
International Journal Of

**Recent Scientific Research**

**ISSN: 0976-3031**

**Volume: 6 (12) December -2015**


# SCREENING OF SIX CITRUS ROOTSTOCKS FOR SALT TOLERANCE AT EMERGENCE AND EARLY SEEDLING STAGE


Fadli A, El Aymani I, Chetto O, Boudoudou D4, Talha A, Benkirane R and Benyahia H



THE OFFICIAL PUBLICATION OF
INTERNATIONAL JOURNAL OF RECENT SCIENTIFIC RESEARCH (IJRSR)
http://www.recentscientific.com/ recentscientific@gmail.com

ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 6, Issue, 12, pp. 7672-7678, December, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

# SCREENING OF SIX CITRUS ROOTSTOCKS FOR SALT TOLERANCE AT EMERGENCE AND EARLY SEEDLING STAGE

Fadli A[1], El Aymani I[2], Chetto O[3], Boudoudou D[4], Talha A[5], Benkirane R[6] and Benyahia H[7]*

[1,2,3,4,5,7] Department of Plant Breeding and Conservation of Phytogenetic Ressources, National Institute for Agricultural Research, Kenitra, Morocco

[1,2,3,4, 6]Department of Biology, Faculty of Science, Ibn Tofail University, Kenitra, Morocco

ARTICLE INFO

*Article History:*

Received 05th September, 2015
Received in revised form 08th October, 2015
Accepted 10th November, 2015
Published online 28st December, 2015

*Key words:*

salinity, citrus, rootstock, emergence, growth, chloride.

ABSTRACT

A greenhouse experiment was conducted to investigate the effects of NaCl-induced stress on emergence and early growth of six citrus rootstock cultivars, namely Rangpur lime (Citrus limonia Osbeck), rough lemon(Citrus jambhiri Lush.), Volkamer lemon (Citrus volkameriana Pasquale), sour orange (Citrus aurantium L.), Swingle citrumelo and citrumelo 4475 (Citrus paradisi Macf. x Poncirus trifoliata (L.) Raf). Seeds of the six rootstocks were sown in a peat-based substrate in a factorial design and irrigated during two months with a nutrient solution containing NaCl at 0 (control), 20 and 40 m M concentrations. The results have shown significant effects of salinity on emergence speed and growth of seedlings by the end of the experimental period, but no effect on final emergence rate. Significant differences were found notably among the cultivars studied suggesting a high tolerance of Rangpur lime and Swingle citrumelo and a low tolerance sour orange and rough lemon. In addition, a considerable accumulation of chloride ions was observed in plant tissue with increasing salinity, which was significantly correlated with final seedling height, biomass reduction and time to 50% emergence. Based on correlation coefficients, shoot chloride concentrations were better linkedtothese parameters than root chloride concentrations and proved to be a more reliable tool for ranking salt tolerance of citrus rootstocks at early growth stages.



## INTRODUCTION

Salinity is one of the most important problems affecting soils in the Mediterranean region. In Morocco particularly, 350.000 ha of irrigated land is considered as salt-affected (Badraoui, 1998), which represent a serious threat for crop production in these areas. One way to overcome this problem is to select a salt-tolerant variety that assures a high yield under saline conditions.

In Citrus, it is well established that the rootstock plays a key role in salt stress tolerance (Hepaksoy, 2000). Indeed, although citrus may suffer from nutritional imbalances and toxicity problems at low salt concentrations (Bernstein, 1965; Maas and Hoffman, 1977), the use of some rootstocks proved to reduce considerably the translocation of saline ions, especially $Cl^-$ to scion shoots and limit salt damages (Storey and Walker, 1998). To classify the tolerance of citrus rootstocks to salinity, many criteria have been used over several decades based on physiological and biochemical parameters that have provided rapid tests to diagnose the varietal salt-tolerance (Ait El Aouad *et al.*, 2015; Fernández-Ballester *et al.*, 2003; Ream and Furr, 1976; Ruiz *et al.*, 1997; Sykes, 2011). These tests include the analyses of growth, nutrition, mineral uptake (accumulation of Na+ and $Cl^-$ in leaves and the K+ use efficiency) and leaf accumulation in proline and sugars. Recently, the advances of in vitro biotechnology have provided novel research approaches to identify new sources of salt tolerance within citrus rootstock species (Beloualy and Bouharmont, 1992; Chetto *et al.*, 2015; Pérez-Tornero *et al.*, 2009). However, little is known about salt tolerance of citrus rootstocks at germination and stand establishment stages. Moreover, the practical value of reported data is generally unknown because the results have never been compared to data that is observed under field conditions.

