



EFFECT OF NANO-SELENIUM AND IRRIGATION WATER QUALITY ON THE QUALITATIVE CHARACTERISTICS OF CITRUS ROOTSTOCKS

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SUMMARY

The presented study aimed to determine the effects of nano-selenium foliar application and types of irrigation water on growth traits of three citrus (*C. aurantium*, *C. volkameriana*, and *C. aurantifolia*) rootstocks. The research started in 2023 at the canopy of the Kerbala-certified citrus nursery, Holy Kerbala Governorate, Iraq. The experiment, laid out in a randomized complete block design, had a split-plot arrangement of three factors and three replications. The first factor was two types of water, i.e., river water and well water, placed in main plots. The second factor (subplot) was the seedlings grown from seeds of three types of Citrus rootstocks, i.e., sour orange, volkameriana, and lime. The third factor (sub-subplot) was the foliar application of nano-selenium with three concentrations (0, 1, and 2 mg L⁻¹). The results showed the river water was superior in the percentage of nitrogen (1.786%) and phosphorus (0.193%), while the well water was better in the content of CAT and POD enzymes (10.76 and 176.7 abs unit min g⁻¹, respectively). Notably also, the cultivar Volkameriana rootstock was superior in the percentage of nitrogen (2.083%) and phosphorus (0.213%). Moreover, the nano-selenium (2 mg L⁻¹) foliar application has a positive effect and exceeded all the doses for various traits.

Keywords: Citrus rootstock (*C. aurantium*, *volkameriana*, and *aurantifolia*), nano-selenium, water quality, growth and biochemical traits

Key findings: Citrus cultivar Volkameriana rootstock proved better with nano-selenium concentration (2 mg L⁻¹) than the other rootstocks and nano-selenium concentrations, providing superior results for various traits.

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INTRODUCTION

Citrus fruits belong to the family Rutaceae and have many genera. From an economic viewpoint, the most important one is the genus *Citrus*, comprising many species (Douai and Fadliya, 2010). It also believed its original habitat was the tropical and subtropical regions between latitudes 40 North and South of the equator, especially the areas of Southeast and Southwest Asia. These countries include Pakistan, India, China, and the Indochina Islands (Cambodia, Vietnam, and Laos), as well as, some areas of Southwest Asia (Hayat *et al.*, 2022).

The cultivated area of citrus amounted to 10,072,197 hectares, with a total production of 158,491,166 tons worldwide. China ranked first in the world for citrus production, followed by Brazil, India, Mexico, the USA, and Spain. As for Arab countries, Egypt came in first place, and seventh globally in citrus production (FAOSTAT, 2021). In Iraq, citrus trees reached 10,355,596, with a production of 226,166,000 tons, and an average production of 21.84 kg/tree. The Salah al-Din Governorate in Iraq ranked first in production, followed by Baghdad and Diyala (CSO, 2023).

The economic, nutritional, medical, and aesthetic importance of citrus trees lies in the fact that the citrus fruit is a prime source of pectin used in food industries. Moreover, its fruit peels, flowers, and leaves serve as sources for extracting essential oils, useful in the food, aromatic material, and soap industries (Ben-Hsouna *et al.*, 2023). Citrus fruits are also distinctly one of the most essential sources of vitamin C and significant amounts of vitamin A, B1, and B2. Likewise, citrus fruits contain Ca, P, Fe, K, Na, and Zn, in addition to natural sugars (80%–90%) of the total soluble solids (TSS) (Morales-Alfaro *et al.*, 2023).

Sour orange is one of the most crucial rootstocks effective to propagate various citrus fruits due to its ease of propagation by seeds, as well as its complete compatibility with most grafts grown on it. It also tolerates increased

ground humidity and salinity up to some extent, with the trees grafted on it having medium to strong growth. Aside from being a good rootstock suitable for medium and heavy soils, other rootstocks exist that are helpful in propagating citrus fruits. The cultivar Volkameriana resulted from the cross-breeding of citron with lime, also considerably as suitable rootstock for grafting some citrus trees on it (Jabarzadeh *et al.*, 2020), with characteristics of resistance to rapid deterioration and gum disease. Grafted fruit cultivars have high-quality features and higher compatibility with most citrus fruits in Iraq (Musa *et al.*, 2008).

