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IMPROVEMENT OF SALINITY TOLERANCE OF CITRUS SCION USING TOLERANT ROOTSTOCKS AND INTERSTOCKS

ALIREZA SHAFIEIZARGAR

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IMPROVEMENT OF SALINITY TOLERANCE OF CITRUS SCION USING TOLERANT ROOTSTOCKS AND INTERSTOCKS

By

ALIREZA SHAFIEIZARGAR

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

March 2014
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DEDICATION

To:

my wife, Mina

my son, Shayan

and

my daughter, Dorsa
Abstract of Thesis Presented to the Senate of Universiti Putra Malaysia in
Fulfillment of the Requirement for the Degree of Doctor of Philosophy

IMPROVEMENT OF SALINITY TOLERANCE OF CITRUS SCION USING
TOLERANT ROOTSTOCKS AND INTERSTOCKS

By

ALIREZA SHAFIEIZARGAR

March 2014

Chairman: Assoc. Prof. Yahya Awang, PhD
Faculty: Agriculture

Soil salinity is one of the most serious environmental threats, that extremely restricts
crop production. One of the most effective strategies to overcome salinity effects is
by growing salt tolerance plant species. Citrus is a commercial fruit crop and grown
exclusively in tropical and sub-tropical zones. It is a glycophyte. The objective of
this study was to evaluate the responses of citrus to salinity stress and to estimate
amelioration of salinity effects by using tolerant rootstocks, diploid and tetraploid
interstocks.

Salinity tolerance of five citrus rootstocks namely Cleopatra mandarin (Citrus reshni
Hort. Ex Tan.), Carrizo citrange [(Citrus sinensis (L.) Osbeck×Poncirus trifoliata
(L.) Raf.], Tiwanica (Citrus taiwanica Tan.& Shimada), Bacraii (Citrus limettioides
× Citrus reticulate) and Shaker [(Citrus limettioides × Citrus reticulate)× Citrus
reshni] during germination was tested at various NaCl concentrations. Salt stress
affected seed germination, emergence spread, percentage of final emergence and
percentage of seedlings survival. At germination stage, Cleopatra mandarin exhibited
higher salt-tolerance than other species. In the subsequent study, the growth
parameters, mineral concentration, physiological and biochemical traits of above-
mentioned citrus species were studied to estimate the degree of salt tolerance. The
results indicated that the lowest Na and Cl concentrations were observed in leaves of
Shaker rootstock. Also results obtained showed that Shaker and Cleopatra mandarin
rootstocks maintained higher RWC and proline content.

To allow the testing of resistant rootstock, a salt sensitive scion cultivar is needed.
For this purpose, salt sensitivity assessment of two locally available citrus cultivars,
Limau Nipis (Citrus aurantifolia Swingle) and Limau Kesturi (Citrus microcarpa
Bunge) were subjected to NaCl salinity. The results demonstrated that cv. Limau
Kesturi was more sensitive to salt stress than cv. Limau Nipis. Therefore, cv. Limau
Kesturi was used as a salinity susceptible cultivar in combination with citrus
rootstocks for further experimentation in determination of suitable rootstock that
could induce salt resistance of the scion. Evaluation of the level of salt tolerance of
Limau Kesturi plants budded on Cleopatra mandarin and Shaker rootstocks revealed
that salt stress decreased leaf N, P, K concentrations and RWC, while Na, Cl,
proline, MDA and H$_2$O$_2$ concentrations of budded Limau Kesturi increased on both
tested rootstocks. The results suggested that the Shaker exhibited higher tolerance to salt stress than the Cleopatra mandarin and therefore can be used as an appropriate rootstock. Based on the changes in leaf mineral contents and biochemical compositions in response of tetraploid and diploid Dez orange cultivars (*Citrus sinensis* (L.) Osbeck grown under saline condition, we noticed that tetraploid Dez orange had induced a higher level of salt tolerance in comparison to diploid Dez orange. Use of tetraploid Dez oranges as interstock for Limau Kesturi showed the tetraploid cultivar generate more tolerance plants against NaCl salt stress and the plant was able to keep acceptable concentrations of mineral contents, proline, MDA and H$_2$O$_2$.

