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Characterization in Greenhouse Conditions of Two Salt Tolerant Citrumelo (*Citrus paradisi* Macf. x *Poncirus trifoliata* (L.) Raf.) Cultivars

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Abstract: Increasing salinity in Mediterranean soils and the wide spread of citrus tristeza virus have challenged the use of sour orange (*Citrus aurantium*) and have accelerated the process of seeking alternative rootstocks. In the present study, nine cultivars of citrumelo (*Citrus paradisi* Macf. x *Poncirus trifoliata* (L.) Raf.) were evaluated for salt tolerance. Two month-old seedlings were raised under greenhouse conditions and irrigated with a half strength Hoagland solution supplemented with different concentrations of NaCl, *i.e.*, 0 mM, 35 mM and 85 mM. Tolerance was assessed after two months of stress by measuring stem growth, number of leaves, fresh and dry weight organs and leaf water, chlorophyll and chloride contents. A differential behavior was noticed among the seedlings we studied. When using increased concentration of salt in irrigation water, all the parameters were significantly reduced except for leaf chloride content which highly increased in response to stress. At 85 mM, the cultivar SC2 showed a high tolerance resulting in less apparent leaf symptoms, higher growth and higher leaf chlorophyll content when compared to other seedlings. Similarly, the cultivar C4475-C was shown to be a strong root chloride excluder with less than 2.6% DW (dry weight) chloride accumulation at leaf level. By contrast, our results suggest that C4475-A and C4475-B are salt sensitive cultivars regarding to all the parameters studied while the other citrumelos were considered as moderately tolerant.

Key words: *Citrus*, rootstock, salinity, growth, NaCl, chloride, screening.

1. Introduction

Among the environmental factors which can limit successful production and/or yield of crops worldwide, salinity is considered to be one of the most important along with water deficit. Salinity represents a serious threat for salt-sensitive crops such as *Citrus* sp. [1-3]. In Morocco, almost 35% of irrigated land is considered as salt-affected [4, 5]. Moreover, the irrigation water from aquifers can often contain excessive amounts of soluble salts (Cl^- and/or Na^+) which may raise the electrical conductivity up to 3 dS/m, the critical level for citrus production [6]. In studies carried out in Morocco we showed a high correlation between soil salinity and severity of gummosis on sour orange (*Cirus aurantium*) caused by *Phtophthora* sp. [5, 7, 8]. We also observed that increasing salinity inirrigation water predispose sour orange and troyer citrange (*Citrus sinensis* × *Poncirus trifoliata*) to root rot caused by *Phytophthora prasitica* by specific effect of Cl^-.

The detrimental effects of salinity in citrus were widely reported and have been frequently related to the toxic effect of Cl^- ions [3]. Indeed, it is well

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established that high leaf Cl^- concentrations due to root zone salinity may lead to physiological disturbances and eventually growth and yield reduction [9-11]. However, it is also known that citrus species differ widely in their ability to restrict Na^+ and Cl^- uptake at root level and the translocation of these toxic ions from roots to shoots [3, 12, 13]. Oppenheimer [14] was the first to report the effect of the rootstock in salt-tolerance of citrus species. His works have shown that mature orange trees on sour orange rootstock accumulated less Cl^- in the scion leaves than trees did on Palestine sweet lime (*C. limettioides* Tan.). Later on, studies carried out on different rootstocks have shown Rangpur lime (*Citrus limonia* Osbeck), Sunki mandarin (*Citrus sunki* Hort. ex Tan.) and Cleopatra mandarin (*Citrus reshni* Hort. ex Tan.) are salt-tolerant species, while trifoliate orange (*Poncirus trifoliata* (L.) Raf.) and its hybrids such as Carrizo and Troyer citranges (*Citrus sinensis* (L.) Obseck x *P. trifoliata* (L.) Raf.) were ranked as salt-sensitive [12, 13, 15-19]. However, few studies have directly compared the performance of *Citrus* and trifoliate orange hybrids under saline conditions. Citrumelos, for example, which are hybrids of trifoliate orange and grapefruit (*Citrus paradisi* Macf. x *Poncirus trifoliata* (L.) Raf.), were largely overlooked as potential rootstocks, until superior performance of Swingle citrumelo was demonstrated in field trials in the 1940's [20, 21]. Since then, Swingle citrumelo has become a popular rootstock in many areas. The current success of citrumelo cultivars can be generally attributed to their many desirable characteristics such as tolerance to *Phytophthora* spp., exocortis and particularly to tristeza disease [20]. Nevertheless, the works of Garnsey et al. [22] reported a high tolerance of some citrumelo cultivars to CTV (citrus tristeza virus) but the response was CTV strain-dependant. In this sense, Grisoni et al. [23] investigated the resistance of different rootstocks to a severe strain of CTV and found that citrumelo 1452 may have a moderate to susceptible reaction.

