

Effect of foliar application with paclobutrazol on the growth of rough lemon (*Citrus jambhiri* Lush.) Seedlings under salt stress conditions

Anas Kiwan *⁽¹⁾, Roula Bayerli ⁽²⁾ and Ali Elkhateeb ⁽³⁾

(1). Sweida Research Center, General Commission for Scientific Agricultural Research, Syria.

(2). Department of Horticulture, Faculty of Agriculture, Engineering, Damascus University, Syria.

(3). Lattakia Center, General Commission for Scientific Agricultural Research, Syria.

(*Corresponding author: Anas Kiwan. E-mail: anas.kiwan@damascusuniversity.edu.sy).

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Abstract

This research was carried out at Cyano Research Station, Agricultural Scientific Research Center, Lattakia, General Commission for Scientific Agricultural Research, Syria, during two successive seasons, 2020-2021 and 2021-2022 to study the effect of foliar spraying with paclobutrazol (PBZ) on growth of *Citrus Jambhiri* Lush. seedlings under salt stress conditions. The experiment was designed according to a split-plot design. Five levels of NaCl (0, 8, 16, 24, 32 mS/cm) were applied, and five concentrations of PBZ (0, 250, 500, 1000, 2000 ppm) were foliar sprayed. The results showed that the gradual increase in salt levels resulted a decrease in all the studied parameters. On the other side, the treatment with PBZ improved most of the studied parameters under all studied salt levels. The interaction treatments of PBZ (2000 ppm) and salinity (in all tested levels) showed superiority in average root length (27.54 cm), shoots fresh and dry weight (36.12 g, 14.32 g, respectively), roots fresh and dry weight (31.39 g, 9.89 g, respectively), relative water content (64.82 %), leaf content of chlorophyll a, b and carotenoids (1.40, 0.74 and 0.52 mg/g FW, respectively), proline accumulation (0.92 mg/g), total soluble sugars (0.58 mg/g), leaves concentrations of nitrogen, phosphorus and potassium (4.34, 0.28, 0.74%, respectively) compared with interaction treatments of PBZ (0 ppm) and salinity (16.05 cm for root length, 15.94, 5.69 g for shoots fresh and dry weight, 18.45, 5.51 g roots fresh and dry weight, 52.07% relative water content, 0.97, 0.48, 0.38 mg/g FW chlorophyll a, b and carotenoids, 0.71 mg/g proline, 0.41 mg/g total soluble sugars and 2.84, 0.21, 0.59% NPK concentrations, respectively).

Keywords: Rootstock, Seedlings, *Citrus Jambhiri* Lush., Salinity, Paclobutrazol.

Introduction:

Citrus is considered one of the world's most important fruit crops cultivated for over 4000 years (Yao *et al.*, 2020). Citrus fruits are consumed in large quantities worldwide due to the abundance of nutrients, high nutritional values and various health-promoting effects. In addition, fruit is enriched with water, carbohydrates, vitamins, minerals, and dietary fibers which are important nutrients (Lu *et*

al., 2023). Taxonomists now agree that the Citrus L. genus is part of the Sapindales Berchtold and J. Presl, order in the Rutaceae Family (Stevens, 2016; Schoch *et al.*, 2020).

The salinity of arable land is an increasing problem in many irrigated, arid and semi-arid areas of the world where rainfall is insufficient to leach salts from the root zone, and it is a significant factor in reducing crop productivity (Safdar *et al.*, 2019). Saline soils are defined by Ponnamperna (1984) as those that contain sufficient salt in the root zone to impair the growth of crop plants. However, since salt injury depends on species, variety, growth stage, environmental factors, and nature of the salts, it is difficult to define saline soils precisely.

Salinity is considered a major threat to the citrus cultivation in the world, particularly in semi-arid and arid regions where saline water mostly is used for irrigation (Srivastava and Singh, 2009). Saline water contains inorganic dissolved salts including cations [sodium (Na^+), calcium (Ca^{+2}) and magnesium (Mg^{+2})] and anions, [chloride (Cl^-), sulfate (SO_4^{-2}) and bicarbonate (HCO_3^-)], these ions generate an electrical field in the solution so that the concentration of salts correlates linearly with electrical conductivity (EC) of the water (Grattan *et al.*, 2015). High EC (saline conditions >4 dS/m) disrupt root membrane integrity, reduce nitrate (NO_3^-) uptake by Cl^- (compete with NO_3^- during uptake and translocation to leaf), alter the activities of nitrogen (N) assimilating enzymes, and reduce relative growth rate (Ashraf *et al.*, 2018). Plants respond to salt stress by increasing root/canopy ratio and chlorophyll content and inducing changes in the leaf anatomy to prevent leaf ion toxicity, and maintain leaf water, turgor and photosynthesis (Acosta-Motos *et al.*, 2017).

Thus, it is direly important to develop salinity mitigation approaches, with the ultimate aim of enhancing the crop yield and salt tolerance potential for food security. For this purpose, the use of plant growth regulators can be one of the possible solutions (Spence and Bais, 2015). The curiosity and use of triazole compounds rose among biologists due to rich multi-protectants and plant growth regulator properties (PGR), which can induce tolerance against hostile environmental conditions. Paclobutrazol (PBZ; (2 RS, 3 RS)-1-(4-chlorophenyl)-4,4 dimethyl-2-(1 H-1,2,4-triazol-1-yl)-pentane-3-ol) possess PGR properties and its application, either in soil drenching or by foliar methods, enhances photosynthetic pigments, regulates stomatal functioning and triggers accumulation of compatible solutes (Fletcher *et al.*, 2000; Plaza-Wüthrich *et al.*, 2016; Sopher *et al.*, 1999). PBZ also has a role in the alleviation of ionic toxicity, lipid peroxidation and oxidative damage by improving the antioxidative machinery under abiotic stresses (Jaleel *et al.*, 2007; KishoreKumar, 2006; Manivannan *et al.*, 2008; Sharma *et al.*, 2011; Soumya *et al.*, 2017; Tuna, 2014).

