

Effects of Salinity on three Mandarin Cultivars grafted on two different Rootstocks

Efectos de Salinidad de tres Cultivares de Mandarina en dos Patrones diferentes

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Abstract

Citrus, one of the most important fruit crops in the world and also they are sensitive to salt stress. The negative effects of stresses often lead to reductions in fruit yield and quality. To assess the effects of salinity on some growth traits, a greenhouse test was performed with the cultivars ‘Mihowase’, ‘Primosole’ and ‘W. Murcott’ as grafted on ‘Cleopatra’ and ‘Swingle citrumelo’ as rootstocks. The experiment was conducted at the Agrarian Experimental Station of National Institution for Agricultural Innovation in Donoso-Huaral, ubicada 90 km north of Lima. The plants were irrigated with water plus NaCl with an Electrical Conductivity of 0.5 or 4.5 dS/m as salt stress. The variables under evaluation were leaf losses, fresh and dry weight of stem, leaves and roots as well as relative water content in the plants. The results showed that the rootstocks ‘Cleopatra’ was more tolerant than ‘Swingle citrumelo’. The cultivars used as scions affected both rootstocks in all the evaluated traits being more notorious in the amount of feeding roots. Selection of mandarin trees for production should take in consideration the combination scion/rootstock.

Keywords: Mandarin, Salt stress, rootstocks, ‘Cleopatra’, ‘Swingle citrumelo’

Resumen

Cítricos, es uno de los más importantes cultivos de frutales en el mundo y son susceptibles a sales. Los efectos negativos de las sales generalmente reducen producción y calidad de fruta. Para evaluar los efectos de la salinidad en algunos parámetros de crecimiento, un experimento en invernadero fue conducido con los cultivares ‘Mihowase’, ‘Primosole’ y ‘W. Murcott’ en los patrones ‘Cleopatra’ y ‘Swingle citrumelo’ El experimento fue conducido en la Estación Experimental Agraria (EEA) del Instituto Nacional de Innovación Agraria (INIA), Huaral-Donoso a 90 km al norte de Lima. Las plantas fueron irrigadas con una solución salina ClNa y con una conductividad eléctrica de 0.5 o 4.5 dS/m. Las variables evaluadas fueron caídas de hojas, peso fresco y seco de tallos, hojas y raíces y contenido de agua por las plantas. Los resultados mostraron que el patrón ‘Cleopatra’ es más tolerante que ‘Swingle citrumelo’. Las variedades afectaron todas las variables

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de los dos patrones, siendo más notorio en la cantidad de pelos absorbentes de las raíces. La selección de plantas de mandarinas para producción debería considerar combinación variedad/patrón.

Palabras clave: Mandarina, estrés salino, patrón, ‘Cleopatra’, “Citrumelo Swingle”

Introduction

High salt concentration either in the soil or water affects growth, yield and quality of major fruit crops. Salinity effects are well known under arid and semiarid conditions due to lack of sufficient water and higher evapotranspiration rates (Zahra et al., 2020).

High levels of salts affect biochemical and physiological processes in the cells. These effects are observed initially on osmosis regulation followed by ion toxicity and imbalance in nutrient absorption. During the initial state of salt stress, the plants present reduced capacity to absorb water by the roots, cell membrane damages, reduced ability to detoxify oxygen radicals, reduced photosynthesis and stomata aperture (Siddiqui et al., 2018; Acosta et al., 2017). High levels of B^+ , Cl^- and Na^+ ions on leaves present symptoms of toxicity as yellowing of the borders and under the presence of high temperatures and winds, loss of older leaves is very common. Absorption of a specific ion, or combination with others, might induce imbalance in plant nutrition, as has been observed in Citrus (Etehadpour et al., 2020).