Nowadays, the recourse to germplasm banks and the management of genetic resources such as those offered by citrumelo rootstocks are necessary considering the critical current situation of citrus in the Mediterranean region. Indeed, the recent spread of quick-decline isolates of CTV in the Mediterranean basin have limited the use of sour orange which has been historically the most utilized rootstock in this area. The latter provides a wide soil adaptability and superior horticultural performance, notably under stressed conditions [24, 25]. Thus, new sources of CTV tolerance with similar or better salinity tolerance than sour orange are needed.

In the present study, the authors investigated the tolerance of nine citrumelo accessions to salinity by using a fast standardized and reproducible screening test in order to appraise their suitability in salt-affected soils.

2. Materials and Methods

2.1 Plant Material and Growth Conditions

The experiment was carried out at the Regional Center for Agricultural Research in Kenitra (Morocco) during the season 2011-2012. Ten rootstock cultivars belonging to the germplasm collection of INRA (National Institute for Agricultural Research) Kenitra, and including nine citrumelo accessions (*Citrus paradisi* Macf. x *Poncirus trifoliata* (L.) Raf.) were investigated for their properties of salt stress tolerance (Table 1). Rangpur lime, which is known to be a salt-tolerant rootstock [26] was also included in the experiment to accurately estimate the tolerance of the other rootstocks.

Healthy mature fruits of all rootstocks were harvested in the experimental fields of the institute. Seeds were extracted, washed and air-dried in shade, then germinated in 60 × 40 cm trays filled with peat. The experiment was carried out during the late summer in a greenhouse when temperature ranged from 25 °C to 40 °C and relative humidity varied between 40% and 60%.

Table 1 List of the rootstock cultivars used in the experiment.

Rootstock accession	Origin	ICVN[a] or SRA[b]	Code
Citrumelo 4475 AB6A4	SRA INRA/Cirad Corse	ICVN 0110140/SRA 732	C4475-B
Citrumelo 4475 B2G3	SRA INRA/Cirad Corse	ICVN 0110145/SRA 928	C4475-C
Citrumelo 4475 BB6A5	SRA INRA/Cirad Corse	ICVN 0110141/SRA 733	C4475-A
Citrumelo 5798502	CRC Riverside		C502
Citrumelo 5798506	CRC Riverside		C506
Sacaton citrumelo B230057	SRA INRA/Cirad Corse	ICVN 0110144/SRA 843	CS
Citrumelo winter Haven B231431	SRA INRA/Cirad Corse	ICVN 0110147	CWH
Swingle Citrumelo 741	CRC Riverside		SW2
Swingle Citrumelo F92255	CRC Riverside		SW1
Rangpur lime	CRC Riverside		RL

[a]International citrus variety numbering.
[b]Agronomical research station numbering.

After two months of growth, uniform seedlings presenting 8 to 10 leaves were uprooted from the nursery and transferred into 0.5 L plastic pots in a mixture of peat and sterilized sand at 1 : 1 ratio [27]. The seedlings were then irrigated regularly twice a week using a half-strength Hoagland solution [28]. Each plant received 100 mL.

2.2 Application of Saline Treatment

Salt stress treatments were carried out for seven weeks. Salt stress was applied by supplementing the nutrient solution with NaCl at two different concentrations, 35 mM and 85 mM respectively. To avoid osmotic shock, salt was added gradually by three-day intervals until reaching desired levels. Control plants were watered only with half strength Hoagland solution. The 100 mL we used allowed leaching of the saline solution from the pot and avoiding salt accumulation.

2.3 Evaluation of Salt Tolerance

2.3.1 Estimation of Leaf Injury

The response of the seedlings to salt stress was determined by recording the occurrence of symptoms of leaf injury after seven weeks. All seedlings were visually evaluated and a 0-6 score was given to each plant according to the scale of Goell [29]. The score was given on the basis of the severity of injury symptoms, i.e., chlorosis, wilting and defoliation.

2.3.2 Growth Measurement and Number of Leaves

Stem height and the number of leaves were measured for each plant at initial time (H_i, L_i) and after seven weeks of saline treatments (H_f, L_f). SGR (stem growth rate) and the PRNL (percent reduction of the number of leaves) were estimated from these parameters according to the following equations:

$$SGR = \frac{Hf - Hi}{Hi} \quad (1)$$

$$PRNL = \left[\frac{dL_{control} - dL_{treated}}{dL_{control}} \right] \times 100 \quad (2)$$

Where dL is the difference between the final number and the initial number of leaves.

