The Pennsylvania State University

The Graduate School

College of Agricultural Sciences

THE USE OF GRAFTING FOR IMPROVING SALINITY TOLERANCE IN TOMATO PLANTS

A Dissertation in

Horticulture

by

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Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

May 2017
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ABSTRACT

THE USE OF GRAFTING FOR IMPROVING SALINITY TOLERANCE IN TOMATO PLANTS

Soil salinity is one of the major abiotic stresses causing yield reductions in agriculture worldwide. Tomato is one of the most economically important crops in the world. Tomato is adapted to a wide variety of climates, but its cultivation is concentrated in warm and dry areas, where salinity is a problem. It has been difficult to develop salt-tolerant tomato lines through breeding programs because of the large number of small-effect genes involved in salinity tolerance. Grafting was proposed as a method to improve tomato tolerance to salinity by combining the salt-tolerant rootstocks with the scions that have the desired fruit quality and yield characteristics. Therefore, in this project, we are examining the role of grafting in improving salinity tolerance in tomato plants.

The first experiment in our project was designed to examine if different scion/rootstock combinations have different survival rates and growth performance. We examined 66 tomato grafting combinations, including 7 self-grafts. We found that survival rates were different among the combinations, with ‘Arnold’ (AR), ‘Taurino’, and ‘Maxifort’ having the highest survival rates. Most self-grafted plants had lower survival rates than the hetero-graft combinations. Genotypes with excellent survival rates (AR, Maxifort, Rocky Top and Phoenix) were selected for the salinity screening experiment.

In our screening experiment (Chapter 3), the aim was to screen 21 wild and cultivated genotypes with 4 NaCl treatments (0, 50, 100, 150 mM NaCl) for 16 days to choose genotypes that could be potential rootstocks in the grafting experiment. There was a substantial genotypic variation in the percent reduction in shoot dry weight (DW) under 50 and 100 mM NaCl treatments. Plant vigor and response to salinity were key criteria.
for selecting plants in this experiment. ‘Ironman’ genotype was the most vigorous genotype under all NaCl treatments. Other genotypes (such as LA3120) were less vigorous under salinity but limited Na\(^+\) transport to the shoot, whereas ‘LA1630’ genotype transported more Na\(^+\) to the shoot. Genotypes that showed high vigor and/or low shoot DW reduction under NaCl treatments were chosen as potential rootstocks for our grafting experiments.

In the grafting experiment, seven rootstocks and one scion ‘Moneymaker’, that showed low reduction in shoot DW and/or high vigor under NaCl treatments in the screening experiment, were used in grafting. Plants were treated with 0 or 75 mM NaCl for 25 days. Na\(^+\), K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\) concentrations were measured in the leaves and roots. In salinized plants, percent reduction of shoot DW varied among graft combinations, ranging from ~4% in Moneymaker/Resistar (M/RS) to ~40% in Moneymaker/Ironman (M/Im). The vigor of the rootstock affected the overall growth of the plants under NaCl treatment. ‘Moneymaker’ grafted onto ‘AR’ rootstock was the most vigorous combination and its vigor seemed to be the main factor for its good performance under salinity conditions. ‘Moneymaker’ grafted onto ‘LA1630’ had the highest leaf Na\(^+\) concentration, whereas those grafted onto ‘LA3120’ had the lowest Na\(^+\) concentration in the leaf. Rootstock clearly had a key role in controlling the salt tolerance mechanism of the scion.

To the best of our knowledge, no previous studies were conducted to test whether Na\(^+\) and K\(^+\) concentrations change across the graft union. Therefore, our sap experiment was planned to measure Na\(^+\) and K\(^+\) ion concentrations in the stem sap below the graft union (below-graft segment) and above the graft union (above-graft segment). Plants in this experiment were grown for 10 days under 75 mM NaCl treatment. Most grafted plants had lower Na\(^+\) concentrations in their sap than in non-grafted plants. In all hetero-grafts, sap Na\(^+\) concentration was also higher in the below-graft segment than in the above-graft segment. Self-grafts, however, had similar Na\(^+\) concentrations in the
two positions. In all graft combinations, $K^+ : Na^+$ ratio was always higher in the above-graft segment than in the below graft segment.

These studies show that the extent and mechanisms of salt tolerance vary among commercial tomato cultivars, and that grafting of different but compatible genotypes helps restrict $Na^+$ movement to the shoot. Salt-tolerant rootstocks from this project can be used by farmers to improve tomato growth under saline conditions.


<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of Figures .......................................................... viii</td>
</tr>
<tr>
<td>List of Tables ............................................................... xi</td>
</tr>
<tr>
<td>Acknowledgements ................................................................... xii</td>
</tr>
</tbody>
</table>

CHAPTER 1 General introduction .................................................. 1
  Tomato production ................................................................. 1
  Causes and types of salinity .................................................... 3
  Plants go through two distinct phases of growth when exposed to salt ........ 3
  Mechanisms of salt tolerance .................................................. 5
  Salt effects on plants ............................................................. 7
  Membrane transporters ......................................................... 11
  Plant grafting for salinity tolerance ........................................ 14
  References .............................................................................. 17

CHAPTER 2 Evaluation of the growth performance and survival of tomato scion-rootstock combinations ........................................... 23
  Introduction ............................................................................ 23
  Methods ................................................................................. 25
    Plant material and growing conditions .................................. 25
    Grafting process .................................................................. 26
    Healing conditions ................................................................ 27
    Experimental design ........................................................... 27
    Measurements ....................................................................... 28
  Results .................................................................................... 28
  Discussion ............................................................................... 29
  References .............................................................................. 32
  Figures .................................................................................... 33

CHAPTER 3 Evaluation of salt tolerance in cultivated and wild tomato genotypes .......... 36
  Introduction ............................................................................ 36
  Methods ................................................................................. 38
    Plant material and treatments ............................................. 38
    Determination of the salt tolerance mechanism ..................... 42
    Cation analysis ..................................................................... 42
  Results .................................................................................... 42
  References .............................................................................. 53
  Tables ..................................................................................... 57
  Figures .................................................................................... 63

CHAPTER 4 Examining the Use of Grafting for Improving Salinity Tolerance in Tomato Plants .......................................................... 69
Introduction........................................................................................................................................69
Materials and Methods....................................................................................................................73
Experiment 1: Grafting for better tolerance to salinity .................................................................73
  Cation analysis ............................................................................................................................76
  Experimental design..................................................................................................................76
Experiment 2: Sap ion concentrations ..........................................................................................76
  Experimental design..................................................................................................................78
  Statistical analysis .....................................................................................................................78
Results...............................................................................................................................................78
  Plant Growth ..................................................................................................................................79
  Ion concentration and partitioning ..............................................................................................79
  Water relations and leaf gas exchange parameters .......................................................................81
Sap Na\(^+\) and K\(^+\) analysis..........................................................................................................81
Discussion.........................................................................................................................................83
  The vigor of the rootstock affects the overall plant growth ..........................................................83
  Reduction in shoot DW under salinity stress is not caused by the osmotic effect of salinity ..........84
  Cultivated genotypes as rootstocks are better at maintaining higher selectivity for K\(^+\) and Ca\(^{2+}\) over Na\(^+\) than M/M .................................................................86
  Rootstock plays a dominant role in controlling salt concentrations in the scion .....................86
  Plants grafted onto wild rootstocks accumulated higher rates of Na\(^+\) in the leaf ....................89
  An inverse relationship found between the percent reduction in shoot DW and root to shoot ratio ..................................................................................................................................................90
  Most graft combinations had lower sap Na\(^+\) in the above-graft than in the below-graft segment .................................................................................................................................................91
References.........................................................................................................................................94
Tables...............................................................................................................................................97
Figures............................................................................................................................................101

CHAPTER 5 OVERALL CONCLUSIONS/SUMMARY ......................................................................113

References.........................................................................................................................................117
LIST OF FIGURES

Fig 2. 1: Heat map of the survival rate (%) at week-3 post grafting in different tomato scion and rootstock combinations.........................................................33

Fig 2. 2: Heat map of survival rate (%) at harvest time (anthesis) in different tomato scion and rootstock combinations.................................................................34

Fig 2. 3: Heat map of average stem diameter (mm) at harvest time in different tomato scion and rootstock combinations.................................................................34

Fig 2. 4: Heat map of average number of expanded leaves at harvest in different tomato scion and rootstock combinations.................................................................35

Fig 2. 5: Heat map of average shoot dry weight (g) at harvest time in different tomato scion and rootstock combinations.................................................................35

Fig 3. 1: Reduction of shoot dry weight in tomato genotypes grown for 16 days in the presence of 50, 100, or 150 mM NaCl compared to control plants of the same genotype .................................................................63

Fig 3. 2: Scatterplots of the shoot dry weight (g) plotted for control plants versus salt-treated plants grown with 50 mM NaCl (A), 100 mM NaCl (B), 150 mM NaCl (C) ...........64

Fig 3. 3: Root to shoot ratio in tomato genotypes grown for 16 days in control, 50, 100, or 150 mM NaCl. ........................................................................................................64

Fig 3. 4: Plant height change between 7 and 16 days of salt treatment in control, 50, 100, or 150 mM NaCl. ........................................................................................................65

Fig 3. 5: Na\(^+\) concentration in the 4th leaf of eight tomato genotypes grown for 16 days in the presence of 50, 100, or 150 mM NaCl .................................................................66

Fig 3. 6: Na\(^+\) concentration in the roots of salt-treated tomato genotypes grown for 16 days in the presence of 50, 100, or 150 mM NaCl.................................................................66

Fig 3. 7: Relationship between percent reduction in shoot DW and leaf Na\(^+\) concentrations in genotypes treated with 50, 100, or 150 mM NaCl after 16 days of salt treatment. ........................................................................................................67

Fig 3. 8: Relationship between leaf K\(^+\) and Na\(^+\) concentrations in genotypes treated with 100 mM NaCl after 16 days of salt treatment .................................................................67

Fig 3. 9: Relationship between leaf K\(^+\) and Na\(^+\) concentrations in genotypes treated with 150 mM NaCl after 16 days of salt treatment .................................................................68
Fig. 3.10: Relationship between leaf Ca$^{2+}$ and Na$^+$ concentrations in genotypes treated with 150 mM concentrations of NaCl after 16 days of salt treatment.

Fig. 4.1: Shoot dry weight of Moneymaker (M) grafted onto different rootstocks in 75 mM NaCl-treated versus control plants grown for 25 days on half-strength Hoagland’s solution.

Fig. 4.2: Scatterplot of the shoot dry weight (g) plotted for control plants vs salt-treated plants (75 mM NaCl).

Fig. 4.3: Percent reduction of shoot dry weight in Moneymaker (M) scions grafted onto different rootstocks in 75 mM NaCl-treated relative to control tomato plants grown for 25 days on half-strength Hoagland’s solution.

Fig. 4.4: Root to shoot ratio of Moneymaker (M) scions grafted onto different rootstocks in 75 mM NaCl-treated versus control plants grown for 25 days on half-strength Hoagland’s solution.

Fig. 4.5: Na$^+$ concentration (mg/g DW) in the 5th leaf and roots of the different graft combinations in 75 mM NaCl-treated plants grown for 25 days on half-strength Hoagland’s solution.