The Citrus plants are considered sensitive to the salts present in the soil or irrigation water. Levels of salts in the soil over 2.0 dS/m or 1.0 dS/m in the irrigation water might reduce yield by 13.0 % to 13.5 % for an increase of 1.0 dS/m (Khoshbakht et al., 2018). The ions B^+ , Na^+ and Cl^- when present in the citrus leaves at the levels of 0.005 % to 0.17 % of dry matter, 0.04 % and 0.7 % respectively are considered toxic (Farhangi & Torabian, 2017). Toxicity symptoms are observed when the levels of Cl^- ion in the leaves are 1.0 % dry weight and 0.1 % to 0.25 % for Na^+ . It will induce yield reduction when the Cl^- in leaves is 0.2 % dry weight (Syverten et al., 1988).

Most fruit crops are grafted and are available in different combinations of scion and rootstocks to improve fruit quality, early production or to overcome environmental and biological constraints. Citrus rootstocks are available for many years like sweet orange, sour orange and “Lima Rangpur” (*Citrus reticulata* var. *austera* x *Citrus limon*), ‘Cleopatra’ (*Citrus reticulata*) and new ones like ‘Swingle citrumelo’ or CPB 445 (Duncan grapefruit Macfad x *Poncirus trifoliata* L.), C 22, C 35 and HRS 942 from specific breeding programs in USA, Spain and Brazil (Gonzales, 2017). Citrus rootstocks present mechanisms of salt tolerance in the process of absorption and translocations of Cl^- and Na^+ ions present in the soil to the pattern. “Lima Rangpur” excludes Cl^- and sequesters Na^+ in the roots, ‘Cleopatra’ mandarin excludes both ions and trifoliolate orange (*Poncirus trifoliata*) excludes Na^+ and translocates Cl^- to the pattern (Khoshbakht et al., 2018). The pattern might affect the absorption and translocations of ions by the rootstocks (Hasanuzzaman et al., 2021; Vardi et al., 1988).

New citrus cultivars are available as scion and rootstocks and their combinations are under continuing evaluation. The purpose of this study was to evaluate under salt stress the early cultivars ‘Mihowase’ and ‘Primosole’ as scions and the late cultivar ‘W. Murcott’, that have not been previously evaluated, on the two most commonly used rootstocks: ‘Cleopatra’ y ‘Swingle citrumelo’. These new grafted mandarin combinations might extend the window of supply for export and local market.

Materials and Methods

The experiment was conducted under greenhouse condition, from October to December of 2019, at the Agricultural Experimental Station in Donosohuaral, 90 km north of Lima at 11° 31' 17" S and 77° 14' 6" W and 130 masl. The average temperature was 25.5° C, relative humidity 88.5 % and natural light conditions. The plants were provided by the tree nursery AgroViperos located in the Sta. Rosa valley near the Agricultural Experimental Station. The plants were grown for 9 months in a greenhouse in 6.0 kg plastic

bag container with a substrate with EC = 0.05 dS/m, pH = 7.7, OM = 10.5 % and CEC = 12.68. The scions were: ‘Mihowase’ (*C. unshiu* Milho), ‘Primosole’ (*C. ushiu* Milho x *C. reticulata*) and ‘W. Murcott’ (*C. reticulata* x *C. sinensis*) grafted on the rootstocks ‘Cleopatra’ (*C. reshni*) and ‘Swingle citrumelo’ (*C. paradise* x *Poncirus trifoliata* L. Raf). The treatments were allocated in a complete block design with 3 replications and 3 plant per replication. It was evaluated the number of leaves loss at 45 and 90 days, fresh and dry weight of roots, stem and leaves, and water loss at 90 days. Plant water content was estimated by the differences between fresh weight less dry weight. Statistical analysis of the data was done with the SPSS v.22 Statistic program and the means were compared with the Duncan test ($P < 0.05$).

Results

Leaf loss

‘Cleopatra’ rootstocks showed less leaves loss (18.13 %) when it is compared at the low and high salt treatments; however, ‘Swingle citrumelo’ showed higher leaf loss (31.65 %). More leaf loss was observed at the second period of evaluation, 45 to 90 days, in both rootstocks. The cultivars used as scions affected the leaf loss of both rootstocks. ‘Cleopatra’ rootstocks were not affected by the ‘Mihowase’ scion but presented more leaf loss with the ‘Primosole’ and

‘W. Murcott’ scion. ‘Swingle citrumelo’ roots showed less leaf loss with ‘Mihowase’ scion but similar with ‘Primosole’ and ‘W. Murcott’ (Table 1 and Figure 1). Similar results have been obtained when engrafted and ungrafted rootstocks respond different to salt stress, where engrafted are more susceptible.