For other crops, the utility of germination-based tests is a controversial issue. Some investigators (Ben Naceur *et al.*, 2001; Greenway, 1965) have suggested that salinity response of seedlings is a good indicator of behavior and yield of adult

**Corresponding author:* **Benyahia H**
Department of Plant Breeding and Conservation of Phytogenetic Ressources, National Institute for Agricultural Research, Kenitra, Morocco

plants in saline environements. Conversely, other authors (Follett *et al.,* 1981; Foolad and Lin, 1997; Kingsbury and Epstein, 1984) have reported the absence of any clear relationship between the results from germination tests and field productivity. To explain this controversy, Sharma and Goyal (2003), have proposed their hypothesis: Because the germination is usually performed in the laboratory using Petri dishes or a saline solution on filter paper, which doesn't reproduce field conditions, these types of germination tests are unsuitable. This fact suggests that the tests based on emergence rate which are performed under field or greenhouse conditions are more appropriate to diagnose salt-tolerance. From a technical point of view, these tests are easy to run, and can cover a large number of samples with rapid results.

Given the importance of seed germination and establishment for the success of later developmental stages, we proposed in this study to investigate the injurious effects of salinity induced by the addition of sodium chloride (NaCl) to a diluted nutrient solution on seedling emergence, vegetative growth and seedling $Cl^-$ concentration of six popular citrus rootstocks. The main objective was to examine the hypothesis that the application of salt stress at early developmental stages may reveal reliable differences among citrus rootstocks regarding salt tolerance and to investigate whether $Cl^-$determination may be a useful indicator of salt tolerance at these stages.

## MATERIALS AND METHODS

The present study was carried out at the Regional Center for Agricultural Research in Kenitra (Morocco). For emergence and growth of seedlings, a sandy, non calcareous and non-saline soil was collected from the experimental field of the station. Seeds of six citrus rootstocks, i.e. Rangpur lime, Swingle citrumelo, citrumelo 4475, rough lemon, volkamer lemon and sour orange (Table 1) were randomly selected and any seed exhibiting external damage was discarded. The experiment was conducted in a greenhouse in which the day-night temperature and relative humidity ranged from 20-35°C and from 40-60%, respectively. The seeds were sown in 60x40 cm plastic trays filled with peat at a depth of 2 cm. Finally, a 1 cm-thick layer of peat was added in each tray over the seeds. Irrigation was applied immediately using water and every two days thereafter using a modified Hoagland solution at one-tenth concentration (Zekri and Parsons, 1989). Control seeds received only the nutrient solution, whereas the salinity treatments contained in addition NaCl at 20 and 40 mM concentrations.

The experiment was carried out in a split-plot design with salinity levels as the main plots and rootstock genotypes as subplots. Each treatment was replicated three times and fourteen seeds of each rootstock were used per replication. Obtained data were statistically tested by ANOVA method to determine significant differences among rootstocks and treatments. Duncan's multiple range test was used as well when F test was significant at $P< 0.05$ for mean separation. The software used for statistical analysis is SAS 9.0.

The number of emerged seedlings was counted daily for two months after the first seedling appeared. A seed was considered emerged when the seedling appeared at the surface of the growing medium. Number of days to emergence of the first seedling (EFS), number of days between the emergence of the first and last seedlings (emergence spread) and final percent emergence (FPE) were also calculated from the daily counts: FPE = $(N_f / N_t) \times 100$ Where $N_f$ is the number of emerged seedlings at the end of the experiment and $N_t$ is the total number of seeds sown at the beginning.

To evaluate the effect of salinity on growth, seedling height was regularly measured every week after the appearance of the first seedlings using a ruler. Once the number of emerged plants stabilized (two months after sowing), plants were harvested, washed free of container growing medium, briefly rinsed with distilled water and blotted dry. Samples of 14 seedlings representing repetitions of the same rootstock and the same treatment were separated into roots and shoots and weighed immediately. For determination of dry weight, the tissues were oven-dried for 48 hours at 70°C. Each part was then weighed, ground and stored for chloride determination. Percent reduction of biomass (%R) was estimated relatively to control as follows: %R = ((Control – Treated) / Control) * 100

Chloride was extracted from shoots and roots using hot water and determined by titration with silver nitrate (Cotlove, 1964). Percent accumulation of chloride (%A) was estimated relatively to control according to the following equation: %A = ((Treated – Control) / Control) * 100

**Table 1** List of the citrusrootstock genotypes used in the experiment

| Common name | Scientific name | Code |
|---|---|---|
| Rangpur lime | Citrus limonia Osbeck | RAN |
| Swingle citrumelo | Citrus paradisi Macf. x Poncirus trifoliata (L.) Raf. | SC |
| Citrumelo 4475 | Citrus paradisi Macf. x Poncirus trifoliata (L.) Raf. | C4475 |
| Rough lemon | Citrus jambhiri Lush. | RL |
| Volkamer lemon | Citrus volkameriana Pasquale | VOL |
| Sour orange | Citrus aurantium L. | SO |

## RESULTS

### *Effect of Salinity on Seedling Emergence*

Seedlings of all genotypes survived the highest salinity level after two months of treatment with no apparent toxicity symptoms. The analysis of emergence speed reveals the existence of a lag phase before the apparition of the first seedlings, which is more or less long depending on the rootstock and salt concentration (Table 2). At control conditions, rough lemon seedlings emerged first beginning 23 days after sowing. Rangpur lime and citrumelo 4475 seedlings emerged later starting at day 25. Volkamer lemon and sour orange seedlings were the slowest to emerge beginning at day 27. For most rootstocks, the addition of 20 mM NaCl to the irrigation solution did not affect clearly EFS. In contrast, a slight delay of emergence was observed in all rootstocks as salt concentration increased to 40 mM except for Swingle citrumelo which seedlings emerged simultaneously to control (26 days after sowing). Statistical analyzes showed a significant effect of the factor 'Rootstock' ($P$ < 0.05) on EFS, but no significant effects of the factor 'Salinity' and the interaction 'Salinity x Genotype'.

