(59.1 mg. g<sup>-1</sup> fresh weight, 29.2 g. seedling<sup>-1</sup>, 4.7 branches. plant<sup>-1</sup>) Whereas, the highest average was recorded when irrigating with the level of salinity (0) dS. M<sup>-1</sup> (76.7 mg.g<sup>-1</sup> fresh weight, 42.5g. seedling<sup>-1</sup>, 7.1 branches. plant<sup>-1</sup>) Which significantly excelled the rest of the treatments, and perhaps the reason for the increase in vegetative growth in the low levels of salinity of irrigation water is the need for seedlings of small amounts of salt represented by nutrient ions that contribute to increasing their growth [5]. The reason for the decrease may be due to the negative effect of salinity on leaf size and structure, which leads to a decrease in the number of chloroplasts [1], It is also attributed to the low nitrogen content of the leaves at the same saline level, which is included in the composition of chlorophyll (a and b), while nitrogen was higher in the rest of the treatments, or the salinity works to demolish chlorophyll and slow the speed of its formation and increase the effectiveness of the chlorophyll-dissolving enzyme chlorophyllase and then decrease Chlorophyll content [29] or this is due to what was mentioned by [17], that the most important negative effect of salinity is the excessive production of oxygen radicals ROS, which causes the oxidation of the internal structures of the chloroplasts. It negatively affects the photosynthesis process. The decrease in the chlorophyll content of citrus leaves with

the increase in the salinity level has been indicated by many researchers including [13], [14] and [31]. The decrease in the dry weight of the vegetative system with the increase in salinity levels is due to the effects of salinity in lowering the water potential of the growing medium, which leads to a decrease in water absorption or its reverse loss by the roots [35]. These results are in agreement with [18] in Cleopatra mandarin and charizocitrands, [20] in Citrus aurantium and (Citrus macrophylla) and with [12] in local oranges and [14] in ten citrus rootstocks. The results of tables (1-3) represented by the characteristics (chlorophyll pigment in leaves, dry weight of the vegetative, number of branches) indicate the positive significant effect of antioxidants, where salicylic acid recorded the highest average (72.9 mg. g<sup>-1</sup> fresh weight, 40.1 g. seedlings<sup>-1</sup>, 7.1 branches plant<sup>-1</sup>) Then ascorbic (70.8 mg. g<sup>-1</sup> fresh weight, 36.9 g. seedling<sup>-1</sup>, 5.6 branches. plant<sup>-1</sup>) then Proline (66.1 mg. g<sup>-1</sup> fresh weight, 36.0 g. seedling<sup>-1</sup>, 6.0 branches. plant<sup>-1</sup>) then the control (61.9 mg. g<sup>-1</sup> fresh weight, 34.2 g. seedling<sup>-1</sup>, 5.3 branches. plant<sup>-1</sup>) respectively, The effect of salicylic acid may be due to its being a widespread phenolic compound in plants that contributes to the regulation of physiological processes in the plant, and the external preparation of SA affects the process of ion entry, transport and transpiration [24]. The

reason is also due to the synergistic effect shown by some phenolic compounds with other growth regulators such as indole acetic acid IAA, which have a confirmed role in the process of cell division and enlargement, thus increasing vegetative growth [25]. The effect of salicylic acid in increasing the content of chlorophyll in leaves is due to the fact that salicylic acid plays an important role in preserving the plastids from demolition as a result of the increased production of free radicals as a result of salt stress and it raises the levels of antioxidants, especially Peroxidase, Catalase, Dismutase, Superoxide, which maintains the plastids and pigments. from decomposition due to environmental stress, [23] In addition, salicylic acid works to increase the photosynthetic pigments and the auxiliary pigments, which are a means of protection by affecting the reactions of the second photosystem, where it works to reduce the effect of the oxidizing oxygen radicals of chlorophyll on the one hand and reduce the activity of chlorophyll demolition enzymes, thus increasing its stability under salt stress conditions. The results are with [12] in orange seedlings and [2] in olive seedlings and [8] in sour orange seedlings, and the effect of salicylic acid is consistent with what was found by [19] and [26]. Also, the reason for the increase in the dry weight of the vegetative system is due to the fact that salicylic acid

works to encourage growth, thus increasing water absorption, which reflected positively on the increase in weight. , the photosynthesis process, and the mechanics of opening and closing stomata [21]. The reason may be due to the ability of salicylic acid to improve the growth features of plants exposed to salt stress due to its clear effect in stimulating the photosynthesis process by preserving the enzymes involved in this process and the permeability of plasma membranes and increasing the photosynthetic pigments in leaves [22] and then increasing the synthesis and accumulation of substances. This may be reflected in the accumulation of dry matter in the vegetative system of the seedling.

