



**UNIVERSITI PUTRA MALAYSIA**

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

**ALIREZA SHAFIEIZARGAR**

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BERILMU BERBAKTI

**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 reticulata*) and Shaker [(*Citrus limettioides* × *Citrus reticulata*)× *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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub>.

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.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

## **MENINGKATKAN TOLERANSI SCION LIMAU TERHADAP KEMASINAN MENGGUNAKAN POKOK PENANTI DAN INTERSTOK**

Oleh

**ALIREZA SHAFIEIZARGAR**

**Mac 2014**

**Pengerusi: Prof. Madya. YahyaAwang, 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 suasana yang masin. Limau ialah sejenis tanaman buah-buahan komersil dan lazimnya hidup dalam zon tropika dan sub-tropika. Tanaman ini adalah sejenis tumbuhan glikofit. Objektif kajian ini adalah untuk menilai gerakbalas limau terhadap kemasinan dan untuk menganggarkan pemulihan scion terhadap kesan kemasinan dengan menggunakan pokok penanti diploid dan bahan interstok tetraploid.

Toleransi kemasinan lima spesies pokok penanti citrus, iaitu Cleopatra mandarin (*Citrus reshni* Hort. Ex Tan.), Carrizo citrange [(*Citrus sinensis* (L.) Osbeck x *Poncirus trifoliata* (L.) Raf.] , Tiwanica (*Citrus taiwanica* Tan. & Shimada), Bacraii (*Citrus limettioides* x *Citrus reticulata*) dan Shaker (*Citrus limettioides* x *Citrus reticulata*) x *Citrus reshni*] semasa percambahan dan pertumbuhan awal anak benih telah diuji pada pelbagai kepekatan NaCl. Kemasinan telah memberi kesan yang nyata terhadap percambahan, sebaran percambahan, peratusan percambahan akhir dan peratusan hidup anak benih. Pada peringkat percambahan, Cleopatra mandarin didapati mempunyai tahap toleransi kemasinan tertinggi berbanding dengan spesies yang lain. Dalam ujikaji berikutnya, parameter pertumbuhan, kandungan mineral, ciri fisiologi dan biokimia spesies limau telah dikaji bagi menilai darjah toleransi terhadap kemasinan seterusnya. Keputusan menunjukkan bahawa daun Shaker mengandungi Na dan Cl terendah berbanding dengan pokok penanti yang lain. Keputusan juga menunjukkan bahawa Shaker dan Cleopatra mandarin mengekalkan kandungan RWC dan prolin yang tinggi.

Bagi membolehkan ujikaji untuk menentukan pokok penanti yang tahan kemasinan, satu kultivar sion 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

seterusnya untuk digabungkan dengan pokok penanti dalam ujikaji seterusnya dalam penentuan pokok penanti yang boleh mengaruhi toleransi sion terhadap ketegasan garam. Penilaian tahap toleransi pokok cantuman Limau Kesturi terhadap ketegasan garam yang didorong oleh Cleopatra mandarin dan Shaker telah menunjukkan bahawa ketegasan garam merendahkan kandungan N, P, K dan RWC manakala telah meningkatkan kandungan Na, Cl, prolin, MDA dan H<sub>2</sub>O<sub>2</sub> anak cantuman Limau Kesturi pada kedua-dua pokok penanti. Bagaimanapun, keputusan ini juga mencadangkan bahawa Shaker mempunyai tahap toleransi yang lebih tinggi terhadap tegasan garam berbanding dengan Cleopatra mandarin. Oleh itu spesis ini merupakan pokok penanti yang lebih sesuai. Berdasarkan kepada perubahan terhadap kandungan mineral dan biokimia dalam limau Kesturi yang dicantum pada Dez Orange [*Citrus sinensis* (L.) Osbeck] tetraploid dan diploid dan ditanam dalam suasana yang masin, kami mendapati bahawa Dez orange tetraploid telah meningkatkan toleransi scion (Limau Kesturi) terhadap ketegasan garam berbanding dengan Dez orange diploid. Penggunaan Dez Orange tetraploid sebagai interstok untuk Limau Kesturi menunjukkan bahawa kultivar tetraploid adalah lebih tahan terhadap kemasinan dan pokok ini berupaya mengekalkan paras mineral, prolin, RWC, MDA dan H<sub>2</sub>O<sub>2</sub>.