Fig. 4.6: K$^+$ concentration (mg/g DW) in the 5th leaf and roots of the different graft combinations in the presence of 75 mM NaCl.

Fig. 4.7: K$^+$: Na$^+$ ratio in the 5th leaf and roots of the different graft combinations in the presence of 75 mM NaCl.

Fig. 4.8: Relationship between leaf Na$^+$ and leaf K$^+$ concentrations (mg/g DW) in all graft combinations after 25 days of growth in 75 mM NaCl.

Fig. 4.9: Ca$^{2+}$ concentration (mg/g DW) in the 5th leaf and roots of the different graft combinations in the presence of 75 mM NaCl.

Fig. 4.10: Ca$^{2+}$: Na$^+$ ratio in the 5th leaf and roots of the different graft combinations in the presence of 75 mM NaCl.

Fig. 4.11: Relationship between root Ca$^{2+}$ and percent reduction in shoot dry weight in all graft combinations after 25 days of growth in 75 mM NaCl.

Fig. 4.12: Mg$^{2+}$ concentration (mg/g DW) in the leaf and roots of the different graft combinations in the presence of 75 mM NaCl.

Fig. 4.13: Mg$^{2+}$: Na$^+$ ratio in the leaf and roots of the different graft combinations in the presence of 75 mM NaCl.
Fig. 4. 14: Leaf relative water content (%) of Moneymaker (M) scions grafted onto different rootstocks in 75 mM NaCl-treated versus control plants grown for 25 days on half-strength Hoagland’s solution...

Fig. 4. 15: Percent reduction in stomatal conductance over time in the different graft combinations in 75 mM NaCl-treated plants relative to control during three weeks of 75 mM NaCl treatment...

Fig. 4. 16: Percent reduction of shoot dry weight in Moneymaker (M) scions grafted onto different Rootstocks in 75 mM NaCl-treated relative to control tomato plants grown for 10 days on half-strength...

Fig. 4. 17: Stem xylem sap Na$^+$ concentrations (mg/L) above the graft-union (A), below the graft-union (B), and in stem segments of non-grafted (NG) plants. ‘MM’ was grafted onto ‘AR’, ‘LA1630’, ‘LA3120’, ‘MM’ (self-grafts), or RS in plants grown for 10 days in the presence of 75 mM NaCl...

Fig. 4. 18: Stem xylem sap K$^+$ concentrations (mg/L) above the graft union (A), below the graft union (B), and in the stem segments of non-grafted (NG) plants. ‘MM’ was grafted onto ‘AR’, ‘LA1630’, ‘LA3120’, ‘MM’ (self-grafts), or ‘RS’ in plants grown for 10 days in the 75 mM NaCl and control treatment.

Fig. 4. 19: Stem xylem sap K$^+$:Na$^+$ ratio above the graft-union (A), below the graft union (B), and in the stem segments of non-grafted (NG) plants of ‘MM’ grafted onto ‘AR’, ‘LA1630’, ‘LA3120’, ‘MM’ (self-grafts), or ‘RS’ in plants grown for 10 days in the presence of 75 mM NaCl...

Fig. 4. 20: Shoot dry weight in grafted (G) and non-grafted (NG) plants grown for 10 days in 75 mM NaCl treatment...

Fig. 4. 21: Shoot dry weight in grafted (G) and non-grafted (NG) plants grown for 10 days in the control treatment...

Fig 5. 1: Divisions for classifying crop tolerance to salinity; adapted from Maas, 1993
LIST OF TABLES

Table 2. 1: Genotypes used in grafting in the experiment. .........................................................26

Table 3. 1: Genotypes used in this screening experiment at 0, 50, 100, and 150 mM NaCl. ..39

Table 3. 2: Two-way Analysis of Variance (ANOVA), for the effect of genotype and NaCl
treatment (0, 50, 100, 150 mM) on leaf and root ion concentrations. .........................57

Table 3. 3: Two-way Analysis of Variance (ANOVA), for the effect of genotype and NaCl
treatment (0, 50, 100, and 150 mM) on leaf and root elemental ratios. ......................58

Table 3. 4: Two-way Analysis of Variance (ANOVA), for the effect of genotype and NaCl
treatment (0, 50, 100, and 150 mM) on the growth variables of the genotypes...........59

Table 3. 5: Correlation matrix for leaf ion concentration, growth and physiological traits
of 8 genotypes grown under salts stress (50 mM NaCl). ............................................60

Table 3. 6: Correlation matrix for leaf ion concentration, growth and physiological traits
of 8 genotypes grown under salt stress (100 mM NaCl).............................................61

Table 3. 7: Correlation matrix for root ion concentration, growth and physiological traits
of 8 genotypes grown under salts stress (100 mM NaCl). ............................................62

Table 4. 1: Genotypes used in grafting in the experiment. .....................................................73

Table 4. 2: One-way Analysis of Variance (ANOVA) for the effect of genotype on ion
centrations and ratios, growth variables, and relative water content (RWC) ..........97

Table 4. 3: Correlation matrix for leaf ion concentration, growth and physiological traits
of 8 genotypes grown under salts stress (75 mM NaCl) .............................................98

Table 4. 4: Correlation matrix for root ion concentration, growth and physiological traits
of 8 genotypes grown under salt stress (75 mM NaCl)..............................................99

Table 4. 5: Two-way Analysis of Variance (ANOVA), for the effect of genotype (grafted or
non-grafted) and position under (0 and 75 mM NaCl) on sap ion concentrations.
The three positions were above-graft segments, below-graft segments, and non-
grafted stem segments. .................................................................................................99

Table 4. 6: One-way Analysis of Variance (ANOVA), for the effect on sap ion
concentrations on the sap position in genotypes grown under 75 mM NaCl. ..........100
ACKNOWLEDGEMENTS

I’m really thankful for having the opportunity to study at the Pennsylvania State University. Words are not enough to express my sincere gratitude to Dr. Kathleen Brown for her unremitting support throughout my PhD studies and research. Without her guidance, motivation, and patience, this work wouldn’t have come to light. Indeed, it has been an honor (and a pleasure) to work under her creative, enthusiastic, and brilliant mentorship. Thank you for everything!

I’m also grateful to Dr. Michael Orzolek for accepting me as a PhD student and for his complete trust in me and in my work. I’m really appreciating his continuous encouragement and his generous support to me since the early beginning of my PhD journey.

I would like to thank my committee member Dr. Jack Watson for his warm encouragement and support. I knew Dr. Watson when I took one of his courses. In his class I knew that he’s not only a great teacher but also a wonderful person. I’m so lucky to have him in my PhD committee. I’m also very lucky to have Dr. Elsa Sanchez in my committee. Dr. Elsa was always supportive and never saved any effort to help me with my research requirements.

I also want to thank all members of Brown and Lynch Lab, for their nonstop help and their amazing cooperation with me that added significantly to my graduate studies experience. Molly Hanlon, I greatly appreciate your excellent assistance and support to me. Thank you for being such a wonderful friend.

I’d also like to thank our lab manager, Bob Snyder, and our greenhouse manager, Scott DiLoreto, for being always helpful and accommodating to my repeated requests for my research experiments.
Last but not least, I would like to thank my parents and my husband for believing in me and for their continuous support in everything in my life. To my sweet baby girl “Haya”, although you made it harder on me to finish my work, I’m million times happier with you; that’s for sure.

Finally, I would like to convey my deepest appreciation for the financial support of the Egyptian Ministry of Higher Education, which has generously funded me and my studies for the first 4 years in the PhD program, and also for the Pennsylvania State University for the financial assistance during my 5th year of studies.

Reham
CHAPTER 1
General introduction

Salinity is an important stress that dramatically affects agricultural productivity in more than 20% (45 million hectares) of irrigated land areas worldwide (Munns 2011). Irrigated lands are only 15% of total cultivated lands, but they produce one-third of the world’s food (Munns 2011). Total global land area affected with salts, including saline and sodic soils, comprise over 800 million hectares (FAO, 2005, Rengasamy 2006). Even though saline soils are more prominent in arid and semi-arid regions, they are found in every climatic zone of the world (Plett and Møller 2010; Tuteja 2007; Williams 1999, 2001).

Lands affected with salinity are expected to expand in the future due to the global climatic changes (Cabot et al. 2014; Williams 1999, 2001). Climate change is expected to change global average temperature, evaporative demands, and patterns of rainfall. In addition, large proportions of the semi-arid lands will get warmer and drier which might increase evapotranspiration and salinity problems (Yeo 1999, IPCC, 2007). Salinity is estimated to decrease arable lands by 50% in the middle of the 21st century. More seriously, salinity is causing deterioration of 1% of the world’s agricultural lands (about 2 million hectares) each year (Tuteja 2007).

Tomato production

Worldwide tomato production was almost 160 million tons in 2011, which makes tomato the seventh most important crop following maize, rice, wheat, potatoes, soybeans, and cassava (Bergougnoux 2013). Although tomato plants are adapted to climatic conditions of many areas around the world, most of its production is from countries with dry and warm conditions (> 30% from countries around the Mediterranean Sea such as Spain, Egypt, and Turkey, and 20% of the production is from California) (Cuartero et al. 1999). Those areas, however, are also subjected to natural
soil-forming processes which produce soil salinization in addition to the secondary salinization problems caused by the poor irrigation practices (Cuartero et al. 1999).

Salinity seriously affects tomato growth at every stage from germination to maturity (Marcelis and Hooijdonk 1999; Romero-Aranda, Soria, and Cuartero 2001). Although wild relatives of cultivated tomatoes are relatively more tolerant, it has been difficult to develop salt tolerant lines because of the large number of small-effect genes involved in salinity tolerance. Therefore, conventional breeding programs are not efficient for the selection of salt-tolerant tomato genotypes (Colla et al. 2006; Cuartero et al. 2006; Roy, Negrão, and Tester 2014).

Using genetic transformations has led to some improvements in salinity tolerance by transferring one or more genes. However, the complexity of the trait and the detrimental side effects made this task difficult (Colla et al. 2010; Roy et al. 2014). For example, transgenic tomato lines with the yeast gene HAL1 had an increase in the K\(^+\): Na\(^+\) ratio compared to the wild type. However, shoot fresh and dry weights of those plants were reduced to about half when grown in the absence of salt, as compared to the wild type (Gisbert et al. 2000). Similar effects were seen in other plant species (such as in Arabidopsis and tobacco), when they were genetically modified (Huang et al. 2000).

The effect of genetic engineering with the purpose of improving plant tolerance or decreased Na\(^+\): K\(^+\) ratios to salinity may not be good enough to meet growers’ expectations of having plants with better growth and yield under salinity conditions. In fact, farmers are more interested in the absolute plant growth and yield. For instance, a less-vigorous, salt-tolerant plant may not be preferred over a salt-sensitive plant that remained more vigorous than the salt-tolerant genotype even after losing up to 50% of its weight (Flowers 2004).

Plant genetic engineering technology will undoubtedly help in improving our understanding about the cellular mechanisms underlying salt tolerance. However,
because of the complexity of the trait, coming up with a tolerant crop by engineering sensitive crops for salinity tolerance might take a long time to develop (Flowers 2004). Therefore, there still is a need for the use of other technologies to improve tomato tolerance to salinity.