Nano-fertilization technology is one of the modern methods used to increase the quantity and quality of the yield, as it deals with tiny particles with dimensions of 1–100 nanometers (El-Saadony *et al.*, 2021). Selenium is also a nano-element, which mainly participates in increasing the enzymatic antioxidants' activity. Likewise, it acts as a catalyst for these antioxidants, especially the enzyme glutathione peroxidase, converting the toxic compound hydrogen peroxide, H_2O_2 , resulting from the effect of salt stress, into water molecules, H_2O (Hassanuzzaman *et al.*, 2010). Characteristically, selenium has associations with amino acids, especially methionine and cysteine acids, forming selenium proteins. These have a superior ability of cell membranes to withstand salt stress conditions and prevent the catabolism of plant metabolic compounds (Sheikhalipour *et al.*, 2021).

Salt stress is one of the critical abiotic environmental factors greatly affecting the growth and productivity of plants, as an increased soil salinity has become one of the chief hindrances, causing reductions in areas allocated for agriculture worldwide. Specifically, the arid and semi-arid areas with little rainfall and relatively high temperatures lead to an increased rate of evaporation and a high rate of transpiration of agricultural production (Zhao *et al.*, 2021). The presented study aimed to identify the best citrus

rootstock along with the nano-selenium concentration and their effects on growth traits under the influence of salt-stress conditions.

MATERIALS AND METHODS

Plant material and experimental design

The reported study sought to determine the effects of nano-selenium foliar application and types of irrigation water on growth traits of three citrus rootstocks (*C. aurantium*, *C. volkameriana*, and *C. aurantifolia*), held in 2023 at the canopy of a Kerbala-certified citrus nursery, Holy Kerbala Governorate, Iraq. The seeds' planting of three citrus cultivar rootstocks, sour orange, Volkameriana, and lime, transpired under the plastic tunnels covered with transparent polyethylene in the wire plant canopy topped with green saran at a shading rate of 50% throughout the experiment duration. Various service operations, comprising combating and removing bushes, proceeded the same for all treatments according to the programs followed by the station. The seeds of three citrus rootstocks, planted in plastic pots with a capacity of 10 kg, bore filling with soil consisting of silty and peat moss at a ratio of 5:1. Samples of the soil used and two types of irrigation water reached analysis for physical and chemical properties in the laboratory at the University of Kerbala, Kerbala, Iraq (Table 1) (Page *et al.*, 1982).

The experiment layout in a randomized complete block design had split-plot arrangements with three factors and three replications. The first factor was two types of

water, i.e., river water and well water, placed in main plots. The second factor (subplot) was the seedlings grown from seeds of three types of citrus rootstocks, i.e., sour orange, Volkameriana, and lime. The third factor (sub-subplot) was the foliar application of nano-selenium with three concentrations (0, 1, and 2 mg L⁻¹).

The number of treatments reached 18, with five pots per treatment per replication and three seeds per rootstock planted in each pot. The treatments, divided into two groups, had each group containing nine treatments distributed randomly. The seedlings of the first group received river-water irrigation, while the second group acquired well-water irrigation throughout the research period. When the grown seedlings reached five true leaves, they received regular irrigation continually. In addition, abundant irrigation continued for seedlings on the day before the foliar application of nano-selenium to increase the humidity. This works to swell the guard cells and open the stomata, making it easier to absorb the nano-selenium. Foliar spraying of the citrus seedlings with nano-selenium commenced in the early morning, at required concentrations used for both groups, using a 1.5-liter hand sprayer after adding a drop of a diffuser used to break the surface tension of water. The spraying process ensued four times, with a two-week interval between each process, starting from July 1st to August 15th. After the last spraying, the seedlings remained as is for a month to ensure the stability of the element concentrations in them. On November 1, samples collected from the seedlings of both groups underwent analysis and documentation of the results.

Table 1. Chemical and physical characteristics of the soil and water used in the experiment.