Plant dry weight

‘Cleopatra’ rootstock showed 18.13 % plant dry weight loss and ‘Swingle citrumelo’ 9.52 % under 4.5 dS/m salt concentration. They also observed the dry weight loss in stem + leaves and in the roots of both rootstocks with more weight loss in roots. The tree cultivars used as scions reduced dry weight loss of both rootstocks but ‘W. Murcott’ increased the weight loss of stem + leaves and roots on ‘Swingle Citrumelo’ (Table 2, Figure 2, Figura 3, and Figura 4).

Plant water loss

Plant water content was estimated by the difference between fresh weight less dry weight. The plants of ‘Cleopatra’ rootstock losses 26.7 % and ‘Swingle citrumelo’ 5.59 % of water, respectively. The three cultivars used as scion reduce this water loss in ‘Cleopatra’ but increased on ‘Swingle citrumelo’ when compared to the rootstocks alone (Table 2 and Figure 5).

Table 1. Duncan test of the effects of salts stress on the leaves loss of each scion/rootstock combination a 45 and 90 days.

Number of days		0-90		0-45		45-90	
Electric conductivity (dS/m)		0.5	4.5	0.5	4.5	0.5	4.5
Variety/pattern	Cleopatra	14.67 a	17.33 ab	8.00 d	8.67 d	6.67 abc	8.67 abcd
	Mihowase/Cleopatra	17.33 ab	16.33 a	0.33 a	1.67 ab	14.67 def	17.00 def
	Primosole/Cleopatra	21.33 ab	26.33 ab	3.33 bc	4.00 bc	17.33 def	23.00 f
	W. Murcott/Cleopatra	19.00 ab	23.99 ab	4.00 bc	10.00 de	9.67 abcde	19.00 ef
	S. Citrumelo	20.00 ab	26.33 ab	9.67 d	11.33 ef	10.33 bcde	15.00 cdef
	Mihowase/S. Citrumelo	17.33 ab	17.00 ab	4.67 c	3.00 bc	12.67 cde	14.00 cdef
	Primosole/S. Citrumelo	15.33 a	19.00 ab	15.00 g	17.33 h	0.33 a	1.67 ab
	W. Murcott/S. Citrumelo	20.33 ab	26.67 ab	8.00 d	12.67 f	12.67 cde	14.00 cdef
	Coefficient of Variation (CV)		22.65		11.4		25.2

*Means with the same letter are not statistically different with the Duncan test ($P < 0.05$)