**Causes and types of salinity**

Salinity is classified into two categories based on its cause, primary (naturally-occurring salinization) and secondary (anthropogenic) salinity (Lawrie 2007). Primary salinity is caused by weathering of rocks, intrusion of sea water to lands, rain, wind-transported materials from land or lakes, and capillary rise of saline shallow groundwater. Secondary salinity, however, is caused by human activities such as overuse of fertilizers, over irrigation and poor drainage systems, and extensive removal and replacement of deep rooted species by shallow rooted species. The use of poor quality water for irrigation and flooding with water rich in salt are other causes of secondary salinization (Rengasamy 2006; Williams 2001).

Soils are affected by one of two categories of salt: sodic and saline soils. Sodic soils are dominated by high carbonate or bi-carbonate ions, pH above 8.5, high sodium absorption ratio (SAR), and poor soil structure, while saline soils are dominated by chloride or sulfate ions, pH below 8.5, lower SAR, and higher electrical conductivity (EC) than sodic soils (Flowers and Flowers 2005). Soils are considered saline when their EC reaches 4 dS.m\(^{-1}\) or more (Parvaiz and Azooz 2013; Plett and Møller 2010).

**Plants go through two distinct phases of growth when exposed to salt**

Plants are divided into halophytes and glycophytes based on their capability of growing under salt conditions. Halophytes such as Atriplex can grow in soils with high salt concentrations. Glycophytes or non-halophytes, including all agricultural crops, cannot tolerate growing under high salt conditions (Flowers and Flowers 2005; Parvaiz and Azooz 2013). Most glycophytes cannot efficiently exclude salts from the roots resulting
in salt accumulation to toxic levels in the transpiring leaves. Some of the glycophytes that can exclude salts cannot compartmentalize salts as efficiently as halophytes (Munns 2002).

Plants go through two phases when they grow under salinity conditions, the osmotic and the ionic phases. In the osmotic (water-deficit) phase, a rapid growth reduction occurs due to the osmotic effect of salt in the outside medium on the roots ability to take up water (Munns and Tester 2008). The mechanisms that control plant growth response in the osmotic phase are related to water stress effects, since Na\(^+\) and Cl\(^-\) are not toxic in the growing tissues in that stage (Munns 2005).

The mechanism for reducing shoot growth and development in the osmotic phase is not very well known. In the first few days, there is evidence that hormonal signals from the roots, rather than water signals, are responsible for the growth reduction in salinized environment (Munns 2005). Abscisic acid is a strong candidate for the chemical signal, since it increases in xylem sap after salt stress; however, there is no certain evidence yet that it is the only hormone that responds to salt. More recently, it was proposed that long distance signaling such as Ca\(^{2+}\) waves, reactive oxygen species (ROS) signaling, or long distance electrical signaling can be other mechanisms involved in the plant response during the osmotic phase (Maischak et al. 2010; Mittler et al. 2011; Munns and Tester 2008; Roy et al. 2014).

The ionic phase, however, has a slower effect on the plants and finally results in the senescence of the old mature leaves (Munns and Tester 2008; Munns 2002). In the ionic (salt-specific) phase, salts may reach toxic levels in the older leaves that have been accumulating salt for a longer time, more than what plants can compartmentalize in the vacuoles (Munns and Tester 2008; Munns 2002). If older leaves die faster than the production of new ones, the rate of photosynthesis will be reduced and the whole plant growth will be reduced (Munns and Tester 2008; Munns 2002, 2005).
In summary, at the early stage of salt stress, osmotic stress has an rapid and temporary effect on plants than ionic stress while later growth reduction is caused by the salt accumulation in the leaves (Munns 2005).

**Mechanisms of salt tolerance**

Plants have evolved adaptations to environmental stresses (Agarwal et al. 2013). Salt tolerance is the plant ability to cope with the negative consequences of salt and to continue growth under salinity stress (Parida and Das 2005). The strategies plants use to acclimate to salt stress are the same for most of the glycophytes. As salt affects plants in different ways, plants also adapt to salinity through three different main mechanisms: osmotic, salt exclusion, and tissue tolerance mechanisms (Roy et al. 2014). The mechanism of osmotic tolerance is not clear yet and very little is known about it. However, some authors have suggested that osmotic tolerance might involve long distance signaling, ROS or Ca$^{2+}$ waves (Maischak et al. 2010; Mittler et al. 2011; Munns and Tester 2008; Munns 2005; Roy et al. 2014). It was reported by Munns and Tester, 2008, that the increase in osmotic tolerance is related to the reduction in cell expansion inhibition, increased production of new leaves, the decreased stomatal closure, and the increase in osmotic adjustment (Munns and Tester 2008).

On the other hand, plants in the ionic phase tolerate salt stress through two main strategies, by minimizing the entry of salts to the plant (salt exclusion mechanisms) or by minimizing the salt concentration in the cytosol (tissue tolerance mechanism) (Plett and Møller 2010; Tester and Davenport 2003). As salts are carried into the shoot via the transpiration stream, they gradually build up in the leaves as water evaporates. For this reason, older leaves tend to have much higher salt concentrations than younger ones and suffer salt damage and premature leaf senescence (Munns and Tester 2008).

Through the salt exclusion mechanism, transport of Na$^+$ and Cl$^-$ ions to the leaves is minimized to avoid salt accumulation in the shoot. Most plants prevent the vast majority of salts in soil solution from entering the roots, while permitting approximately
2% of the salts to be transported in the xylem (Deinlein et al. 2014; Munns 2002).
Exclusion refers to exclusion (or removal) of Na\(^+\) from root epidermal cells, reduction of
Na\(^+\) loading to the xylem, and/or maximizing Na\(^+\) retrieval from xylem before reaching
the leaves (Munns and Tester 2008; Roy et al. 2014). The plasma membrane H\(^+\)/Na\(^+\)
antiporter (SOS1) plays a role in the extrusion of Na\(^+\) from root epidermal cells. Salts can
be re-translocated in the phloem from the shoot to the root; however, this is a relatively
small amount compared to salts imported to the leaves in the xylem (Munns and Tester
2008). Salt exclusion appears to be a good indicator of tolerance to salinity (Møller et al.
2009; Shavrukov et al. 2010). However, the latter is not always associated with salt
exclusion (Genc, McDonald, and Tester 2007).

If plants fail to minimize salt entry, they can increase their ability to tolerate the
accumulated salts in the leaves by the tissue tolerance mechanism, i.e.
compartmentalization of salt ions into the vacuoles which helps reduce salt
concentrations in the cytoplasm. This may require the synthesis of compatible solutes
and other compounds that have a role in osmotic adjustment (Roy et al. 2014). In this
mechanism, salt ions such as Na\(^+\) and Cl\(^-\) are sequestered in the vacuoles, while K\(^+\) and
other organic solutes accumulate in the cytosol and in the other organelles to balance
the osmotic pressure caused by the ions in the vacuole. Examples of low molecular
weight compounds that accumulate in the cytoplasm are proline, mannitol, sorbitol, and
glycine betaine. These compounds are known as osmolytes or compatible solutes
(Munns, James, and Läuchli 2006; Parvaiz and Azooz 2013). The benefit of accumulating
such compounds in the cytosol is to provide a water potential gradient for water uptake
and to maintain cell turgor (Munns and Tester 2008; Munns 2002, 2005; Parvaiz and
Azooz 2013; Roy et al. 2014).

Increased osmotic and tissue tolerance will both result in the maintenance of growth
under certain Na\(^+\) tissue level of accumulation. These tolerance mechanisms could be
distinguished from each other by studying the effects of salt on the younger versus the
older leaves. If the plant still has the ability to produce new leaves, that is a sign of osmotic stress tolerance. Increased survival of older leaves is a sign of tissue tolerance (Munns and Tester 2008). It is important to mention that each of these mechanisms entails some energy cost to the plants. However, energy cost from excluding salts, or intercellular compartmentalization is relatively small in comparison to osmotic adjustment or the synthesis of organic solutes (Munns 2002).

In summary, plants use different strategies to minimize the damage caused by NaCl on the leaves. Plants 1) Minimize the entry of salt ions to the roots; 2) Increase the efflux of salts from the roots back to the rhizosphere; 3) Reduce loading of salt ions into the xylem and increase their retrieval from the xylem; 4) Compartmentalize salt ions in vacuoles and synthesize compatible solutes; 5) Recirculate salt ions in the phloem, although it has a very small share in the exclusion of salts from the leaves; 6) Allocate salts to older leaves or secrete them to the surface of the leaves (Tester and Davenport 2003).

Salt effects on plants

Most plants are less sensitive to salinity during the germination stage but more sensitive after seedling emergence (Maas 1993). Salinity delays and inhibits germination. For example, tomato seeds take 50% and 100% more time to germinate when treated with 80 mM and 190 mM NaCl, respectively, than in the control treatment (Cuartero et al. 1999). Nevertheless, germination is recovered when seeds are rinsed with deionized water (Gaylord and Egan 2008). Delayed germination seems to result from the decreased water uptake by the seeds because of the osmotic effect of salt (Cuartero et al. 1999; Gaylord and Egan 2008).

Another detrimental aspect of salinity on plants is its effect on the uptake and translocation of other essential ions (Maathuis 2013). Salinity decreases Ca$^{2+}$, K$^+$ and Mg$^{2+}$ uptake (Botella et al. 1997; Rengel 1992; Yu, Wang, and Wang 2012), and reduces K$^+$ translocation when its concentration is low in the growing medium (Botella et al.
1997; Shabala and Cuin 2008). In addition, \( \text{Na}^+ \) inhibits binding of \( \text{Ca}^{2+} \) to the transporters in the plasma membrane, resulting in decreased calcium influx and increased efflux from the roots (Munns and Tester 2008; Rengel 1992; Shabala, Shabala, and Volkenburgh 2003).

All primary biological processes such as photosynthesis, protein synthesis, and lipid metabolism are negatively affected during salt stress (Ashraf and Harris 2013; Parida and Das 2005). Photosynthesis is reduced because of the reduction in water potential and the accumulation of \( \text{Na}^+ \) and \( \text{Cl}^- \) ions in chloroplasts along with the effect of salt on carbon metabolism and photophosphorylation. High levels of \( \text{Na}^+ \) are toxic because \( \text{Na}^+ \) interferes with \( \text{K}^+ \) nutrition, which reduce \( \text{K}^+ \)-stimulated enzyme activities, photosynthesis and metabolism (Ward, Hirschi, and Sze 2003). \( \text{Na}^+ \) and \( \text{Cl}^- \) ions reduces photosynthetic performance, though the exact mechanism is not fully clarified (Ashraf and Harris 2013). Salt stress induces oxidative stress in chloroplasts, which results in structural alteration of thylakoids. This alteration might be the reason for the reduced photosynthetic performance (Parida and Das 2005). In addition, because PSII plays a major role in the photosynthetic activity, some work was done to investigate the effect of salt on PSII. Studies showed that at low salt treatments, PSII activity was not affected, whereas it was reduced at higher salt treatment (Ashraf and Harris 2013; Parida and Das 2005). Other studies showed no effect of salt on PSII (Ashraf and Harris 2013; Parida and Das 2005; Parvaiz and Azooz 2013).