At the end of the experiment, plants were harvested and divided into roots, stems and leaves for biomass determination. Fresh weigh of each part was immediately measured, whereas dry weight was determined after oven-drying tissue at 60 °C for 48 h [30].

2.3.3 Physiological Analyzes

After seven weeks of treatment, leaf chlorophyll Content was estimated using a portable chlorophyll meter (SPAD)-502 device (Minolta, Osaka, Japan). chloride were extracted from dry leaf tissue using hot water and determined by titration according to the method of Cotlove [31], whereas *LWC* (leaf water

content) was calculated from LFW (leaf fresh water) and LDW (leaf dry water) weights as follows:

$$LWC = \left[\frac{LFW - LDW}{LDW} \right] \times 100 \quad (3)$$

Most of the parameters listed above were estimated relatively to control using RP (relative percentage) and PR (percentage of reduction):

$$RP = \left(\frac{Treated}{Control} \right) \times 100 \quad (4)$$

$$PR = \left(\frac{Control - Treated}{Control} \right) \times 100 \quad (5)$$

2.4 Experimental Design and Statistical Analysis

The experiment was carried out in a split-plot design with six replications by rootstock and treatment. The salinity factor was placed in the main plot and the rootstock factor in subplot. Collected data were transferred to SAS software and subjected to analysis using a two-way ANOVA. Means were separated by Duncan's multiple range test.

3. Results and Analysis

3.1 Effect of Salt Stress on Leaf Injury

Leaf symptoms of damages were observed in all treated plants 30 to 45 days after the beginning of the experiment. These symptoms began generally with necrosis at leaf tips then progressed inward towards petioles. It noted also that injury began at lower leaves and thereafter progressed to upper leaves.

Based on statistical results, a clear difference regarding the salt tolerance was observed depending on seedling cultivars and salt levels. At 85 mM NaCl, most of C4475-B seedlings showed severe necrosis and defoliation symptoms which was reflected by an average SSI (symptom severity index) of 5.8 (Table 2), whereas the occurrence of injured leaves was much lesser in Rangpur lime which showed the lowest SSI (4.2). By contrast, control plants showed no salt stress symptom throughout the treatment period. The average SSI ranged at these conditions from 1 to 1.5 and no significant difference was found among cultivars. Using 35 mM NaCl solution, an intermediate response was found in all cultivars we tested. However,

Table 2 Severity of leaf injury symptoms according to the scale of Goell (1969).

Rootstocks	Symptom severity index[a]		
	Control	35 mM	85 mM
C4475-A	1.5[a]	3.3[a]	5.3[ab]
C4475-B	1.5[a]	3.3[a]	5.8[a]
CWH	1[a]	2.3[b]	4.7[ab]
SC1	1[a]	3.5[a]	5.5[ab]
CS	1.5[a]	3.5[a]	5.0[ab]
SC2	1.3[a]	3.5[a]	4.5[ab]
C502	1.2[a]	3.8[a]	4.8[ab]
C506	1.2[a]	3.7[a]	5.3[ab]
C4475-C	1.3[a]	3.5[a]	5.5[ab]
RL	1.3[a]	3.0[ab]	4.2[b]
Analysis of variance[b]			
R		***	
T		*	
R × T		NS	

[a]Means followed by the same letter in same rows do not differ significantly at $P \leq 0.05$ (one-way-ANOVA, separated by Duncan test).

[b]The factors R and T refer respectively to rootstock and treatment. Significant effects are indicated by * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$, and NS indicates not significant difference.

at this salt concentration, CWH was found to be more tolerant than Rangpur lime.

3.2 Effect of Salt Stress on Growth and Number of Leaves

NaCl caused a significant reduction in all growth parameters we considered. As shown in Figs. 1 and 2, a significant decline in stem growth was found with increasing salt concentration in the irrigation water. A high genotypic difference was also found between seedlings in their response to salinity although the interaction rootstock × salt treatment was not significant.

As compared to their respective controls, seedlings of SC2 showed the greatest tolerance at both salt treatments, whereas those of C4475-A and C4475-B were the most sensitive. For instance, under 35 mM treatment, SRGR (stem relative growth rate) values were respectively 96%, 58% and 57% for SC2, C4475-B and C4475-A. The corresponding values at 85 mM NaCl were 52%, 31% and 24%.

Fig. 1 Effect of salt stress on growth of *Rangpur lime* (a) and *Sacaton citrumelo* (b) seedlings. (T0) Control; (T1) 35 mM NaCl; (T2) 85 mM NaCl.