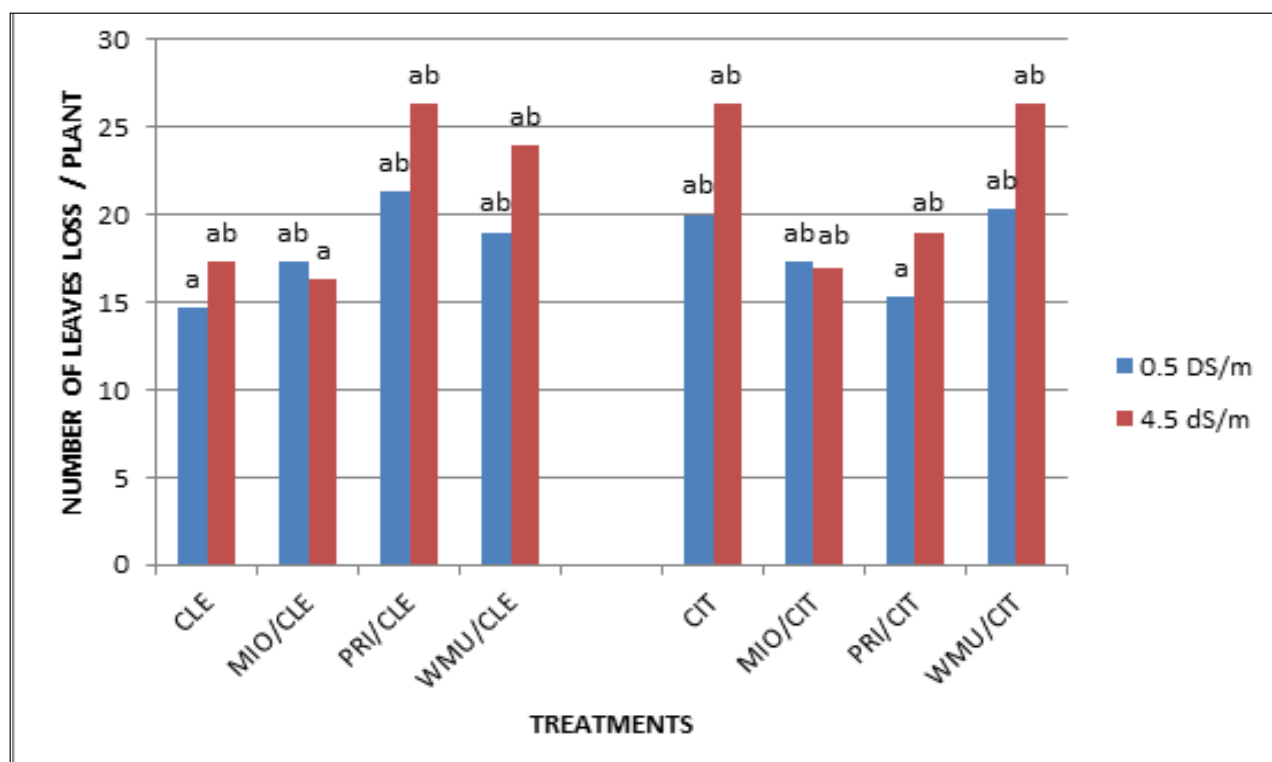


Figure 1. Effects of salt stress on leaves loss / plant of each scion/rootstock combination at 90 days. *CLE (‘Cleopatra’), MIO (‘Mihowase’), PRI (‘Primosole’), WMU (‘W. Murcott’), CIT (‘Swingle Citrumelo’).

Table 2. Effects of salt stress on plant dry weight and plant water content for each scion/rootstock combination.

Variety/pattern	Weight part of the plant (g)		Total Plant		Stem+Leaves		Roots		Stem+Leaves/Roots		Water content in plant	
	Electric conductivity (dS/m)		0.5	4.5	0.5	4.5	0.5	4.5	0.5	4.5	0.5	4.5
	Cleopatra	20.8 ab	17.87 a	16.0 a	11.23 a	6.27 a	4.77 ab	2.79 e	2.33 d	18.7 ab	13.7 abcd	
Mihowase/Cleopatra	61.4 f	61.83 f	44.5 gh	17.0 hi	25.17 ijklm	19.13 fg	1.51 abc	1.47 abc	33.33 fghij	30.00 abcd		
Primosole/Cleopatra	47.43 de	45.2 de	41.73 gh	18.0 fg	22.77 ghijklm	22.0 ghijkl	1.13 a	1.14 a	24.4 bcdef	23.63 cdefg		
W.Murcott/Cleopatra	65.77 fg	61.97 f	60.13 jkl	19.0 jk	28.93 mno	27.27 klm	1.29 ab	1.27 ab	36.23 ijk	34.37 ghij		
S. Citrumelo	25.83 abc	23.27 ab	25.87 cd	20.0 bc	11.83 bcd	10.27 abcd	1.29 a	1.19 ab	19.67 abcd	18.57 abcd		
Mihowase/S. Citrumelo	45.43 de	41.93 cde	35.87 de	21.0 de	18.43 efgh	16.53 defg	1.56 abc	1.51 abc	24.37 cdefg	18.57 abd		
Primosole/S. Citrumelo	70.47 g	62.47 f	63.17 kl	22.0 no	34.27 nop	27.2 klm	1.26 a	1.12 ab	35.10 hij	30.37 fghi		
W. Murcott/S. Citrumelo	64.33 fg	49.87 de	41.6 fg	23.0 j	28.2 ghijkl	22.1 klmn	1.31 ab	1.26 ab	33.9 fghij	25.87 defgh		
Coefficient of Variation (CV)	19.3		8.8		16.5		22.2		19.01			