Salinity decreases stomatal conductance resulting in decreased \( \text{CO}_2 \) availability for the carboxylation reactions, which also reduces photosynthesis. However, the extent of salinity effects on photosynthesis depends on salt concentration and plant species and genotype (Ashraf and Harris 2013; Stepien and Johnson 2009; Sudhir and Murthy 2004). Reduced stomatal conductance is an early response to salinity because of the osmotic stress even before \( \text{NaCl} \) toxic effects starts. Therefore, it was suggested to use stomatal
conductance as a measure to screen for salinity tolerance before salt builds up in leaves (Munns et al. 2006; Munns and James 2003).

Other effects of salinity on plants include the inhibition of leaf expansion (secondary to the reduction in leaf turgor) which compromises carbon acquisition and reduces yield (Neumann, Volkenburgh, and Cleland 1988). In addition, as salinity increases photorespiration, disrupts photosynthesis, and changes the hemostasis of cells, and ROS production is increased (Miller et al. 2010). Under salt stress conditions, there is an imbalance between the ROS produced and scavenged. Therefore, those unquenched ROS interact with organic molecules that impair lipids, inhibit enzymes, and oxidize proteins. ROS might lead to cell death under severe salt stress conditions (Miller et al. 2010; Mittler et al. 2011).

As reviewed by Munns, 2002, plants go through time-dependent changes when exposed to salt. After a sudden salt treatment, plant growth is reduced transiently and rapidly (Munns 2002). This change in the growth rate is solely due to changes in water relations in plants but not to salt-specific effects. Several minutes later (30 minutes or more), leaf growth recovers again (depending on the salt concentration) to reach a steady state of growth. The same growth reduction happens in roots, but roots remarkably recover and reach to the steady state sooner. A salt-specific effect on roots can occur at this stage because of salt-induced Ca$^{+2}$ deficiency, especially in genotypes with less Ca$^{+2}$ uptake rate. After days of salt treatment, leaf and root growth settle down to a reduced steady state of growth.

Within days or weeks (depending on salt concentration, genotypic abilities to regulate salt uptake, and environmental conditions), symptoms of salt injury in salt-sensitive genotypes start to show in the older leaves. Salts build up either in cell walls (which dehydrates the cells) or in the cytoplasm (which inhibits enzyme activities). Munns and Passioura, 1984, indicated that in barley plants, when the content of ions in the cells reached their maximum and when net uptake rates became zero, salt ions built up in
the cell walls. In addition, Romero-Aranda et al., 2001, showed that different genotypes of tomato differed in their tolerance strategies under salt stress (Romero-Aranda et al. 2001). For example, ‘Moneymaker’ accumulated less Na$^+$ in the leaves which means less Na$^+$ was transported to the leaves. Whereas, ‘Daniela’ cultivar accumulated more Na$^+$ in the leaves, which indicates better tissue tolerance (vacuolar sequestration).

Over weeks, in the more tolerant genotypes, salts are excluded from the transpiration stream or compartmentalized in vacuoles. In sensitive genotypes, however, old leaves show progressively increased chlorosis symptoms, which eventually causes leaf death. If the new leaves are produced faster than the loss of the older ones, there will be enough photosynthates for the plants to enter the reproductive stage.

In summary, cells lose their turgor and shrink in the first few seconds/minutes. Cells regain their turgor over hours; however, leaf and root cell elongation starts to decrease. The decrease in cell elongation and division leads to smaller new leaves. Leaf growth is usually more affected by salinity than root growth and older leaves are the ones that start showing symptoms of salt-specific effects. After weeks of salt treatment, lateral shoots are inhibited while some old leaves might be dead. In months, differences in growth between sensitive and tolerant genotypes become apparent as many leaves show injury symptoms and the whole plant might die if salt concentrations are sufficiently high (Munns 2002).

Finally, one of the most detrimental effects of salinity on agriculture is its effect on crop yield. Yield of most of the crops is reduced by salinity. The relative crop yield usually declines significantly, and approximately linearly, after a threshold of salt has been exceeded (Maas and Hoffman 1977). In some plant species, plants might die before approaching the reproductive growth stage (Maas 1993).
Membrane transporters

Many plant membrane transporters play important roles in plant tolerance to abiotic and biotic stresses (Deinlein et al. 2014). Under salt stress conditions, plants have different transporters that are important for the regulation of Na\(^+\) and K\(^+\) transport through membranes (Almeida, Katschnig, and de Boer 2013; Deinlein et al. 2014). Na\(^+\) enters the roots and might cross the plasma membrane through voltage independent ion channels (Munns and Tester 2008) or transporters (Horie et al. 2007). Plants control Na\(^+\) homeostasis through different membrane transporters and antiporters such as: 1) membrane transporters that control the accumulation of Na\(^+\), 2) antiporters that extrude Na\(^+\) to the soil from the roots such as SOS pathway (Almeida et al. 2013; Shi et al. 2000), 3) antiporters for sequestering Na\(^+\) in the vacuoles such as NHX1 antiporters, and 4) transporters that remove Na\(^+\) from the transpiration stream to avoid its accumulation in the photosynthetic tissues such as HKT transporters (Almeida et al. 2013; Xue et al. 2011).

HKT transporters

HKT transporters (high affinity potassium transporters), act as Na\(^+\)-selective uniporters and as Na\(^+\)/K\(^+\) symporters (Apse and Blumwald 2007). HKT transporters are the most studied transporters. They are located in xylem parenchyma and root epidermal cells. Studies on the role of HKT in the long distance transport of Na\(^+\) revealed that HKT is involved in the recirculation and exclusion of Na\(^+\) (Almeida et al. 2013). A study by Berthomieu et al., 2014, revealed that AtHKT1 was involved in the recirculation of Na\(^+\) from the shoot to the root in Arabidopsis. AtHKT1 was probably controlling Na\(^+\) accumulation in the phloem sap and unloading in the roots. In addition, AtHKT1 plays another role in the exclusion of salts by regulating Na\(^+\) unloading from the xylem sap into xylem parenchyma cells of the roots, thus protecting plant leaves from excessive accumulation of salts via transpiration stream (Sunarpi et al. 2005). However, the role of
HKT in Na\textsuperscript{+} recirculation is not clear yet and more research is needed for understanding its involvement on Na\textsuperscript{+} recirculation (Almeida et al. 2013).

HKT genes have possible role in Na\textsuperscript{+} influx and retrieval from the xylem and in K\textsuperscript{+} transport (Plett and Møller 2010). HKT2 transporters show up-regulation in their expression under K\textsuperscript{+} limiting conditions (Almeida et al. 2013). For instance, a study on rice showed that OsHKT2;1 was mediating Na\textsuperscript{+} influx to the roots under K\textsuperscript{+} starvation conditions (Horie et al. 2007). Maintenance of low Na\textsuperscript{+}:K\textsuperscript{+} ratio in the leaves as a result of increased K\textsuperscript{+} concentrations is often considered a major component of salinity tolerance in plants during salt stress (Munns and Tester 2008). For this reason, HKT was tested for its relation with K\textsuperscript{+} levels in the xylem. Overexpression of AtHKT1:1 in the root stele reduced shoot Na\textsuperscript{+} accumulation while it increased K\textsuperscript{+} shoot concentration which consequently increased plants tolerance to salinity (Møller et al. 2009). Therefore, it was concluded that the increased K\textsuperscript{+} concentration is a result of the reduced Na\textsuperscript{+} concentration in the shoot (Møller et al. 2009).

Studies have shown the importance of HKT transporters in salinity tolerance of many plant species. Therefore, HKT transporters are attracting researchers to engineer salinity tolerant plants (Almeida et al. 2013). Although HKT transporters’ role in the regulation of Na\textsuperscript{+} and K\textsuperscript{+} homeostasis is well known, the mechanisms underlying HKT regulation and expression is not well studied. However, some mechanisms found to be involved in the regulation of the HKT genes and proteins include ROS, cytokinins, and abscisic acid Insensitive 4 (ABI4), which is an ABA responsive transcription factor (Almeida et al. 2013).

**NHX transporters**

NHX is a vacuolar Na\textsuperscript{+}/H\textsuperscript{+} antiporter which move Na\textsuperscript{+} into the vacuoles in exchange for H\textsuperscript{+} (Plett and Møller 2010). NHX transporters are involved in improving plant tolerance to salinity (Apse and Blumwald 2007). These transporters play a role in the compartmentalization of Na\textsuperscript{+} into vacuoles to alleviate its excess accumulation in the
cytosol. This mechanism is controlled by Na\(^+\)/H\(^+\) antiporters driven by the H\(^+\) gradient as a driving force for Na\(^+\) sequestration into the vacuoles under salt stress conditions. NHX transporters are subdivided into the vacuolar NHX (class 1) or endosomal NHX2 (class 2). Most plant types have both types (Apse and Blumwald 2007; Yamaguchi et al. 2013).

Vacuolar and endosomal NHXs are involved in the tolerance of a range of plant species to salinity. Arabidopsis plants overexpressing vacuolar antiporter NHX1 had a maintained growth and development under high salt concentrations (Apse et al. 1999). In addition, transgenic tomato plants overexpressing LeNHX2 (an endosomal class II) grew better and showed improved tolerance to NaCl (Apse and Blumwald 2007). Those tomato transgenic plants had a lower cytosolic K\(^+\) which increased K\(^+\) uptake by plants rather than Na\(^+\) uptake. This resulted in better cellular K\(^+\)-homeostasis, and hence improved plant tolerance (Huertas et al. 2013). On the other hand, LeNHX2 knockdown tomato plants had a significant inhibition in their growth and development in response to salt stress (Martínez-Rodríguez et al. 2008; Yamaguchi et al. 2013).

**SOS transporters**

Na\(^+\) efflux from the cytosol across the plasma membrane in roots to the soil solution is mediated by Salt Overly Sensitive (SOS1) transporters. *AtSOS1* of Arabidopsis was found to be upregulated in response to salt stress and expressed within the stele in the root and in the epidermal cells of the root tip (Plett and Møller 2010). It has an essential role in promoting the efflux of Na\(^+\) at the plasma membrane of the root cells (Shi et al. 2000; Yamaguchi et al. 2013). Consequently, SOS1 is essential for Na\(^+\) and K\(^+\) homeostasis in the shoot and the xylem (Shi et al. 2000; Zhang and Shi 2013). Therefore, knockdown plants of *SOS1* genes were found to accumulate more Na\(^+\) than wild types, especially under higher salt treatments (Shi et al. 2000; Yamaguchi et al. 2013).

*SOS1* has an important role in the partitioning of Na\(^+\) ions between plant organs. Thus, it protects the photosynthetic tissues from the harmful effects of Na\(^+\) ions. It was found that tomato plants expressing *SOS1S1* under salt conditions retain Na\(^+\) in the stems,
preventing it from reaching the leaves (Olías, Eljakaoui, Li, et al. 2009). SOS1S1- silenced plants were found to be more sensitive to salt stress because of the decreased K⁺ homeostasis, as a result of the increased Na⁺ in the leaves (Olías, Eljakaoui, Pardo, et al. 2009).

Interestingly, other cation/anion exchangers such as the CHX family of exchangers might play a role under salinity stress. CHX are known as the largest families of K⁺ transporters in flowering plants (Rosario 2013). In sos1 mutant Arabidopsis, there was still a little exchange activity of the Na⁺/H⁺ at the isolated plasma membrane in the roots under salinity conditions, indicating the presence of additional exchanges on those membranes (Apse and Blumwald 2007; Qiu et al. 2003).