Secara keseluruhannya, bukti yang direkodkan daripada kajian ini membuktikan bahawa antara pokok penanti limau yang telah diuji, pokok penanti Shaker, dan 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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I certify that a Thesis Examination Committee has met on 4 March 2014 to conduct the final examination of Alireza Shafieizargar qp" jku"vj guku"gpvkng f"õImprovement of salinity tolerance of citrus scion using tolerant rootstocks and interstocksö" 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.

Members of the Thesis Examination Committee were as follows:

Siti Aishah Binti Hassan, PhD  
Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

Mohd Ridzwan bin Abdul Halim, PhD  
Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Internal Examiner)

Mahmud T.M Mohammed, PhD  
Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Internal Examiner)

Muhammad Ashraf, PhD  
Professor  
University of Agriculture Faisalabad  
Pakistan  
(External Examiner)

---

**NORITAH OMAR, PhD**

Associate Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 21 April 2014

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

**Yahya B. Awang, PhD**

Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Abdul Shukor Juraimi, PhD**

Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

**Radziah Othman, PhD**

Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

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**BUJANG BIN KIM HUAT, PhD**

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## TABLE OF CONTENTS

	Page
<b>ABSTRACT</b>	<b>iii</b>
<b>ABSTRAK</b>	<b>v</b>
<b>ACKNOWLEDGEMENTS</b>	<b>vii</b>
<b>APPROVAL</b>	<b>viii</b>
<b>DECLARATION</b>	<b>x</b>
<b>LIST OF TABLES</b>	<b>xvii</b>
<b>LIST OF FIGURES</b>	<b>xviii</b>
<b>LIST OF ABBREVIATIONS</b>	<b>xx</b>
<b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	<b>1</b>
1.1 Background	1
1.2 Objectives	2
<b>2 REVIEW OF LITERATURE</b>	<b>3</b>
2.1 Causes of salinity	3
2.1.1 Primary cause	3
2.1.2 Secondary salinization	3
2.1.3 Global warming and soil salinization	3
2.2 Salinity effects on plants	4
2.2.1 Effects of salinity on plant growth	5
2.2.2 Effects of salinity on plant mineral concentration	6
2.2.3 Effects of salinity on water relations	8
2.2.4 Effects of salinity on photosynthesis	9
2.2.5 Effect of salinity on chlorophyll content	10
2.2.6 Effect of salinity on seed germination	11
2.3 Salt tolerance in plants	12
2.3.1 Mechanisms of salt tolerance	12
2.4 Citrus	17
2.4.1 Taxonomy and morphology of citrus	17
2.4.2 Citrus cultivars	18
2.4.3 Citrus rootstocks	18
2.4.4 Citrus interstock	19
2.4.5 Citrus propagation	19
2.5 Effects of salinity on citrus	19
<b>3 IMPACTS OF SALINITY ON SEEDLING EMERGENCE AND EARLY GROWTH OF CITRUS ROOTSTOCKS</b>	<b>24</b>
3.1 Introduction	24
3.2 Materials and methods	25
3.2.1 Plant material and growth condition	25

3.2.2	Salinity treatments	25
3.2.3	Growth measurements	25
3.2.4	Experimental design and data analysis	26
3.3	Results	26
3.3.1	Emergence of the first seedlings	26
3.3.2	Emergence spread of citrus rootstocks seedlings	28
3.3.3	Percentage of final emergence of seedlings	28
3.3.4	Percentage of survival of seedlings	28
3.3.5	Effect of salinity on seedlings growth indices	31
3.4	Discussion	35
3.5	Conclusion	36
<b>4</b>	<b>ASSESSING CITRUS ROOTSTOCKS FOR SALINITY TOLERANCE</b>	<b>38</b>
4.1	Introduction	38
4.2	Materials and methods	39
4.2.1	Plant material and growth condition	39
4.2.2	Salinity treatments	41
4.2.3	Mineral contents	42
4.2.4	Vegetative growth parameters	42
4.2.5	Relative water content	42
4.2.6	Proline assay	43
4.2.7	Experimental design and data analyses	43
4.3	Results	43
4.3.1	Mineral contents	43
4.3.2	Leaf number	50
4.3.3	Shoot height	51
4.3.4	Root length	51
4.3.5	Relative water content	53
4.3.6	Proline	53
4.4	Discussion	54
4.5	Conclusion	58
<b>5</b>	<b>EFFECTS OF SALINITY ON SOME PHYSIOLOGICAL AND NUTRITIONAL INDICATORS OF TWO MALAYSIAN CITRUS CULTIVARS</b>	<b>60</b>
5.1	Introduction	60
5.2	Materials and methods	61
5.2.1	Plant material and growth condition	61
5.2.2	Salinity treatments	61
5.2.3	Mineral contents	61
5.2.4	Vegetative growth	61
5.2.5	Relative water content	61
5.2.6	Proline assay	62
5.2.7	Experimental design and data analyses	62
5.3	Results	62