Soil characteristics			Water characteristics		
Properties	Value	Unit	Dissolved ions	River water	Well water
Sand	745	g kg ⁻¹	E.C	1.7	5.3
Silty	62	g kg ⁻¹	pH	7.91	7.85
Clay	193	g kg ⁻¹	Na ⁺	7.72	31.67
E.C	1.25	dS m ⁻¹	K ⁺	0.20	0.11
pH	8.10	-----	Ca ⁺⁺	0.45	3.89
N.NH ₃	45.3	mg kg ⁻¹	Mg ⁺⁺	0.18	5.48
P	0.003	%	Cl ⁻	3.87	5.89
K	11.16	mg kg ⁻¹	HCO ₃ ⁻	1.53	2.25
SO ₄	17.91	ppm	HCO ₃ ⁻	3.10	6.74

Data recorded

Nitrogen (%) in the leaves gained measuring by using the Micro Kjeldahl device (Jackson, 1958). The phosphorus (%) in the leaves also obtained measurement using the Spectrophotometer EMC-11-Uv-visible device at a wavelength of 620 nm ammonium (Page *et al.*, 1982). The chlorophyll content in leaves (mg 100 g⁻¹ fresh weight) incurred estimation according to the method of Ranganna *et al.* (1983). The activity of CAT enzyme in leaves (abs unit min g⁻¹) reached estimation using a spectrophotometer EMC-11-Uv-visible at a wavelength of 240 nm (Kadhum and Hadwan, 2021). Measuring the effectiveness of POD enzyme in leaves (abs unit min g⁻¹) followed the method according to Nezh (1985).

Statistical analysis

All the recorded data underwent the analysis of variance using the Genstat 12.1 program. The means' further comparison and separation used the least significant difference (LSD_{0.05}) test (Al-Rawi and Khalaf-Allah, 2000).

RESULTS AND DISCUSSION

Nitrogen (%) in leaves

Irrigation water types significantly affected the citrus seedlings, with the river water causing an increase in the level of nitrogen percentage (1.786%) in the leaves compared with irrigating with well water (1.411%) (Table 2). The elevated nitrogen content in citrus leaves from the fresh river water's influence could refer to its enhancing plant metabolic processes, boosting the plant's ability to absorb and use the nitrogen available in the soil and water (García-Márquez *et al.*, 2020).

Meanwhile, the rootstocks also had a notable influence on nitrogen percentage, with the citrus cultivar Volkameriana rootstock recording the highest percentage of nitrogen element (2.083%), compared with the lowest percentage of this element in the cultivar lime rootstock (1.290%) (Table 2). The superiority of citrus cultivar Volkameriana in leaf nitrogen content can be due to several factors, including the unique genetic make-up of the genotype and its effect on the structure of the leaves

Table 2. Effect of the irrigation water quality, citrus cultivars, nano-selenium, and their interactions on the nitrogen (%) in citrus rootstocks.

Water types	Citrus types	Nano-Selenium (ml L ⁻¹)			Water types x Citrus types
		0	1	2	
River water	Sour	1.548	1.635	1.542	1.575
	Volkameriana	2.240	2.226	2.312	2.260
	Lime	1.451	1.545	1.576	1.524
Well water	Sour	1.230	1.320	1.260	1.270
	Volkameriana	1.906	1.869	1.946	1.907
	Lime	1.025	1.060	1.084	1.056
Triple interaction		LSD _{0.05}			LSD _{0.05}
		0.231			0.248
Means (Nano-Selenium)		1.567	1.609	1.620	LSD _{0.05}
					N.S
					Means (Water types)
Water types x Nano-Selenium	River water	1.747	1.802	1.810	1.786
	Well water	1.387	1.416	1.430	1.411
LSD _{0.05}		0.275			0.301
					Means (Citrus types)
Citrus types x Nano-Selenium	Sour	1.389	1.477	1.401	1.422
	Volkameriana	2.073	2.048	2.129	2.083
	Lime	1.238	1.303	1.330	1.290
LSD _{0.05}		0.078			0.067

and the absorption of nutrients. Environmental conditions and the cultivation methods may also have a role in this case, as the surrounding conditions can affect the leaves' composition and their nutritional content and, thus, can also affect the nitrogen content in citrus leaves (Jahromi *et al.*, 2012).