Citrus spp. is among the most salinity-sensitive of all agricultural crops (Maas, 1993). Previous old records of the threshold for EC (saturated-soil, ECs) in citrus was about 1.4 dS/m where fruit yields decreased by about 13% for each 1.0 dS/m increase in ECs above this value (Maas, 1993). Moreover, EC values over 3 dS/m and sodium adsorption ratio (SAR) over 9 in saturated soil extract are characterized as critical for the survival of citriculture cultivation (Ziogas *et al.*, 2021). Similarly, Anjum *et al.* (2008) indicated that EC values up to 2 dS/m have no negative effects on citrus seedlings. However, salt stress negatively affects relative growth rate, net assimilation rate, nutrient uptake and utilization in citrus (Anjum *et al.*, 2008; Mahmoud *et al.*, 2020). The highest growth rate, leaf area and survival rate of grafted citrus trees decreased with increasing tissue Na^+ and Cl^- concentrations (Simpson *et al.*, 2014).

It was found that Vitex trifolia Linn. var. simplicifolia Cham died when the NaCl concentration reached 450 mM (20 days after salt stress) (Yin *et al.*, 2021). In a study using 7 citrus rootstocks with osmotic potentials of -0.10 , -0.20 , and -0.35 MPa, at the osmotic potential of -0.10 MPa, sour orange, rough lemon, and Milam lemon could not exclude either Na^+ or Cl^- from their leaves (Zekri and Parsons, 1992). According to Cole (1985), four salinity-level treatments (range from 2 to 5 meq

$L^{-1} Cl^{-}$) were applied to mature Washington Navel orange trees grown on Rough Lemon. The fruit yield reduction started after 4 meq $L^{-1} Cl^{-}$ treatment. When uniform seedlings of Rough Lemon and Swingle citrumelo (SC) were grown with or without 50 mM NaCl for 42 days, salinity reduced leaf chlorophyll and plant transpiration rate (Ep) more in ROUGH LEMON than Swingle citrumelo (Gonzalez *et al.*, 2012; Ziogas *et al.*, 2021). Rough Lemon (*Citrus jambhiri* Lush.), native to northeastern India, performs well under deep sandy soil. The root system can reach 4.6 m in depth. Additionally, mature trees on ROUGH LEMON have good drought tolerance. Rough Lemon is considered to have an intermediate salt tolerance (Al-Yassin, 2004).

Sharma *et al.* (2011) investigated the effects of putrescine (Put) and paclobutrazol (PBZ) on the salt-sensitive citrus rootstock, Karna khatta, under NaCl-induced stress. The study revealed that PBZ and/or Put treatments significantly improved various physiological parameters, such as membrane integrity, water content, photosynthetic rate, and pigment content. Additionally, the treatments enhanced antioxidant enzyme activities and led to reduced sodium and chloride concentrations in leaf tissues. These findings indicate that PBZ and/or Put treatments play a crucial role in enhancing salt tolerance in Karna khatta by regulating ion absorption and accumulation, as well as improving antioxidant defenses.

The application of paclobutrazol (PBZ) has been shown to mitigate the adverse effects of salt stress in peach and mango plants, as demonstrated in studies by Abou El-Khashab *et al.* (1997) and Kishor *et al.* (2009). When PBZ is externally applied, it exerts a significant influence on various developmental and physiological processes of plants experiencing salt stress. Specifically, PBZ treatment has been observed to stimulate root and stem growth, as reported by Manivannan *et al.* (2008). Additionally, PBZ plays a crucial role in regulating water relations, which is essential for maintaining cellular turgor and water uptake under saline conditions, as highlighted in the research by Sharma *et al.* (2011).

Moreover, the application of PBZ positively affects the antioxidant defense system of plants facing salt stress, leading to increased activities of antioxidant enzymes. Studies conducted by Kishor *et al.* (2009) have highlighted the significant enhancement of antioxidant enzymes, which play a vital role in scavenging reactive oxygen species and protecting plant cells from oxidative damage. Furthermore, PBZ treatment has been found to enhance photosynthetic pigments, as demonstrated in research by Jaleel *et al.* (2007), which contributes to the maintenance of photosynthetic efficiency and carbon assimilation under saline conditions.

Overall, the application of PBZ emerges as a promising strategy to mitigate the negative impact of salt stress on various physiological and biochemical processes in plants. By promoting growth, improving water relations, bolstering antioxidant defenses, and enhancing photosynthetic efficiency, PBZ demonstrates its potential as an effective tool to enhance salt tolerance and alleviate the detrimental effects of salinity on important crop species.

The objective of the research was to develop an exhaustive search for information regarding the importance of PBZ on the growth of Rough Lemon (*Citrus jambhiri* Lush.) seedling under Salt stress conditions.

Materials and methods

This research was carried out at Cyano Research Station, Agricultural Scientific Research Center, Lattakia, General Commission for Scientific Agricultural Research, Syria (located at 35.37 N 35.994 E, with an altitude of 125 m). Rough Lemon (RL) seedling were used as plant material. Seedlings were grown and developed in a greenhouse for two successful seasons, 2020-2021 and 2021-2022. Seeds were extracted from mature fruits on February 14, 2021, then sown in a basin containing a mixture of red soil, sand, and organic matter (1:1:1). The seeds germinated on March 22, 2021. 750

seedlings with good growth characteristics were selected and transferred to 2-liter black polyethylene bags after five true leaves appeared on the plant (on July 3, 2021).

One-year-old rootstock seedlings were subjected to irrigation with NaCl treatment (0, 8, 16, 24, 32 mS/cm) two times a week. Pacloputrazol (0, 250, 500, 1000, 2000 ppm) was foliar sprayed once a week. Salinity and PBZ treatments continued for two months. The search treatments were as follows: non-treated control, NaCl (8, 16, 24, 32 mS/cm), PBZ (250, 500, 1000, 2000 ppm), NaCl (8 mS/cm) + PBZ (250, 500, 1000, 2000 ppm), NaCl (16 mS/cm) + PBZ (250, 500, 1000, 2000 ppm), NaCl (24 mS/cm) + PBZ (250, 500, 1000, 2000 ppm), NaCl (32 mS/cm) + PBZ (250, 500, 1000, 2000 ppm). The study included 25 treatments, where each treatment consisted of three replicates, and each replicate contained 10 plants.