5.3.1	Mineral contents	62
5.3.2	Vegetative growth	64
5.3.3	Relative water content	65
5.3.4	Proline content	66
5.4	Discussion	66
5.5	Conclusion	68
<b>6</b>	<b>GROWTH AND PHYSIOLOGY OF LIMAU KESTURI ON CLEOPATRA MANDARIN AND SHAKER ROOTSTOCKS UNDER SALINE CONDITIONS</b>	<b>69</b>
6.1	Introduction	69
6.2	Materials and methods	70
6.2.1	Plant material and growth condition	70
6.2.2	Salinity treatments	71
6.2.3	Mineral contents	71
6.2.4	Scion shoot length	71
6.2.5	Relative water content	71
6.2.6	Proline content	72
6.2.7	MDA determination	72
6.2.8	H <sub>2</sub> O <sub>2</sub> determination	72
6.2.9	Experimental design and data analysis	72
6.3	Results	73
6.3.1	Leaf mineral contents of scion	73
6.3.2	Scion shoot length	77
6.3.3	Relative water content	78
6.3.4	Proline content	78
6.3.5	MDA content	79
6.3.6	H <sub>2</sub> O <sub>2</sub> content	80
6.4	Discussion	80
6.5	Conclusion	84
<b>7</b>	<b>COMPARATIVE STUDIES BETWEEN DIPLOID AND TETRAPLOID DEZ ORANGE [<i>CITRUS SINENSIS</i> (L.) OSB.] UNDER SALINITY STRESS</b>	<b>85</b>
7.1	Introduction	85
7.2	Materials and methods	86
7.2.1	Plant materials and growth conditions	86
7.2.2	Salinity treatments	87
7.2.3	Mineral contents	87
7.2.4	Proline content	87
7.2.5	MDA determination	87
7.2.6	H <sub>2</sub> O <sub>2</sub> determination	88
7.2.7	Experimental design and data analysis	88
7.3	Results	88



7.3.1	Mineral contents	88
7.3.2	Proline content	96
7.3.3	MDA content	96
7.3.4	H <sub>2</sub> O <sub>2</sub> content	96
7.4	Discussion	97
7.5	Conclusion	99
<b>8</b>	<b>EFFECT OF DEZ ORANGE [<i>CITRUS SINENSIS</i> (L.)OSB.] AS INTERSTOCK ON THE SALT TOLERANCE OF LIMAU KESTURI (<i>CITRUS MICROCARPA</i> BUNGE) UNDER SALINE CONDITION</b>	<b>100</b>
8.1	Introduction	100
8.2	Materials and methods	101
8.2.1	Plant material and growth condition	101
8.2.2	Salinity treatments	103
8.2.3	Scion shoot length	103
8.2.4	Mineral contents	103
8.2.5	Net photosynthesis	103
8.2.6	Relative water content	103
8.2.7	Proline content	103
8.2.8	MDA determination	103
8.2.9	H <sub>2</sub> O <sub>2</sub> determination	103
8.2.10	Experimental design and data analysis	104
8.3	Results	104
8.3.1	Mineral ion contents	104
8.3.2	Scion shoot length	108
8.3.3	Net photosynthesis	109
8.3.4	Relative water content	109
8.3.5	Proline content	111
8.3.6	MDA content	111
8.3.7	H <sub>2</sub> O <sub>2</sub> content	111
8.4	Discussion	113
8.5	Conclusion	116
<b>9</b>	<b>SUMMARY, CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH</b>	<b>117</b>
9.1	Summary and conclusion	117
9.2	Recommendations for future research	119
	<b>REFERENCES</b>	<b>120</b>
	<b>APPENDICES</b>	<b>139</b>
	<b>APPENDIX A</b>	<b>139</b>
	<b>A-1 Nutrient solution</b>	<b>139</b>
	<b>APPENDIX B</b>	<b>140</b>
	<b>BIODATA OF STUDENT</b>	<b>148</b>
	<b>LIST OF PUBLICATIONS</b>	<b>149</b>