The results further revealed foliar application of nano-selenium on the leaves did not significantly affect the nitrogen content in leaves of citrus rootstocks. On the triple interaction between these factors, the coefficients differed among them and reached significance. The interaction of river water, cultivar Volkameriana, and nano-selenium (2 mg L⁻¹) outperformed (2.312%) the rest of the combinations, with the lowest percentage of nitrogen in leaves (1.025%) obtained in the interaction of the factors, i.e., well water, cultivar lime, and nano-selenium (0 mg L⁻¹).

Phosphorus (%) in leaves

In citrus rootstock leaves, irrigation water types substantially modified the phosphorus content, and the river water gave a higher percentage (0.193%) than the irrigation with well water, which showed the lowest

phosphorus percentage (0.154%) (Table 3). Fresh water contains unique amounts of phosphorus dissolved in it, and by irrigating citrus with fresh water, phosphorus absorption is easier from the soil through the plant's roots and transferred to leaves. Therefore, using fresh water in irrigation increases the availability of phosphorus to plants, which contributes to raising the phosphorus content in citrus leaves and improving citrus' growth and quality (Graham *et al.*, 1987).

Moreover, the cultivar rootstocks remarkably affected this phosphorus percentage (Table 3). The cultivar Volkameriana rootstock revealed the highest percentage (0.213%) in the leaves compared with two other cultivar rootstocks. The lowest phosphorus (%) resulted in the cultivar lime rootstock (0.140%). The superiority of citrus cultivar Volkameriana in leaf phosphorus content may be due to multiple factors, including its ability to absorb and store more phosphorus from the soil, leading to a higher phosphorus content in its leaves. Additionally, the surrounding conditions, such as soil and climate, could be more suitable for this type of citrus (Obreza *et al.*, 2008).

Table 3. Effect of the irrigation water quality, citrus cultivars, nano-selenium, and their interactions on the phosphorus (%) in citrus rootstocks.

Water types	Citrus types	Nano-Selenium (ml L ⁻¹)			Water types x Citrus types
		0	1	2	
River water	Sour	0.147	0.238	0.188	0.191
	Volkameriana	0.206	0.227	0.252	0.229
	Lime	0.132	0.164	0.185	0.160
Well water	Sour	0.114	0.155	0.159	0.142
	Volkameriana	0.177	0.197	0.221	0.198
	Lime	0.096	0.123	0.143	0.121
Triple interaction		LSD _{0.05}			LSD _{0.05}
		0.010			0.005
Means (Nano-Selenium)		0.145	0.184	0.191	LSD _{0.05}
					0.004
Means (Water types)					
Water types x Nano-Selenium	River water	0.162	0.210	0.208	0.193
	Well water	0.129	0.159	0.174	0.154
LSD _{0.05}		0.006			0.005
Means (Citrus types)					
Citrus types x Nano-Selenium	Sour	0.130	0.196	0.174	0.167
	Volkameriana	0.191	0.212	0.237	0.213
	Lime	0.114	0.144	0.164	0.140
LSD _{0.05}		0.007			0.003

The significant effect of the nano-selenium spraying was evident on citrus cultivar rootstock seedlings (Table 3). The nano-selenium concentration (2 mg L^{-1}) proved superior in getting more phosphorus in citrus leaves (0.191%) than the control treatment (0.145%). The use of selenium spraying in citrus cultivation has a considerable role in increasing the leaves' phosphorus content. Phosphorus stimulates the activity of particular enzymes within plants that help absorb phosphorus from the soil and transfer it to plant leaves. Thus, spraying with nano-selenium can enhance the phosphorus availability in citrus trees (Samynathan *et al.*, 2023). For the triple interaction among the factors, it was visible that the river water, Volkameriana variety, and nano-selenium (2 mg L^{-1}) had the supreme average (0.252%) compared with the lowest content (0.096%) obtained from the interaction of well water, lime variety, and nano-selenium (0 mg L^{-1}).