Preparation of salt solution concentrations:

Preparation of an 8 mS/cm salt solution:

8 millisiemens per centimeter (mS/cm) = 8×640 parts per million (ppm) = 5120 parts per million = 5120 mg/L = 5.12 g/L. Therefore, 5.12 grams of pure sodium chloride were weighed on a sensitive balance and dissolved in one liter of distilled water (where: 1 mS/cm = 1 dS/m = 640 ppm). Similarly, the remaining salt solution concentrations used in the study were calculated and prepared.

Preparation of PBZ concentrations:

Concentration of Paclobutrazol in the commercial preparation = 250 g/L = 250,000 mg/L = 250,000 ppm.

Concentration of 250 ppm: Using the formula: $C_1 \times V_1 = C_2 \times V_2$, substituting: $250,000 \times V_1 = 250 \times 1000$, thus $V_1 = 1$ ml. Hence, 1 ml of the commercial preparation was taken and topped up with distilled water to reach 1 liter. Similarly, the remaining PBZ concentrations used in the study were calculated and prepared in the same context.

Studied Parameters:

Morphological Parameters:

At the end of the experiment, plant height, root length, shoots fresh and dry weight, roots fresh and dry weight, were recorded. Dry weights for shoots and roots were measured after exposing fresh tissues to temperature at 70°C for 48 h according to Balal *et al.* (2011).

Physiological Parameters:

Chlorophyll and carotenoid contents were estimated following the procedure described by Harborne (1973). The relative water content (RWC) in the leaves was estimated according to Santos *et al.* (2013) using the following equations:

$$RWC\% = [(W_f - W_d) / (W_t - W_d)] \times 100.$$

Where's: W_f : the leaves fresh weight. W_t : the leaf's turgid weight, and W_d : the leaves dry weight.

The free proline content in the ethanol-soluble fractions was determined by a colorimetric method using ninhydrin (Bates *et al.*, 1973). The total soluble sugar content in the ethanol soluble fractions was determined by a modified method of Irigoyen *et al.* (1992).

Nitrogen and phosphorous concentrations were calorimetrically estimated using the spectrophotometer (Jones *et al.*, 1991; Evenhuis and Dewaard, 1976). Potassium concentration was determined using the flame photometer (Tandon, 2005). All values of macronutrients were expressed as a percentage (%) per dry weight (DW) of leaves.

Experiment design and statistical analysis

The experiment was designed according to the split-plot design. The experiment included 25 treatments, each treatment was repeated 3 times, and each replicate contained 10 plants. Data were analyzed using a two-way analysis of variance, and the least significant difference test was used to compare the means at 95% confidence level using GenStat version 12.

Results:**morphological parameters**

The gradual increase in salt levels resulted in a gradual decrease in plant length. The treatment with PBZ reduced plant length. The interaction treatments of PBZ (2000 ppm) and salinity (in all tested levels) showed a significant decrease in plant length compared with all studied treatments. The highest value (27.7 cm), however, was observed in control plants. On the other hand, the lowest value (0.06 cm) was observed in the interaction treatment of PBZ (2000 ppm) and NaCl (32 mS/cm) (Tab. 1). Increasing salt application doses significantly decreased all studied morphological indicators.

Table (1): Effect of PBZ treatment on plant length (cm) under saline stress conditions

Salt (mS/cm)	PBZ (ppm)					
	0	250	500	1000	2000	Mean
0	27.70 ^a	26.06 ^b	25.50 ^c	21.06 ^e	21.00 ^e	24.26 ^A
8	21.78 ^d	20.06 ^f	19.57 ^g	17.44 ^h	16.03 ⁱ	18.98 ^B
16	21.04 ^e	13.94 ^k	13.27 ^l	12.62 ^m	9.29 ^o	14.03 ^C
24	15.50 ^j	10.95 ⁿ	8.70 ^p	7.85 ^q	5.84 ^s	9.77 ^D
32	12.69 ^m	7.11 ^r	5.26 ^t	4.18 ^u	0.06 ^v	5.86 ^E
Mean	19.74 ^A	15.62 ^B	14.46 ^C	12.63 ^D	10.44 ^E	
LSD _{0.05}	Interaction: 0.4290		Salt: 0.1919		PBZ: 0.1919	

The capital letters indicate significant differences between the treatment means (saline and paclobutrazol), while the lowercase letters indicate significant differences for interactions at a 95% confidence level.

Meanwhile, the treatment with paclobutrazol improved most of the studied parameters under all studied salinity levels (Except plant length). Interaction between NaCl and PBZ in relation to the root length was a statistically significant. On the other side, the highest root length (49.70 cm) was observed as in the combination treatment of NaCl (0 mS/cm) and PBZ (2000 ppm). Meanwhile, the lowest root length (6.75 cm) was observed when NaCl (32 mS/cm) was added in combination with PBZ (0 ppm) (Tab. 2).

Table (2): Effect of treatment with PBZ treatment on root length (cm) under saline stress.

Salt (mS/cm)	PBZ (ppm)					
	0	250	500	1000	2000	Mean
0	25.00 ^f	33.50 ^d	35.25 ^c	42.50 ^b	49.70 ^a	37.19 ^A
8	19.75 ^h	21.65 ^g	21.75 ^g	31.25 ^e	32.25 ^{de}	25.33 ^B
16	15.25 ^j	17.50 ⁱ	18.50 ^{hi}	21.65 ^g	22.00 ^g	18.98 ^C
24	13.50 ^{kl}	14.50 ^{ijkl}	14.50 ^{ijkl}	18.50 ^{hi}	18.75 ^{hi}	15.95 ^D
32	6.75 ⁿ	9.40 ^m	13.35 ^l	13.90 ^{ijkl}	15.00 ^{jk}	11.68 ^E
Mean	16.05 ^E	19.31 ^D	20.67 ^C	25.56 ^B	27.54 ^A	
LSD _{0.05}	Interaction: 1.633		Salt: 0.730		PBZ: 0.730	

The capital letters indicate significant differences between the treatment means (saline and paclobutrazol), while the lowercase letters indicate significant differences for interactions at a 95% confidence level.

