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Investigating salt tolerance in citrus rootstocks under greenhouse conditions using growth and biochemical indicators

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ABSTRACT

Citrus is ranked among the most sensitive crops to salinity. This constrainst affects plant morpho-physiology and may lead to yield declines. To assess the effects of salinity on some physiological and biochemical traits, an in vivo screening test was performed under controled saline conditions using different citrus rootstocks i.e. citrumelo 57-98-502, Swingle citrumelo F9-22-55 (80-11), citrumelo 57-98-506, Swingle citrumelo 74-1, citrumelo Winter Haven B2, Carrizo citrange 28608, Troyer citrange C35B6A11, Troyer citrange B2 31655, citrumelo 4475 B2G3, citrumelo 4475 B B6A5, citrumelo 4475 A B6A4, citrumelo Sacaton 30057, Gou-Tou SRA 506, Volkamer lemon B2 28613 andTroyer citrange.Plants were grown on a sand substratum and subjected to three salt treatments including 0 (control), 2 and 5 g.l-1 NaCl during 90 days. Physiological responses to salt stress were evaluated at the end of this period. Results showed that all studied parameters were affected by salinity. High salt concentrations caused a considerable reduction of growth parameters such as fresh and dry weights of shoots and roots, especially in citrumelo 57- 98-502, Swingle citrumelo swingle F9-22-55 (80-11), Carrizo citrange 28608 andcitrumelo 4475 BB6A5. In some rootstocks such as citrumelo 4475 B2G3, citrumelo 4475 B B6A5 and citrumelo 4475 A B6A4, these changes were associated witha decrease in leaf chlorophyll content. In addition, we noted a significant accumulation of proline in the leaves of rootstocks as the salinity of the irrigation solution increased, particularly in citrumelo 4475 A B6A4 and citrumelo Sacaton 30057. We concluded that these osmolytes may play a key role in Sentivity or tolerance of citrus rootstocks to salinity

Key words: citrus, rootstock, salt stress

INTRODUCTION

Soil salinity is a major environmental stress causing important losses of crop yields worldwide. This problem is more frequent in the arid and semi-arid regions (Munns, 2002) where citrus is grown extensively and where salt concentrations in soils and irrigation waters are high enough to inhibate the development of this crop (Maas, 1993 ; Story, 1998)

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Salinity is known to limit plant growth and productivity (Abbas et al., 2010 ; Bhantana and Lazarovitch, 2010 ; Siringam et al., 2012) and influence plant physio-biochemical processes such as photosynthetic rate (Hayat et al., 2010), transpiration rate (Cambolle et al., 2011), stomatal conductance (Perez-Perez et al., 2009), the rational use of water (Grewal, 2010) and sugars (Noreen and Achraf, 2009). Citrus species are particularly sensitive to salts (Maas, 1993). However, there is a great variability in the ability to tolerate salinity depending on the rootstock used (Levy et al., 1999) and the grafted variety (Lloyd et al., 1990). The most used rootstock in the mediterranean region, including Moroccan orchards, is sour orange (Citrus aurantium) due to its wide adaptability to soil types, its better affinity with most commercial varieties and its good resistance to Phytophthora gummosis (Loussert, 1989; Benyahia, 2004). But under salt condition the resistance of this rootstocks to Phytophthora gummosis and root rot are affected (Benyahia et al 2004; Benyahia, 2007). Nevertheless, the sensitivity of many citrus varaitie grafted on Citrus aurantium or sour orange to Tristeza – considered as the most destructive viral disease of citrus – is a big threat for citrus industry in this area. Therefore, the search and development of new sources of salt tolerance has became a big priority for citrus research programs nowadays (Ollitrault et al., 2000; Benyahia 2007, Benyahia et al., 2011).The most detrimental effects of saline soils are due to the presence of chloride ions which are assimilated by the rootstock (Maas, 1993). The selection of tolerant rootstocks to salinity is generally made on the basis of their ability to exclude chloride and Sodium ions (Ream and Furr, 1976 ; Walker, 1986) accumulated in their foliage (Levy and Syvertsen, 2004 ; Maas, 1993). Moreover, the tolerance can be expressed by a satisfactory tree growth and fruit yield under saline conditions (Castle et al., 1993). The aim of the present experiment is to study the physiological and biochemical responses and identify possible indicators to assess and/or predict the degree of tolerance of fifteen citrus rootstocks when grown at saline conditions.

Material and Methods

Plant material and growth conditions

The experiment was conducted in a greenhouse located at El Menzeh Experimental Station of the National Institut for Agricultural Research in Kenitra (Morocco). Mature healthy 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 53x53 cm trays filled with peat. 300 ml of water was given every day after to each tray. After two months of growth, uniform seedlings of approximately 10 cm length with 4 to 6 leaves were uprooted from the nursery and transferred into 0.5 L plastic pots in a mixture of sterilized sand at 1:3 to 2:3 ratios. These seedlings were then placed in a greenhouse under high temperature and irrigated twice a week with a half-strength Hoagland solution (Hoagland and Arnon, 1950). 100 ml was given to each pot.

Salt treatment:

The salt chosen for this experiment is sodium chloride. This choice is based on the fact that NaCl is the most frequent salt in soils and irrigation waters of the Gharb region where citrus is grown extensively (Fetouhi, 1981; Fekhaoui, 1993; Benyahia, 1998, 2007). Salt treatment was applied two weeks after transplanting the seedlings (time needed for adaptation to the new environmental conditions) and consisted in the addition of NaCl to a half-strength Hoagland solution (Hagland and Arnon, 1950) at different concentrations (0 g/l (control), 2 g/l and 5 g/l). The salt was added gradually (during one week) to the irrigation solution until reaching final concentrations of 2 and 5 g/l to ensure a better seedling adaptation to stress conditions and avoid osmotic chock. Irrigations were performed twice a week in addition to a water leaching that was applied once in two weeks to avoid salt accumulation. The experimental design used was a split-split-plot with three replications. This design consisted of three treatments and three blocks (replications) with the treatment factor in the main plots and the rootstock factor in the subplots.