*Means with the same letter are not statistical different at the Duncan test (P<0.05)

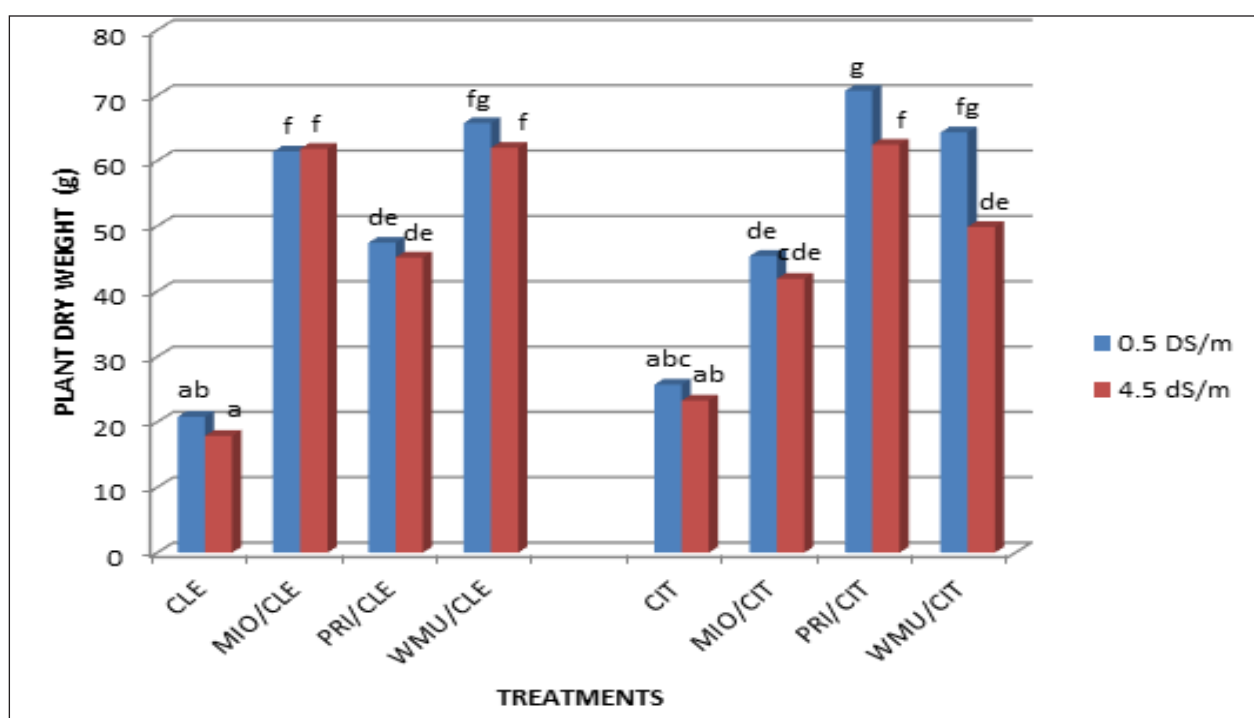
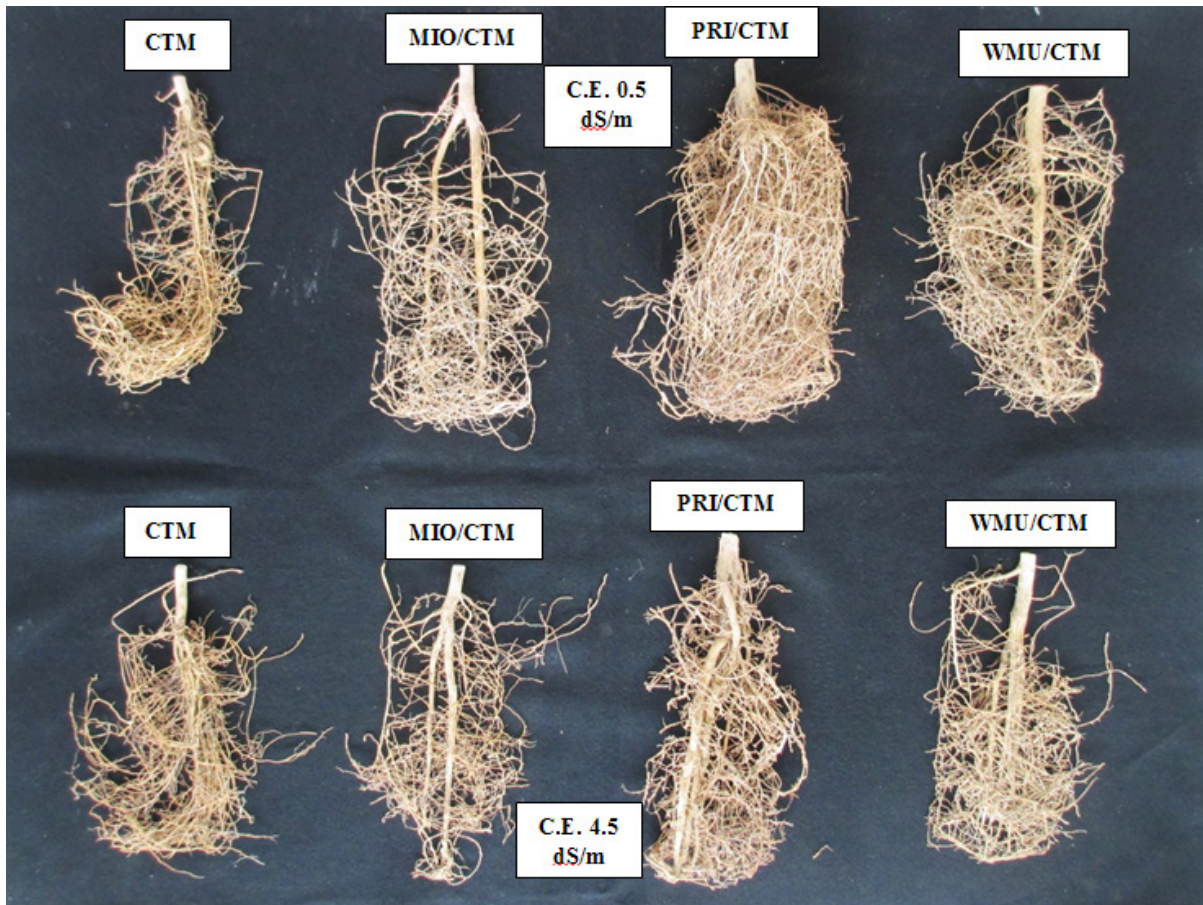