**Plant grafting for salinity tolerance**

Plant grafting has been known for more than two thousand years (Pina and Errea 2005). Grafting has made many contributions to scientific research by solving many long distance signaling and substance translocation questions (Wang 2011). Grafting has traditionally been used mainly to propagate fruit trees, but grafting in vegetables was introduced for the purpose of controlling soil borne diseases and nematodes (Bithell et al. 2012; Goldschmidt 2014; Pina and Errea 2005).

Grafting of herbaceous crops was started in Asia, particularly in Japan and Korea in the late 1920s (Rivero, Ruiz, and Romero 2003). The first graft was performed by a farmer in Japan who grafted a watermelon (*Citrullus lanatus*) on a squash (*Cucurbita moschata*) rootstock for disease resistance and growth benefits (Tateishi 1927). Grafting of vegetables for commercial production was introduced to Europe late in the 20th century. Recently, it was introduced to North America as it did attract greenhouse and organic producers because of less need for soil fumigation (Kubota et al. 2008).

Grafting is the technique of joining two plant parts together; i.e. the branches or stems from one plant onto the root of another, to form a single plant (Wang 2011). The upper
part of the plant is the scion, whereas the lower part is the rootstock (Wang 2011).

Grafting could improve plant growth and yield if scion-rootstock combinations are tested and chosen carefully. Success of grafting is affected by pests/diseases, environment, taxonomic factors, genetic factors, the developmental stages of scions and rootstocks, and the environment after grafting (Moore and Walker 1983; Pina and Errea 2005; Wang and Kollmann 1996; Wang 2011).

Recently, researchers investigated the important role of grafting in improving plant tolerance to abiotic stresses (Schwarz et al. 2010; Sánchez-Rodríguez et al. 2014). Since salinity is one of the significant stresses that negatively impact plant growth and yield, many studies were designed to test the effect of grafting in improving plant’s tolerance to salinity (Santa-Cruz et al. 2002; Estañ et al. 2005; Martinez-Rodriguez et al. 2008; Asins et al. 2010; Gioia et al. 2013). Rootstock and scion genotypes that are able to reduce the uptake and transport of salt to the shoot are known as ‘salt excluders’, whereas those genotypes that take up more salts and compartmentalize them in the vacuoles of the roots or the leaves are known as ‘salt includers’ (Perez-Alfocea et al. 1996).

It has been shown that grafted tomato plants can improve tolerance to salinity when salt-tolerant rootstocks are used (Estañ et al. 2005; Martinez-Rodriguez et al. 2008). For instance, ‘Moneymaker’ (MM) grafted onto the ‘Pera’ rootstock had a better yield despite higher leaf Na$^+$ and Cl$^-$ concentrations compared to self-grafted ‘MM’ plants. The higher leaf ion concentration seemed to contribute to the osmotic potential of the leaf. Therefore, the authors suggested that ‘Pera’ cultivar utilizes the includer mechanism to tolerate salinity (Stanta-Cruz et al. 2001).

It seems that the role of grafting in improving plant tolerance to salinity is not solely limited to the ability of rootstocks to tolerate saline conditions. In a study by Santa-Cruz et al., 2002, the rootstock ‘Kindya’ showed a better ability to regulate plant ion uptake with includer scion ‘UC-82B’ than with excluder scion ‘MM’. Therefore, it was concluded
that the scion’s tolerance characteristics might be important for the plant tolerance (Santa-Cruz et al. 2002).

Self-grafted ‘MM’ plants showed higher leaf Na\(^+\) and lower root Na\(^+\) concentrations than in MM/Radja (a salt excluder rootstock) or MM/Pera (a salt includer rootstock) when treated with high salt concentration (150 mM). The results showed that ‘MM’ was less able to store Na\(^+\) in the root when it is grafted on its own root than onto other rootstocks (Martinez-Rodriguez et al. 2008).

Despite the abundance of literature on the use of grafting in improving tolerance to salinity, we still need more detailed physiological studies and growth performance on grafted tomato plants under salinity conditions. We need to examine how grafting onto salt-tolerant rootstocks can affect ion concentrations in the leaf and the roots and the mechanisms associated with their tolerance. We need to examine the differences in the growth and tolerance of the scion when wild and cultivated rootstocks are used with different mechanisms of tolerance. Therefore, in this thesis, we will be testing the potential of grafting to improve salinity tolerance in tomatoes and the mechanisms that are responsible for that improvement.
References


Cuartero, J., Bolarín, M. C., Asins, M. J., & Moreno, V. (2006). Increasing salt tolerance in the


CHAPTER 2
Evaluation of the growth performance and survival of tomato scion-rootstock combinations

Introduction

Grafting technology is less often practiced in vegetable production as compared to fruit tree production. Vegetable grafting, however, is constantly expanding due to its numerous benefits including better acquisition of nutrients and water and the improved plant resistance to diseases and abiotic stresses (Martínez-Ballesta et al. 2010). For these reasons, grafting of tomato plants can be a beneficial management practice that leads to better tomato growth and yield (Abdullah Ibrahim, Mahmoud Wahb-Allah, Hesham Abdel-Razzak 2014). Indeed, vegetable grafting success is dependent on many factors, particularly the right choice of the rootstock and scion genotypes (Davis et al. 2008).

Grafting incompatibility is described as the interruption in the development of a new grafted plant or as the loss of vascular regeneration between the scion and the rootstock (Gülen 2005; Santamour 1988). Therefore, any interruptions in the formation of the union whether it is caused by mechanical, environmental, anatomical, or physiological reasons, will affect the success rate of the grafted plants (Goldschmidt 2014; Martínez-Ballesta et al. 2010; Moore and Walker 1981; Pina and Errea 2005). In addition, under- or over- growth of scion relative to the rootstock could negatively affect the success of the grafted plants (Huarachi Morejon 2013; Martínez-Ballesta et al. 2010).

Graft union development in compatible plants comprises a number of consecutive events including isolation layer formation, adhesion of scions and rootstocks, wound
callus formation, vascular differentiation and the reconnection of vascular bridges (Wang 2011). Scion-rootstock combinations do not always form a successful grafted plant (Pina and Errea 2005) and success of the grafted plants depends on the success of all those events mentioned above. If the vascular connection between the scion and the rootstock is not well established because of any of these factors, this might result in an incompatible grafting combination (Wang 2011).

In a study examining the compatibility of different grafting combinations, ‘Celebrity’ and ‘CLN 3212A’ tomato scions were grafted onto ‘Maxifort’ or onto Solanum torvum rootstocks derived from seeds or cuttings. Grafting onto ‘Maxifort’ resulted in a 100% survival rate, whereas self-grafted ‘Celebrity’ and ‘CLN 3212A’ plants had survival rates of 70% and 80%, respectively. Interestingly, survival rates for scions grafted onto Solanum turvom derived from seeds were higher (80-100% survival) than those derived from cuttings (50% survival) (Petran and Hoover, 2014).

With so many factors affecting the success and survival of the different tomato graft combinations, it is important to identify genotypes that perform well when grafted before those graft combinations can be used in commercial production. Therefore, we hypothesize that some grafting combinations may outperform others in terms of plant survival and growth. Acquiring such information will help plant growers choose the combinations with better growth and discard the ones with poor graft compatibility or vigor.

The overall aim of this research project is to test the use of different rootstocks on the growth and tolerance of the tomato plants under salinity stress conditions. The aims of this experiment are to (1) test whether different grafting combinations will have different survival rates, (2) investigate the effects of grafting on plant growth and development.
Methods

Plant material and growing conditions

In this experiment, twelve tomato cultivars were grafted and examined for their growth performance and compatibility. Tomato (S. lycopersicum) cultivars used in the experiment are indicated in Table 2.1. ‘Redline’, ‘Charger’, ‘Finishline’, ‘Phoenix’, ‘Florida 91’, ‘Plum Regal’, ‘Mountain Fresh Plus’ were used as scions, whereas ‘Rocky Top’, ‘Beaufort’, ‘Arnold’, ‘Maxifort’, ‘Taurino’, ‘Florida 91’, ‘Plum Regal’, ‘Mountain Fresh Plus’ and ‘Charger’ were used as rootstocks. All studied genotypes are of the determinate type, except rootstocks ‘Taurino’, ‘Beaufort’, ‘Maxifort’, and ‘Arnold’, that were developed to have a vigorous indeterminate growth.

Seeds of the scions and rootstocks were sown in 36-cell seed starter trays containing the soilless media Sunshine Advanced Mix #4 (Sun Gro Horticulture, Agawam, MA, USA) which is composed of sphagnum peat moss, and grown in the greenhouse. Plants were irrigated daily and fertilized with a 20N 10P 20K fertilizer (Peters professional, Everris NA, Inc., Dublin, OH) at a rate of 150 ppm N. The experiment was conducted under natural light conditions in a greenhouse at The Pennsylvania State University, University Park, PA, USA (40° 48° N, 72° 51° W).
Table 2. 1: Genotypes used in grafting in the experiment.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Code</th>
<th>Source</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arnold</td>
<td>AR</td>
<td>Syngenta Seeds, Boise, Idaho, USA</td>
<td>Vigorous, disease-resistant rootstock.</td>
</tr>
<tr>
<td>Rocky Top</td>
<td>RCK</td>
<td>Syngenta Seeds, Boise, Idaho, USA</td>
<td>High yield of large, high quality fruits under greenhouse and high tunnel growing conditions.</td>
</tr>
<tr>
<td>Finishline</td>
<td>FN</td>
<td>Syngenta Seeds, Boise, Idaho, USA</td>
<td>Disease resistance characteristics, including verticillium Wilt and fusarium Wilt (race 1, 2, and 3).</td>
</tr>
<tr>
<td>Florida 91</td>
<td>FL</td>
<td>Syngenta Seeds, Boise, Idaho, USA</td>
<td>Resistance to alternaria stem canker, gray leaf spot, fusarium wilt, and verticillium wilt. Fruits of ‘Florida 91’ set in high temperatures in the summer.</td>
</tr>
<tr>
<td>Redline</td>
<td>RL</td>
<td>Syngenta Seeds, Boise, Idaho, USA</td>
<td>Vigorous plant that has large fruits and good resistance to fusarium wilt.</td>
</tr>
<tr>
<td>Charger</td>
<td>CH</td>
<td>SeedWay Company, Hall, New York, USA</td>
<td>High yield of good-flavor fruits, resistant to alternaria stem canker, and verticillium wilt.</td>
</tr>
<tr>
<td>Plum Regal</td>
<td>PR</td>
<td>SeedWay Company, Hall, New York, USA</td>
<td>Productive genotype that is resistant to late blight and verticillium wilt.</td>
</tr>
<tr>
<td>Mountain Fresh Plus</td>
<td>MT</td>
<td>SeedWay Company, Hall, New York, USA</td>
<td>Vigorous, productive, good flavor and great resistance to verticillium wilt, nematodes, and fusarium wilt (races 1 and 2).</td>
</tr>
<tr>
<td>Taurino</td>
<td>TA</td>
<td>SeedWay Company, Hall, New York, USA</td>
<td>Vigorous rootstock and resistant to corky root rot.</td>
</tr>
<tr>
<td>Beaufort</td>
<td>BEU</td>
<td>Seminis Company, Oxnard, CA, USA</td>
<td>Vigorous rootstock, tolerant to cold stress, and resistant to corky root rot.</td>
</tr>
<tr>
<td>Maxifort</td>
<td>MX</td>
<td>Johnny’s Selected Seeds, Winslow, ME</td>
<td>Highly vigorous rootstock and resistant to tobacco mosaic virus, verticillium wilt, fusarium root rot, nematodes, and corky root rot.</td>
</tr>
<tr>
<td>Phoenix-XP 01408233</td>
<td>PHO</td>
<td>Seminis Company, Oxnard, CA, USA</td>
<td>Sets fruits in very high temperatures during the summer. Resistant to verticillium race1, alternaria stem canker, and gray leaf spot.</td>
</tr>
</tbody>
</table>

**Grafting process**

Four weeks after planting, most of the plants had an average of three to four true leaves (4-5 week-old seedlings). Grafting was then performed using the cleft grafting method (Wang 2011). In this method, rootstocks were cut horizontally below the cotyledons using a razor blade, and then a vertical cut of 1.5 cm deep was made. Scions are cut into
a wedge, and then inserted into the rootstock and securely attached with plastic tubing. The scions were either grafted onto roots derived from a different plant from the same genotype, or onto roots from a different genotype.