Analysis of tolerance indicators

Morphological parameters :

The changes in morphological aspects of the experimental seedlings were regularly observed from the start of treatment to the end of the experiment. Indeed, foliar damage, height and relative growth were used as indicators to assess salinity tolerance. We took particularly consideration of plant organ formation and the apparition of toxicity symptoms. Our main objective was to discern the impact of salt on plant development and note the changes that would be associated to salt stress tolerance.

Intensity of chlorosis:

The resistance to foliar damage, as a selection criterion, is based on the fact that the absence of chlorosis is usually indicative of the plant ability to exclude salts and therefore is a characteristic of tolerant genotypes. The intensity of toxicity symptoms was evaluated according to the scale established by Goell (1969).

Growth measurements:

Tip growth was estimated by the calculation of linear growth rate (LGR) as follows:

LGR= (Hf -Hi)/ Hi

Where Hi and Hf refers respectively to initial and final seedling height.

Growth rate was expressed relatively to control as following:
RGR = 100 × (LGR treated/ LGR control)

Estimation of biomass production:

After harvesting, plants were divided into leaves, stems and roots. Roots were rinsed with running water and dried using filter paper. Each plant part was then put in a bag and weighed before and after oven-drying at 80°C for 48 hours to determine the fresh and dry weights. The salinity tolerance was estimated by determining the relative reduction percentage of fresh and dry weights (% of control) which is an accurate indication of the plant relative vigor under salt stress conditions.
% Reduction = 100 x (Control – Treated) / Control)

Biochemical parameters

Leaf proline and soluble sugars contents were determined for collected seedling samples of each treatment and each rootstock genotype.

Determination of chloride content:

Chloride were extracted from dry leaf tissue using hot water and determined by titration according to the method of Cotlove (1965).

Determination of proline content:

Proline was determined according to the method of Monneveux and Nemmar (1986). The absorbance was then measured at 528 nm and obtained values were expressed in mg.g-1 DW using the following equation:
Y = 0,1043 × X Where X is the optical density.

Determination of soluble sugar content:

We used the method of Dubois et al. (1956). The optical density is measured at 585 nm. The obtained values were expressed in mg.g-1 DW using the equation of the standard curve: Y=4,3918 × (X – 0,194). Where Y refer to total soluble sugar content and X refer to the absorbance.

Statistical Analysis

Collected data were statistically analyzed by ANOVA method using the General Linear Models of SAS software. 0.05 was accepted as a significant probability (Steel et al., 1997). To stabilize variances, proportional data (growth rate) were transformed using the arcsine squareroot method (Gomez and Gomez, 1984; Sokal and Rohlf 1995). Duncan's multiple range test was also used to discern significant differences between means. Simple correlation coefficients between studied variables were developed using the same statistical software.

Results

Effect of salt stress on growth parameters

NaCl caused a significant reduction in all growth parameters we considered (P<0,001). The reduction was more evident at higher NaCl concentration (5g/l). Indeed, shoot and root fresh and dry weights, plant height were gradually decreased as the concentration of NaCl increased in the irrigation solution (Table 3, 4 and 5).

Effect of salt stress on leaf injury

The monitoring of pathological symptoms shows that the severity of the foliar toxicity differs depending rootstocks and increases in conjunction with the concentration of salt stress. The statistical results showed a great variation of response among the genotypes studied which was reflected by a significant effect (P < 0,001) of the factor 'Rootstock' on all parameters. At 2 g/l NaCl concentration, the leaves of the genotypes citrumelo 57-95-502 (1), citrumelo 4475 B B6A5 (F12) and citrumelo 4475 A B6A4 (F13) showed a slight chlorosis and yellowing

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

Rootstock	Code (INRA)	Origin
Citrumelo 57-98-502	1	CRC Riverside
Swingle citrumelo F9-22-55 (80-11)	2	CRC Riverside
Citrumelo 57-98-506	3	CRC Riverside
Swingle citrumelo 74-1	4	CRC Riverside
Citrumelo Winter Haven B2	F1	SRA INRA/Cirad Corse
Carizo citrange 28608	F7	SRA INRA/Cirad Corse
Troyer citrange C35B6A11	F8	SRA INRA/Cirad Corse
Troyer citrange B2 31655	F9	SRA INRA/Cirad Corse
Citrumelo 4475 B2G3	F11	SRA INRA/Cirad Corse
Citrumelo 4475 B B6A5	F12	SRA INRA/Cirad Corse
Citrumelo 4475 A B6A4	F13	SRA INRA/Cirad Corse
Sacaton citrumelo 30057	F14	SRA INRA/Cirad Corse
Gou-Tou SRA 506	F23	SRA INRA/Cirad Corse
Volkamer lemon B2 28613	F25	SRA INRA/Cirad Corse
Troyer citrange (Témoin Maroc)	F33	INRA Morocco

symptoms starting at tips and around edges. By contrast, the leaves of citrumelo 4475 B2G3 (F11), Sacaton citrumelo 30057 (F14), Gou-Tou SRA 506 (F23) and volkamer lemon B2 286131 (F25) remained healthy and maintained a light green color. When NaCl concentration was raised to 5g/l of irrigation solution, some genotypes showed a pronounced leaf chlorosis, namely citrumelo 57-98-502 (1), Swingle citrumelo F9-22-55 (80-11) (2) and citrumelo 4475 A B6A4 (F13) or a complete chlorosis as it was the case for citrumelo 4475 B B6A5 (F12) (Table 2).