### **2.3. Effect of salt stress on root traits**

The results of the two tables (4.5) indicate that there are significant differences between the levels of salinity of irrigation water in the averages of (Dry weight of the root system, number of secondary roots). It was noted that there is a decrease in the average of root traits by increasing the salinity of the irrigation water to 6 dS.m<sup>-1</sup>. The control treatment recorded the highest average (22.0 g. seedlings<sup>-1</sup>, 29.5 roots), and these results agreed with [18], [20] and [14] in a trait of a decrease in the dry weight of the roots due to the effect of high salinity levels. The results of the same two tables indicate that there

are significant differences between the levels of antioxidants in the average of root traits (dry weight of the root system, number of secondary roots), where salicylic acid treatments excelled and gave the highest values (28.2 root, 21.6 g. seedlings<sup>-1</sup>), then proline, then ascorbic, then straight control .This may be due to the effect of salicylic acid in increasing cell division in the

apical meristem of the root [32] and its role in improving dry matter accumulation during the development of unfolded storage organs [16].The increase in the dry weight of the roots as a result of treatment with salicylic acid was indicated by[2] in olive seedlings and [8] in *Citrus aurantium*.

**Table (1): Effect of salt stress on the rate of chlorophyll pigment in leaves (mg. g<sup>-1</sup> fresh weight).**

Antioxidants	salinity levels				average
	S0	S1	S2	S3	
A0	71.4	65.4	58.4	52.5	61.9
A1	74.6	70.0	62.6	57.4	66.1
A2	79.3	74.1	67.1	62.5	70.8
A3	81.3	76.3	69.8	64.2	72.9
average	76.7	71.5	64.5	59.1	
L.S.D 0.05	S=1.553	A=1.553	S*A=n.s		

**Table (2): Effect of salt stress on the average dry weight of the vegetative growth (gm. seedlings<sup>-1</sup>)**

Antioxidants	salinity levels				average
	S0	S1	S2	S3	
A0	40.6	38.5	31.2	26.4	34.2
A1	41.5	40.4	33.4	28.6	36.0
A2	42.6	41.6	34.2	29.5	36.9
A3	45.5	44.3	38.3	32.3	40.1
average	42.5	41.2	34.3	29.2	
L.S.D 0.05	S=1.872	A=1.872	S*A=n.s		

**Table (3): The effect of salt stress on the average number of branches (branch. plant<sup>-1</sup>)**

Antioxidants	salinity levels				average
	S0	S1	S2	S3	
A0	6.2	6.0	5.0	4.2	5.3
A1	7.2	6.5	5.7	4.7	6.0
A2	7.0	6.0	5.3	4.2	5.6
A3	8.0	7.8	7.0	5.7	7.1
average	7.1	6.6	5.8	4.7	
L.S.D 0.05	S=0.72	A=0.72	S*A=n.s		

**Table (4): Effect of salt stress on the average dry weight of the root system (g. seedlings<sup>-1</sup>).**

Antioxidants	salinity levels				average
	S0	S1	S2	S3	
A0	19.3	18.5	16.2	14.2	17.1
A1	22.5	21.4	18.7	17.2	20.0
A2	21.4	20.5	17.6	15.7	18.8
A3	24.7	22.7	20.5	18.6	21.6
average	22.0	20.8	18.3	16.4	
L.S.D 0.05	S=0.563	A=0.563	S*A=n.s		

**Table (5): The effect of salt stress on the average number of secondary roots .**

Antioxidants	salinity levels				average
	S0	S1	S2	S3	
A0	27.2	22.2	20.8	18.7	22.2
A1	29.3	24.8	23.5	22.0	24.9
A2	29.0	24.3	23.2	21.0	24.4
A3	32.5	30.2	26.7	23.3	28.2
average	29.5	25.4	23.5	21.3	
L.S.D 0.05	S=0.703	A=0.703	S*A=1.406		



#### **4. Conclusions**

1- Irrigation of Pomelo seedlings with water of a salinity level of (0,2) ds.m<sup>-1</sup> gave good vegetative growth and did not show symptoms of nutrient deficiency compared to seedlings irrigated with water of a level of (4.6) ds.m<sup>-1</sup>, which had a negative effect In the traits of vegetative and root growth, in addition to the accumulation of sodium and chloride ions in the tissues of seedlings instead of potassium ions and nitrates, which had a significant impact on the deterioration of the traits of vegetative growth in later studies.

2- The results showed that spraying seedlings with salicylic acid was more effective and effective in improving vegetative and root growth indicators and reducing the harmful effect of salinity in irrigation water, followed by spraying with ascorbic and then proline.

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