Table (3) illustrated the effect of treatment with PBZ on shoots fresh and dry weight under salt stress conditions. The gradual increase in salt concentrations resulted in gradual decrease in shoots fresh and dry weight.

Treatment with PBZ (2000 ppm) significantly increased shoots fresh and dry weight, with the superiority to the interaction treatment of NaCl (0 mS/cm) and PBZ (2000 ppm) (72.95 and 32.05 g, for shoots fresh and dry weight, respectively). Meanwhile, the lowest values (7.80 and 2.48 g, for shoots fresh and dry weight, respectively) were observed in the interaction treatment of NaCl (32 mS/cm) and PBZ (0 ppm).

Roots' fresh and dry weight also showed similar results. The highest root fresh (60.34 g) and dry (20.7 g) weights were observed in the interaction treatment of NaCl (0 mS/cm) and PBZ (2000 ppm).

Table (3): Effect of treatment with PBZ treatment on shoots fresh and dry weight (g) under saline stress conditions

parameter	Salt (mS/cm)	PBZ (ppm)					
		0	250	500	1000	2000	Mean
fresh weight	0	25.85 ^f	53.85 ^d	65.20 ^c	67.25 ^b	72.95 ^a	57.02 ^A
	8	18.65 ⁱ	20.70 ^h	21.20 ^{gh}	33.65 ^e	52.80 ^d	29.40 ^B
	16	15.00 ^j	15.00 ^j	15.50 ^j	19.65 ^{hi}	22.75 ^g	17.58 ^C
	24	12.40 ^k	12.95 ^k	14.00 ^k	18.10 ⁱ	18.10 ⁱ	15.11 ^D
	32	7.80 ^l	8.70 ^l	12.25 ^k	12.95 ^k	14.00 ^k	11.14 ^E
	Mean	15.94 ^E	22.24 ^D	25.63 ^C	30.32 ^B	36.12 ^A	
	LSD _{0.05}	Interaction: 1.896		Salt: 0.848		PBZ: 0.848	
dry weight	0	9.86 ^f	23.30 ^c	27.45 ^b	27.45 ^b	32.05 ^a	24.02 ^A
	8	6.74 ^{ghij}	7.07 ^{gh}	7.21 ^{gh}	15.58 ^e	18.95 ^d	11.11 ^B
	16	5.19 ^{hijkl}	5.25 ^{hijkl}	5.59 ^{hijkl}	6.90 ^{ghi}	8.96 ^{fg}	6.38 ^C
	24	4.19 ^{klm}	4.52 ^{jklm}	4.62 ^{ijklm}	6.25 ^{hijk}	6.70 ^{ghij}	5.26 ^D
	32	2.48 ^m	3.41 ^{lm}	3.71 ^{lm}	4.51 ^{jklm}	4.96 ^{hijkl}	3.82 ^E
	Mean	5.69 ^D	8.71 ^C	9.72 ^C	12.14 ^B	14.32 ^A	
	LSD _{0.05}	Interaction: 2.298		Salt: 1.028		PBZ: 1.028	

Table (4): Effect of treatment with PBZ treatment on roots fresh and dry weight (g) under saline stress conditions

parameter	Salt (mS/cm)	PBZ (ppm)					
		0	250	500	1000	2000	Mean
fresh weight	0	29.50 ^f	43.87 ^c	46.23 ^b	47.54 ^b	60.34 ^a	45.50 ^A
	8	19.65 ^{ijk}	20.36 ^{hi}	21.69 ^h	32.02 ^e	35.20 ^d	25.78 ^B
	16	18.10 ^{klm}	18.60 ^{jklm}	18.65 ^{ijklm}	19.88 ^{ij}	24.04 ^g	19.85 ^C
	24	14.66 ^{no}	15.29 ⁿ	17.62 ^m	19.43 ^{ijkl}	19.64 ^{ijk}	17.33 ^D
	32	10.35 ^q	12.72 ^p	13.45 ^{op}	14.97 ^{no}	17.73 ^{lm}	13.84 ^E
	Mean	18.45 ^E	22.17 ^D	23.53 ^C	26.76 ^B	31.39 ^A	
	LSD _{0.05}	Interaction: 1.743		Salt: 0.779		PBZ: 0.779	
dry weight	0	9.82 ^d	14.00 ^c	15.05 ^{bc}	16.05 ^b	20.70 ^a	15.12 ^A
	8	5.73 ^{efghi}	6.79 ^{efg}	7.11 ^{ef}	9.92 ^d	10.86 ^d	8.08 ^B
	16	5.16 ^{ghi}	5.23 ^{ghi}	5.23 ^{ghi}	6.18 ^{efgh}	7.22 ^e	5.80 ^C
	24	4.31 ⁱ	4.70 ^{hi}	5.13 ^{ghi}	5.43 ^{fghi}	5.55 ^{efghi}	5.02 ^D
	32	2.53 ^j	4.12 ^{ij}	4.16 ^{ij}	4.42 ⁱ	5.14 ^{ghi}	4.07 ^E
	Mean	5.51 ^D	6.97 ^C	7.33 ^C	8.40 ^B	9.89 ^A	
	LSD _{0.05}	Interaction: 1.714		Salt: 0.767		PBZ: 0.767	

The capital letters indicate significant differences between the treatment means (saline and paclobutrazol), while the lowercase letters indicate significant differences for interactions at a 95% confidence level.

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The interaction treatment of NaCl (32 mS/cm) and PBZ (0 ppm) showed the least values for roots fresh (10.35 g) and dry (2.53 g) weight (Tab. 4).