According to Table 2, the number of days between the emergence of the first and the last seedlings (emergence spread) was not affected by salinity, but a considerable variation of the time to 50% emergence (T50) was observed. In contrast to ES, both factors 'Genotype' and 'Salinity' had significant effects on T50 ($P< 0.001$) as well as their interaction ($P < 0.05$). For the no salt control treatment, T50 ranged from 30 to 33 days, whereas it reached up to 36 days under the 40 mM NaCl treatment. Minimum tolerance was observed in citrumelo genotypes (up to 4 days later than control) and maximum tolerance was observed in Rangpur lime seedling which displayed equal T50 valuesto control when exposed to saline treatments. We should note also that the addition of NaCl to the irrigation solution did not affect significantly the final percent emergence (FPE). Indeed, although statistical analysis showed some differences between root stock in this regard, high emergence rates were obtained (above 70%) whatever the salt concentration applied and the genotype tested.

control seedlings of sour orange and volkamer lemon were almost 1, 5 cm higher than seedlings subjected to 40 mM NaCl treatment. Surprisingly, the evolution of hypocotyl height for Rangpur lime under saline treatments followed similar patterns to the ones observed for control seedlings of this root stock. Under non-stressed conditions, shoot and root biomass differed among roots tocks (Table 3 and 4). Generally, the values recorded at his level for sour orange were the highest and those of Rangpur lime were the lowest. The additions of NaCl to the Irrigation solution caused a significant reduction in shoot and root biomass with a variable extent depending on rootstock.

At 20 mM concentration, the seedlings of rough lemon and Swingle citrumelo displayed the highest shoot/root fresh/dry weight values, whereas those of Rangpur lime showed the lowest. However, when compared to control, the highest sensitivity was found in sour orange which was expressed bya 36 % reduction in shoot FW, 49% reduction in root FW, 32% reduction in shoot DW and 39% reduction in root DW.

**Table 2** Effect of salinity on emergence of six citrus rootstocks (EFS) Days to emergence of the first seedlings (T50) Time to 50% emergence. (ES) Emergence spread (FPE) Final percent emergence (T0) Control (T1) 20 mM NaCl (T2) 40 mM NaCl

| | EFS$^z$ (days) | | | T50$^z$ (days) | | | ES$^z$ (days) | | | FPE$^z$ (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T0 | T1 | T2 | T0 | T1 | T2 | T0 | T1 | T2 | T0 | T1 | T2 |
| RAN | 25 a$^y$ | 25 a | 26 a | 31 ab | 30 b | 31 c | 11 b | 18 a | 14 ab | 90 ab | 98 a | 93 a |
| CS | 26 a | 28 a | 26 a | 31 ab | 34 a | 35 ab | 21 a | 16 a | 19 a | 93 ab | 86 a | 86 ab |
| C4475 | 25 a | 26 a | 27 a | 32 ab | 32 ab | 36 a | 17 ab | 12 a | 19 a | 81 b | 74 b | 71 b |
| RL | 23 a | 25 a | 26 a | 30 b | 32 ab | 33 bc | 14 ab | 14 a | 16 ab | 95 ab | 95 a | 93 a |
| VOL | 27 a | 26 a | 28 a | 31 ab | 32 ab | 33 bc | 11 b | 13 a | 11 b | 90 ab | 90 a | 88 a |
| BIG | 27 a | 29 a | 31 a | 33 a | 35 a | 35 ab | 14 ab | 19 a | 11 b | 100 a | 93 a | 93 a |
| | Analysis of variance$^x$ | | | | | | | | | | | |
| G | | * | | | *** | | | NS | | | *** | |
| S | | NS | | | *** | | | NS | | | NS | |
| G x S | | NS | | | NS | | | NS | | | NS | |

$^z$Mean of three replications; $^y$For each rootstock, superscripts indicate mean separation within columns by Duncan's multiple range test at 0.05 level; $^x$G and S refer respectively to the factors'Genotype' and 'Salinity'. Significant effects are indicated by asteriks : * = $P< 0.05$, ** = $P< 0.01$ and *** = $P< 0.001$, and NS indicates not significant results.