Table 2: Effect of salt stress on symptom severity

Rootstocks	Intensity of chlorosis		
	0g/l NaCl	2g/l NaCl	5g/l NaCl
1	1,0 a	2,3 ab	3,7 ab
2	1,0 a	2,7 a	3,5 abc
3	0,7 ab	0,5 cde	2,7 bcde
4	0,3 b	0,0 e	1,5 de
F1	0,2 b	0,3 cde	2 cde
F7	0 b	0,2 de	2 cde
F8	0 b	0,2 de	2,2 bcde
F9	0 b	0 e	1,3 e
F11	0 b	1 abcde	3 abcd
F12	0,2 b	2 abc	4,3 a
F13	0 b	1,8 abcd	3,5 abc
F14	0 b	1,3abcde	2,2 bcde
F23	0 b	0,8 bcde	1,7 de
F25	0,3 b	1,3abcde	2 cde
F33	0 b	0,3 cde	1,3 e

Means followed by the same letter in the same rows do not differ significantly at P≤0,05 (0ne-way ANOVA, separated by Duncun test).

Effect of salt stress on linear growth

As shown in Table 3, maximum reduction was observed in the rootstocks citrumelo 4475 B B6A5 (F12) and Troyer citrange (F33). By contrast, we noted that the genotypes Sacaton citrumelo 30057 (F14), Swingle citrumelo 74-1 (F4) and citrumelo Winter Haven B2 (F1) reached similar growth rates to their respective controls when subjected to the 2 g/l NaCl treatment. Nevertheless, when NaCl concentration was increased to 5 g/l, Swingle citrumelo F9-22-55 (80-11) (F2) and Swingle citrumelo 74-1 (F4) were the genotypes which maintained the maxiumum relative growth when compared to control. Fresh and dry biomass were significantly (P < 0,001) decreased in response to saline treatments in all rootstocks (Table 4 and 5) with a higher impact of the 5 g/l NaCl treatment. However the interaction between 'rootstock' and 'salt treatment' factors was non-significant. In general, the maximum reduction was found in citrumelo 57-98-502 (F1), Swingle citrumelo F9-22-55 (80-11) (F2), Carrizo citrange 28608 (F7) and citrumelo 4475 B B6A5 (F12), whereas the minimum reduction was shown in citrumelo 4475 A B6A4 (F13).

Effect of salt stress on chloride content

The application of salt stress caused a significant accumulation of chloride ions in plant organs, which is more or less evident depending on salt concentration increases in the irrigation solution (Table 6). For roots and leaves, the greatest accumulation was found in Troyer citrange C35B6A11 (F8), wheres for stems, the genotypes citrumelo 4475 B B6A5 (F12), citrumelo 4475 A B6A4 (F13) and Volkamer lemon B2 28613 (F25) resulted in the highest values. In contrast, leaf and root chloride concentrations were the lowest in the genotypes Gou-Tou 506 (F23), Swingle citrumelo 74-1 (F4) and citrumelo Winter Haven B2 (F1), whatever the stress level applied.

Effect of salt stress on proline and soluble sugar contents

Saline treatments induced a considerable proline accumulation in the leaves of all rootstocks except for citrumelo 4475 B B6A5 (F12) (Table 7). A higher accumulation was observed under the 5 g/l NaCl treatment than the 2 g/l NaCl treatment. According to the results shown in table 8, maximum values were recorded in volkamer lemon B2 28613 (F25), followed by Troyer citrange (F33) and Sacaton citrumelo 30057 (F14). In contrast, the genotype citrumelo 4475 B B6A5(F12) resulted in the lowest values. Similarly to proline, soluble sugars acccumulated in the leaves of all studied rootstocks in response to salt stress (Table 7). Maximum concentrations were shown in citrumelo 4475 A B6A4(F13) while minimum concentrations were found in citrumelo 57-98-502 and Gou-Tou SRA 506 (F23).

Discussion

Table 3: Effect of salt stress on linear growth

Rootstocks	Linear Growth Rate (%)			Relative Linear Growth Rate (% of control)	
	0g/l NaCl	2g/l NaCl	5g/l NaCl	2g/l NaCl	5g/l NaCl
1	54,84bc	57,34abcde	47,19bcd	88,02 ab	75,20bcd
2	63,07abc	56,31abcdef	63,25a	97,27 ab	96,18a
3	68,48a	60,06abcd	57,61ab	85,65 b	83,06bc
4	66,97a b	64,48a	60,21a	104,18 a	90,08ab
F1	62,08abc	62,95ab	56,24abc	99,94 ab	81,65bc
F7	58,37abc	50,64cdef	42,45de	90,83 ab	71,15bcd
F8	58,04abc	51,89bcdef	46,70bcde	89,07 ab	75,80bcd
F9	57,95abc	55,16abcdef	43,29de	90,57 ab	77,02bcd
F11	57,57abc	57,09abcde	43,05de	95,37 ab	71,24bcd
F12	57,26abc	49,13def	34,25e	85,01 b	60,61d
F13	51,70c	45,18f	44,93cde	91,37 ab	57,77ab
F14	58,26abc	56,95abcde	44,52cde	104,34 a	55,97bcd
F23	51,14c	46,40ef	43,19de	90,48 ab	53,18bc
F25	67,41ab	61,07abc	56,80abc	96,13 ab	61,71bc
F33	51,75c	47,15ef	47,19e	91,26ab	43,64cd

**Means followed by the same letter in the same rows do not differ significantly at P≤0,05 (0ne-way-ANOVA, separated by Duncun test).*

It is a well-established fact that plants growing on a saline medium remain stunted due to the reduction of cell elongation and cell division and that both of those processes are controlled by auxins which synthesis is delayed by salinity (Loreto et al, 2003;. Ndayiragije and Lutts, 2006). In the present study, the genotypes citrumelo 57-98-506, Swingle citrumelo 74-1 and Volkamer lemon B2 28613 maintained a relatively high growth under saline conditions and consequently can be described as salt tolerant, by contrast to the genotype citrumelo 4475 B B6A5 which proved to be salt sensitive. These results are consistent with the findings of Fadli et al. (2014) which also ranked Swingle citrumelo 74-1 as salt tolerant and citrumelo 4475 B B6A5 as salt sensitive among several citrumelo accessions. In a previous study carried out on sour orange, Ruiz et al. (2001) attributed the decrease in growth to the osmotic effect of salt stress. In our case, we noted that treated plants maintained approximatively an equal water content to that of control plants which suggest a possible osmotic adjustment. According to Walker et al. (1981), one of the main reasons of growth reduction is the decrease in photosynthetic ability due to the loss of turgor. However, this fact was not valid for sour orange in which growth reduction was not related to the decrease in turgor (Fernandez-Ballester et al., 1998). Another finding of the present study was the inhibitory effect of salinity on biomass yield as shown in all rootstocks studied. This effect was more pronounced at high salt concentration and may be explained by disturbances in physiological and biochemical activities under saline conditions (Craine, 2005 ; Munns et al., 2006) which may arise due to the reduction in leaf area and loss of foliage (Romero Aranda et al., 1998 ; Dong et al., 2007).