**Healing conditions**

Grafted plants were kept under complete darkness in the healing chamber for 7 days until the grafting union was completely healed. The healing chamber was constructed of PVC pipes and covered with two layers of plastic, one was transparent and the other was black. The healing chamber’s bench was covered with a capillary mat and was constantly saturated with water to maintain a relative humidity of 87-90%.

Plants were then moved to another chamber covered with a transparent plastic sheet. The plastic sheet was gradually opened in the following three days by rolling it up and light intensity was increased gradually to acclimate the plants to the lower humidity (84%) and higher light intensity before moving the plants to the greenhouse. Temperature in the growth chamber was kept at 23° C.

Ten days after grafting, plants were transplanted to bigger pots (16.5 cm d x 12.9 cm h) containing the same growth medium and were moved to the greenhouse. Grafted plants were covered with shade cloth for the first 5 days after transplanting to avoid any photo damage to the grafted plants. Plants were fertilized using slow-release fertilizer at a rate of 17.5 g/pot (Osmocote plus, Everris NA, Inc., Dublin, OH), and grown under natural light conditions and temperatures ranging between 21°- 34° C. Seeds of the first replicate were sown in June of 2012, and 10 days separated each of the three replicates. The experiment was completed by the end of October.

**Experimental design**

The experiment was conducted in a randomized complete block design. Rootstock and scion combinations were randomized within each replicate (block) with three blocks. Block is the replication in time. Duration of 10 days was spaced between blocks. Each
replicate consisted of 5 plants per each grafting combination treatment. The number of grafted plants was 330 plants per block (66 grafting combinations, 5 grafts/graft combination).

**Measurements**

The following measurements were taken to evaluate the survival rate and the growth performance of the graft combinations after moving the plants out of the grafting chamber and at the harvest time. Plants were harvested at anthesis and the shoot was cut from the root for shoot weight measurement. The measurements included: Survival rate; number of surviving plants which were counted two times, at 3 weeks after grafting and at harvest time (time to 1st anthesis), number of leaves that were counted 3 weeks after grafting and at harvest time, stem diameter (mm) which was taken at harvest time, and shoot dry weight (g).

**Results**

In this experiment, 66 tomato graft combinations were studied to evaluate their survival rate and growth. Different graft combinations showed different survival rates at 3 weeks post grafting (Fig.2.1). ‘TA’ rootstock consistently resulted in the highest survival rate regardless the genotype used as a scion (Fig. 2.1), followed by plants grafted on ‘MX’ then ‘AR’. In contrast, ‘PR’ as a rootstock gave the lowest rates with a wide variation in the survival rates ranging from 60% in MT/PR to 100% in FL/PR (Fig. 2.1). Except with FN/FN, ‘FN’ scions grafted onto different rootstocks resulted in the highest 3-week survival rates. In contrast, ‘MT’ scions showed the lowest survival rate among all studied hetero-grafts (Fig.2.1).

At harvest time, ‘MX’ rootstocks had the highest survival rate followed by ‘TA’ and ‘AR’ (Fig. 2.2). ‘RL’ scion had the highest survival rates compared to other scions grafted onto the different rootstocks. Except for self-grafts, scions of ‘FN’ and ‘RL’ maintained the
highest rates of survival. ‘MT’ scions continued to show the lowest survival rates compared to other scions grafted onto different rootstocks (Fig. 2.2).

Different graft combinations had comparable stem diameters (Fig. 2.3). Most scions grafted onto ‘BEU’ had high stem diameters compared to their stem diameter when grafted onto different rootstocks. Stem diameter of ‘PR’ scion grafted onto different rootstocks was lower than the average (5.3), and was lower than most of the combinations. Despite the poor survival of ‘MT’ scions grafted onto different rootstocks, surviving plants resulted in the highest total number of leaves 3 weeks after grafting and at harvest (Fig. 2.4, data not shown for leaf number at 3-weeks post-grafting). The number of leaves was comparable for all studied graft combinations at 3 weeks after grafting and at harvest (Fig. 2.4, data not shown for leaf number at 3-weeks post-grafting). There were no major variations in the shoot DW among the rootstock-scion combinations (Fig. 2.5). Plants grafted onto FL rootstock, particularly MT/FL and CH/FL, gave the highest shoot DW (Fig. 2.5).

**Discussion**

The initial formation of the graft union does not necessarily guarantee that grafted plants will be successful. Graft failure can occur at any stage during the plant growth, starting from the healing phase of the graft union until later growth stages (Goldschmidt 2014). The purpose of this experiment was to examine if different scion-rootstock combinations would result in different survival rates. Growth parameters including stem diameter, leaf number, and shoot weight were measured to examine the growth performance of the surviving plants.

In our experiment, different tomato scion-rootstock combinations resulted in different survival rates. Scion genotypes grafted onto ‘TA’, ‘MX’, and ‘AR’ had the highest survival rates, 3 weeks after grafting and at harvest. Grafting one scion onto a particular rootstock can result in better survival rates than those obtained from a different scion.
grafted onto the same rootstock, and vice versa. For example, FN/FL resulted in a 100% survival rate compared to 60% survival rate in MT/FL.

Some graft combinations (e.g. CH/MT) maintained a 100% survival rate until harvest, while other graft combinations (e.g. PR/TA and PHO/MT) had initial 100% survival rates at 3-weeks post grafting then decreased to 80% and 73.3%, respectively, at harvest. Therefore, the success of different grafts should be evaluated throughout a long-term assessment and at different stages of the plant growth.

Interestingly, none of the self-grafts had a 100% survival rate, except for CH/CH. RL/RL and FN/FN plants had the lowest survival rates (53.3 and 46.7%, respectively) at harvest. These findings are in agreement with those reported by Petran and Hoover, 2014, where self-grafted ‘Celebrity’ and ‘CLN 3212A’ plants had lower survival rates (70% and 80%, respectively) compared to 100% survival rates when scions of these genotypes were grafted onto ‘Maxifort’ (Petran and Hoover 2014).

Stem diameter of ‘PR’ scions grafted onto different rootstocks was lower than the average (5.3), and was lower than most of the combinations. ‘PR’ scion grafted onto ‘AR’, ‘RCK’, or ‘PR’ initially gave lower number of leaves at 3-weeks post grafting than most combinations (data not shown). The same graft combinations, however, developed more leaves later in the experiment. At harvest, ‘MT’ scion had the highest number of leaves especially when grafted onto ‘MX’, ‘MT’, ‘CH’, and ‘AR’. In general, all graft combinations, including those with initially low leaf numbers, had comparable numbers of leaves. The reason for that is that some of the graft combinations had more delay in development after grafting, but caught up later, or declined later.

No major differences in the shoot DW were noticed among the surviving combinations. Although FN/FN had the lowest survival rate and the smallest stem diameter, surviving plants had a comparable number of leaves to the average of all the other combinations. Shoot DW of FN/FN was also similar to the average of shoot DW of all combinations. On the other hand, RL/RL had a lower shoot DW than the average of all genotypes (6.4 vs. 7
g), whereas, hetero-grafted RL/MT had shoot DW higher than the average (7.9 vs. 7 g). In a study by Leonardi and Giuffrida, 2006, the authors found that self-grafted ‘Rita’ tomato plants had lower shoot DW than those grafted onto ‘Beaufort’ rootstock (Leonardi and Giuffrida 2006).

Factors that affect the success of the scion/rootstock graft combination are quite complex, and are still not very well understood. Plants may be impacted by a physiological rejection between the rootstock and scion (Goldschmidt 2014). Over-growth or under-growth of the scion or the rootstock may also affect the success of the graft combination (Huarachi Morejon 2013; Martínez-Ballesta et al. 2010). Rootstocks or scions may be affected by a viral infection (Goldschmidt 2014). It has been reported that phloem irregularities in the graft union can cause incompatibility between the rootstock and the scion (Goldschmidt 2014). Soumelidou et al., 1994, reported that the wound of the graft union in apples interfered with auxin crossing through the graft union (Soumelidou Katerina et al. 1994). Auxins were found to be essential for vascular differentiation and reconnection at the graft union (Yin et al. 2012). Therefore, the vascular tissues of the apples’ graft union did not differentiate leading to a reduced flow of water and nutrients from the rootstock to the scion.

This work has highlighted the survival rates and growth performance of grafted tomato plants when different scion and rootstock genotypes are used in grafting. Careful selection of rootstocks and scions is fundamental to guarantee successful graft combinations with better survival rate. ‘AR’, ‘RCK’, ‘MX’, and ‘PHO’ that showed high survival rates in the present experiment will be evaluated for their tolerance to salinity in a following screening experiment (Chapter 3).


Fig 2. 1: Heat map of the survival rate (%) at week-3 post grafting in different tomato scion and rootstock combinations. Red color represents higher survival rates. Yellow color represents lower survival rates.
Fig 2.2: Heat map of survival rate (%) at harvest time (anthesis) in different tomato scion and rootstock combinations. Red color represents higher survival rate and yellow color represents lower survival rate.

<table>
<thead>
<tr>
<th>Scion</th>
<th>RL</th>
<th>PHO</th>
<th>FN</th>
<th>PR</th>
<th>MT</th>
<th>FL</th>
<th>CH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>93.3</td>
<td>100</td>
<td>93.3</td>
<td>73.3</td>
<td>86.7</td>
<td>93.3</td>
<td>73.3</td>
</tr>
<tr>
<td></td>
<td>86.7</td>
<td>66.7</td>
<td>80</td>
<td>100</td>
<td>100</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>93.3</td>
<td>73.3</td>
<td>100</td>
<td>80</td>
<td>100</td>
<td>48.7</td>
</tr>
</tbody>
</table>

Fig 2.3: Heat map of average stem diameter (mm) at harvest time in different tomato scion and rootstock combinations. Red color represents higher stem diameters and yellow color represents lower stem diameters (n = 7-15 depending on the number of surviving plants).

