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Impact of exogenous ascorbic acid and putrescine on vegetative, root system morphology and chemical composition of clementine Mandarin saplings.

Taghreed Ali Hilal[1] & Thamer H. R. Al-Falahy[2*]

[1]Horticulture Office, Ministry of Agriculture, Iraq
[2]Department of Horticulture, Agriculture College, University of Anbar, Iraq.

*Email: ag.thamer.hameed@uoanbar.edu.iq

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Abstract

Spraying with antioxidants and polyamines is one of the techniques that contribute to improving vegetative growth and the architectural structure of the roots, in addition to improving the chemical content of the plant. The objective of the study was to enhance the traits related to vegetative growth, root development, and chemical composition of one-year-old Clementine mandarin saplings. This was achieved by applying ascorbic acid through foliar application at different concentrations (0,300, 600, 900 mg/L) and utilizing three concentrations of putrescine (0,75,150 mg/L). The results showed that most of the growth indicators in terms of main shoot number, main shoot length, vegetative dry weight, taproot length, secondary lateral roots number, secondary lateral roots length, roots system dry weight, chlorophyll, and vitamin C content in leaf, and shoots carbohydrate content were significantly increased with increasing concentration. The ascorbic acid is more effective at 900 mg/L than other concentrations, whereas putrescine was more effective at 150 mg/L, contrasted with 75 mg/L and control.

Keywords

antioxidants; foliar spray; polyamines; putrescine; root growth; vegetative system

Introduction

Mandarin orange (*Citrus reticulate* Blanco), also known as mandarin or mandarine. It is a rounded fruit citrus tree. It is an evergreen fruit tree regarded as the smallest species in the Citrus genus in height and size because of the quantity and thinness of the branches it produces, giving it a drooping appearance (1). One of the prevalent and productive varieties in Iraqi orchards is the clementine mandarin, distinguished by its medium to moderately large growth and short, elongated, pointed leaves lacking auricles or thorns (2). The early-ripening fruits are juicy and medium in size. A small bundle can be seen on the fruits, which are dark orange. At maturity, the peel is attached to the pulp (3).

One of the most important citrus fruits in Iraq for local consumption is mandarin. A total of 241,549 mandarin fruit trees were projected to exist, with an average yield of 18.6 kg per tree. Meanwhile, 4,494 tons of mandarin were produced worldwide. Baghdad Governorate ranked first in terms of production, with an estimated 1,380 tonnes produced at a rate of (30.7%) Diyala province of Iraq came in second with 1,199 tonnes produced at a rate of (26.7%), and Salah al-Din province ranked third with 1,159 tonnes produced at a rate of (25.8%) of Iraq's total production (4).

Ascorbic acid is one of the many essential and vital roles that antioxidants, such as those found in plants, play in metabolic processes, cell growth, and differentiation; they also provide support to numerous hormones and enzymes found in plants, including auxins, gibberellins, ethylene, and abscisic acid (5). It is crucial for the chelation of various oxygen-free radical forms, including singlet oxygen($^1O_2$), superoxide anion($O2^{-}$) hydrogen peroxide (H_2O_2), hydroxyl radicals($^{\cdot}OH$) and per hydroxyl radicle ($HO2^{\cdot}$) which emerge during different metabolic processes and in stressful environments like extreme heat, cold, dryness, salinity, and extreme light, even when these free radicals are left without scavenge or chelating it leading to disrupts plasma membranes, oxidation of lipids as well as proteins, many enzymes inhibition, and damage to plasma membranes can result in cell death, which might injure plant tissues (6, 7).

Modern research in improving plant growth and increasing production efficiency has placed significant emphasis on the utilization of various plant growth regulators, including polyamines; among these regulators, putrescine, a low molecular weight organic compound containing two amines groups, holds considerable significance, putrescine exhibits multiple activities and demonstrates high biological effectiveness leading scientists to recognize it as a plant growth regulators, it plays diverse roles and elicit distinct biological and physiological responses to the maintenance of cell membranes stability (8,9). Additionally, putrescine plays a vital role in regulating plant defense mechanisms against various environmental stresses (10).