According to many authors, chloride uptake is the most reliable criterion for evaluating NaCl induced damage and ranking citrus rootstocks with respect tosalinity tolerance (Chapman, 1968 ; Zekri et al., 1992 ; Cooper et al., 1961).In our study, leaf analysis revealed a clear accumulation of chloride ions which increased with increasing salinity level. In addition,the comparison among rootstocks investigated have shown Troyer citrange as a chloride accumulator and obviously a salt sensitive rootstock, which is consistent with the findings of Levy and Shamhevet (1990). Similar results were also reported by Bañuls et al. (1990) who attributed the severity of leaf damage, abscission and photosynthesis disturbances in this rootstock to chloride uptake. Furthermore, it was established that NaCl toxicity is frequently associated to high leaf chloride amounts (Walker et al., 1984).

Similarly to chloride, proline and soluble sugars accumulated in response to salt stress with different rates depending on genotype and salt concentration. Among the rootstocks studied, the greatest proline accumulation was observed in Volkamer lemon B2 28613 and Troyer citrange, whereas the lowest accumulation was observed in citrumelo 4475 BB6A5. On the other hand, Troyer citrange B2 31655, citrumelo 4475 B2G3 and citrumelo 4475 A

Figure 1: Appearance of citrus rootstock seedlings after two months of treatment

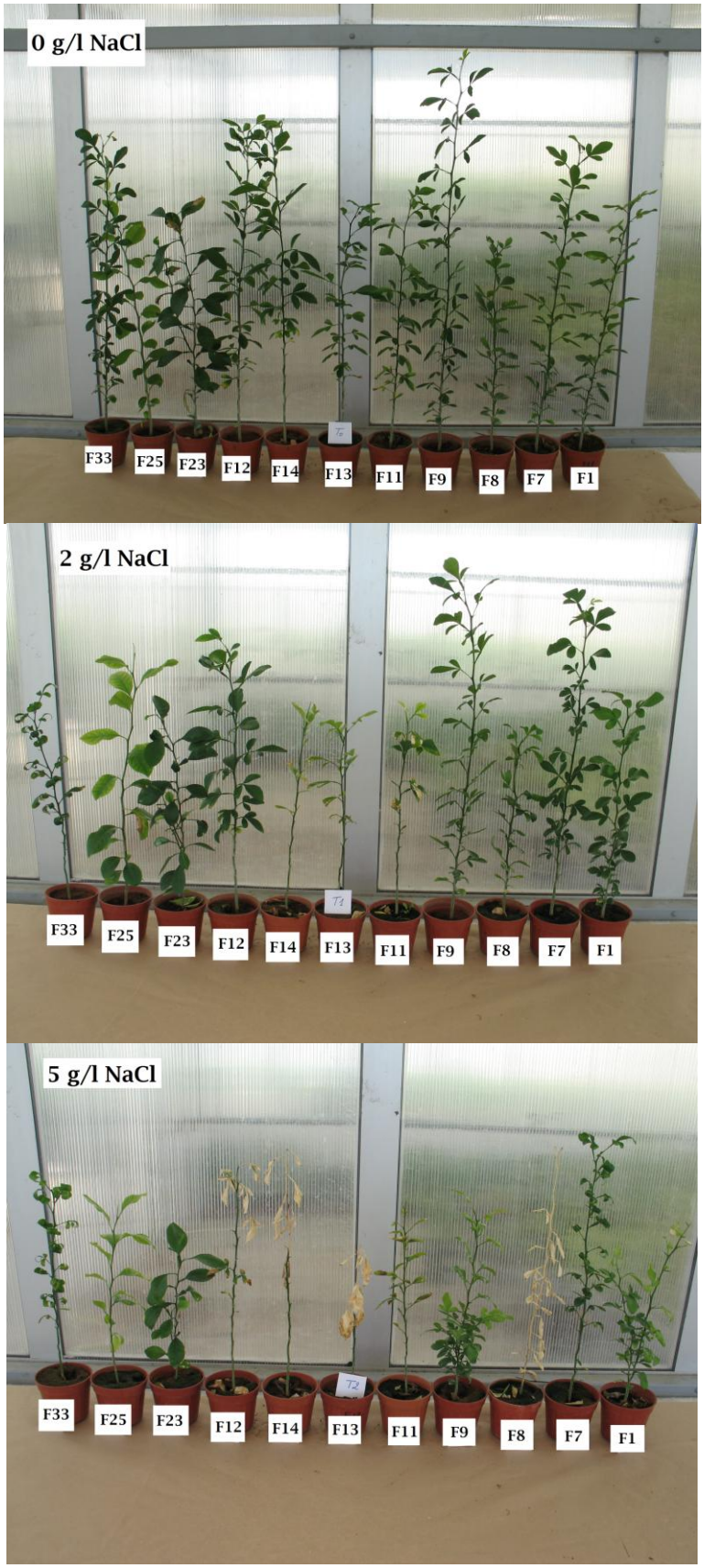

Table 4: Variations in plant fresh weight in response to salt treatments

Root stocks	Leaves (g)			Stem (g)			Roots (g)		
	0g/l NaCl	2g/l NaCl	5g/l NaCl	0g/lNaCl	2g/l NaCl	5g/l NaCl	0g/lNaCl	2g/lNaCl	5g/l NaCl
1	4,049cde	2,975def	1,523efg	5,309a	3,495cde	2,264cd	6,499 ab	4,267 ab	2,727de
2	4,041cde	2.360f	1.800def	5,591a	3,834cde	4,108ab	5,674 ab	4,506 ab	3,986bcd
3	3,012 f	3,014def	1,675efg	5,023a	4,869abcd	3,679abc	4,950 bc	6,253 a	4,454abcd
4	3,290 ef	2,754ef	2,029cdef	2,211b	1,824e	1,442d	2,182 c	2,113 b	1,504e
F1	4,706bcd	3,931bcde	3,070abcd	6,286a	6,071abcd	4,432ab	7,579 ab	6,253 a	4,647abc
F7	3,870cdef	3,925bcde	2,705abcde	6,089a	5,738abcd	3,827abc	8,082ab	6,056 a	2,916cde
F8	3,773def	4,462abc	2,130cdef	4,938a	4,992abcd	3,652abc	6,173ab	7,150 a	3,781bcd
F9	4,599bcd	4,870ab	3,356abc	6,837a	7,184a	4,170ab	7,005ab	6,079 a	4,404abcd
F11	4,608bcd	3,246cdef	1,244fg	6,975a	6,347abc	4,023abc	7,975ab	6,510 a	4,230abcd
F12	4,460bcd	3,221cdef	0,489g	6,190a	4,983abcd	2,667bcd	7,043ab	4,136 ab	2,975cde