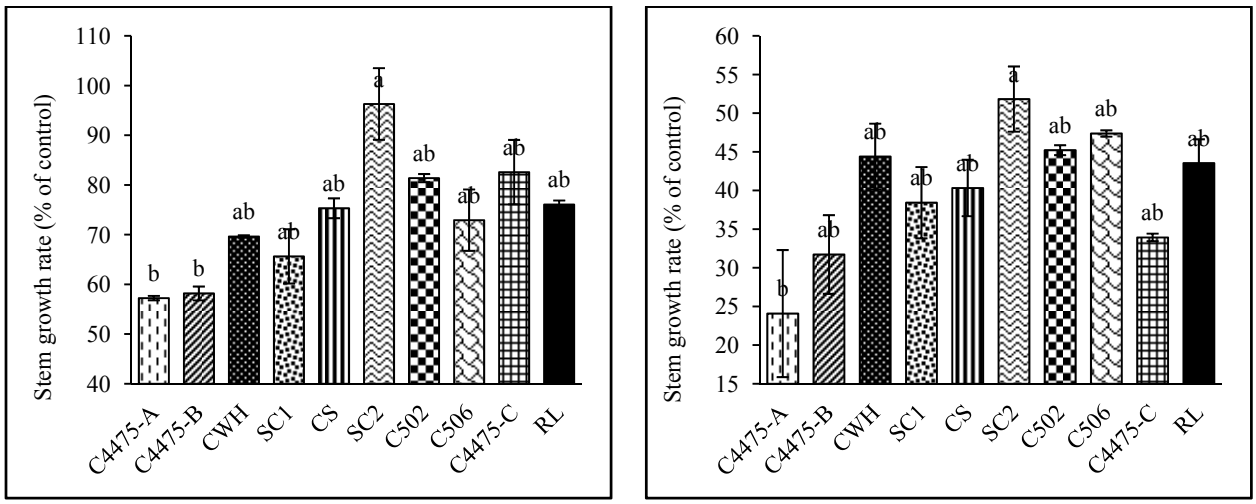

Fig. 2 Effect of salt treatments on stem growth rate in the ten rootstocks studied expressed as % of control plants. (a) 35 mM NaCl; (b) 85 mM NaCl. Means represented by the same letter do not differ significantly at $P \leq 0.05$ (one-way-ANOVA, separated by Duncan test). Vertical bars indicate the mean values ± SE ($n = 6$).

ANOVA analysis showed that both plant FW (fresh weight) and *DW* (dry weight) were significantly decreased in response to salt stress but the impact was more or less important depending on the cultivar (Table 3). Also, the reduction in biomass was quite variable depending on plant organ. Indeed, a reduction gradient was observed at high salt concentration which could be summarized as following from the most affected to the least affected: leaves > roots > stem.

Among rootstocks, SC2 showed less biomass reduction at whole plant level. Relatively to its control, this rootstock displayed a 40.2%, 16.9% and 19.2% reduction in fresh leaf, stem and root weight respectively and a 9.5%, 19.5% and 22.8% reduction in dry leaf, stem and root weight. However, at low salinity level, RL seedlings were found to be more tolerant than SC2 seedlings resulting in no fresh weight reduction at stem level (-1%), 4.1% reduction in stem dry weight and 4.3% reduction in leaf fresh weight. The corresponding values for SC2 were 11.6%, 17.8% and 19.3% respectively. By contrast, the highest reduction in fresh and dry biomass was observed in C4475-A and C4475-B cultivars whatever the organ studied.

The number of leaves also considerably declined in response to high salt stress ($P < 0.001$). However, no significant difference was found among cultivars at 35 mM NaCl (Fig. 3). By contrast, at high salt concentration (85 mM), the comparison of PRLN (percent reduction of the number of leaves) means for the different cultivars studied revealed the presence of three statistically different groups:

Group 1, which included C4475-C that showed more than 250% reduction in the number of leaves when compared to control;

Group 2, composed of C.4475-B and C506 cultivars that showed a moderate reduction ranging from 150% to 200% relatively to control;

Group 3, that included all other seedling cultivars for which the values of PRNL were less than 150%.

Table 3 Effect of salt treatments on fresh and dry biomass expressed relatively to control values. (T1) 35 mM NaCl; (T2) 85 mM NaCl.