## LIST OF TABLES

<b>Table</b>	<b>Page</b>
3-1 Effect of salinity levels and rootstocks on first seedlings, emergence spread, percentage of final emergence and percentage of seedlings survival	27
3-2 Effect of salinity levels and rootstocks on fresh plant weight, plant dry matter, shoot length, length of major root and stem diameter	31
4-1 Characteristics of the citrus rootstocks	40
4-2 Effect of rootstock and NaCl salinity on N, P, K, Na and Cl contents of leaves and roots	44
4-3 Effect of rootstock and NaCl salinity on leaf number, shoot height, root length, RWC and proline content	50
5-1 Mean square and significant levels of cultivar and NaCl salinity level on leaf K, Na, Cl, K/Na ratio, leaf number, shoot height, root length, RWC and proline content	63
6-1 Effect of rootstock and NaCl salinity on N, P, K, Na, Cl contents and K/Na ratio	73
6-2 Effect of rootstock and NaCl salinity on scion length, RWC, proline, MDA and H <sub>2</sub> O <sub>2</sub> contents	78
7-1 Mean square and effect of rootstock and NaCl salinity level on N, P, K, Na, Cl, K/Na, proline, MDA and H <sub>2</sub> O <sub>2</sub> contents	89
8-1 Grafted citrus plants used in the study	102
8-2 Effect of rootstock and NaCl salinity on contents of N, P, K, Cl, Na and K/Na ratio	105
8-3 Effect of rootstock and NaCl salinity on scion length, Net photosynthesis, RWC, Proline, MDA and H <sub>2</sub> O <sub>2</sub> contents	108
A-1 Nutrients composition of the solution used in the study	139
B-1 ANOVA for first seedlings, emergence spread, percentage of final emergence, percentage of seedlings survival, fresh plant weight, plant dry matter, shoot length, length of major roots and stem diameter	140
B-2 ANOVA for N, P, K, Na and Cl contents of leaves and roots, leaf number, shoot height, root length, RWC	141
B-3 ANOVA for leaf K, Na, Cl, K/Na ratio, leaf number, shoot height, root length, RWC and proline content	143
B-4 ANOVA for N, P, K, Na, Cl contents, K/Na ratio, scion length, RWC, proline, MDA and H <sub>2</sub> O <sub>2</sub> contents	144
B-5 ANOVA for N, P, K, Cl, Na and K/Na ratio, scion length, Net photosynthesis, RWC, Proline, MDA and H <sub>2</sub> O <sub>2</sub> contents	146