Physiological Parameters:

Results in Figs 1, 2, 3, and 4 demonstrate the effect of PBZ treatment on photosynthetic pigments under salt stress conditions. The gradual increase in salt concentrations resulted in a significant decrease in the leaf content of photosynthetic pigments. Treatment with PBZ resulted in a significant increase in the leaf content of photosynthetic pigments. The lowest content of photosynthetic pigment

(0.18 mg/g, 0.23 mg/g, 0.06 mg/g, and 0.4 mg/g for chlorophyll a, b, carotenoids, and total chlorophyll) was observed when NaCl (32 mS/cm) was applied in combination with PBZ (0 ppm). On the other hand, the highest content (1.74 mg/g, 1.23 mg/g, 0.61 mg/g and 2.96 mg/g for chlorophyll a, b, carotenoids and total chlorophyll) was observed in the interaction treatment of NaCl (0 mS/cm) and PBZ (2000 ppm).

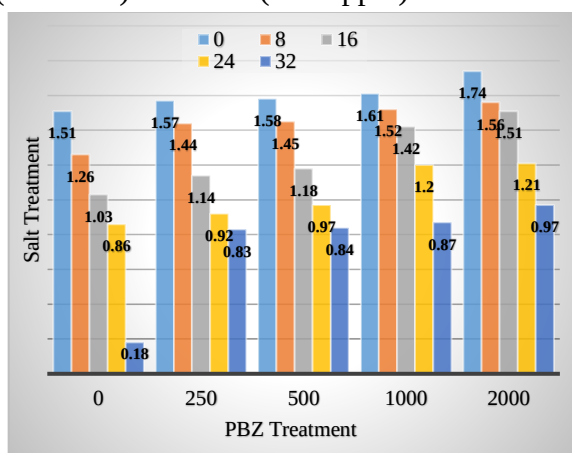


Figure (1): Effect of treatment with PBZ treatment on chlorophyll a (mg/g) under saline stress conditions

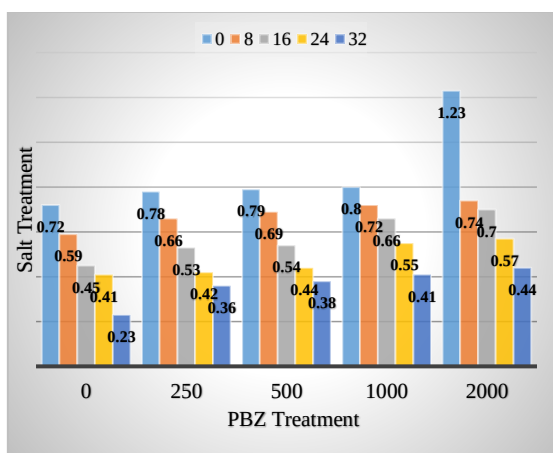


Figure (2): Effect of PBZ treatment on chlorophyll b (mg/g) under saline stress conditions

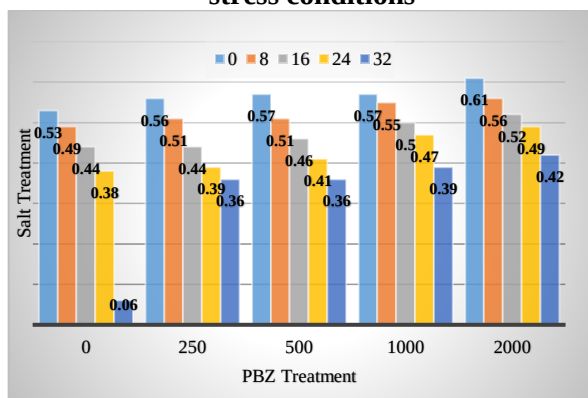


Figure (3): Effect of PBZ treatment on carotene (mg/g) under saline stress conditions

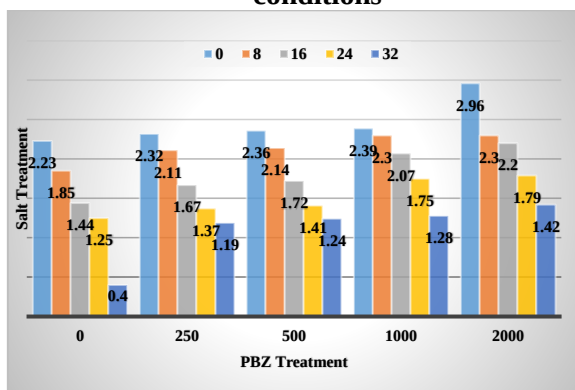


Figure (4): Effect of PBZ treatment on total chlorophyll (mg/g) under saline stress conditions

Data tabulated in Table (5) exhibit the effect of NaCl, PBZ, and their combinations on relative water content (RWC%). The gradual increase in salt levels resulted in a significant decrease in RWC%. Moreover, the treatment with PBZ significantly increased RWC, and the highest value (75.68 %), however, was recorded when PBZ was sprayed at 2000 ppm with 0 mS/cm of NaCl. On the other side, the lowest value (32.14%) was observed in the combination treatment of NaCl (32 mS/cm) and PBZ (0 ppm).

Table (5): Effect of treatment with PBZ treatment on relative water content (%) under saline stress conditions

Salt (mS/cm)	PBZ (ppm)					
	0	250	500	1000	2000	Mean
0	68.04 ^{cd}	71.43 ^b	72.33 ^b	75.00 ^a	75.68 ^a	72.50 ^A
8	62.86 ^{gh}	64.71 ^{ef}	65.63 ^e	68.18 ^{cd}	69.05 ^c	66.09 ^B
16	51.85 ^j	52.08 ^j	60.60 ⁱ	63.64 ^{fg}	67.60 ^d	59.15 ^C
24	45.45 ⁿ	48.28 ^{lm}	49.67 ^{kl}	61.11 ⁱ	61.76 ^{hi}	53.25 ^D
32	32.14 ^q	40.00 ^p	42.55 ^o	46.88 ^m	50.00 ^k	42.31 ^E
Mean	52.07 ^E	55.30 ^D	58.16 ^C	62.96 ^B	64.82 ^A	
LSD _{0.05}	Interaction: 1.422		Salt: 0.636		PBZ: 0.636	

The capital letters indicate significant differences between the treatment means (saline and paclobutrazol), while the lowercase letters indicate significant differences for interactions at a 95% confidence level.