Rootstock	Fresh weight[a] (% lower than control)						Dry weight[a] (% lower than control)					
	Leaves		Stem		Roots		Leaves		Stem		Roots	
	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
C4475-A	18.7^{a}	55.4^{a}	14.0^{ab}	35.7^{ab}	29.7^{a}	49.0^{a}	16.8^{a}	39.3^{ab}	34.7^{ab}	46.7^{a}	34.0^{abc}	51.9^{a}
C4475-B	22.9^{a}	63.1^{a}	27.9^{ab}	46.9^{a}	34.7^{a}	45.0^{a}	21.2^{a}	41.2^{ab}	32.2^{ab}	39.6^{abc}	39.3^{ab}	48.1^{ab}
CWH	34.0^{a}	48.4^{a}	36.8^{a}	47.1^{a}	38.9^{a}	41.5^{a}	27.2^{a}	29.2^{ab}	39.2^{a}	42.2^{ab}	41.6^{a}	43.6^{ab}
SC1	21.0^{a}	51.3^{a}	3.6^{b}	20.5^{b}	22.1^{a}	39.9^{ab}	1.7^{a}	21.4^{ab}	14.4^{bc}	25.7^{bc}	25.8^{abc}	44.3^{ab}
CS	24.5^{a}	49.2^{a}	13.0^{ab}	32.7^{ab}	25.2^{a}	41.9^{a}	10.6^{a}	24.7^{ab}	18.8^{abc}	32.8^{abc}	26.9^{abc}	43.7^{ab}
SC2	19.3^{a}	40.2^{a}	11.6^{ab}	16.9^{b}	15.9^{a}	19.2^{b}	5.5^{a}	9.5^{b}	17.8^{abc}	19.5^{c}	18.3^{c}	22.8^{c}
C502	27.5^{a}	51.5^{a}	22.4^{ab}	31.4^{ab}	23.4^{a}	41.6^{a}	18.5^{a}	31.4^{ab}	30.3^{ab}	35.7^{abc}	28.5^{abc}	43.4^{ab}
C506	21.2^{a}	53.5^{a}	17.5^{ab}	34.3^{ab}	14.2^{a}	40.3^{ab}	13.3^{a}	32.0^{ab}	30.6^{ab}	41.0^{ab}	23.3^{bc}	46.7^{ab}
C4475-C	16.6^{a}	50.1^{a}	17.7^{ab}	28.5^{ab}	21.5^{a}	27.9^{ab}	5.4^{a}	24.6^{ab}	27.1^{ab}	31.8^{abc}	24.3^{abc}	30.9^{bc}
RL	4.3^{a}	40.1^{a}	-1.0^{b}	33.9^{ab}	24.9^{a}	40.5^{ab}	7.6^{a}	44.3^{a}	4.1^{c}	34.3^{abc}	25.2^{abc}	42.0^{ab}
Analysis of variance[b]												
R	***		***		***		***		***		***	
T	NS		*		*		NS		**		**	
R × T	NS		NS		NS		NS		NS		NS	

[a]Means followed by the same letter in same rows do not differ significantly at $P \leq 0.05$ (one-way-ANOVA, separated by Duncan test).

[b]The factors R and T refer respectively to rootstock and treatment. Significant effects are indicated by * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$, and NS indicates not significant difference.

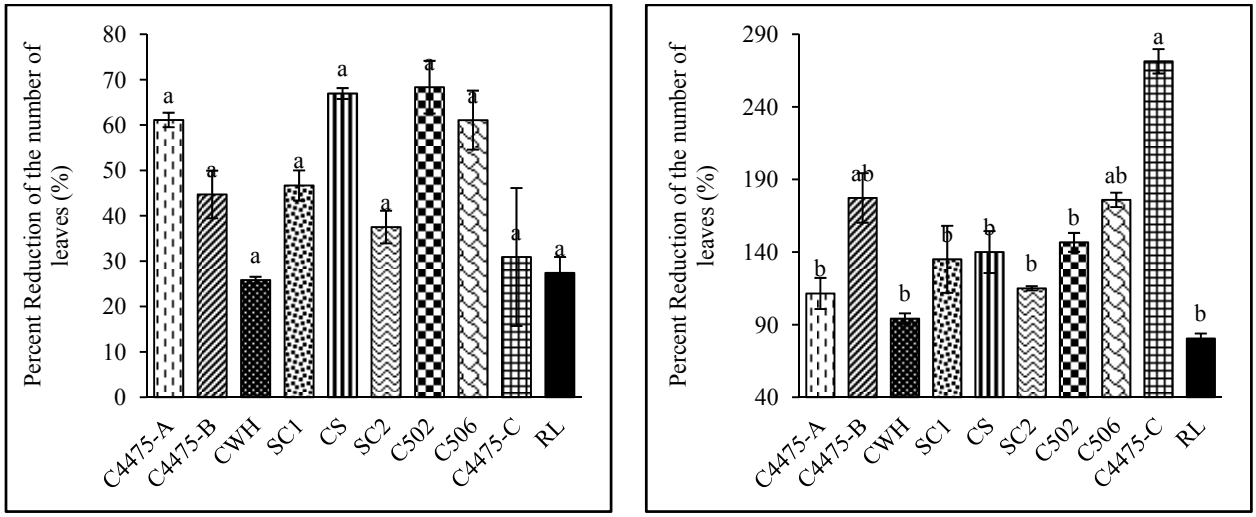

Fig. 3 Reduction in the number of leaves in response to salinity expressed relatively to control. (a) 35 mM NaCl; (b) 85 mM NaCl. Means represented by the same letter do not differ significantly at $P \leq 0.05$ (one-way-ANOVA, separated by Duncan test). Vertical bars indicate the mean values ± SE ($n = 6$).