F13	3,375ef	4,292abcd	2,273bcdef	5,253a	6,706ab	4,190ab	6,122ab	6,900 a	4,925ab
F14	4,532bcd	2,484f	2,387abcdef	7,795a	5,154abcd	4,539a	8,781a	5,707 a	5,723a
F23	6,833a	5,395a	3,668a	5,147a	4,513bcd	2,879abcd	5,791ab	5,275 a	3,253bcd
F25	5,271b	4,509abc	3,519ab	7,615a	4,864abcd	3,331abc	6,630ab	6,293 a	4,604abc
F33	4,819bc	4,276abcd	2,685abcde	7,001a	5,392abcd	4,202ab	8,797a	6,749 a	3,547bcd

**Means followed by the same letter in the same rows do not differ significantly at P≤0,05 (0ne-way-ANOVA, separated by Duncun test).*

Table 5: Variations in plant dry weight in response to salt treatments

Roots tocks	Leaves (g)			Stem (g)			Roots (g)		
	0g/lNaCl	2g/l NaCl	5g/l NaCl	0g/l NaCl	2g/l NaCl	5g/l NaCl	0g/l NaCl	2g/lNaCl	5g/lNaCl
1	3,653ced	1,500f	0,570de	2,658ab	1,491 cd	0,902ef	2,367bc	1,684ab	0,997c
2	2,622fg	1.765ef	1.262bcde	3,934a	1,817bcd	1,867abcd	3,257ab	1,607ab	1,492abc
3	1,970g	2,332bcdef	0,489^{e}	2,987ab	2,473abcd	1,720abcd	2,351bc	2,517a	1,641ab
4	2,992ef	2,145abcdef	1,499abc	1,162b	0,818 d	0,548f	0,899c	0,784b	0,430d
F1	4,333bc	3,566ab	1,663ab	4,454a	1,909bcd	2,019abc	4,735a	1,636ab	1,652ab
F7	3,516cdef	3,243abcd	1,166bcde	3,463a	2,830abc	1,723abcd	3,912ab	2,362a	1,167bc
F8	3,247def	3,186abcd	1,504abc	2,737ab	2,338abcd	1,811abcd	3,028ab	2,345a	1,266bc
F9	3,690cde	3,049abcde	2,241a	3,908a	3,700a	2,435a	3,211ab	2,648a	2,037a
F11	4,034bcd	2,261bcdef	0,711cde	3,666a	3,057abc	1,763abcd	3,420ab	2,772a	1,515abc
F12	3,771cde	2,755bcdef	0,375^{e}	3,457a	2,536abc	1,157def	3,331ab	1,908ab	1,103bc
F13	3,087ef	1,959def	0,459^{e}	2,825ab	3,241ab	1,909abcd	2,502bc	2,637a	1,534abc
F14	4,315bc	2,130cdef	1,416abcd	4,212a	2,575abc	2,074ab	3,718ab	2,324a	1,981a
F23	6,544a	4,307a	1,814ab	2,510ab	2,015abcd	1,222cdef	2,345bc	2,062ab	1,126bc
F25	4,915b	3,469abc	1,844ab	3,631a	2,118abcd	1,363bcde	2,787ab	2,389a	1,685ab
F33	3,299def	3,196abcd	1,833ab	3,754a	2,731abc	1,745abcd	3,719ab	2,685a	1,456abc

**Means followed by the same letter in the same rows do not differ significantly at P≤0,05 (0ne-way-ANOVA, separated by Duncun test).*

B6A4 displayed the maximum leaf sugar content. The accumulation of total soluble sugars is a common phenomenon in conditions of stress (William et al., 2000; Murakeozy., et al, 2003). The ability of proline and soluble sugar production in response to salt stress was reported by many authors (Gomez Cadenas et al.,1998; Garg et al., 2002) and which