Concerning proline accumulation, the maximum recorded value (0.96 mg/g FW) was observed when NaCl was applied at 32 mS/cm and PBZ was sprayed at 2000 ppm. Meanwhile, the lowest value (0.12 mg/g FW) was recorded in non-treated control (Table, 6). Total soluble sugars also showed similar results. The combination treatment of NaCl (32 mS/cm) and PBZ (2000 ppm) significantly increased total soluble sugars (0.88 mg/g) comparing with all other treatment and with control (fig. 5).

The increase in salt concentration resulted in a reduction in the nitrogen, phosphorus, and potassium levels in the leaves. In contrast, treatment with PBZ increased the concentration of NPK comparing with untreated control plants, with the superiority to the combination treatment of NaCl (0 mS/cm) and PBZ (2000 ppm) which resulted in the highest concentrations (5.87%, 0.37% and 0.93% for NPK, respectively). The lowest concentrations (0.73%, 0.12%, and 0.33% for NPK, respectively), however, were recorded in the interaction treatment of NaCl (32 mS/cm) and PBZ (0 ppm) (Table, 7).

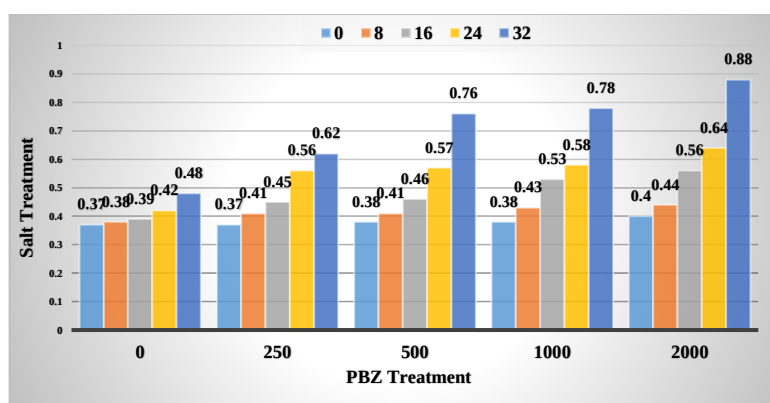


Figure (5): Effect of treatment with PBZ treatment on total soluble sugars (mg/g) under saline stress conditions

Table (6): Effect of treatment with PBZ treatment on proline (mg/g FW) under saline stress conditions

Salt (mS/cm)	PBZ (ppm)					Mean
	0	250	500	1000	2000	
0	0.12 ^q	0.54 ^p	0.62 ^o	0.82 ^{mn}	0.87 ^l	0.60 ^E
8	0.81 ⁿ	0.88 ^l	0.89 ^k	0.90 ^{ij}	0.91 ^{hij}	0.88 ^D
16	0.83 ^m	0.91 ^{ghij}	0.91 ^{ghij}	0.91 ^{fghi}	0.92 ^{efgh}	0.89 ^C
24	0.90 ^{jk}	0.91 ^{fghi}	0.92 ^{efg}	0.92 ^{def}	0.93 ^{cd}	0.92 ^B
32	0.91 ^{fghi}	0.92 ^{cde}	0.93 ^c	0.94 ^b	0.96 ^a	0.93 ^A
Mean	0.71 ^E	0.83 ^D	0.86 ^C	0.90 ^B	0.92 ^A	
LSD _{0.05}	Interaction: 0.011		Salt: 0.005		PBZ: 0.005	

The capital letters indicate significant differences between the treatment means (saline and paclobutrazol), while the lowercase letters

Discussion

Exposure of plants to salt stress reduces stomatal density, induces stomatal closure, and leads to impaired gas exchange and reduced photosynthesis (Orsini *et al.*, 2011; Shabala *et al.*, 2013; Shabala *et al.*, 2012; Taylor *et al.*, 2012). Salt treatment results in decreased plant length due to increased osmotic stress and reduced water availability, leading to decreased cell elongation (David and Nilsen, 2000). Additionally, PBZ, a plant growth regulator, reduced plant length compared to the control. This is because gibberellic acid promotes root and stem elongation, and its synthesis is influenced by inhibitors like paclobutrazol, resulting in decreased plant length (Bose *et al.*, 2013; Cavatte *et al.*, 2012; Chang *et al.*, 2019; Fletcher and Hofstra, 1990).

The results revealed a decrease in shoot fresh weight with increasing salt concentrations, attributed to reduced growth rates (Nafees *et al.*, 2023). Salt stress reduces water content, leading to decreased

nucleic acids and chlorophyll, increased abscisic acid, and inhibited gibberellin activity (Sherif and Asaad, 2014). Decreased water potential weakens root water uptake (Taiz and Zeiger, 2006).

The salt treatment resulted in reduced fresh weight and length of the root, attributed to saline conditions affecting roots, altering their growth, water and ion uptake, and the production of plant hormones like auxins. Salt stress negatively impacts root growth due to reduced cell expansion, water potential, water loss, and toxic effects from sodium and chloride ion accumulation, leading to root cell death (Hoopkins and Muner, 2008).

Salt treatment led to a decrease in both shoot and root dry weight. This decrease may be attributed to reduced photosynthetic efficiency due to salt accumulation in the soil, leading to reduced water availability and an imbalance in ion and nutrient uptake. Sodium ion accumulation in the leaves can inhibit photosynthesis, resulting in decreased water potential and reduced cell water content (David and Nilsen, 2000). The reduction in the dry weight of the root mass may be due to the adverse effects of increased salt levels, leading to reduced water and ion uptake by the roots due to osmotic and ionic effects. Consequently, this negatively affects photosynthesis and reduces the translocation of metabolic products to the roots (Farooq *et al.*, 2022). On the other hand, the treatment with paclobutrazol increased shoot and root fresh and dry weight, as well as root length. Paclobutrazol enhances stomatal density (Waqas *et al.*, 2017) due to increased abscisic acid concentration (Fletcher *et al.*, 2000), leading to improved gas exchange and photosynthetic capacity. Elevated cytokinin levels, primarily synthesized in the root apices, result in increased root growth (Ko *et al.*, 2014; Sopher, 1999). Moreover, paclobutrazol reduces lipid peroxidation under salt stress, enhancing plant tolerance (Waqas *et al.*, 2017) - a finding consistent with Pan *et al.* (2013) on rice, Tuna (2014) on potatoes, and Jungklang *et al.* (2015) on tulips under various non-biological stresses.