3.3 Effect of Salt Stress on Leaf Physiological Traits

3.3.1 Water Content

Fig. 4 shows the changes in LWC (leaf water content) with respect to salt treatments. At 35 mM NaCl, almost all genotypes maintained more than 80% LWC as compared to their respective controls, in contrast to 85 mM NaCl treatment which caused an important desiccation of leaves. However, the leaves of the cultivar SC1 showed a considerable reduction of water content even at low salinity (68%). ANOVA results revealed that both rootstock and salt treatment factors had significant effects on LWC ($P < 0.01$) as well as their interaction ($P < 0.05$). The authors should also note that RL showed a different behavior than other rootstocks tested, resulting in a slight increase in LWC (succulence) under salt stress compared to control condition. The relative LWC values for the latter were 105% and 109% respectively under 35 mM and 85 mM NaCl treatments.

3.3.2 Chlorophyll Content

The LCC (leaf chlorophyll content) patterns in response to salt stress were similar to the ones of LWC (Fig. 4). At both salt concentrations, RL performed better than all citrumelo cultivars. However, when using a moderate salinity treatment, we noted a much greater tolerance of C4475-C and SC2 cultivars which reached respectively 90% and 85% the control values of LCC. By contrast, C4475-A, CWH, CS and C502 cultivars showed the lowest values at this level (respectively 76%, 72%, 77% and 77%). At 85 mM NaCl, differences were higher. For instance, RL resulted in 79% LCC of the control, which corresponds to three fold the average value obtained in the most sensitive cultivar, C4475-B (25%). The authors should note also that SC2 maintained higher leaf chlorophyll content in high salt stress condition even though it was not significantly different from other cultivars according to Duncan's multiple range test.

3.3.3 Chloride Content

The concentration of Cl^- in leaves extracted after seven weeks of treatment was significantly ($P < 0.001$) increased under saline conditions (Table 4). Indeed, control seedlings of all genotypes showed low levels of leaf Cl^- content which ranged from 0.72% to 1.37% DW, whereas the seedlings treated with 85 mM NaCl showed an accumulation of Cl^- in their leaves ranging from 2.48% to 3.22% DW which indicates a difference of three to four fold between the two treatments.