Table 6: Effect of salt stress on chloride content

Root stocks	Leaves (g)			Stem (g)			Roots (g)		
	0g/lNaCl	2g/l NaCl	5g/l NaCl	0g/l NaCl	2g/l NaCl	5g/l NaCl	0g/l NaCl	2g/lNaCl	5g/lNaCl
1	51,92a	155,92a	192,34bcde	33,04ab	57,82a	80,24abc	42,48ab	57,82ab	66,08ab
2	38,94bcdefg	113,28bcde	175,82bcde	28,32ab	43,66bc	59,00cde	42,48ab	62,54ab	69,62ab
3	29,50g	100,30cde	169,92cde	29,50ab	37,76cde	54,28de	37,76b	46,02b	53,10b
4	34,22efg	83,78ef	156,94de	29,50ab	41,30bcd	56,52de	47,20ab	44,84b	68,76ab
F1	38,94bcdefg	103,84cde	158,12de	30,68ab	40,12bcde	55,46de	42,48ab	44,84b	50,74b
F7	44,84abcd	123,90bcd	234,82ab	29,50ab	40,12bcde	55,46de	54,28a	61,36ab	75,52ab
F8	47,20ab	127,44abc	258,42a	25,96b	36,58de	51,92e	50,74ab	49,56b	99,12a
F9	36,58cdefg	107,38cde	189,98bcde	25,96b	34,22e	57,82cde	44,84ab	56,64ab	79,06ab
F11	35,40defg	141,60ab	206,50abcde	28,32ab	46,02b	76,70bcd	55,46a	80,24a	87,32ab
F12	34,22efg	95,58cde	212,93abcde	31,86ab	42,48bcd	96,76ab	53,10ab	60,18ab	89,68ab
F13	30,68fg	90,86de	214,76abcd	29,50ab	41,30bcd	100,30a	50,74ab	62,54ab	82,60ab
F14	40,12bcdef	115,64bcde	177,00bcde	30,68ab	44,84b	63,72cde	50,74ab	64,90ab	83,78ab
F23	38,94bcdefg	57,82f	148,68e	35,40a	37,76cde	64,90cde	46,02ab	46,02b	53,10b
F25	42,48bcde	122,72bcd	202,96abcde	35,40a	42,48bcd	92,04ab	49,56ab	55,46ab	67,26ab
F33	46,02abc	156,94a	228,92abc	29,50ab	37,76cde	62,54cde	53,10ab	71,98ab	80,24ab

[a] *Means followed by the same letter in the same rows do not differ significantly at P≤0,05 (0ne-way-ANOVA, separated by Duncun test).*

Table 7: Influence of salt stress on leaf proline and soluble sugar contents

Rootstocks	Proline (µg·g^{-1} FW)			Sugars (mg.g^{-1} FW)		
	0g/l NaCl	2g/l NaCl	5g/l NaCl	0g/l NaCl	2g/l NaCl	5g/l NaCl
1	0,338 ab	0,347 ab	0,289 ab	0,865 ab	0,549 ab	0,493 b
2	0,270 b	0,143 b	0,239 ab	0,903 ab	0,628 ab	0,676 ab
3	0,328 ab	0,330 ab	0,330 ab	1,089 ab	0,758 ab	0,982 ab
4	0,268 b	0,323 ab	0,248 ab	1,892 a	0,490 ab	1,038 ab
F1	0,312 ab	0,327 ab	0,292 ab	0,852 ab	0,908 ab	1,250 ab
F7	0,298 ab	0,331 ab	0,305 ab	0,724 b	0,848 ab	1,061 ab
F8	0,309 ab	0,352 a	0,354 a	1,507 a	0,931 ab	0,670 ab
F9	0,333 ab	0,292 ab	0,347 ab	1,251 ab	0,690 ab	1,332 ab
F11	0,333 ab	0,359 a	0,316 ab	1,275 ab	0,895 ab	1,348 ab
F12	0,332ab	0,369 a	0,087 b	1,028 ab	1,296 a	1,206 ab
F13	0,351 a	0,352 a	0,264 ab	0,960 ab	0,924 ab	1,693 ab
F14	0,360 a	0,342 ab	0,363 a	1,202 ab	0,450 b	2,705 a
F23	0,334 ab	0,335 ab	0,320 ab	0,921 ab	0,768 ab	0,743 ab
F25	0,270 b	0,360 a	0,374 a	1,018 ab	0,855 ab	0,669 ab
F33	0,313 ab	0,352 a	0,374 a	1,273 ab	0,988 ab	1,028 ab

was also demonstrated in our study, and is considered as a salt tolerance reaction (Singh et al., 1973). Indeed, both molecules are thought to play a key role in osmotic adjustment under saline conditions (Ashraf and Harris, 2004). However, many authors agree that most part of this regulation process is ensured by sugars (Popp and Smirnoff, 1999 ; Atienza et al., 2004 ; Mohanty et al., 2002 ; Martino et al., 2003).

CONCLUSION

The analysis of salinity effects in young seedlings of citrus rootstocks based on growth and biochemical indicators showed that all parameters considered were affected after two months of treatment. Moreover, the variability of these effects highlighted a wide range of physiological and biochemical traits which can be used as effective tools for quick assessment of salt tolerance at a large scale. According to the results obtained, citrus rootstocks used do not have the same behavior when subjected to salt stress. Indeed, salinity induced a reduction of chlorophyll content and an accumulation of chloride ions at leaves which seem to be both possible causes of salt sensitivity in citrus rootstocks. Based on this fact, Troyer citrange was ranked as salt sensitive as compared to the other rootstocks. On the other hand, Troyer citrange B2 31655, citrumelo 4475 B2G3 and citrumelo 4475 A B6A4 proved to be salt tolerant by accumulating high amounts of soluble sugars at their leaves suggesting a possible implication of these solutes in osmotic adjustment.

Conflict of Interests:

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

1. Abbas W, Ashraf M, Akram NA 2010. Alleviation of salt-induced adverse effects in eggplant (Solanum melongena L.) by glycinebetaine and sugar beet extracts. Sci Hortic 125:188-195.
2. Ashraf, M. and Harris, P.J.C. 2004. Potential biochemical indicators of salinity tolerance in plants. Plant Science, 166, pp. 3-16.
3. Ashraf, M., M. H. Bokhari and S. N. Chishti. 1992. Variation in osmotic adjustment of accessions of lentil (Lens culinaris Medic.) in response to drought stress. Acta Bot. Netherlands., 41:51-62.
4. Atienza, S. G., P. Faccioli, G. Perrotta, G. Dalfino, W. Zschiesche, K. Humbeck, A. M. Stanca and L. Cattivelli. 2004. Large-scale analysis of transcripts abundance in barley subjected to several single and combined abiotic stress conditions. Plant Sci., 167: 1359-1365.
5. 5. Banuls J., Legaz F. & Primo Millo E., 1990. Effects of salinity on uptake and distribution of chloride and sodium in some Citrus scion-rootstock combinations. J. Hortic. Sci.,65,715-724.
6. Benyahia, H. 1998. Effet de la salinité sur le développement des maladies à Phytophthora des agrumes au Maroc. Thèse de troisième cycle, Université Cadi Ayyad, Faculté des Sciences Semlalia, Marrakech, 170pp.
7. Benyahia, H. 2007. “Amélioration de la Résistance des Porte-Greffes D’Agrumes Vis-à-Vis des Contraintes Biotiques et Abiotiques.” Ph.D. thesis, Mohamed Ben Abdellah University.
8. Benyahia, H., Ait Haddou, M. M., Jrifi, A., and Lamsettef, Y. 2004. “Effet de la Salinité de L’Eau D’Irrigation sur la Colonisation des Racines des Porte-Greffes D’Agrumes par Phytophtora Parasitica.” Fruits 59: 101-108.
9. Benyahia, H., Beniken, L., Omari, F. Z., Bnazzouze, A., Handaji, N., Msatef, Y., and Olitrault, P. 2011. “Evaluation of the Resistance of Few Citrus Rootstocks to Alkalinity by Applying a Faste Test of Screening.” African Journal of Agricultural Research 6 (4): 780-784.
10. Bhantana P, Lazarovitch N 2010. Evapotranspiration, Crop coefficient and growth of two young pomegranate (Punica granatum L.) varieties under salt stress. Agri Water Manage 97:715-722.
11. Cambrolle J, Redondo-Gomez S, Mateos-Naranjo E, Luque T, Figueroa ME 2011. Physiological responses to salinity in the yellow-horned poppy, Glaucium flavum. Plant Physiol Biochem 49:186-194.
12. Castle, W.S., Tucker, D.P.H., Krezdorn, A.H., Youtsey, C.O., 1993. Rootstocks for Florida citrus: Rootstock selection, the first step to success. Second Edition, SP-42. Institute of Food and Agricultural Sciences, Gainesville, FL, p. 92. University of Florida.
13. Chapman H.D., Reuther W., Batchelor L.D. & Weber H.J., 1968. The mineral nutrition of Citrus. In: Reuther W., ed. The Citrus industry. Vol. 2. Berkeley, USA: University of California, 127-289.
14. Cooper, W.C. 1961. Toxicity and accumulation of salts in Citrus trees on various rootstocks in Texas .Proceeding of the Florida State Horticultural Society 74 : 95-104.
15. Cotlove, E. 1965. “Determination of Cl- in Biological Material.” In Methods of Biochemical Analysis, edited by Glick D. New York: Interscience.
16. Craine, J. M. 2005. Reconciling plant strategy theories of Grime and Tilman. J. Ecol., 93:1041-1052.
17. Dong, Y., T. Ji and S. Dong. 2007. Stress responses to rapid temperature changes of the juvenile sea cucumber (Apostichopus japonicus Selenka). J. Ocean Uni. Chin., 6:275-280.
18. Dubois M., Gilles K.A., Hamilton J.K., Rebecs P.A., Smith F. 1956. Colorimetric method for détermination of sugars and related substances. Anal. Chem., 28 (3) : 350-356.
19. Fadli, A., Chetto, O., Talha, A., Benkirane, R., Morillon, R. and Benyahia, H. 2014. Characterization in greenhouse conditions of tolerant citrumelo (Citrus paradisi Macf. x Poncirus trifoliata (L.) Raf.) cultivars. Journal of life sciences, 8, pp. 955-966.