Overall, evidences recorded from this study proved that among the rootstocks tested, Shaker, and tetraploid interstocked plants are more tolerant to salinity stress and therefore can be introduced as new source of plant materials for salinity tolerance in the citrus industry.
MENINGKATKAN TOLERANSI SCION LIMAU TERHADAP KEMASINAN MENGGUNAKAN POKOK PENANTI DAN INTERSTOK

Oleh

ALIREZA SHAFIEIZARGAR

Mac 2014

Pengerusi: Prof. Madya. Yahya Awang, PhD
Fakulti: Pertanian

Kemasinan tanah adalah salah satu daripada ancaman alam sekitar yang paling serius dan telah menghadkan pengeluaran pertanian. Salah satu strategi yang paling berkesan untuk mengatasi masalah kemasinan ini ialah dengan menanam spesies tumbuhan yang tahan suasan masin. Limau ialah sejenis tanaman buah-buahan komersil dan lazimnya hidup dalam zon tropika dan sub-tropika. Tanaman ini ialah sejenis tumbuhan glikofit. Objektif kajian ini adalah untuk menilai gerak balas limau terhadap kemasinan dan untuk menganggarkan pemulihan scion terhadap kesan kemasinan dengan menggunakan pokok penanti diploid dan bahan interstok tetraploid.


Bagi membolehkan ujikaji untuk menentukan pokok penanti yang tahan kemasinan, satu kultivar scion diperlukan. Untuk ini, penilaian sensitiviti terhadap ketegasan garam dua kultivar tempatan, Limau Nipis (Citrus aurantifolia Swingle) dan Limau Kesturi (Citrus microcarpa Bunge) telah didedahkan kepada ketegasan garam NaCl. Keputusan menunjukkan bahawa Limau Kesturi adalah lebih sensitif berbanding dengan Limau Nipis. Oleh itu, Limau Kesturi telah digunakan sebagai bahan ujikaji...

Secara keseluruhannya, bukti yang direkodkan daripada kajian ini membuktikan bahawa antara pokok penanti limau yang telah diuji, pokok penanti Shaker, dan pokok yang di’intersto’kan dengan bahan tetraploid mempunyai tahap toleransi terhadap ketegasan garam yang lebih tinggi. Dengan itu, teknik dan bahan tanaman ini boleh diperkenalkan sebagai sumber baru untuk mengurangkan kesan kemasinan dalam industri limau.
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Finally I would like to thank my family, my patient wife (Mina), my amiable children (Shayan and Dorsa) who were my spiritual supports during my study and struggle to make the best future for them.
I certify that a Thesis Examination Committee has met on 4 March 2014 to conduct the final examination of Alireza Shafieizargar on his thesis entitled “Improvement of salinity tolerance of citrus scion using tolerant rootstocks and interstocks” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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DECLARATION

Declaration by graduate student

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ABA</td>
<td>Abscisic acid</td>
</tr>
<tr>
<td>DM</td>
<td>Dray matter</td>
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<tr>
<td>DW</td>
<td>Dry weight</td>
</tr>
<tr>
<td>EC</td>
<td>Electrical conductivity</td>
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<tr>
<td>FW</td>
<td>Fresh weight</td>
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<tr>
<td>H$_2$O$_2$</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>IAA</td>
<td>Indole acetic acid</td>
</tr>
<tr>
<td>K/Na</td>
<td>Potassium to sodium ratio</td>
</tr>
<tr>
<td>MDA</td>
<td>Malondialdehyde</td>
</tr>
<tr>
<td>mM</td>
<td>Millimolar</td>
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<tr>
<td>nmol</td>
<td>Nanomole</td>
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<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RWC</td>
<td>Relative water content</td>
</tr>
<tr>
<td>TW</td>
<td>Turgid weight</td>
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<tr>
<td>µmol</td>
<td>Micromole</td>
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</table>
CHAPTER 1