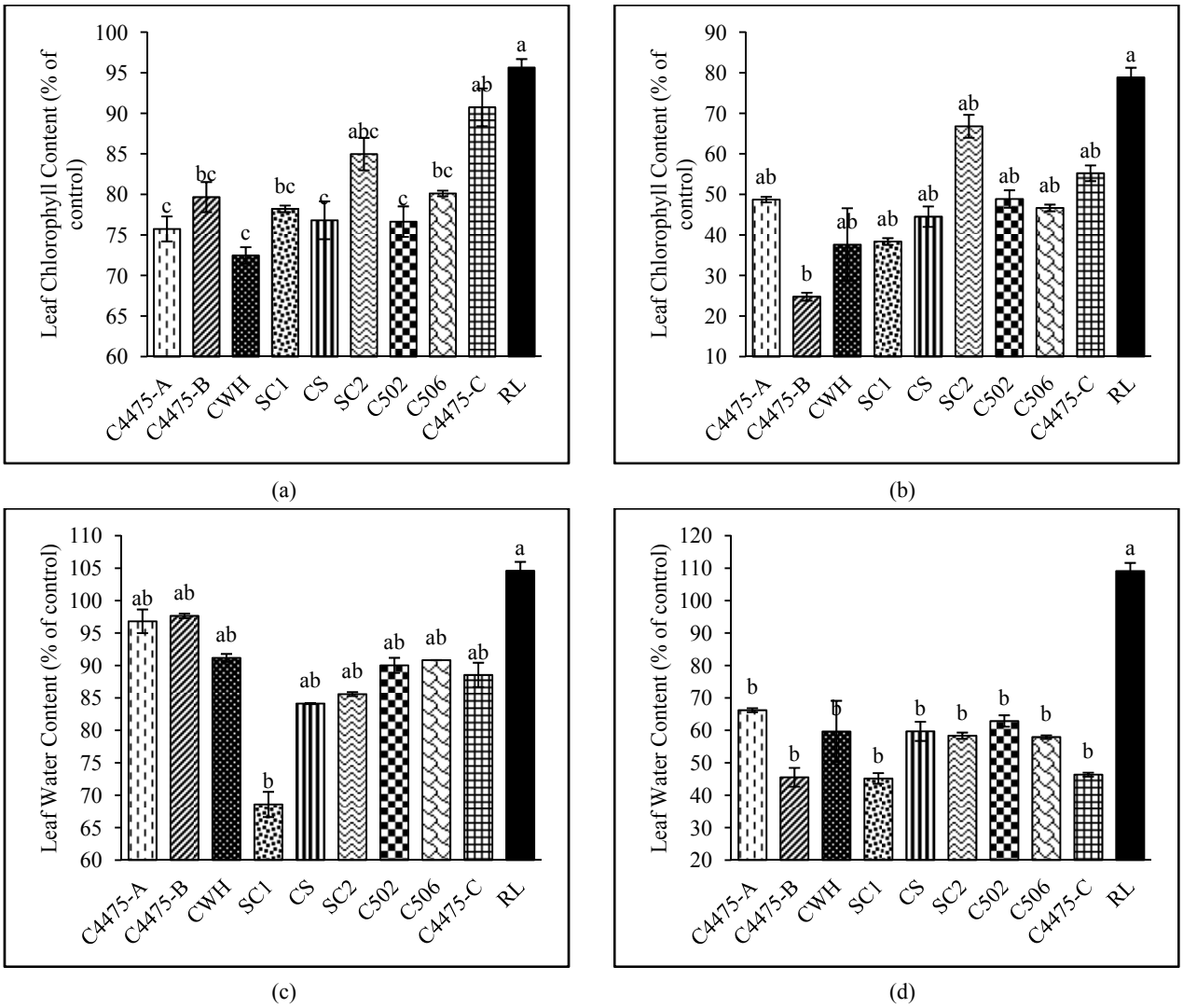

Fig. 4 Changes in leaf water and chlorophyll contents expressed relatively to control values in response to saline treatments. (a, c) 35 mM NaCl. (b, d) 85 mM NaCl. Means represented by the same letter do not differ significantly at $P \leq 0.05$ (one-way-ANOVA, separated by Duncan test). Vertical bars indicate the mean values ± SE ($n = 6$).

Similarly to the other traits studied, the comparison among the seedlings for leaf chloride contents reveals significant differences depending on the salt treatment concentration that was applied. As shown in the Table 4, many cultivars groups were identified using Duncan's multiple range test. Generally, RL and C506 seedling cultivars exhibited a lower leaf Cl^- accumulation compared to other cultivars whatever the condition is, whereas the greatest accumulation was observed for CWH. The authors should note also that C506 showed the highest leaf accumulation of Cl^- when exposed to high salt level (85 mM), although this cultivar showed intermediate values at low salinity (35 mM).

4. Discussion

Salt present in the irrigation solution considerably affected seedling growth and physiology in all the cultivars even at low concentration. Salt stress symptoms are related to cellular toxicity and manifest as chlorosis, leaf tip burn and defoliation. Such symptoms have been reported in earlier studies and have been associated with the accumulation of toxic ions such as chloride, sodium and boron in plant tissue [10]. Cl^- was reported to be the most harmful element

Table 4 Effect of saline treatments on the accumulation of chloride ions in leaves of the ten rootstocks studied expressed as % of dry weight.

Rootstocks	Leaf chloride content[a] (%DW)		
	Control	35 mM	85 mM
C4475-A	1.37[a]	1.82[bc]	3.00[ab]
C4475-B	0.93[bc]	2.19[ab]	2.70[bcd]
CWH	1.05[b]	2.46[a]	3.13[a]
SC1	0.94[bc]	2.21[ab]	2.91[abc]
CS	0.93[bc]	2.05[abc]	2.95[abc]
SC2	0.78[c]	2.19[ab]	2.88[abc]
C502	0.84[bc]	1.70[bc]	2.91[abc]
C506	0.83[bc]	1.96[abc]	3.22[a]
C4475-C	0.77[c]	1.93[bc]	2.58[cd]
RL	0.72[c]	1.63[c]	2.48[d]
Analysis of variance[b]			
R	***		
T	***		
R × T	*		

[a]Means followed by the same letter in same rows do not differ significantly at $P \leq 0.05$ (one-way-ANOVA, separated by Duncan test).

[b]The factors R and T refer respectively to rootstock and Treatment. significant effects are indicated by * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$, and NS indicates not significant difference.

for leaves [32, 33]. In the present study, RL, which was used as a reference seedling, maintained low Cl^- content in leaves and obviously showed less toxicity symptoms. Conversely, SC2 exhibited the least toxicity symptoms among citrumelo cultivars although its leaves accumulated moderate amounts of Cl^-.