<table>
<thead>
<tr>
<th>Scion</th>
<th>RL</th>
<th>PHO</th>
<th>FN</th>
<th>PR</th>
<th>MT</th>
<th>FL</th>
<th>CH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5.2</td>
<td>5.6</td>
<td>5.3</td>
<td>5.0</td>
<td>5.6</td>
<td>5.6</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>5.4</td>
<td>5.9</td>
<td>5.3</td>
<td>4.8</td>
<td>4.9</td>
<td>4.9</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>5.2</td>
<td>5.4</td>
<td>5.3</td>
<td>4.8</td>
<td>4.9</td>
<td>4.9</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>5.1</td>
<td>5.1</td>
<td>5.4</td>
<td>4.8</td>
<td>4.9</td>
<td>4.9</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>5.1</td>
<td>5.1</td>
<td>5.4</td>
<td>4.8</td>
<td>4.9</td>
<td>4.9</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>5.4</td>
<td>5.1</td>
<td>5.4</td>
<td>4.8</td>
<td>4.9</td>
<td>4.9</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>5.2</td>
<td>5.2</td>
<td>4.8</td>
<td>4.9</td>
<td>4.9</td>
<td>4.7</td>
</tr>
</tbody>
</table>
Fig 2. 4: Heat map of average number of expanded leaves at harvest in different tomato scion and rootstock combinations. Red color represents higher leaves number and yellow color represents lower leaves number (n = 7-15 depending on the number of surviving plants).

Fig 2. 5: Heat map of average shoot dry weight (g) at harvest time in different tomato scion and rootstock combinations. Red color represents higher shoot weight and yellow color represents lower shoot weight (n = 7-15 depending on the number of surviving plants).
CHAPTER 3  
Evaluation of salt tolerance in cultivated and wild tomato genotypes

Introduction

Salinity is the accumulation of water-soluble salts in the upper portion of the soil profile (or regolith) to an extent which affects environmental health and agricultural production (Rengasamy 2006). In more than 100 countries worldwide, soils are affected by salinity either naturally or induced by irrigation (Rengasamy 2006). More seriously, soil salinization problems are increasing, mostly because of poor agricultural practices (Tester and Davenport 2003).

Tomato is a major annual vegetable crop, ranked the seventh most essential crop species in the world with an approximate total production of 160 million tons in 2011 (Bergougnoux 2013; Cuartero et al. 1999). Although tomatoes are adapted to a wide variety of climates, production is concentrated in warm and dry areas (Cuartero et al. 1999) where the naturally occurring soil-forming processes (primary salinization) may cause production of saline and gypsiferous soils. In addition, improper irrigation practice leads to secondary salinization due to salinization of soils and water resources (Cuartero et al. 1999; Williams 1999).

Salinity has negative consequences on most of the physiological and biochemical processes of plants. Slower growth rates are early responses to salinity while higher salt concentrations can extensively reduce yield quality and quantity and may lead to plant death (Cuartero et al. 2006; Munns and Termaat 1986; Parida and Das 2005). Salt stress encompasses both osmotic and ionic stresses. Consequently, reduced water potential as well as ion imbalance and toxicity are detrimental effects of salinity on plants (Munns and Tester 2008; Munns 2002; Parida and Das 2005).

Salt tolerance is defined as the plants’ ability to grow and to complete their life cycle successfully under salt stress conditions (Parida and Das 2005). In general, there are three main types of plant adaptations to salinity stress: osmotic stress tolerance, ion exclusion, or tissue
tolerance to accumulated ions (Munns and Tester 2008). Although great efforts are directed to increase plants’ tolerance to salinity, only a small number of tomato breeding lines were found to be partly tolerant to salinity due to the many genes involved in salinity tolerance that have small effect compared to the environmental variation (Cuartero et al. 2006).

Researchers have used certain criteria to evaluate genotypic differences for salinity tolerance (Genc et al. 2007; Munns and Tester 2008; Munns et al. 2002, 2012). In a study by Munns and James, 2003, salinity tolerance of durum wheat (salinized for 3-4 weeks) was defined based on biomass production. In the same study, the authors found a correlation between Na\(^+\) exclusion and salt tolerance. However, in bread wheat plants, salt tolerance was achieved by the combination of both salt exclusion and tissue tolerance to the accumulated salt (Genc et al. 2007). Moreover, high K\(^+\) accumulation in the cytoplasm relative to that of Na\(^+\) contributes to salinity tolerance in wheat (Munns and James 2003; Munns and Tester 2008).

Among other physiological parameters, stomatal conductance was found to be the most sensitive to salinity, followed by CO\(_2\) assimilation (Munns and James 2003; Munns et al. 2002). This is because stomatal conductance is reduced in the leaves due to root signals even before NaCl builds up in the leaves (Munns and James 2003; Munns and Tester 2008; Richard A. James, Anna Rita Rivelli, Rana Munns 2002).

Generally, cultivated tomatoes are classified as moderately sensitive to salinity stress (Maas and Hoffman 1977). However, wild tomato germplasm was found to be a potential genetic source for salt tolerance (Bernacchi et al. 1998; Bolarín and Fernández 1991). Some tolerance mechanisms such as regulation of Na\(^+\) accumulation and K\(^+\): Na\(^+\) ratio of leaves and stems were found in both the cultivated and the wild germplasm (Saranga et al. 1993).

In a study on different wild tomato species, the ‘PE2’ accession, which belongs to the S. pimpinellifolium species, was found to be more salt tolerant than S. pennelli, S. peruvianum, and S. hirsutum (Bolarín and Fernández 1991). In addition, ‘PE2’ was more tolerant than accessions from other species (S. lycopersicum, S. cheesmanii, S. pennelli and S. peruvianum) as its dry matter at 40% of artificial seawater in relation to 2% control treatment showed the least reduction in relative weight (Cuartero, Yeo, and Flowers 1992). However, despite the marked
reduction in the relative weight of *S. lycopersicum*, compared to *S. pimpinellifolium* (~77% vs. 41%, respectively), the absolute weight under 40% seawater treatments was comparable between the two species. Therefore, *S. pimpinellifolium* can be a good candidate for increasing salt tolerance of *S. lycopersicum* especially since it is closely related to the cultivated tomato species.

Due to the increasing problem of soil salinity all over the world, there is a great need to find new ways to improve tomato tolerance to salinity. Grafting onto salt tolerant rootstocks could help with this problem while maintaining the desired fruit quality and yield. Therefore, evaluation of tomato germplasm for salinity tolerance is a mandatory initial step to choose the potential rootstocks for the grafting experiments. We hypothesize that identification of the salt tolerant genotypes will be necessary for their planned use as rootstocks and/or scions in plant grafting under saline conditions.

Therefore, the aim of this experiment was to choose salt tolerant genotypes under greenhouse conditions in order to use them as rootstocks or scions in plant grafting. In addition, ion contents were measured in the leaves and roots with the aim of understanding the tolerance mechanism of the plants with the least growth reduction and with the most vigorous plants.

**Methods**

**Plant material and treatments**

In order to examine a range of genetic variability for salinity tolerance, 21 tomato genotypes were used in this experiment (Table 3.1).
### Table 3.1: Genotypes used in this screening experiment at 0, 50, 100, and 150 mM NaCl.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Type</th>
<th>Source</th>
<th>Reason for choice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arnold ‘AR’</td>
<td>Rootstock</td>
<td>Syngenta Seeds, Boise, Idaho, USA</td>
<td>Developed by seed companies for the purpose of grafting for better vigor and disease resistance.</td>
</tr>
<tr>
<td>Maxifort ‘MX’</td>
<td>Rootstock</td>
<td>Johnny’s selected seeds, Winslow, ME</td>
<td>Developed by seed companies for the purpose of grafting for better vigor and disease resistance.</td>
</tr>
<tr>
<td>Ironman ‘Im’</td>
<td>Rootstock</td>
<td>Syngenta Seed company (Minnetonka, MN 55305 1526, USA)</td>
<td>Developed by seed companies for the purpose of grafting for better vigor and disease resistance.</td>
</tr>
<tr>
<td>Resistar ‘RS’</td>
<td>Rootstock</td>
<td>Hazera seed company (Coconut Creek, FL, USA),</td>
<td>Developed by seed companies for the purpose of grafting for better vigor and disease resistance.</td>
</tr>
<tr>
<td>Moneymaker ‘MM’</td>
<td>S. lycopersicum</td>
<td>Johnny’s Seeds (Winslow, Maine, USA)</td>
<td>Reported as salt-tolerant genotype in some studies (Cuartero and Flower 1992; Cuartero et al. 1992; Martinez-Rodriguez et al. 2008), whereas it was reported as salt-sensitive genotype in other study (Santa-Cruz et al. 2001).</td>
</tr>
<tr>
<td>Rocky Top ‘RCK’</td>
<td>Hybrid</td>
<td>Johnny’s Seeds (Winslow, Maine, USA)</td>
<td>Chosen because it was very compatible with many scions and rootstocks in our previous experiment. Has a high yield of large, high quality fruits.</td>
</tr>
<tr>
<td>Massada ‘MAS’</td>
<td>Hybrid</td>
<td>Johnny’s Seeds (Winslow, Maine, USA)</td>
<td>Resistant to some diseases and claimed to taste better than many greenhouse tomato varieties.</td>
</tr>
<tr>
<td>SC*DRO138TX ‘SC’</td>
<td>Hybrid</td>
<td>Johnny’s Seeds (Winslow, Maine, USA)</td>
<td>Rootstock variety chosen to be tested for salinity tolerance.</td>
</tr>
<tr>
<td>‘Phoenix-XP’ ‘PHO’</td>
<td>Hybrid</td>
<td>Johnny’s Seeds (Winslow, Maine, USA)</td>
<td>Chosen because it was compatible with some rootstocks in our previous experiment. Sets fruits in high temperatures in summers. Disease resistant.</td>
</tr>
<tr>
<td>LA2711 (Edkawy)</td>
<td>S. lycopersicum</td>
<td>Tomato Genetics Resource Center (Davis, CA, USA)</td>
<td>Egyptian cultivar. Has a degree of salt tolerance (Cuartero et al. 1992).</td>
</tr>
<tr>
<td>LA3120</td>
<td>S. lycopersicum</td>
<td>Tomato Genetics Resource Center (Davis, CA, USA)</td>
<td>Stress tolerant (particularly to heat).</td>
</tr>
<tr>
<td>LA3320</td>
<td>S. lycopersicum</td>
<td>Tomato Genetics Resource Center (Davis, CA, USA)</td>
<td>Stress tolerant (particularly to heat).</td>
</tr>
<tr>
<td>LA2375</td>
<td>S. lycopersicum</td>
<td>Tomato Genetics Resource Center</td>
<td>To be tested for salinity tolerance.</td>
</tr>
<tr>
<td>Accession</td>
<td>Species</td>
<td>Source</td>
<td>Description</td>
</tr>
<tr>
<td>-----------</td>
<td>---------------</td>
<td>---------------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>LA2662</td>
<td><em>S. lycopersicum</em></td>
<td>Tomato Genetics Resource Center (Davis, CA, USA)</td>
<td>Stress tolerant (particularly to heat).</td>
</tr>
<tr>
<td>LA3465 (Walker)</td>
<td><em>S. lycopersicum var. cerasiforme</em></td>
<td>Tomato Genetics Resource Center (Davis, CA, USA)</td>
<td>Considered a salt-susceptible genotype (Nveawiah-yoho et al. 2013).</td>
</tr>
<tr>
<td>LA1310</td>
<td><em>S. lycopersicum var. cerasiforme</em></td>
<td>Tomato Genetics Resource Center (Davis, CA, USA)</td>
<td>Reported as salt-tolerant in the tomato genetics resource center website.</td>
</tr>
<tr>
<td>LA722</td>
<td><em>S. pimpinellifolium</em></td>
<td>Tomato Genetics Resource Center (Davis, CA, USA)</td>
<td>Reported tolerance to salinity (Foolad and Chen 1999).</td>
</tr>
<tr>
<td>LA1697</td>
<td><em>S. pimpinellifolium</em></td>
<td>Tomato Genetics Resource Center (Davis, CA, USA)</td>
<td>To be tested for salinity tolerance.</td>
</tr>
<tr>
<td>LA1630</td>
<td><em>S. pimpinellifolium</em></td>
<td>Tomato Genetics Resource Center (Davis, CA, USA)</td>
<td>Chosen because it was annotated by the Tomato Genetics Resource Center as salt tolerant.</td>
</tr>
<tr>
<td>Finishline ‘FN’</td>
<td>Hybrid</td>
<td>Syngenta Seeds, Boise, Idaho, USA</td>
<td>Had a high survival rate when was grafted in chapter 2. It does have some disease resistance characteristics.</td>
</tr>
<tr>
<td>LA3847</td>
<td><em>S. lycopersicum</em></td>
<td>Tomato Genetics Resource Center (Davis, CA, USA)</td>
<td>Has heat tolerance and disease resistance characteristics.</td>
</tr>
</tbody>
</table>