Polyamines have played an important role in the growth and development of citrus trees and physiological processes, especially cell division, growth and development of roots, the morphological and structural composition of the shoot, and development of flowering primordia, fruit setting, and development. Furthermore, their role in impeding leaf senescence and enhancing the capacity of the photosynthesis process (11-13). The study aims to explore the effects of ascorbic acid as an antioxidant and polyamines like putrescine on mandarin sapling growth and evaluate the synergistic effect between these two factors.

Materials and Methods

Experiment has been achieved on one-year-old mandarin Clementine saplings, uniform in size and growth as much as possible, which were brought from one of the government stations certified (Al-Hindiyah /Karbala, 70 miles south of Baghdad) in the lath house of the Department of Horticulture and Landscape Gardening, College of Agriculture, University of Anbar from beginning of spring in April to December 2023 to explore the effect of feeding with ascorbic acid as well as putrescine on developmental characteristics of mandarin saplings cv. Clementine. 108 saplings as homogeneous in growth as possible with age of one year old, grafted onto sour orange rootstock (*Citrus aurantium* L.), cultivated in polyethylene bags (20 cm x 17 cm) and were transferred to larger polyethylene bags with dimensions (30 cm x 25 cm) fooled with mixture of soil and beat moss (3:1). Saplings received adequate fertilizers with NPK (20: 20: 20 + TE) was added during March, May and September at a rate of 30 g. sapling -1 divided into two batches for each month, saplings irrigated via drip irrigation system in addition to sprinkler irrigation with shading with green nets (saran) in the scorching summer, viz. (June, July and August), in order to safeguard saplings from extreme high waves expected during these periods (14,15), saplings received uniform cultural practices, viz. removing weeds and controlling agricultural pests as needed, saplings were sprayed with the pesticide Devimethrin5, which contains the active ingredient (Alphacypermethrin 5%) with concentration 1ml/L-1 to management leafminers, after noticing their infestation, control continued at a rate of one spray every two weeks during the beginning of April, and the process of spraying the pesticide was resumed during the mid of September at a rate of one spray every two weeks. Soil analysis was done to explore physical and chemical properties, as shown in (Table 1)

The experiment employed two variables as a factor: the first, treatment with the antioxidant (ascorbic acid) at four levels 0 (spraying with water only), 300, 600, and 900 mg/L symbolized with L0, L1, L2, and L3, respectively, the second factor included spraying with putrescine at three concentration (0, 75, 150 mg/L) symbolized by P0, P1, and P2, respectively. The study was conducted using a randomized complete block design (RCBD) with three replications, each replicate containing three saplings; The following characteristics were measured as per the protocol.

Main shoot number increment (shoot/sapling): After calculating the number of main branches on the main stem of mandarin saplings before implementing the trial

Table1. Some physio-chemical traits of the soil.

Soil texture/ Loam clay										
pH	**Soil texture/ Loam clay EC (1:1)** dS/m	**CEC** C.mol/l	**O.M** g/kg soil	**Sand** g/kg soil	**Loam** g/kg soil	**Clay** g/kg soil	**N** mg/ kg	**P** mg/ kg	**K** mg/ kg	**CaCO3** g / kg
7.4	2.24	25.35	14	42.2	30.2	29.2	69.2	15.7	218.7	179.0

at the beginning of April 2023, then calculating them at the end of the trial at the beginning of December of the same year, and by calculating the difference between the first and second readings, the rate of increase is obtained.

Main shoot length (cm): It was measured where the branch connected to the stem, using a tape measure, in December 2023.

Vegetative dry weight (g): This measurement was taken for one sapling from each replication in early December. The vegetative system was separated from the root system and thoroughly rinsed with water; after drying, the samples were subjected to a constant temperature of 65°C until the weight remained stable, according to (17)

Taproot length (cm): After removing the saplings in December 2023, for one sapling from each replicate, the soil was carefully removed from the root area, the root system was washed carefully, then dried with a cloth, and then the length of the taproot was measured from where it connects to the base of the stem to the terminal end of the root. (18)

Secondary lateral roots number (root/sapling): In December 2023, saplings were uprooted (one sapling from each replicate), the soil was removed from around the root system, and it was carefully washed with water; the number of main adventitious roots was counted according to (18)

Secondary lateral roots length (cm): The length of the adventitious roots of saplings was measured from where they connected to the base of the stem until the end of the root, as mentioned in (18)

Roots system dry weight (g): After uprooting the saplings in December 2023 and after washing them from the surrounding soil several times, the roots were subjected to a temperature of 65°C, as stated in (17), until the constant weight.