Chlorophyll content

The irrigation water types showed significant differences for chlorophyll content in citrus

seedling leaves (Table 4). However, the river irrigation water displayed superiority in giving the maximum chlorophyll content ($55.41 \text{ mg } 100 \text{ g}^{-1}$ fresh weight) compared with irrigating with well water ($48.37 \text{ mg } 100 \text{ g}^{-1}$ fresh weight). The river water could contribute to increasing the chlorophyll content as a result of its positive impact on plant health. This is by reducing the plant's exposure to the influence of excess salts, which may negatively affect its ability to absorb water and nutrients. Similarly, irrigation water with low salinity helps maintain a balance. Salts inside the plants contribute to improving photosynthesis and boost chlorophyll production in the leaves of citrus rootstocks (Alak and Al-Sabagh, 2020).

The citrus' different rootstocks also had a relevant effect on the rate of chlorophyll content in leaves (Table 4). The cultivar lime rootstock appeared with the highest rate ($55.03 \text{ mg } 100 \text{ g}^{-1}$ fresh weight) compared with the lowest rate ($47.92 \text{ mg } 100 \text{ g}^{-1}$ fresh weight) obtained in the sour orange rootstock. The superiority of the lime rootstock in leaf chlorophyll content could come from several factors, including genetic make-up of the genotype and environmental conditions. These

Table 4. Effect of the irrigation water quality, citrus cultivars, nano-selenium, and their interactions on the chlorophyll content ($\text{mg } 100 \text{ g}^{-1}$ fresh weight) in citrus rootstocks.

Water types	Citrus types	Nano-Selenium (ml L^{-1})			Water types x Citrus types
		0	1	2	
River water	Sour	50.76	51.66	51.34	51.25
	Volkameriana	53.38	57.15	57.72	56.09
	Lime	56.66	59.50	60.51	58.89
Well water	Sour	43.99	44.46	45.31	44.59
	Volkameriana	48.14	48.75	51.19	49.36
	Lime	48.64	51.97	52.87	51.16
Triple interaction		LSD _{0.05}			LSD _{0.05}
		4.07			2.53
Means (Nano-Selenium)		50.26	52.25	53.16	LSD _{0.05}
					1.70
Water types x Nano-Selenium	River water	53.60	56.11	56.52	Means (Water types)
	Well water	46.93	48.39	49.79	55.41
LSD _{0.05}		2.20			48.37
					2.01
					Means (Citrus types)
Citrus types x Nano-Selenium	Sour	47.38	48.06	48.32	47.92
	Volkameriana	50.76	52.95	54.46	52.72
	Lime	52.65	55.74	56.69	55.03
LSD _{0.05}		3.00			2.06

citrus cultivars have distinct genetic structures that make some efficient at photosynthesis and producing more chlorophyll than other cultivars. Furthermore, the promising citrus cultivars can use nutrients more efficiently, including nitrogen, phosphorus, and potassium, which are essential components in the chlorophyll synthesis. The cultivar lime rootstock could be adaptable to environmental conditions, contributing to enhanced production. Distinctive chlorophyll content was prominent while assessing the nitrogenized nutrition of citrus rootstocks using chlorophyll concentrations in the leaves (Esposti *et al.*, 2003).

Significant differences were also occurring among the spray treatments of nano-selenium for the total chlorophyll content in citrus seedling leaves (Table 4). The nano-selenium concentration (2 mg L⁻¹) was superior with the premier rate of chlorophyll content in leaves (53.16 mg 100 g⁻¹ fresh weight) versus the control treatment of nano-selenium (0 mg L⁻¹) giving the bottom rate (50.26 mg 100 g⁻¹ fresh weight). Foliar application of nano-selenium may lead to an increased chlorophyll content in citrus leaves. It could be because the nano-selenium can act as a catalyst for photosynthesis in plants, boosting the efficiency of using light in photosynthesis and, thus, raising the chlorophyll production.