Moreover, the rise in salt concentrations resulted in a decline in the relative water content. This decrease in water content can be attributed to the reduced water potential between the soil solution and the root cell mass, leading to a decreased water flow and uptake by the roots (Ma *et al.*, 2021). These findings align with previous research by Sousa *et al.* (2018) on citrus, Gucci *et al.* (1997) on olive. Conversely, the application of paclobutrazol (PBZ) resulted in an increase in the relative water content. This observation is consistent with the findings of Jungklang and Saengnil (2012), who reported that PBZ helps maintain leaf water content, possibly due to its role in increasing leaf thickness and, consequently, improving water retention (Jungklang *et al.*, 2017).

The decline in leaf pigment content resulting from salt treatment can be attributed to either pigment degradation or reduced biosynthesis (Lee *et al.*, 2013). Conversely, the partial improvement in leaf pigment content observed in plants treated with paclobutrazol under salt stress conditions may be linked to increased cytokinin levels (Ko *et al.*, 2014; Sopher, 1999). Cytokinins play a significant role in regulating chlorophyll and carotenoid biosynthesis, thereby preserving chlorophyll and protecting the plant from harmful light during development under stressful conditions (Dobránszki and Mender-Drienyovszki, 2014; Li *et al.*, 2008; Talla *et al.*, 2016). These findings are consistent with the research of Sharma *et al.* (2011) and Murkute *et al.* (2005) on citrus, Kishorekumar *et al.* (2007) on potatoes. Proline and total soluble sugars act as vital osmoprotectants in response to salt stress (Ruffino *et al.*, 2009; Ruiz-Carrasco *et al.*, 2011). They play key roles in improving metabolic status, cellular turgor, and sugar transport across cell membranes. The increase in total soluble sugars at higher salt concentrations may be attributed to the inhibition of carbohydrate-hydrolyzing enzymes, leading to their accumulation and raising cellular osmotic pressure, which counteracts the external osmotic pressure induced by salt stress (Hasegawa *et al.*, 2000).

Under salt stress conditions, the rise in proline concentration may result from impaired protein synthesis or increased degradation, leading to its accumulation in plant tissues. Additionally, the

effect of salinity in enhancing the accumulation of free amino acids, with proline being one of the most accumulated amino acids under such conditions, contributes to its higher content (Wynjones *et al.*, 1978). This accumulation of proline can be attributed, in part, to the presence of abscisic acid, which acts as a chemical signal from the roots and is transported to the vegetative mass, leading to proline accumulation in leaves (Alcazar *et al.*, 2011). Proline serves as a protective compound against macromolecules, safeguards enzymes from stress-induced damage, and partially promotes root growth (Tuasamu, 2009). Moreover, combined treatment with salt and paclobutrazol was found to increase the content of proline and total soluble sugars (Waqas *et al.*, 2017). The treatment with paclobutrazol also increased proline concentration, possibly due to its interference with enzymes involved in the cytokinin pathway, resulting in gibberellin synthesis inhibition, increased ABA and cytokinin levels, and decreased ethylene production (Farooq *et al.*, 2022). These findings align with prior research by Waqas *et al.* (2017) and Sharma *et al.* (2011) on citrus, as well as Kishorekumar *et al.* (2007) on potatoes.

Furthermore, the different salt concentrations led to a decrease in nitrogen, phosphorus, and potassium concentrations in the leaves. This decrease may be attributed to competitive effects with sodium at uptake sites in the roots or selective loss in nutrient uptake. Additionally, the decrease could

Table (7): Effect of treatment with PBZ treatment on macronutrients (%) under saline stress conditions

parameter	Salt (mS/cm)	PBZ (ppm)					
		0	250	500	1000	2000	Mean
N	0	4.19 ^{cd}	4.71 ^b	4.73 ^b	4.96 ^b	5.87 ^a	4.89 ^A
	8	3.82 ^{defg}	3.87 ^{def}	4.04 ^{de}	4.65 ^{bc}	4.67 ^{bc}	4.21 ^B
	16	3.33 ^{ghij}	3.41 ^{fghi}	3.57 ^{efgh}	3.86 ^{def}	4.19 ^{cd}	3.67 ^C
	24	2.12 ^l	2.91 ^k	3.08 ^{ijk}	3.70 ^{efg}	3.77 ^{defg}	3.12 ^D
	32	0.73 ^m	1.72 ^l	2.04 ^l	2.85 ^k	3.21 ^{hijk}	2.11 ^E
	Mean	2.84 ^D	3.33 ^C	3.50 ^C	4.00 ^B	4.34 ^A	
	LSD_{0.05}	Interaction: 0.485		Salt: 0.217		PBZ: 0.217	
P	0	0.28 ^{de}	0.32 ^{bcd}	0.33 ^{bc}	0.34 ^{ab}	0.37 ^a	0.33 ^A
	8	0.24 ^{fgh}	0.25 ^{ef}	0.28 ^{de}	0.29 ^{cde}	0.30 ^{bcd}	0.27 ^B
	16	0.21 ^{ghi}	0.21 ^{ghi}	0.21 ^{fghi}	0.25 ^{efg}	0.28 ^{de}	0.23 ^C
	24	0.19 ⁱ	0.19 ^{hi}	0.20 ^{hi}	0.22 ^{fghi}	0.22 ^{fghi}	0.21 ^D
	32	0.12 ^j	0.19 ⁱ	0.19 ⁱ	0.19 ^{hi}	0.20 ^{hi}	0.18 ^E
	Mean	0.21 ^D	0.23 ^C	0.24 ^{BC}	0.26 ^{AB}	0.28 ^A	
	LSD_{0.05}	Interaction: 0.041		Salt: 0.018		PBZ: 0.018	
K	0	0.80 ^f	0.83 ^d	0.91 ^c	0.91 ^b	0.93 ^a	0.88 ^A
	8	0.75 ^j	0.77 ⁱ	0.78 ^h	0.81 ^e	0.83 ^d	0.79 ^B
	16	0.64 ⁿ	0.66 ^m	0.66 ^m	0.75 ^j	0.79 ^g	0.70 ^C
	24	0.41 ^s	0.45 ^q	0.47 ^p	0.67 ^l	0.68 ^k	0.54 ^D
	32	0.33 ^v	0.40 ^u	0.40 ^t	0.42 ^r	0.49 ^o	0.41 ^E
	Mean	0.59 ^E	0.62 ^D	0.65 ^C	0.71 ^B	0.74 ^A	
	LSD_{0.05}	Interaction: 0.004		Salt: 0.002		PBZ: 0.002	