**Table 3**Variation in Shoot and root fresh weight (FW) in response to saline treatments. (T0) Control (T1) 20 mM NaCl (T2) 40 mM NaCl (%R) Percent reduction relatively to control

| | Shoot FW (g) | | | | | | Root FW (g) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T0 | | T1 | | T2 | | T0 | | T1 | | T2 | |
| | Mean$^z$ | %R | Mean$^z$ | %R | Mean$^z$ | %R | Mean$^z$ | %R | Mean$^z$ | %R | Mean$^z$ | %R |
| RAN | 2.6 b$^y$ | - | 2.7 b | -4 | 3.2 a | -22 | 0.7 b | - | 0.9 b | -27 | 1.1 b | -43 |
| SC | 4.7 ab | - | 5.1 a | -9 | 4.2 a | 10 | 1.7 ab | - | 1.9 a | -13 | 1.8 a | -10 |
| C4475 | 4.4 ab | - | 4.3 ab | 4 | 3.2 a | 28 | 1.7 ab | - | 1.7 ab | 2 | 1.6 ab | 4 |
| RL | 6.2 a | - | 5.0 a | 19 | 3.5 a | 43 | 2.0 ab | - | 1.9 a | 9 | 1.4 ab | 30 |
| VOL | 5.2 a | - | 4.2 ab | 20 | 3.3 a | 36 | 2.4 ab | - | 1.8 a | 27 | 1.4 ab | 42 |
| SO | 5.8 a | - | 3.7 ab | 36 | 4.0 a | 31 | 3.4 a | - | 1.7 ab | 49 | 1.9 a | 44 |
| | Analysis of variance$^x$ | | | | | | | | | | | |
| G | | | ** | | | | | | ** | | | |
| S | | | ** | | | | | | * | | | |
| G x S | | | NS | | | | | | NS | | | |

$^z$Mean of three replications; $^y$For each rootstock, superscripts indicate mean ; eparation within columns by Duncan's multiple range test at 0.05 level; $^x$G and S refer respectively to the factors'Genotype' and 'Salinity'. Significant effects are indicated by asteriks : * = $P< 0.05$, ** = $P< 0.01$ and *** = $P< 0.001$, and NS indicates not significant results.

### *Effect of Salinity on Seedling Growth*

Salt stress caused a delay in growth of all rootstocks, which was expressed by a reduction in hypocotyl height compared to control (table 1). The reduction started from the first week after emergence of the first seedlings and had a variable extent depending on genotype and salt concentration. Generally, seedlings of sour orange and Volkamer lemon were the most sensitive in this regard. At the end of the treatment period,

**Table 4** Variation in Shoot and root dry weight (DW) in response to saline treatments. (T0) Control. (T1) 20 mM NaCl. (T2) 40 mM NaCl. (%R) Percent reduction relatively to control

| | Shoot DW (g) | | | | | | Root DW (g) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T0 | | T1 | | T2 | | T0 | | T1 | | T2 | |
| | Mean$^z$ | %R | Mean$^z$ | %R | Mean$^z$ | %R | Mean$^z$ | %R | Mean$^z$ | %R | Mean$^z$ | %R |
| RAN | 0.7 b$^y$ | - | 0.8 b | -2 | 0.8 a | -9 | 0.3 b | - | 0.3 a | 1 | 0.3 ab | -7 |
| SC | 1.4 a | - | 1.3 a | 5 | 1.1 a | 23 | 0.5 ab | - | 0.4 a | 15 | 0.4 a | 14 |
| C4475 | 1.2 ab | - | 1.2 ab | 6 | 0.8 a | 34 | 0.4 ab | - | 0.4 a | 13 | 0.4 ab | 21 |
| RL | 1.5 a | - | 1.2 ab | 21 | 0.8 a | 45 | 0.4 ab | - | 0.4 a | 20 | 0.3 ab | 41 |
| VOL | 1.2 ab | - | 1.0 ab | 17 | 0.8 a | 36 | 0.5 ab | - | 0.3 a | 30 | 0.3 b | 45 |
| SO | 1.3 a | - | 0.9 ab | 32 | 0.9 a | 30 | 0.7 a | - | 0.4 a | 39 | 0.4 a | 35 |
| | Analysis of variance$^x$ | | | | | | | | | | | |
| G | | | ** | | | | | | ** | | | |
| T | | | *** | | | | | | *** | | | |
| G x T | | | NS | | | | | | NS | | | |

$^z$Mean of three replications; $^y$For each rootstock, superscripts indicate mean separation within columns by Duncan's multiple range test at 0.05 level; $^x$G and S refer respectively to the factors'Genotype' and 'Salinity'. Significant effects are indicated by asteriks : * = $P< 0.05$, ** = $P< 0.01$ and *** = $P< 0.001$, and NS indicates not significant results.

At 40 mM concentration, the biomass of sour orange seedlings did not undergo any further decrease as compared to control, whereas that of rough lemon and volkamer lemon was severely reduced to reach minimum values as compared to the other rootstocks. For instance, the reduction rates for fresh and dry weights of rough lemon shoots were respectively 43% and 45%, For both salt concentrations and both plant parts, the highest tolerance was observed in Rangpur lime and Swingle citrumelo. In some cases, biomass production seemed even to be stimulated in these rootstocks with increasing salt concentration.