Additionally, nano-selenium foliar application enhances plant growth and increases the availability of nutrients needed to store chlorophyll in leaves, and, thus, can raise chlorophyll content in citrus leaves (Almutairi *et al.*, 2023). One can also conclude that nano-selenium has preserved the chlorophyll pigment from the negative effects of salinity. Depending on the rootstock, it reduced the formation of chlorophyllase, causing the chlorophyll pigment's breakdown due to a decreased percentage of CHO from a decline in photosynthesis (Sevengor *et al.*, 2011, Al-Sabagh *et al.*, 2024). As for interaction effects, evidently, the river water, lime variety, and nano-selenium (2 mg L⁻¹) had the highest average (60.51 mg 100 g⁻¹ fresh weight) compared with the lowest interference (43.99

mg 100 g⁻¹ fresh weight) obtained from the reaction of well water, sour variety, and nano-selenium (0 mg L⁻¹).

Effectiveness of CAT enzyme

The irrigation water types of citrus seedlings showed significant differences for the CAT enzyme (Table 5). The irrigation with well water was better at increasing CAT enzyme (10.76 abs units min g⁻¹) than the irrigation with river water, which provided a decreased CAT rate (8.34 abs units min g⁻¹). The well water contains many compounds, including salts, and thus it exerts a type of salt stress. The increased effectiveness of the CAT enzyme under salt-stress conditions reflects the ability of plants to reduce the harmful effects of salinity, especially oxidative damage resulting from effective oxygen radicals. Consequently, these negatively affect the proteins, enzymes, and plant growth by displacing these oxidative radicals (Abul-Soud and Abd-Elrahman, 2016).

The citrus rootstocks also differed significantly for the CAT enzyme in leaves (Table 5). The cultivar sour orange rootstock showed an increased CAT content in the leaves (10.37 abs units min g⁻¹) compared with the rootstock of cultivars Volkameriana and lime, which showed the lowest rates (9.04 and 9.24 abs units min g⁻¹, respectively). The difference among the citrus roots for the catalase enzyme after exposing to salt stress could refer to the dissimilarity in their genetic makeup. Some citrus roots comprise genetic patterns that make them more capable of stimulating the production of the catalase enzyme to confront the salt stress more effectively than others, indicating the diversity in the response. Past studies also reflected the physiological and biochemical responses of citrus rootstocks, which always varied under salinity stress conditions (Singh *et al.*, 2014). The interaction of the well water, sour variety, and nano-selenium (2 mg L⁻¹) provided the maximum average (11.95 abs units min g⁻¹) compared with the minimum rate (7.34 abs units min g⁻¹) obtained from the interaction of the river water, Volkameriana variety, and nano-selenium (0 mg L⁻¹).

Table 5. Effect of the irrigation water quality, citrus cultivars, nano-selenium, and their interactions on the CAT enzyme content (abs unit min g⁻¹) in citrus rootstocks.

Water types	Citrus types	Nano-Selenium (ml L ⁻¹)			Water types x Citrus types
		0	1	2	
River water	Sour	8.80	8.99	9.71	9.17
	Volkameriana	7.34	7.80	8.22	7.78
	Lime	7.59	8.22	8.39	8.06
Well water	Sour	11.14	11.65	11.95	11.58
	Volkameriana	9.86	10.41	10.61	10.29
	Lime	10.15	10.33	10.76	10.41
Triple interaction		LSD _{0.05}			LSD _{0.05}
		2.29			1.38
Means (Nano-Selenium)		9.15	9.57	9.94	LSD _{0.05} N.S
Means (Water types)					
Water types x Nano-Selenium	River water	7.91	8.34	8.77	8.34
	Well water	10.38	10.80	11.11	10.76
LSD _{0.05}		1.38			1.51
Means (Citrus types)					
Citrus types x Nano-Selenium	Sour	9.97	10.32	10.83	10.37
	Volkameriana	8.60	9.10	9.41	9.04
	Lime	8.87	9.27	9.57	9.24
LSD _{0.05}		1.62			1.00

Effectiveness of POD enzyme

The outcomes indicated that both types of irrigation water caused considerable variations in the POD enzyme content in citrus leaves (Table 6). The irrigation with well water gave a higher level of the POD enzyme (176.7 abs units min g⁻¹) than the lowest value (136.7 abs units min g⁻¹) obtained in the citrus seedlings irrigated with river water. An increased level of salts in the well water can cause an enhancement in the POD enzyme activity in citrus trees due to the harmful effect of salts. The salt levels in soil and irrigation water cause plant stress, eventually boosting the production of harmful compounds in the roots. The POD enzyme is part of the plants' defense system against environmental stress and increases its activity in response to this stress to help remove harmful compounds and mitigate damage. Therefore, increased POD enzyme activity could be a response to elevated salts in irrigation water as part of the plant's response to adapting to environmental stress conditions (Hassan and Ali, 2014).