20. Fekhaoui M., Bennasser L., Seigle-Murandi F & Benoit-Guyod J.L. 1993. Water pollution in the Sebou river basin (Morocco) - preliminary result. First Setac World Congress: Ecotoxicology and environmental chemistry - a global perspective. Lisbon, Portugal, March, 28-31.
21. Fernandez-Ballester G., Vicente M., Dionisio R., Antonio C. 1998. Changes in inorganic and organic solutes in Citrus growing under saline stresses. J. Plant Nutr. 21, p. 2497–2514.
22. Fetouhi, A. 1981. Les sols salés dans les O.R.M.V.A. Garg, A. K., J. K. Ownes and R. J. Wu. 2002. Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stress. Proceeding of the National Academy of Sciences of the United States of America, 99:15898-15903.
23. Goell A. 1969. salinity effects on citrus trees. Proc. First. Intern. Citrus Symp. 1969, 3: pp.1819-1824.
24. Gomez KA., Gomez AA. 1984. Statistical procedures for agricultural research (2nd ed.). NewYork: Wiley, p. 272–356.
25. Gomez-Cadenas A., Tadeo F. R., Primo-Millo E., and Talon M. 1998. Involvment of abscisic acid and ethylene in the responses of citrus seelings to salt shock. Physiologia Plantarum, 103: 475-484.
26. Grewal, H.S., 2010. Response of wheat to subsoil salinity and temporary water stress at different stages of the reproductive phase. Plant Soil 330, 100-113.
27. Hayat S, Hasan SA, Yusuf M, Hayat Q, Ahmad A 2010. Effect of 28- homobrassinolide on photosynthesis, fluorescence and antioxidant system in the presence or absence of salinity and temperature in Vigna radiata. Environ Exp Bot 69:105-112.
28. Hoagland, D. R., and Arnon, D. I. 1950. “The Water-Culture Method for Growing Plants without Soil.” California Agricultural Experiment Station Circular 347: 1-32.
29. Levy Y., Lifshitz J., De Malach Y., David Y. 1999. The response of several Citrus genotypes to high salinity irrigation water. Hort. Sci. 34, p. 878–881.
30. Levy, Y. and J. P. Syvertsen. 2004. Irrigation water quality and salinity effects in citrus trees. Hortic. Rev., 30:37-82.
31. Levy, Y., Shamhevet, J. 1990. Ranking the tolerance of citrus rootstocks by juice analysis. Sci. Hortic.45, 89-98.
32. Lloyd J., Kriedemann P.E. and Aspinall D.,1990. Contrast between Citrus species in response to salinisation: an analysis of photosynthesis and water relations for different rootstock-scion combinations. Physiol. Plant., 78, 236-246.
33. Loreto, F., M. Centritto and K. Chartzoulakis. 2003. Photosynthetic limitations in olive cultivars with different sensitivity to salt stress. Plant Cell Environ., 26:595-601.
34. Loussert R. 1989. Les agrumes. Techniques agricoles méditerranéennes, Lavoisier, Paris.
35. Maas E.V. 1993. Salinity and Citriculture, Tree Physiolopgy 12, pp. 195-216.
36. Martino, C. D., S. Delfine, R. Pizzuto, F. Loreto and A. Fuggi. 2003. Free amino acids and glycinebetaine in leaf osmoregulation of spinach responding to increasing salt stress. New Phytol., 158:455-463.
37. Mohanty, A., H. Kathuria, A. Ferjani, A. Sakamoto, P. Mohanty, N. Murata and A. K. Tyagi. 2002. Transgenics of an elite indica rice variety Pusa Basmati1 harbouring the codA gene are highly tolerant to salt stress. Theor. Appl Genet., 106:51-57.
38. Monneveux P.H., Nemmar M., 1986. Contribution à l’étude de la résistance à la sécheresse chez le blé tendre (Triticum aestivum L.) et chez le blé dur (Triticum durum Desf.): Etude de l’accumulation de la proline au cours du cycle de développement. Agronomie, 6 (6) 583–590.
39. Munns R, Richard AJ, Lauchli A, 2006. Approaches to increasing the salt tolerance of wheat and other cereals. Journal of Experimental Botany, Vol. 57, No. 5, pp. 1025–1043.
40. Munns, R. 2002. Comparative physiology of salt and water stress. (Special issue: Drought stress). Plant, Cell and Environment. 25: 239-250.
41. Murakeozy, E. P., Z. Nagy, C. Duhaze, A. Bouchereau and Z. Tuba. 2003. Seasonal changes in the levels of compatible osmolytes in three halophytic species of inland salinevegetation in Hungary. J. Plant Physiol., 160:395-401.
42. Ndayiragije A. and S. D. Lutts. 2006. Do exogenous polyamines have an impact on the response of a salt sensitive rice cultivar to NaCl? J. Plant Physiol., 163:506-16.
43. Noreen Z, Ashraf M 2009. Assessment of variation in antioxidative defense system in salt-treated pea (Pisum sativum) cultivars and its putative use as salinity tolerance markers. J Plant Physiol 166:1764-1774.
44. Ollitrault, P., Froelicher, Y., Dambier, D., and Seker, M. 2000. Rootstock breeding by somatic hybridisation for the Mediterranean citrus industry. Acta Horticulturae. 157-162.
45. Perez-Perez, J.G., Robles, J.M., Tovar, J.C., Botia, P., 2009. Response to drought and salt stress of lemon 'Fino 49' under field conditions: water relations, osmotic adjustment and gas exchange. Sci. Hortic. 122, 83-90.
46. Popp, M. and N. Smirnoff. 1999. Polyol accumulation and metabolism during water deficit. In: Smirnoff, N. (ed.): Environment and plant metabolism: Flexibility and acclimation. Bios Scientific, Oxford, 199-215.
47. Ream C.L. & Furr J.R., 1976. Salt tolerance of some citrus species, relatives, and hybrids tested

as rootstocks. J. Am. Soc. Hortic. Sci., 101, 265-267.
48. Romero-Aranda R., Moya JL., Tadeo FR., Legaz F., Primo- Millo E., Talon M. 1998. Physiological and anatomical disturbances induced by chloride salts in sensitive and tolerant Citrus: beneficial and detrimental effects of cations. Plant Cell Environ. 21, p.1243–1253.
49. Ruiz-Medrano R., Xoconostle C. & Lucas W.J., 2001. The phloem as a conduit for inter-organ communication. Curr. Opin. Plant Biol., 4, 202-209.
50. Singh TN, Paleg LG, Aspinal D, 1973. Stress metabolism. Variations in reponse to water deficit in the barley plant. Aust. J. Biol. Sci.vol 26, pp. 65-75.
51. Siringam K, Juntawong N, Cha-um S, Boriboonkaset T, Kirdmanee C 2012. Salt tolerance enhancement in indica rice (Oryza sativa L. spp. indica) seedlings using exogenous sucrose supplementation. Plant Omics J 5:5259.
52. Sokal, R.R. and Rohlf, J.F. 1981. Biometry: the principles and practice of statistics in biological research. 2nd ed., W. H. Freeman and Company, San Francisco, 859p.
53. Steel, R.G.D., J.H. Torrie et D.A. Dickey, 1997. Prin-ciples and Procedures of Statistics: A Biometrical Approach. Boston, MA, 666 p.
54. Storey, R., Walker, R.R., 1998. Citrus and salinity. Scientia Horticulturae 78, 39–81.
55. Walker RR., Sedgley M., Blessing MA., Douglas TJ. 1984. Anatomy, ultrastructure and assimilate concentrations of roots of Citrus genotypes differing in ability for salt exclusion. J. Exp. Bot. 35, p. 1481–1494.
56. Walker R.R., Torokfalvy E., Scott N.S. & Kriedemann P.E., 1981. An analysis of photosynthetic response to salt treatment in Vitis vinifera. Aust. J. Plant Physiol., 8, 359374.
57. Walker, R.R. 1986. Sodium exclusion and potassium-sodium selectivity in salt-treated trifoliate orange (poncirus trifoliata) and Cleopatra mandarin (citrus reticulata) plants. Aust. J. plant physiol. 13:393-303.
58. Williams, L.E., R. Lemonie and N. Saucer. 2000. Sugars transporters in higher plants: a diversity of roles and complex regulation trends. Plant Sci, 5:283-290.
59. Zekri M, Lawrance R, Parson R. Salinity tolerance of citrus rootstocks. 1992. Effect on root and leaf mineral concentrations. Plant and Soil; 147: 171-81.

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