In all the relationships forementioned, the correlation coefficients (r) were higher for shootsthan roots.

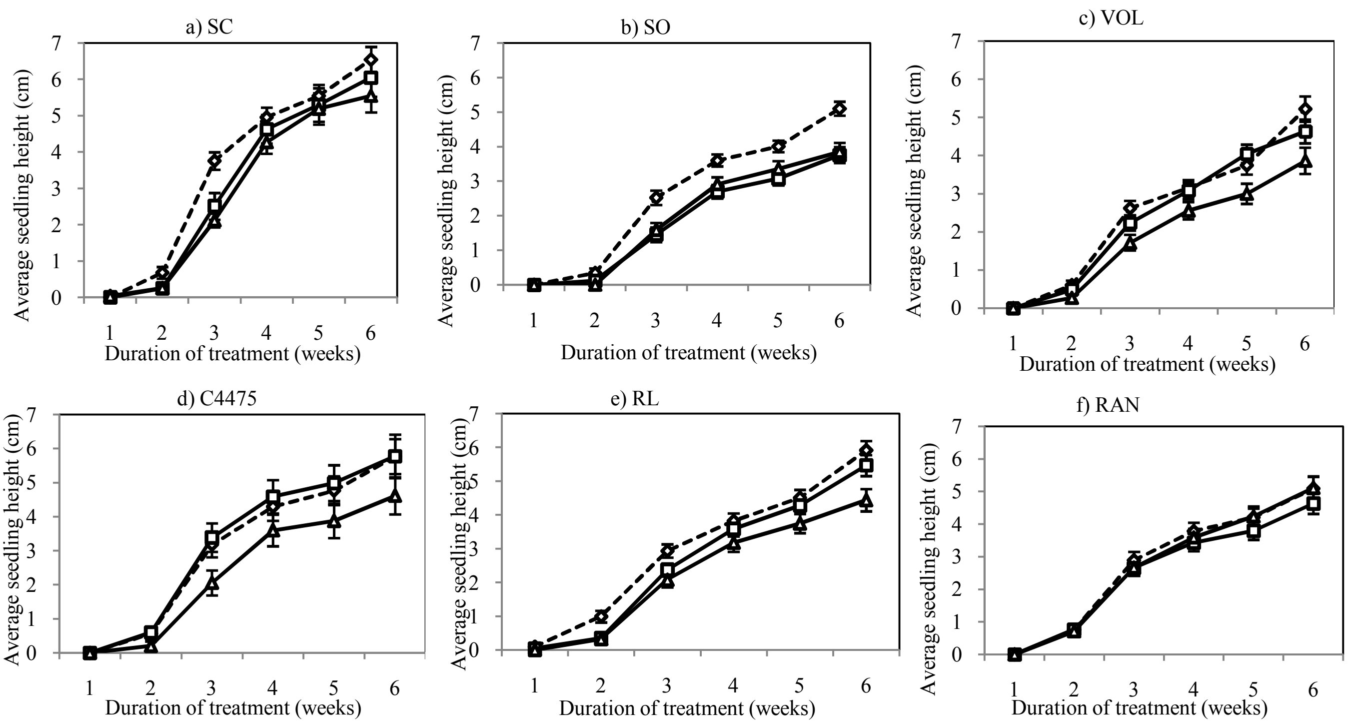


**Figure 1**Evolution of seedling height in response to saline treatments. (◊) Control. (□) 20 mM NaCl. (Δ) 40 mM NaCl

***Effect of salinity on chloride uptake***

In the no salt control treatment, Cl$^-$concentrations in shoots varied between 5.6 to 12.0 mg.g$^{-1}$ DW, whereas the corresponding values for roots ranged between 8.1 and 15.5 mg.g$^{-1}$ DW (**Error! Reference source not found.**). There was an increasing trend in Cl$^-$ content of both plant parts with increasing salinity in the irrigation solution. Statistical analysis of obtained data revealed a highly significant effect ($P < 0.001$) of 'Salinity' factor on shoot and root Cl$^-$ contents, but no significant effects of the 'Genotype' factor and the interaction 'Salinity x Genotype'. At the 40 mM concentration, the genotype that accumulated most Cl$^-$ in shoots was rough lemon resulting in 6 fold the control values. By contrast, the minimum accumulation was observed in Rangpur lime seedlings which resulted in less than 2 fold the shoot Cl$^-$ content of control seedlings. A low Cl$^-$ accumulation was also shown in the shoots of Swingle citrumelo seedlings which displayed an average of 20.8 mg.g-1 DW at 40 mM treatment and a 258% accumulation as compared to control. We should note that mean separation by Duncan's multiple range test did not show any differences among rootstocks regarding shoot and root chloride concentrations at low salinity (20 mM).