## LIST OF FIGURES

<b>Figure</b>		<b>Page</b>
3-1	Experimental pots	26
3-2	Effect of salinity levels on seed germination	27
3-3	Interaction effects of salinity and rootstocks on emergence of the first seedling	28
3-4	Interaction effects of salinity and rootstocks on emergence spread (A), final percentage emergence (B) and seedling survival percentage (C)	30
3-5	Interaction effects of salinity and rootstocks on fresh weight (A) and dry weight (B)	32
3-6	Interaction effects of salinity and rootstocks on shoot length (A), length of major root (B) and stem diameter (C)	34
4-1	Fruits of tested citrus rootstocks used in the study	41
4-2	Young seedlings in nursery bed at TPU.	41
4-3	Interaction effects of salinity and rootstocks on leaf nitrogen (A), root nitrogen (B), and leaf phosphorus (C) content (Mean $\pm$ S.E.; n = 4)	45
4-4	Interaction effects of salinity and rootstocks on root phosphorus (A), leaf potassium (B), and root potassium (C) content (Mean $\pm$ S.E.; n = 4)	47
4-5	Interaction effects of salinity and rootstocks on leaf sodium (A), root sodium (B), and leaf chloride (C) content (Mean $\pm$ S.E.; n = 4)	49
4-6	Interaction effects of salinity and rootstocks on root chloride content (A), shoot height (B), and root length (C) (Mean $\pm$ S.E.; n = 4)	52
4-7	Interaction effects of salinity and rootstocks on RWC (A) and proline content (B)	54
4-8	Leaf necrosis caused by NaCl salinity	56
5-1	Interaction effects of salinity and cultivars on leaf potassium content (A), leaf chloride content (B), and leaf K/Na ratio (C) (Mean $\pm$ S.E.; n = 4)	64
5-2	Interaction effects of salinity and cultivars on leaf number (A) and shoot height (B) (Mean $\pm$ S.E.; n = 4)	65
5-3	Interaction effects of salinity and cultivars on RWC (A) and proline content (B) (Mean $\pm$ S.E.; n = 4)	66
5-4	Effect of salinity on plant growth at 30 days after stress initiation	67
6-1	Method of creating a budded tree. Placing a scion bud on the rootstock (A), joining of rootstock and scion bud (B), growth of scion bud (C&D) and schema - a grown budded tree (D)	71
6-2	Effects of salinity and rootstocks on N (A), P (B) and K (C) contents (Mean $\pm$ S.E.; n = 4)	75
6-3	Effects of salinity and rootstocks on Na (A), Cl (B) contents and K/Na ratio (C) (Mean $\pm$ S.E.; n = 4)	77
6-4	Effects of salinity and rootstocks on relative water content (A) and proline content (B) (Mean $\pm$ S.E.; n = 4)	79
6-5	Effects of salinity and rootstocks on MDA (A) and H <sub>2</sub> O <sub>2</sub> (B) contents (Mean $\pm$ S.E.; n = 4)	80
7-1	Dez orange Cultivars: Tetraploid (A) and Diploid (B)	87
7-2	Effects of salinity and Dez orange cultivars on leaf nitrogen (A), root nitrogen (B), leaf phosphorus (C) and root phosphorus (D) content (Mean $\pm$ S.E.; n = 4)	91
7-3	Interaction effects of salinity and Dez orange cultivars on leaf potassium	93

	(A), root potassium (B), leaf sodium (C) and root sodium (D) content (Mean $\pm$ S.E.; n = 4)	
7-4	Interaction effects of salinity and Dez orange cultivars on leaf chloride (A), root chloride content (B), leaf K/Na ratio (C) and root K/Na ratio (D) (Mean $\pm$ S.E.; n = 4).	95
7-5	Interaction effects of salinity and Dez orange cultivars on proline (A), MDA (B) and H <sub>2</sub> O <sub>2</sub> content (D) (Mean $\pm$ S.E.; n = 4)	97
8-1	Technique of creating an interstocked citrus plant. Preparation of interstock (A), inserting of interstock into rootstock (B), graft wrapping (C) and joining of rootstock and interstock (D).	102
8-2	Longitudinal and transverse incision of joining of cambium layers (A and B) photographed by stereomicroscope, scion growth on interstock (C), grown interstocked citrus (D).	102
8-3	Effects of salinity and Dez orange interstocks on nitrogen (A), phosphorus (B) and potassium (C) content of leaves of interstocked Limau Kesturi (Mean $\pm$ S.E.; n = 4)	106
8-4	Effects of salinity and Dez orange interstocks on chloride (A) and sodium (B) contents of leaf of interstocked Limau Kesturi (Mean $\pm$ S.E.; n = 4)	107
8-5	Effects of salinity and Dez orange interstocks on scion length (A), net photosynthesis (B) and RWC (C) of interstocked Limau Kesturi (Mean $\pm$ S.E.; n = 4)	110
8-6	Interaction effects of salinity and Dez orange interstocks on proline (A), MDA (B) and H <sub>2</sub> O <sub>2</sub> (C) contents of interstocked Limau Kesturi (Mean $\pm$ S.E.; n = 4)	112

## LIST OF ABBREVIATION

ABA	Absciscic acid
DM	Dray matter
DW	Dry weight
EC	Electrical conductivity
FW	Fresh weight
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
IAA	Indole acetic acid
K/Na	Potassium to sodium ratio
MDA	Malondialdehyde
mM	Millimolar
nmol	Nanomole
ROS	Reactive oxygen species
RWC	Relative water content
TW	Turgid weight
μmol	micromole



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## 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 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<sup>-1</sup> (1dS m<sup>-1</sup> 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<sup>+</sup>, K<sup>+</sup>, Mg<sup>+2</sup>, Ca<sup>+2</sup>, Cl<sup>-</sup>, SO<sub>4</sub><sup>-2</sup>, HCO<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>-2</sup> and NO<sub>3</sub><sup>-</sup> 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 interstock 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 of 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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