Leaf chlorophyll content (mg/100gm fresh weight): It was estimated by taking samples of sapling leaf for each treatment within the replicates in mid-November of 2023. Chlorophyll was determined according to (19).

Leaves Vit. C content (mg/100gm fresh weight): Through titration, determination of Vit. C in mandarin leaves with a strong oxidizing agent and titration with (2,6 -dichlorophenol indophenols), as explained in (20).

Shoots carbohydrate content (%): It was measured in December 2023, according to what was mentioned by (21).

Data were subjected to statistical analysis using the GenStat software program to compare the means, and the least significant difference (LSD) method was employed at a significance level of 0.05 (16).

Results

Main shoots number increment: The statistical results presented in Table 2 revealed a notable rise in main shoots, which was observed with higher levels of ascorbic acid and putrescine, the highest values (5.33 and 5.12 shoots per sapling) for ascorbic acid and putrescine respectively. Moreover, when considering the interaction between the factors, A_3P_1 yielded (6.50 shoots per sapling), while the control (2.16 shoots per sapling).

Table 2. Impact of foliar application with ascorbic acid, putrescine, and their interaction on main shoots number increment, main shoots length, and vegetative dry weight.

Putrescine (P)	Ascorbic Acid (A)				Mean
	A0 = control	A1 = 300 mg/l-	A2 = 600 mg/l	A3 =900 mg/l	
Main shoots number increment (shoot/sapling)					
P_0= control	2.16	3.33	3.66	4.00	3.29
P_1= 75 mg/l	3.33	4.16	5.50	6.50	4.87
P_2 = 150 mg/l	4.50	4.83	5.66	5.50	5.12
Mean	3.33	4.11	4.94	5.33	
L.S.D 0.05	A	P	A×P		
	0.44	0.38	0.76		
Main shoots length (cm)					
P_0= control	16.36	17.50	16.17	17.67	16.92
P_1= 75 mg/l	18.22	24.17	29.73	32.30	26.10
P_2 = 150 mg/l	18.50	25.67	36.00	30.07	27.56
Mean	17.69	22.44	27.30	26.68	
L.S.D 0.05	A	P	A×P		
	1.03	0.89	1.79		
Vegetative dry weight (g)					
P_0= control	51.73	53.77	55.47	55.30	54.07
P_1= 75 mg/l	54.80	57.27	58.33	79.97	62.59
P_2 = 150 mg/l	61.37	71.00	74.13	82.80	72.33
Mean	55.97	60.68	62.64	72.69	
L.S.D 0.05	A	P	A×P		
	1.30	1.12	2.25		

LSD = Least significant difference at 5% probability

Length of main shoots: Outputs of Table 2 cleared that there are significant differences between the treatments when spraying mandarin saplings with ascorbic acid, as treatment A_2 achieved the highest value, without a significant difference from A_3, which recorded (27.30 and 26.68 cm), respectively, while A_0 gave the lowest values (17.69 cm). Putrescine showed a marked effect on shoot length; P_2 was unique in achieving the highest values, reaching (27.56 cm), while P_0 gave the lowest values (16.92 cm). In the same context, a combination of ascorbic acid and putrescine showed an increase in shoot length, A_2P_2 recorded (36.00 cm), while A_0P_0 gave the lowest values (16.36 cm).

Vegetative dry weight: Data in Table 2 indicated that ascorbic acid significantly affected the dry weight. A_3 was unique in achieving the highest values, which were recorded (72.69 g/sapling), with an increase rate of 29.87% compared to the lowest values in A_0, which reached (55.97g/sapling). Likewise, putrescine achieved the highest dry weight, especially P_2, which gave (72.33 g/sapling), with an increase rate of 33.77% compared to treatment P_0, which gave the lowest values, reaching (54.07 g/sapling). As for the interaction, A_3P_2 achieved the highest dry weight (82.80 g/sapling) in comparison with A0P0 (51.73 g/sapling).