INTRODUCTION

1.1 Background

All the environmental stresses such as extreme temperatures, mineral deficiency, salinity and low water availability induce potential injuries on plant species (Langridge et al., 2006). Two types of environmental stresses are biotic (infection and/or competition by other organisms) and abiotic (light, high and low temperature, drought, salinity, radiation, etc.) that change the normal physiological function of plants (Khayatnezhad et al., 2011). Salinity is one of the major important abiotic stresses, limiting crop production in arid and semi-arid regions, where soil salt content is naturally high and precipitation can be insufficient for leaching (Asghari, 2008). The USDA Salinity Laboratory defines a saline soil as having an electrical conductivity of the saturation extract (ECe) of 4 dS m\(^{-1}\) (1 dS m\(^{-1}\) is approximately equal to 10 mM NaCl) or more. ECe is the electrical conductivity of the ‘saturated paste extract’, that is, of the solution extracted from a soil sample after being mixed with sufficient water to produce a saturated paste (Yadav et al., 2011). High concentrations of soluble salts such as chlorides of sodium, calcium and magnesium contribute to the high electrical conductivity of saline soils. NaCl contributes to most of the soluble salts in saline soil (Chinnusamy et al., 2006). Various ions such as Na\(^+\), K\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), Cl\(^-\), SO\(_4^{2-}\), HCO\(_3^-\), CO\(_3^{2-}\) and NO\(_3^-\) are involved in soil salinization but most commonly, the stress is caused by high Na and Cl concentrations in the soil solution. Na ion particularly causes the dispersion of the soil and Cl ion causes high toxicity and nutrient imbalances in plants (Hasegawa et al., 2000). However, the severity of salt damage has been found to be dependent on the meteorological conditions, soil type, species and cultivar, growth stages of the plant, time interval between irrigations, amount of water distributed and time of exposure to saline water (Parida and Das, 2005; Munns and Tester, 2008).

One strategy to overcome problem of salinity is by selecting salt tolerant genotypes. For this, researchers require an understanding of relative tolerance of crops and their sensitivity, morphological and physiological traits that contribute to salinity tolerance; the ameliorative effects of nutrition and other treatments on growth, mineral uptake, photosynthesis and active constituents of salt-stressed plants; alleviate the mechanisms of salt resistance in different plants (Omami, 2005; Said-Al Ahl and Omer, 2011). For many fruit tree plants such as grapevine and citrus, chloride ion is more toxic than sodium ion, because Na is maintained in the tissue of roots while chloride ion accumulated in aerial organs of plant, negatively impacting on photosynthesis (Asghari, 2008). The osmotic part of salinity is produced by excess ions such as sodium and chloride in the medium that decrease the osmotic potential of soil and hence water absorption by root of plant. Excessive uptake ions reduces the osmotic potential of the plant (Parida and Das, 2005). To escape with the damage of ion toxicity, the plants generally compartmentalized harmful ions in their vacuole and/or in less salt sensitive tissues. Parallel to this, adjustment of the cytoplasmic compartment is accomplished through production of compatible osmolytes such as proline (Ghotb Abadi et al., 2010).
Citrus belongs to the genus *Citrus* L. and *Rutaceae* family, originating in tropical and subtropical Southeast Asia. These fruits are economically important in a large scale production for both fresh fruit and processed products. Although Citrus (*Citrus spp.*) is classified as salt-sensitive, there is great variation in the ability of citrus trees to tolerate salinity depending on rootstock, thus selection among the rootstocks should lead to increasing salt tolerance. Among a very limited study, a few reports showed that there is a lack of positive effects of natural rootstocks and interstocks on citrus grown under saline conditions. A more extensive study is therefore necessary especially when it involves citrus species or cultivars that are unique for a particular country or region. Information generated through such studies on the effects of salinity on physiological and biochemical aspects of citrus could lead to identification of salt tolerant cultivars and rootstocks. Polyploid interstocks may increase salinity tolerance of sensitive citrus cultivars. Because the tree breeding is a time-consuming process, interstocking technique could be an efficient and effective alternative to improve citrus productivity. Thus, the primary objective of the present study was to assess the significance of tolerant rootstocks and tetraploid interstocks in ameliorating the adverse effects of salt stress on citrus scions. The results obtained could be beneficial in improving citrus production practices, as well as in giving new directions in citrus research in the future.

### 1.2 Objectives

The objective of this study was:

i. To determine differences in salinity tolerance among citrus rootstocks at seed germination stage.

ii. To characterize the growth and physiological responses of citrus rootstocks and cultivars to different levels of NaCl salinity.

iii. To evaluate the role of citrus rootstocks in alleviating salinity effects on citrus scions.

iv. To explore the growth and physiological responses of diploid and tetraploid of Dez orange to different levels of NaCl salinity.

v. To determine whether the use of salt tolerant rootstock and interstock can alleviate the problem of salinity of salt intolerant scion.
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Melon Varieties After Short Exposure to Calcium and Potassium Chloride Salts. *Journal of Plant Nutrition* 15 (6-7), 959-975.