The **Error! Reference source not found.** shows the relationship between Cl$^-$ accumulation in plant tissue, emergence and growth parameters as expressed by Pearson correlation. As shown in this table, the correlation strength varied according to the Cl$^-$ accumulating organ. Shoot Cl$^-$ content was correlated positively with EFS and T50 and negatively with all growth parameters. Similarly, root Cl$^-$ content was correlated significantly with shoot and root biomass and T50, but not with final seedling height and EFS. relationships of FPE and ES with Cl$^-$ build up in plant tissue. Further more, poor relationships were found between FPE and the decrease in seedling growth at stand establishment stage under saline conditions (data not shown). Seedling growth was better correlated with EF Sas suggested by significant r values of -0.428, -0.336 and -0.403 respectively for final seedling height, shoot FW and shoot DW.

## DISCUSSION

The main objective of the study was to examine whether the application of salt stress at germination and early developmental stages may reveal reliable differences among citrus rootstocks regarding salt tolerance. This hypothesis was verified on six cultivars that showed contrasting behaviors when grown under saline conditions. The results have shown clearly that NaCl added to the irrigation solution delayed seedling emergence, particularly by depressing the time to 50% emergence. However, the final emergence rate was not affected by the increase in NaCl concentration. Similar findings were reported in citrus (Zekri, 2001) and other crops(Katerji *et al.*, 2012; Sharma and Goyal, 2003). According toMobayen and Milthorpe (1978), there was no reduction in final germination rate of trifoliate orange - which is known to be salt sensitive – under the concentration 100 mol.m$^{-3}$, while final germination of Cleopatra mandarin seeds was reduced by 20% and that of Bakraie mandarin (C. reticulata Blanco) seeds by 53%. In contrast to emergence rate, the delay in emergence under the effect of salinityis well reported in citrus rootstocks. The study of (Zekri, 1993), for example, has shown that an increase in salt concentration of the nutrient solution may lead to an emergence delay of 3 to 5 days depending on the rootstock. The same author attributed this delay to (i) the osmotic effect of the soil solution which, if severe enough, may retard or prevent the intake of water by seeds, or (ii) to the toxic effect of salinity on the development of embryo and seedling. Later, the findings of (Alaoui *et al*., 2013) on reversibility of salt effect on germination of different wheat cultivars showed that the osmotic effect is the most dominant. Nevertheless, the toxic effect occurred at high salt concentrations. This fact suggests that the application of higher NaCl concentrations than the ones used in our study might have influenced the final emergene rate.

Salinity adversely affected growth of most rootstocks (except for Rangpur lime) at stand establishment without causing apparent toxicity symptoms. According to (Ackerson and Youngner, 1975), the effects of salinity on shoot and root growth may occur before saline ion accumulation in the shoots would have built up to high levels. This may be a plausible explanation for our observation since the accumulation of saline ions is known to be the main cause of salt induced damage(Levy and Syvertsen, 2004). Furthermore, it is reported that the early formative stages of shoots and leaves are very sensitive to salinity because they are governed by cell division, which is limited under saline conditions due to poor supply of assimilates (Kriedemann, 1986). Thus, growth reduction at early developmental stages of citrus rootstocks seems to be related to a nutritional imbalance rather than a toxic effect of saline ions.

Based on reduction in seedling biomass, maximum tolerance was found in Rangpur lime and Swingle citrumelo, whereas the highest sensitivity was observed in rough lemon and sour orange. This ranking confirmed that obtained by Hassan and Galal (1989) and Seday *et al.*(2014) on older seedlings. Our results are also in agreement with our previous findings on salt tolerance of nine citrumelo accessions at seedling stage (Fadli *et al*., 2014) which have shown Swingle citrumelo as salt-tolerant and citrumelo 4475 as salt-sensitive. However, the reduction rates observed in our study were lower than those obtained by Zekri (1993) who conducted a similar experiment on various citrus rootstocks and reported a 51 to 89% reduction as compared to control. These variations may be explained by differences in growth conditions such as temperature and relative humidity.

There was no good relationship between emergence under saline conditions and reduction in seedling biomass at stand establishment. By contrast, we observed that some varieties combined high emergence with a low salt-tolerance. Thus, this study doesn't support the hypothesis that postulates the existence of a correlation between emergence and the potential of a citrus rootstock variety to tolerate salinity. Similar findings were reported for various crops (Katerji *et al*., 2012).

The present study has also shown that the accumulation of $Cl^-$ ions in shoots and roots is mainly controlled by the rootstock. Indeed, significant differences were found between the genotypes studied in their ability to exclude $Cl^-$ as observed previously on older citrus plants (Ream and Furr, 1976; Walker and Douglas, 1983). When expressed relatively to control, the best $Cl^-$ excluding ability was found in Rangpur lime which showed almost the same shoot $Cl^-$ concentration as control whatever the treatment applied,whereas the highest accumulation was recorded in Rough lemon. These data supported previous works concluding that Rangpur lime is an effective $Cl^-$excluder (Grieve and Walker, 1983; Sykes, 2011)and rough lemon is a poor excluder (Xu *et al.*, 1999; Zekri, 1993; Zekri and Parsons, 1992).