Taproot length: Statistical analysis in Table 3 illustrated there is a significant effect when spraying ascorbic acid, and this effect appeared clearly when measuring the length of the taproot; A_3 achieved the highest value (19.66 cm), with an increase rate of 76.79% compared to A_0 (11.12 cm). The results also showed the significant effect of spraying with putrescine, which appeared clearly through treatment P_2 (20.68 cm), with an increase rate of 82.68% compared to P_0 (11.32 cm). The synergistic effect between ascorbic acid and putrescine had an obvious effect; A_3P_2 gave (24.77 cm), compared to A0P0 (7.43 cm).

Secondary lateral roots number: From observing the results in Table (3), it is clear that spraying with ascorbic acid had a significant effect on the number of adventitious roots, and this effect increased with increasing levels of spraying, A_3 spraying was unique in achieving the highest value and with a significant superiority over the rest of the levels, and it reached (11.11 roots/sapling) with an increase of 40.81% compared to treatment A0, which gave the lowest values (7.89 roots/sapling). Spraying with putrescine also has a significant effect; P_2 gave (11.08 roots/sapling), with an increase (52.82%) compared to treatment P0, which gave the lowest values (7.25 roots/sapling). It was noted that combination had an obvious impact, and this was demonstrated by the A_3P_2, which achieved the highest values roots/sapling), without a significant difference from the A_2P_2 and A3P1, compared to the lowest values A_0P_0 (4.33 roots/sapling).

Secondary lateral roots length: Data in Table 3 revealed that an increase in the length of secondary roots as a result of spraying with ascorbic acid, A_3 was unique in achieving the highest values (15.44 cm), with an increase of 49.90%, compared to treatment A_0, which gave the lowest values

Table 3. Impact of foliar application with ascorbic acid, putrescine and their interaction on tap root length, secondary lateral roots number, secondary lateral roots length and roots system dry weight.

Putrescine (P)	Ascorbic Acid (A)				Mean
	A0 = control	A1 = 300 mg/l	A2 = 600 mg/l	A3 =900 mg/l	
Tap root length (cm)					
P_0= control	25.43	29.13	31.70	31.00	29.32
P_1= 75 mg/l	28.17	28.40	34.07	40.20	32.71
P_2 = 150 mg/l	33.77	39.40	42.20	48.27	40.91
Mean	29.12	32.31	35.99	39.82	
L.S.D 0.05	A	P	A×P		
	1.49	1.29	2.59		
Secondary lateral roots number (root/sapling)					
P_0= control	8.33	10.67	12.67	15.33	11.75
P_1= 75 mg/l	11.33	14.00	14.67	15.00	13.75
P_2 = 150 mg/l	16.00	16.67	19.00	22.00	18.42
Mean	11.89	13.78	15.44	17.44	
L.S.D 0.05	A	P	A×P		
	1.21	1.05	2.10		
Secondary lateral roots length (cm)					
P_0= control	16.44	18.67	20.67	23.55	19.83
P_1= 75 mg/l	19.33	22.00	22.89	29.78	23.50
P_2 = 150 mg/l	24.11	27.33	23.78	24.67	24.97
Mean	19.96	22.67	22.44	26.00	
L.S.D 0.05	A	P	A×P		
	1.00	0.87	1.74		
Roots system dry weight (g)					
P_0= control	21.13	23.98	29.23	29.53	25.97
P_1= 75 mg/l	24.63	28.53	32.43	33.23	29.71
P_2 = 150 mg/l	27.30	31.10	35.27	32.67	31.58
Mean	24.35	27.87	32.31	31.81	
L.S.D 0.05	A	P	A×P		
	0.93	0.80	1.61		

LSD = Least significant difference at 5% probability

(10.30 cm). Spraying with putrescine also showed a significant effect on this trait. P_2 achieved (14.97 cm), with an increase (66.33%), compared to the lowest values in the P_0 (9.00 cm). As for the effect of the combination of the two study factors, A3P1 achieved the highest values (19.78 cm) compared to A_0P_0, which gave the lowest values (6.44 cm).