Figure 2. Effects of salt stress on plant dry weight (g) for each scion/rootstock combination at 90 days. *CLE ('Cleopatra'), MIO ('Mihowase'), PRI ('Primosole'), WMU ('W. Murcott'), CIT ('Swingle citrumelo').



*CLE ('Cleopatra'), MIO ('Mihowase'), PRI ('Primosole'), WMU ('W. Murcott'), CIT ('Swingle Citrumelo')
 Figure 3. Effects of salt stress on roots for each scion/rootstock combination at 90 days.



*CLE ('Cleopatra'), MIO ('Mihowase'), PRI ('Primosole'), WMU ('W. Murcott'), CIT ('Swingle Citrumelo')
 Figure 4. Effects of salt stress on roots for each scion/rootstock combination at 90 days.

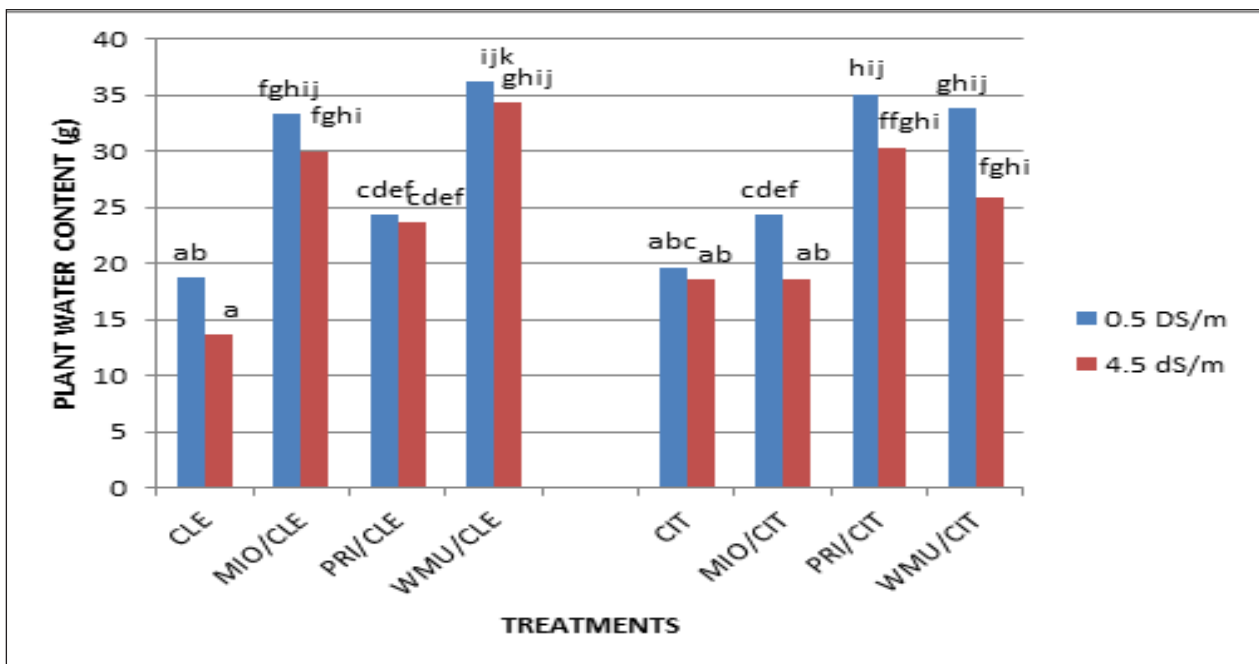


Figure 5. Effects of salt stress on plant water content (g) for each scion/rootstock combination at 90 days. *CLE (Cleopatra), MIO (Mihowase), PRI (Primosole), WMU (W. Murcott), CIT (Swingle Citrumelo).

Discussion

Leaf loss

The results on leaf defoliation showed that the ‘Cleopatra’ rootstock and the three scions grafted in this rootstock presented less leaves loss than in ‘Swingle citrumelo’ rootstock and the three scions in this rootstock. “Rangpur lime” and ‘Cleopatra’ mandarin are mentioned as the most salt resistance and ‘Swingle citrumelo’ and trifoliolate orange as the most susceptible. ‘Cleopatra’ resistance to salts is associated with its capacity to limit the accumulation of Cl^- in leaves. This response of leaves loss was most significant at the first 45 days treatment. Similar results have been observed when the plants were exposed to the salinity treatment from the first day and they were not applied gradually to allow plant adaptation to the new conditions (Simpson et al., 2014). The cultivar ‘Mihowase’ as scion grafted on both rootstocks reduced more leaves loss than the other two cultivar used as scions. Similar results have been reported where the effects of the scion grafted on a rootstock can be linear or quadratic depending on the rootstock (Brito, 2014). The defoliation observed on the older leaves, more than 2 months old, did not show symptoms of injury like leaf bronzing and/or leaf tip yellowing probable because the leaves had less than 0.7 % Cl^- dry weight; minimum level of Cl^- in leaves showed up visible symptoms (Ferguson & Grattan, 2005).