When plotting seedling biomass as a function of $Cl^-$ concentrations, a strong correlation was found which suggest a cause-effect relationship between the two parameters. Similar results were obtained by Romero-Aranda *et al*.(1998) with one-year-old seedlings of carrizo citrange and Cleopatra mandarin after a six-month saline treatment. At field conditions also, Raveh (2005) reported that once leaf chloride content exceeds a value of 0.7% (dry matter), a linear reduction in shoot growth and fruit yield is expected.

As compared to shoots, roots accumulated more $Cl^-$. However shoot $Cl^-$ concentrations were found to be more correlated with growth than root $Cl^-$ concentrations, which is in agreement with the findings of (Zekri, 2001). These results suggest that shoot $Cl^-$ concentration is a better indicator than root $Cl^-$ concentration in evaluating the injurious effects of salinity and a better parameter in ranking salt tolerance of citrus rootstocks.

## CONCLUSIONS

The previous discussion clearly indicates that the varietal ability to emerge under saline conditions does not represent a potential indicator for identifying salt tolerant citrus rootstocks. Indeed, although our results showed some significant differences between the genotypes investigated regarding emergence speed and similarities with previous studies, the weak relationship found between emergence parameters and salt tolerance makes them poor and insensitive tools for assessment of salt tolerance.

At early seedling stage, the salt tolerance of citrus rootstocks seems to be rather based on:

- The capacity to preserve biomass production under saline conditions assuring a good establishment.
- The capacity to exclude chloride ions from being easily Trans located to shoots.

Thus, future research should focus on these approaches and include them in screening strategies to ensure an early, quick and effective selection of salt tolerant rootstocks within citrus species. Also, given that salt tolerance is not a constant character throughout plant development, growth conditions and developmental stages have to be thoroughly described when studies are performed to rank or screen citrus rootstocks for salt tolerance.

*Acknowledgments*

The authors would like to thank the staff of the Regional Center for Agricultural Research of Kenitra, particularly Dr. Hassan Benaouda, the head of the center, for help and technical assistance. We are also grateful to the National Center for

Scientific and Technical Research (CNRST) for providing a research grant to Anas Fadli.