Simultaneously to symptoms, a considerable growth inhibition was observed which was reflected in decreased plant height and biomass yield. Growth suppression was more apparent in some cultivars such as C4475-A and C4475-B conversely to SC2 and RL which showed respectively the greatest tolerance. Previous works suggest that there are many hypotheses to explain growth inhibition under salt stress conditions. Most of these reports agree that growth reduction may be attributed to Cl^- and Na^+ inhibitory effects [34] and to disturbance in physiological processes of the plant such as photosynthesis and gas exchange [32, 35, 36]. In our case, both hypotheses can be accepted as we found similar patterns for growth inhibition under saline conditions to the ones observed for leaf Cl^- accumulation on one hand and to the decrease in number of leaves, leaf water content and leaf chlorophyll content on the other hand, given that these last three effects may inevitably affect gas exchange and photosynthetic processes [37].

The decrease in chlorophyll content under salt stress conditions has been for long time a controversy for researchers. Different reasons were given, but the most probable is the suppression of specific enzymes that are responsible for chlorophyll biosynthesis and the reduction in magnesium, iron and manganese [38, 39]. On the other hand, the reduction in water content was widely reported and had been described as a consequence of a water imbalance between the apoplast and symplast that leads to turgor decrease, which in turn may cause growth reduction [40]. However, many reports have indicated that tolerant species can adjust their osmotic potential when subjected to salt stress through the accumulation of soluble compounds known as osmolytes and/or osmoprotectants [41, 42]. This hypothesis could be valid for RL which maintained high water content and

simultaneously showed high growth rate and biomass yield at the end of the experiment. It is also important to note that seedlings of the same rootstock proved to be successful in maintaining high levels of proline under salt stress as shown by the findings of Balal et al. [43].

CWH and C506 accumulated much more leaf Cl^- than the other cultivars, whereas the least accumulation was observed in the salt-tolerant rootstock RL followed by C4475-C. These data support previous reports that have shown that RL is an efficient Cl^- excluder [12, 17, 44, 45]. The mechanism of salt exclusion was reported by many authors as an active and energetic process that occurs in the roots and involves molecular synthesis, enzyme induction and membrane transport [46, 47]. According to Storey and Walker [3], Cl^- accumulation in citrus may regulate genes involved in Cl^- membrane transporters. Primary candidate genes for Cl^- transport regulation are, for instance, the recently identified CCC (cation-Cl^- cotransporter) family [48] and members of the CIC (Cl-channel) family [47]. This might be a plausible explanation when analyzing the behavior of the cultivars in which the difference in tolerance was found to be related to the buildup of Cl^- ions in the leaves such as RL and C4475-A. However, the cultivar SC2 showed a remarkable tolerance to salinity, which was even greater than that of RL in terms of growth, although it accumulated high amounts of Cl^- at leaf level. This observation suggests the presence of other tolerance mechanisms involved in growth recovery under salt stress besides Cl^- exclusion. Indeed, many strategies operating at cellular, molecular and whole plant levels and contributing to minimizing osmotic stress or ion disequilibrium or alleviating the consequent secondary effects caused by salt stress were described in previous reports. Among all, compartmentalization of toxic ions in the vacuole appears to constitute the most effective way for cells to handle efficiently high concentrations of salts and prevent their toxic effects in the cytoplasm [49, 50]. Furthermore, other contributory features may enhance salinity tolerance as well such as the osmotic adjustment mechanism described above and/or the regulation of Na^+ entry and translocation in plant tissue [51, 52]. The study of Gonzalez et al. [53], for example, have shown a higher capacity for Na^+ sequestration in root tissue vacuoles of Swingle citrumelo than in Rangpur lime, which could be a rational explanation for our results.

5. Conclusions

The short-term salt stress experiment we performed showed many differences regarding the behavior of tolerance of the citrumelo cultivars we studied. For instance, although Swingle citrumelo is reported to be moderately salt tolerant when grown in the field [20], our results showed that in pots, some specific accessions of this rootstock such as SC2 may exhibit a much higher tolerance to salinity.

In addition, the differences of the cultivars in response to stress were discerned even at low salt concentration. Those results were consistent with findings of previous studies employing older plant material, which proved the effectiveness of our screening test. Therefore, the authors may think of generalizing this test for seeking, in a limited space and time, other sources of tolerance in citrus species or in collections of hybrids obtained through breeding programs. To end the tolerant genotypes we identified could then be used in further experiments for evaluating their compatibility and performance in association with different scion cultivars.

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