Plant dry weight

The presence of salts reduced total plant dry weight in all treatments and this reduction on plant biomass was less on the rootstocks ‘Cleopatra’ and its scion combinations than on the rootstock ‘Swingle citrumelo’ and its scion combinations. This effect of biomass reduction was higher on leaves and stems than on roots. Both rootstocks and their scion combinations presented similar leaves and stem dry weight loss, however the rootstock ‘Cleopatra’ and its scion combination presented less mass loss than the rootstock ‘Swingle citrumelo’ and lower leaves plus stem/root ratio. The decrease of shoot/root ratio is a common response to salt stress, related

to factors associated with water stress (osmotic effect) and constitute a typical mechanism of plant resistance under saline conditions. Similar results have been reported about the effects of salinity on plant mass reduction on different scion/rootstocks combination on orange or grapefruit (Brito et al. 2014; Garcia et al. 2016) but there are lack of information on mandarin. Much attention has been dedicated to understand adverse effects of Na^+ and Cl^- on morphological, physiological, and biochemical processes on citrus and how these ions contribute to plant growth inhibition. Chlorophyll content, net CO_2 assimilation rate, transpiration and stomata conductance significantly decrease in response to salinity. The salt-tolerant ‘Cleopatra’ was less affected on these physiological parameters than the salt-sensitive ‘Swingle citrumelo’ (Mahmoud et al., 2020). ‘Cleopatra’ mandarin is using a donor of salt tolerance in traditional breeding programs, because it possesses the three mechanism of salt tolerance in citrus; chloride exclusion, water saving and accumulation of soluble solids (Garcia et al., 2016; Rodrigues et al., 2019; Mahmoud et al., 2020).

Plant water loss

The two rootstocks, ‘Cleopatra’ mandarin, ‘Swingle citrumelo’, and the scions grafted in these rootstocks presented water loss at 90 days. The ‘Swingle citrumelo’ and the scions grafted on this rootstock showed 48.2 % more water loss than the ‘Cleopatra’ rootstock and the scions grafted in it. Similar results have been presented in previous research on orange (Navarro, 2010) but there are still missing information on plant dehydration on mandarin cultivars and interaction with different rootstocks combinations. Plants dehydration under salinity stress presented lower root hydraulic conductance, leaf and stem water potential, decrease in stomata conductance, leaf ultrastructure disorganization, and photosynthesis decline, due to more difficulty in taking up water from the soil and salt accumulation (Acosta et al., 2017; Simpson et al., 2014). Specific mechanism for salt avoidance, has been suggested for salt – tolerant ‘Cleopatra’ mandarin, like minimization of salt entry in the plant, decrease

of salt concentration in the cytoplasm of the cell (compartmentalization in vacuoles), and accumulation of proline, organic and inorganic solutes that reduces cellular osmotic potential (Ziogas et al., 2021). Proline is widely used in traditional breeding programs to transfer salt tolerant from ‘Cleopatra’.

Conclusions

The combination of mandarin scion/rootstocks showed similar growth and biomass responses to water salinity as previously reported on orange and grapefruit. ‘Cleopatra’ mandarin and the scions grafted in it presented more salt tolerant than ‘Swingle citrumelo’ in all traits under evaluation like: leaves loss, plant dry weight loss, canopy/root ratio decreases and plant water loss. The response to salinity of the scion/rootstocks combinations were also influenced by the scion with being more notorious in the amount of feeding roots. Mandarin agronomic practices should take in consideration the combination scion/rootstock.

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Author contributions

Elaboration and execution, development of methodology, conception and design; editing of articles and supervision of the study have involved all authors.

Conflicts of interest

The signing authors of this research work declare that they have no potential conflict of personal or economic interest with other people or organizations that could unduly influence this manuscript.

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