## References


1. Ackerson R.C. and Youngner V.B., (1975). Responses of bermudagrass to salinity. *Agron. J*. 67 (5), 678–681.
2. Ait El Aouad B., Fadli A., Aderdour T., Talha A., Benkirane R. and Benyahia H., (2015). Investigating salt tolerance in citrus rootstocks under greenhouse conditions using growth and biochemical indicators. Biolife 3 (4), 827–837.
3. Alaoui M.M., El Jourmi L., Ouarzane A., Lazar S., El Antri S., Zahouily M. and Hmyene A., (2013). Effect of salt stress on germination and growth of six Moroccan wheat varieties. *J. Mater. Environ. Sci*. 4 (6), 997–1004.
4. Badraoui M., (1998). Effects of intensive cropping under irrigation on soil quality in Morocco, in: Proceedings of the 16th World Congress of Soil Science, B5-Post Congress Tour in Morocco, AMSSOL, Rabat.
5. Beloualy N. and Bouharmont J., (1992). NaCl-tolerant plants of Poncirus trifoliata regenerated from tolerant cell lines. Theor. Appl. Genet. 83 (4), 509–514.
6. Ben Naceur M., Rahmoune C., Sdiri M., Meddahi M.L. and Selmi M., (2001). Effet du stress salin sur la germination, la croissance et *la* production en grains de quelques variétés maghrébines de blé. Sécheresse 12, 167–174.
7. Bernstein L., (1965). Salt Tolerance of Fruit Crops. Agriculture Research Service, U.S. Dept. Agric. Inf. Bull. 292.
8. Chetto O., Dambier D., Fadli A., Benkirane R., Talha A. and Benyahia H., (2015). Mise au point d'un test in vitro de comportement au sel de quatre génotypes d'agrumes. *J. Appl. Biosci.* 88 (1), 8154–8166.
9. E. Cotlove, 1964. Determination of Chloride in Biological Materials, In: D. Glick (ed.), Methods of Biochemical Analysis. John Wiley & Sons, Inc., pp. 277–391.
10. Fadli A., Chetto O., Talha A., Benkirane R., Morillon R. and Benyahia H., (2014). Characterization in Greenhouse Conditions of Two Salt Tolerant Citrumelo (Citrus paradisi Macf. x Poncirus trifoliata (L.) Raf.) Cultivars. *J. Life Sci.* 8, 955–966.
11. Fernández-Ballester G., García-Sánchez F., Cerdá A. and Martínez V., (2003). Tolerance of citrus rootstock seedlings to saline stress based on their ability to regulate ion uptake and transport. Tree Physiol. 23 (4), 265–271.
12. Follett R.H., Murphy L.S. and Donahue R.L., (1981). Fertilizers and soil amendments. Englewood Cliffs. Prentice-Hall, Inc.
13. Foolad M.R. and Lin G.Y., (1997). Absence of a genetic relationship between salt tolerance during seed germination and vegetative growth in tomato. Plant Breed. 116 (4), 363–367.
14. Greenway H., (1965). Plant response to saline substrates VII. Growth and ion uptake throughout plant development in two varieties of Hordeum vulgare. Aust. *J. Biol. Sci.* 18 (4), 763–780.
15. Grieve A.M., Walker R.R., (1983). Uptake and distribution of chloride, sodium and potassium ions in salt-treated citrus plants. Crop Pasture Sci. 34 (2), 133–143.
16. Hassan M.M. and Galal M.A., (1989). Salt tolerance among some citrus rootstocks. *J. King Saud*. Univ. 1 (1,2), 87–93.
17. Hepaksoy S., (2000). Effect of salinity on citrus. *J. Aegean Agric. Res*. Inst. Turk. 10 (1), 52–72.
18. Katerji N., Mastrorilli M., Lahmer F.Z. and Oweis, T., (2012). Emergence rate as a potential indicator of crop salt-tolerance. Eur. *J. Agron*. 38, 1–9.
19. Kingsbury R.W. and Epstein E., (1984). Selection for salt-resistant spring wheat. Crop Sci. 24 (2), 310–315.
20. Kriedemann P.E., (1986). Stomatal and photosynthetic limitations to leaf growth. Funct. Plant Biol. 13 (1), 15–31.
21. Levy, Y. and Syvertsen J.P., (2004). Irrigation water quality and salinity effects in citrus trees. Hortic. Rev. 30, 37–82.
22. Maas E.V. and Hoffman G.J., (1977). Crop Salt Tolerance - Current Assessment. *J. Irrig. Drain*. Div. 103 (2), 115–134.
23. Mobayen R.G. and Milthorpe F.L., (1978). Citrus seed germination as influenced by water potential and salinity. Proc. *Int. Soc*. Citric. 1, 247–249.
24. Pérez-Tornero O., Tallón C.I., Porras I. and Navarro J.M., (2009). Physiological and growth changes in micropropagated Citrus macrophylla explants due to salinity. *J. Plant Physiol*. 166 (17), 1923–1933.
25. Raveh E., (2005). Methods to assess potential chloride stress in citrus: analysis of leaves, fruit, stem-xylem sap, and roots. Hort technology 15 (1), 104–108.
26. Ream C.L. and Furr J.R., (1976). Salt tolerance of some Citrus species, relatives, and hybrids tested as rootstocks. *J. Am. Soc*. Hortic. Sci. 101 (3), 265–267.
27. Romero-Aranda R., Moya J.L., Tadeo F.R., Legaz F., Primo-Millo E. and 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 (12), 1243–1253.
28. Ruiz D., Martínez V. and Cerdá A., (1997). Citrus response to salinity: growth and nutrient uptake. Tree Physiol. 17 (3), 141–150.
29. Seday U., Gulsen O., Uzun A. and Toprak G., (2014). Response of citrus rootstocks to different salinity levels for morphological and antioxidative enzyme activites. *J. Anim. Plant Sci*. 24 (2), 512–520.
30. Sharma S.K. and Goyal S.S., (2003). Progress in plant salinity resistance research: need for an integrative paradigm. *J. Crop Prod.* 7, 387–407.
31. Storey R. and Walker R.R., (1998). Citrus and salinity. Sci. Hortic. 78 (1-4), 39–81.
32. Sykes S.R., (2011). Chloride and sodium excluding capacities of citrus rootstock germplasm introduced to Australia from the People's Republic of China. *Sci. Hortic*. 128 (4), 443–449.
33. Walker R.R. and Douglas T.J., (1983). Effect of salinity level on uptake and distribution of chloride, sodium and

potassium ions in citrus plants. *Crop Pasture Sci.* 34 (2), 145–153.
34. Xu G., Magen H., Tarchitzky J. and Kafkafi U., (1999). Advances in chloride nutrition of plants. Adv. Agron. 68, 97–150.
35. Zekri M., (2001). Salinity effect on seedling emergence, nitrogen and chloride concentrations, and growth of citrus rootstocks. Proc. Fla. State Hort. Soc. 114, 79–82.
36. Zekri M., (1993). Salinity and calcium effects on emergence, growth and mineral composition of seedlings of eight citrus rootstocks. *J. Hortic. Sci*. 68 (1), 53–62.
37. Zekri M. And Parsons L.R., (1992). Salinity tolerance of citrus rootstocks: Effects of salt on root and leaf mineral concentrations. Plant Soil 147 (2), 171–181.
38. Zekri M. and Parsons L.R., (1989). Growth and root hydraulic conductivity of several citrus rootstocks under salt and polyethylene glycol stresses. Physiol. Plant. 77 (1), 99–106.

**How to cite this article:**

Benyahia H *et al.,* Screening of Six Citrus Rootstocks For Salt Tolerance At Emergence And Early Seedling Stage. *International Journal of Recent Scientific Research Vol. 6, Issue, 12, pp. 7672-7678, December, 2015*

*******

ISSN 0976-3031

9 770976 303009 >