Roots system dry weight (g): The results of Table 3 demonstrated that there was a clear and significant effect on the dry weight of the root system; A_2 achieved the highest values, reaching (31.98 g), without a significant difference from treatment A_3, which gave (31.48 g) and at the same context, Spraying with putrescine showed enhancement in this characteristic, especially P_2, which achieved the highest values(31.92 g), while P_0 gave the lowest values, (24.52 g). The interaction between studied factors had a significant effect on the dry weight, and this was demonstrated by achieving A_2P_2 the highest value, (35.27 g), compared to A0P0, which gave (19.34 g).

Leaf chlorophyll content (mg/100 g fresh weight): Ascorbic acid increasing chlorophyll pigment of the leaf, results in Table 4 indicated that A_3 achieved the highest chlorophyll content 233.56 (mg/100 gm fresh weight), with an increased rate of 8.92%, compared to A_0, which gave 214.42 (mg/100 gm fresh weight), spraying growth regulator "putrescine" stimulate increasing concentration of chlorophyll, especially P_2 which recorded 235.46 (mg/100 gm fresh weight) with an increase 9.32% while P0, gave 215.38 (mg/100 gm fresh weight). As a result of the effect of the interaction, A_3P_2 achieved the highest chlorophyll content, 246.63 (mg/100 gm fresh weight), while A_0P_0 gave 209.94 (mg/100 gm fresh weight).

Leaf Vit. C content (mg/100gm fresh weight): Data in Table 4 illustrated that there was an increase in the content of vitamin C in mandarin leaf; this effect was evident when treatments A_3 and A_2 achieved 140.80 and 138.99 (mg/100 gm fresh weight) respectively, while the lowest values in the A0 which gave 129.89 (mg/100 gm fresh weight). It was also clear that application with putrescine increasing vitamin C content, P_3 and P_2 achieved the highest values, 138.12 and 135.64 (mg/100 gm fresh weight), respectively, while P_0 gave 134.41 (mg/100 gm fresh weight).

Shoots carbohydrate content (%): Statistical analysis in Table (4) showed that application with ascorbic led to a significant increase in the carbohydrate content; treatment A_3 recorded the highest value (9.07%), compared to A_0 (7.29%). Also, due to spraying with putrescine, P_2 was unique in achieving the highest percentage, reaching (9.73%), compared to P_0, which gave (6.68%). Concerning the combination, A_3P_2 gave the highest value (10.66%), while A0P0 gave (6.04%).

Table 4. Impact of foliar application with ascorbic acid, putrescine and their interaction on leaves chlorophyll content, leaves vitamin C content and shoots carbohydrate content.

Putrescine (P)	Ascorbic Acid (A)				Mean
	A0 = control	A1 = 300 mg/l	A2 = 600 mg/l	A3 =900 mg/l	
	Leaves chlorophyll content (mg/100gm fresh weight)				
P_0= control	209.94	211.91	216.97	222.72	215.38
P_1= 75 mg/l	212.92	216.93	221.60	231.33	220.70
P_2 = 150 mg/l	220.39	234.53	240.27	246.63	235.46
Mean	214.42	221.12	226.28	233.56	
L.S.D 0.05	A	P	A×P		
	1.89	1.63	3.27		
	Leaves Vit. C content (mg/100gm fresh weight)				
P_0= control	128.53	132.66	136.17	140.27	134.41
P_1= 75 mg/l	130.80	133.62	136.91	141.23	135.64
P_2 = 150 mg/l	130.34	137.37	143.89	140.90	138.12
Mean	129.89	134.55	138.99	140.80	
L.S.D 0.05	A	P	A×P		
	3.26	2.82	5.64		
	Shoots carbohydrate content (%)				
P_0= control	6.04	6.34	7.13	7.23	6.68
P_1= 75 mg/l	6.94	7.23	7.81	9.31	7.82
P_2 = 150 mg/l	8.91	9.40	9.96	10.66	9.73
Mean	7.29	7.65	8.30	9.07	
L.S.D 0.05	A	P	A×P		
	0.28	0.24	0.49		