The capital letters indicate significant differences between the treatment means (saline and paclobutrazol), while the lowercase letters indicate significant differences for interactions at a 95% confidence level.

result from the osmotic effects of salts in the soil, hindering nutrient movement, or due to reduced growth of root cells responsible for nutrient uptake (Grattan and Grieve, 1992), consistent with Julain (2004) and Ruiz *et al.* (1997). In contrast, the treatment with paclobutrazol led to an increase in nitrogen, phosphorus, and potassium concentrations in plant leaves. This could be due to a close

relationship between ion uptake and water uptake by the roots (Bowling, 1976). Furthermore, paclobutrazol might have influenced absorption mechanisms within the roots, consistent with Atkinson *et al.* (1987) and Atkinson *et al.* (1983), but conflicting with Martin *et al.* (1987). Although the current study and previous research contain some conflicting information, it is evident that paclobutrazol can affect nutrient uptake and/or accumulation in leaves. The decrease in root hydraulic conductivity and changes in nutrient uptake level may be side effects of paclobutrazol treatment, and both correspond to the degree of growth inhibition and changes in root growth and form (Rieger and Scalabrelli, 1990).

Conclusion

The present study revealed that elevated salt levels led to a decrease in all studied parameters. The application of PBZ improved seedlings' growth under all salt levels. The interaction treatment of PBZ (2000 ppm) and salinity resulted in the best morphological and physiological parameters. Based on our analysis, we concluded that foliar application of PBZ at a concentration of 2000 ppm alleviates the detrimental effects of salinity on Rough lemon seedlings.

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تأثير الرش الورقي بالباكلوبوترازول في نمو بادرات أصل الليمون المخرفش (*Citrus jambhiri lush.*) تحت ظروف الإجهاد الملحي

أنس كيوان * ⁽¹⁾ و رولا بايرلي ⁽²⁾ و علي الخطيب ⁽³⁾

(1). مركز بحوث السويداء، الهيئة العامة للبحوث العلمية الزراعية، سورية.

(2). قسم علوم البستنة، كلية الهندسة الزراعية، جامعة دمشق، سورية.

(3). مركز بحوث اللاذقية، الهيئة العامة للبحوث العلمية الزراعية، سورية.

(*) للمراسلة: م. أنس كيوان، البريد الإلكتروني: anas.kiwan@damascusuniversity.edu.sy

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الملخص

نُفذ البحث في محطة بحوث سيانو التابعة لمركز البحوث العلمية الزراعية في اللاذقية، سورية خلال عامي 2020-2021 و 2021-2022، لدراسة تأثير الرش الورقي بالباكلوبوترازول (PBZ) في نمو بادرات أصل المخرفش (*Citrus jambhiri Lush.*) تحت ظروف الإجهاد الملحي. صممت التجربة وفقاً لتصميم القطع المنشقة. حيث تم تطبيق خمس مستويات من كلوريد الصوديوم (0، 8، 16، 24، 32 مللي سيمنز/سم)، وخمس تراكيز من PBZ (0، 250، 500، 1000، 2000 جزء في المليون) أظهرت النتائج أن الزيادة التدريجية في مستويات الملح أدت إلى انخفاض في جميع المعايير المدروسة. ومن ناحية أخرى، أدت المعاملة بالباكلوبوترازول (PBZ) إلى تحسين معظم المعايير المدروسة تحت جميع مستويات الملوحة المدروسة. بينت معاملات التفاعل بين PBZ (2000 جزء في المليون) والملوحة (جميع المستويات المدروسة) تفوقاً في طول الجذر (27.54 سم)، الوزن الرطب والجاف للمجموع الخضري (36.12، 14.32 غ، على التوالي)، الوزن الرطب والجاف للمجموع الجذري (31.39، 9.89 غ، على التوالي)، محتوى الماء النسبي (64.82%)، محتوى الأوراق من كلوروفيل أ (1.40 ملغ/غ وزن طازج)، وكلوروفيل ب (0.74 ملغ/غ وزن طازج)، والكاروتين (0.52 ملغ/غ وزن طازج)، تركيز البرولين (0.92 ملغ/غ)، والسكريات الذائبة الكلية (0.58 ملغ/غ)، وتراكيز النيتروجين والفوسفور والبوتاسيوم في الأوراق (4.34، 0.28، 0.74%، على التوالي) مقارنة بمعاملات التفاعل بين PBZ (0 جزء في المليون) والملوحة (16.05 سم طول الجذر، 15.94 غ و 5.69 غ الوزن الرطب والجاف للمجموع الخضري، 18.45 غ و 5.51 غ الوزن الرطب والجاف للمجموع الجذري، 52.07% محتوى الماء النسبي، 0.97 و 0.48 و 0.38 ملغ/غ لكل من الكلوروفيل أ، ب، والكاروتين، 0.71 ملغ/غ برولين، 0.41 ملغ/غ السكريات الذائبة الكلية، و 2.84% و 0.21% و 0.59% تراكيز NPK، على التوالي).

الكلمات المفتاحية: أصل، بادرات، الليمون المخرفش، ملوحة، باكلوبوترازول.