The results showed citrus rootstocks significantly differed for the POD content in leaves (Table 6). The cultivar sour orange rootstock recorded with an increased POD content in leaves (171.0 abs units min g⁻¹)

versus the cultivars Volkameriana and lime, which had the lowest POD rates (148.4 and 150.7 abs units min g⁻¹, respectively). The difference in the POD enzyme activity in citrus rootstocks could be due to the varying ability of cultivars to adapt to environmental conditions. Each genotype has diverse genetic and physiological features determined by genetic evolution and natural selection, which affect its response to various stress conditions. Some cultivars may be more tolerant to salt than others as a result of their genetic adaptation to salty environments. This adaptation may include distinct regulation of enzyme activity, such as POD, to adapt to salt challenges, while other cultivars may have a completely varied response. Moreover, the differences in POD enzyme activity can be results of disparities in genetic make-up and genes' regulation among the cultivars, causing differences in genotype expression and the enzyme activity after exposure to salt-stress conditions (Singh *et al.*, 2014).

The nano-selenium foliar application meaningfully affected the POD enzyme content in leaves of citrus' different rootstocks (Table 6). The nano-selenium concentration (1 mg L⁻¹) was distinct by giving the highest POD content (164.0 abs units min g⁻¹) compared with the control treatment (0 mg L⁻¹) of nano-

Table 6. Effect of the irrigation water quality, citrus cultivars, nano-selenium, and their interactions on the POD enzyme content (abs unit min g⁻¹) in citrus rootstocks.

Water types	Citrus types	Nano-Selenium (ml L ⁻¹)			Water types x Citrus types
		0	1	2	
River water	Sour	140.8	153.2	160.0	151.3
	Volkameriana	120.9	129.3	134.9	128.4
	Lime	125.0	129.7	136.4	130.4
Well water	Sour	180.7	191.0	200.4	190.7
	Volkameriana	161.4	168.8	175.0	168.4
	Lime	165.2	170.5	177.3	171.0
Triple interaction		LSD _{0.05}			LSD _{0.05}
		20.15			13.10
Means (Nano-Selenium)		149.0	157.1	164.0	LSD _{0.05}
					8.27
Means (Water types)					
Water types x Nano-Selenium	River water	128.9	137.4	143.8	136.7
	Well water	169.1	176.7	184.2	176.7
LSD _{0.05}		12.27			14.23
Means (Citrus types)					
Citrus types x Nano-Selenium	Sour	160.7	172.1	180.2	171.0
	Volkameriana	141.1	149.0	155.0	148.4
	Lime	145.1	150.1	156.8	150.7
LSD _{0.05}		14.31			9.51

selenium, which showed the lowest POD rate (149.0 abs units min g⁻¹). The increased POD enzyme activity could refer to the complex chemical reactions in plants. Nano-selenium seemed to act as an antioxidant, which limits harmful oxidants and protects plants from the damage resulting from environmental stresses, such as drought and salinity. Therefore, it could contribute to the plant's antioxidant activity in stress conditions, and one of these antioxidants was the POD enzyme, incurring an upsurge in the enzyme content (Ikram *et al.*, 2022). A significant interaction was evident between the well water, sour variety, and nano-selenium (2 mg L⁻¹), giving the maximum POD enzyme average content (200.4 abs units min g⁻¹) compared with the minimum content (120.9 abs units min g⁻¹) obtained from the interaction of river water, Volkameriana variety, and nano-selenium (0 mg L⁻¹).

CONCLUSIONS

Based on the results, it was evident that nano-selenium has helped preserved citrus seedlings from the harmful effects of salinity, especially when irrigated with salty well water. It also

increased the average number of leaves, helped the seedlings retain their content of major elements (NPK), as well as reducing the chlorophyll pigment's deterioration and raising the content of enzyme antioxidants, such as CAT and POD.

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