LSD = Least significant difference at 5% probability

Discussion

The role of ascorbic acid in increasing growth indicators, represented by the increment in main shoots number, main shoots length, and dry weight of vegetative system (Table 2) may be attributed to the vital of ascorbic acid in many biochemical operations within the plant, *viz*., growth and cell differentiation, moreover, role as a co-factor to many enzymes and hormones within the plant, such as auxin, gibberellin, and cytokinin (5, 22), which stimulate cell division, growth of lateral shoots, and the leaf expansion as a result of the stimulation of cell division and expansion (23). These results agree with those obtained by (24, 25); they mentioned that using ascorbic acid at concentrations of 2000 and 300 mg/L, respectively, achieved the highest value of olive vegetative traits. The role of foliar spraying with putrescine in increasing the growth indicators described above may be attributed to the important role of putrescine in many functions related to growth , development and reproductive of the plant, moreover, support the plant to adapt to various environmental conditions (26), which helps the plant to grow well, which increases the efficiency of photosynthesis, which stimulates vegetative growth, as putrescine stimulates growth by working in solidarity with some internal hormones such as auxin and cytokinin through its work as a hormonal messenger to increase cell division and differentiation in many physiological processes, as it regulates the plant's sensitivity to the auxin/cytokinin ratio (27, 28), due to auxins play an important role in the elongation and expansion of cells, besides that, cytokinin encourage cell division, according with many researchers have pointed out to the positive role of polyamines, including putrescine, on the cellular functions of the plant, as it increases cell longevity and enhances carbon metabolism, in addition to its effective role in maintaining the effectiveness and stability of proteins and nucleic acids, which have a role in transmitting signals between cells (29-31).

Plant roots play an important and distinct role in absorbing nutrients and establishing the mechanical support for the plants (32) by observing Table (3) regarding the length of the taproot, number of the adventitious roots, consequently increasing roots dry mass, we noticed that spraying saplings with ascorbic acid and putrescine have an activation of roots growth, perhaps it can be ascribed to the reality that application of both substances enhancements canopy characteristics of mandarin saplings and thus its impact on the root characteristics, as a portion of the substances manufactured in the shoot are transferred to the root system to increase its growth and spread (33-35), therefore, it is expected that the dry weight of the root system will increase, and this is what has been proven by many researchers in this field, including (36) when spraying ascorbic acid on 2-year-old olive seedlings, and these results also agreed with (12) when spraying putrescine on seedlings of trifoliate orange (*Poncirus trifoliata*) and with (37) On Carrizo citrange and Volkameriana rootstocks.

The increase of vitamin C in the leaf as a result of spraying with ascorbic acid is probably attributed to the direct absorption of ascorbic through the leaf, and then the saplings maintain levels of this acid throughout the study period, also, the increase in the products of photosynthesis, represented by carbohydrates, may contribute to the increase in ascorbic acid in the leaves (35). On the other hand, putrescine led to an increase in the number of leaves, leaf area, and chlorophyll content, which works to polarize light and activate the photosynthesis process; since sugars are the main products of the photosynthesis process, therefore prospective that vitamin C will accumulate because the precursor of its formation is L-Galactose with the help of the enzyme L-Galactose dehydrogenase (38,39).

Conclusion

We need to have comprehensive knowledge of antioxidants and polyamines, which are considered a main compound that plays a vital role in protecting plants from damage caused by free radicals produced during the metabolic process. Moreover, it is an important co-factor for many enzymes and phytohormones. This study was conducted to investigate the possibility of improving the growth of Clementine mandarin saplings under lath house conditions through spraying with ascorbic acid and putrescine. From the results of this study, it can be concluded that foliar application with ascorbic acid (900 mg/l) and putrescine (150 mg/l) and synergistic effect between them achieved the best growth results, i.e., (increment in main shoots number, main shoots length and dry weight of vegetative system, taproot length, number of the adventitious roots, dry weight of the root system), moreover, chemical content of saplings represented by (chlorophyll content, Vit. C content in leaves and carbohydrate content in main shoots)

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Authors' contributions

Dr.Thamer Al-Falahy was responsible for creating the initial research idea and collecting the literature review to achieve the final idea for this research, as well as performing statistical analysis after collected data to investigate the effect of individual factors solely or interaction between them, moreover comprehensive reading for the final manuscript. Mrs. Taghreed Ali carried out the experiment, data collection, and interpretation of results; moreover, the initial writing of the manuscript also compared the findings with the literature and made the conclusions built into the output of this research.

Compliance with ethical standards

Conflict of interest: : Authors have stated that there are no conflicts of interest.

Ethical issues: There are no ethical issues to declare.

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