Seeds were surface sterilized with 0.5% NaOCl solution for 10 minutes, washed three times with sterile distilled water and sown in rockwool cubes (3.81 cm wide). Those cubes were kept in a germination chamber until seedlings emerged. Plants were irrigated daily with deionized water during the germination period. At 10 days after planting, seedlings were placed in hydroponic tanks filled with 25 l of half-strength Hoagland’s nutrient solution (Hoagland and Arnon 1950). The experiment was conducted under controlled conditions in a greenhouse at The Pennsylvania State University, University Park, PA, USA (40° 85′ N, 77° 82′ W). Artificial light was supplemented from 0800 to 2000 h with 110 μmol photons m⁻² s⁻¹ from 400 W metal halide bulbs (Energy Technics, York, PA, USA).

Salt treatments were applied to the seedlings by adding 0, 50, 100, or 150 mM NaCl to the nutrient solution. To avoid NaCl-induced Ca²⁺ deficiencies, CaCl₂ was added to a concentration calculated using the MINTEQ software version 3.1 (Cramer et al. 1989). It was reported that
Ca^{2+} transport is highly reduced when Na^{+} is added to the growing medium (Cramer et al. 1989). Salt was added in increments of 25 mM NaCl to seedlings 2 weeks after moving the plants to the hydroponic system to avoid salt shock. Additional increments of 25 mM NaCl were added every day until the final salt concentration of each treatment was reached. Control plants were grown in tanks filled with nutrient solution only. Thereafter, plants were grown, with or without NaCl, for 16 more days.

A total of 21 genotypes (one plant per genotype per treatment) were randomly assigned to the hydroponic tanks (4 plants per each tank). The experimental design was a randomized complete block design where the blocks were the tank positions on different benches. Each treatment was replicated three times. As a result, the experiment consisted of 21 tanks per replicate for a total number of 63 tanks in the whole experiment. Blocks consisted of replicates in time and there was a two-week interval between plantings.

Constant aeration was provided by aeration pumps that were connected to bubblers submerged in the hydroponic containers. Solutions were replaced 2 weeks after moving the plants to the hydroponic system and every week after the onset of salt treatment. The electrical conductivities (ECs) were ~1.11, 6.30, 8.06, and 8.93 dS/m for 0, 50, 100, and 150 mM NaCl treatments, respectively, and the pH was maintained at 5.9-6.1 EC and pH readings were recorded using hand-held EC-pH meter (Mettler-Toledo International Inc., Mississauga, Canada). The tanks were rinsed weekly to keep them clean and sanitized with bleach. At harvest, the shoots and roots of each plant were separated and weighed to determine fresh (FW), dry weights (DW) and the root/shoot ratio.

**Measurements included:** Plant height, which was recorded after 7 and 16 days of NaCl treatment, for the calculation of plant height change (cm/day), shoot and root fresh (FW) and dry weights (DW) were measured at harvest (16 days after NaCl treatment), and root to shoot ratio calculated on a dry weight basis. Percent reduction in shoot DW was calculated for each replicate, and then the average percent reduction in shoot DW was calculated.
**Determination of the salt tolerance mechanism**

Eight genotypes, ‘LA722’, ‘RS’, ‘LA3120’, ‘Im’, ‘MM’, ‘LA1630’, ‘LA3465’, and ‘AR’ were selected for further ion analysis of Na\(^+\), K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\) in the 4\(^{th}\) leaf and in the roots. These genotypes had low reductions in shoot DW growth and were vigorous (high shoot DW) under 50 and 100 mM NaCl treatments.

**Cation analysis**

At the end of the experiment, leaf number 4 and the entire root system were kept in separate bags for ion analysis. Samples of roots and leaves were oven-dried at 65\(^\circ\)C for 48 h then powdered by a grinder. Dry powdered samples (0.5 g) were digested in a mixture of H\(_2\)SO\(_4\)–salicylic acid–H\(_2\)O\(_2\)–selenium. Na\(^+\), K\(^+\), Ca\(^{2+}\) and Mg\(^{2+}\) were assessed using an atomic absorption spectrometer (Perkin-Elmer 1100, USA) at the plant analysis lab of the Pennsylvania State University. The K\(^+\) : Na\(^+\) ratio was calculated, since K\(^+\) : Na\(^+\) rather than Na\(^+\) alone has been used as an index of salinity tolerance.

**Results**

The purpose of the experiment was to screen wild and cultivated genotypes with 4 NaCl treatments (0, 50, 100, 150 mM NaCl), in order to choose genotypes that could be potential rootstocks in our grafting experiments (Chapter 4).

**Plant Growth**

The genotypes varied in their response to salt treatment and the rank of responsiveness among genotypes varied with NaCl concentration. The average percent reduction in shoot DW of all genotypes was ~21, 44%, and 61%, at 50, 100, and 150 mM NaCl, respectively. Under 50 mM NaCl treatment, some genotypes including ‘LA722’, ‘MM’, ‘RCK’, and ‘AR’ showed a lower percent reduction in shoot DW than the average (Fig. 3.1). At 100 mM NaCl, ‘Im’, ‘AR’, ‘MM’, and ‘LA1697’ had lower percent reduction in shoot DW than the average (27%, 33%, and 39%, vs. 44% of the average, respectively) (Fig. 3.1).
By plotting shoot DW for plants under control versus NaCl treatments (Fig. 3.2), the responsiveness of each genotype can be visualized in the context of overall plant vigor. Although some genotypes (e.g. LA722) showed little or no decrease in shoot DW with 50 mM NaCl treatment, they still had less shoot DW than other genotypes (e.g. Im, LA3120) that showed a considerable shoot DW reduction with the same salt treatment (Fig. 3.2). Over all NaCl concentrations, ‘Im’ showed a considerable weight reduction with higher salinity but still had a better vigor than other genotypes that showed less weight reduction.

Root to shoot ratio significantly increased with increasing NaCl concentration in most of the genotypes (Fig. 3.3). The root to shoot ratio in ‘AR’, ‘LA3465’, ‘RS’, ‘Im’, and ‘FN’ did not show much difference between 50 mM NaCl and the control treatments. NaCl treatment decreased plant height in most genotypes (data not shown). Change in height between 7 and 16 days of salt treatment significantly varied among the genotypes and with NaCl treatment ($P<0.001$, Table 3.4). Some genotypes such as ‘LA722’ and ‘LA1630’, had less growth as compared to the control, whereas others, such as ‘LA3320’ and ‘LA3120’, had minimal or no changes in their rate of growth with 50 mM NaCl treatment (Fig. 3.4).

**Ion concentration and compartmentalization**

Genotypes that showed high vigor and/or low shoot DW reduction under NaCl treatments were chosen for measuring Na$^+$, K$^+$, Ca$^{2+}$, and Mg$^{2+}$ concentrations in the leaves and roots. The average leaf Na$^+$ concentration of all genotypes was ~25, 34, and 37 mg/g DW at 50, 100, and 150 mM NaCl treatments, respectively (Table 3.2). Although leaf Na$^+$ concentrations were significantly affected by genotype and treatment, there was a lack of interaction between genotype and NaCl treatment (Table 3.2). ‘Im’ leaves had the lowest Na$^+$ concentration (~19 mg/g DW) at 50 mM NaCl, while ‘AR’ showed the lowest concentration at 100 mM (~20 mg/g DW) and at 150 mM NaCl (~27 mg/g DW) (Fig. 3.5). On the other hand, plants of ‘LA722’ and ‘LA1630’ had the highest leaf Na$^+$ concentrations under all NaCl treatments (Fig. 3.5).
There was a significant NaCl treatment effect on root Na\(^+\) concentration; however, no genotype effect was found (Table 3.2). Despite that, Na\(^+\) concentration in the roots of plants grown with 50 mM NaCl varied from ~22 mg/g DW in LA1630 to ~38 mg/g DW in AR. At higher NaCl concentrations, no marked differences in root Na\(^+\) concentration were noted among genotypes (Table 3.2). Under 50 and 100 mM NaCl, the average Na\(^+\) concentrations in the roots of all genotypes were slightly higher than in the leaves (~28 and 37 vs. ~25 and 34 mg/g DW, respectively), but were similar at 150 mM NaCl.

No significant relationship was found between leaf Na\(^+\) concentration and percent reduction in shoot DW within any of the individual NaCl treatments ($P=0.299$, $r^2=0.177$ at 50 mM NaCl, $P=0.599$, $r^2=0.059$ at 100 mM NaCl), but there was a significant relationship with all the three salt treatments combined ($P=<0.001$, Fig. 3.7). Our results showed significant genotype and NaCl treatment effects on leaf ion concentrations, but Ca\(^{2+}\) had a genotype effect only at $P=0.07$ (Table 3.2). However, there was no significant interaction between genotype and NaCl treatments for cation concentrations or ratios. In order to avoid the reduction in Ca\(^{2+}\) activity caused by Na\(^+\) in the growing solution, we supplemented our nutrient solution with extra Ca\(^{2+}\), calculated using a software program called MINTEQ, (Munns and Tester 2008). This adjustment was intended to ameliorate Na\(^+\) effects on Ca\(^{2+}\) uptake by the plants. It was reported that Ca\(^{2+}\) transport is highly reduced when Na\(^+\) is added to the growing medium (Cramer, Epstein, and Lauchli 1989). Root Na\(^+\) was significantly affected by NaCl treatment ($P<0.001$), but K\(^+\) and Ca\(^{2+}\) concentrations in the root were not affected by either the NaCl treatment or the genotype, whereas root Mg\(^{2+}\) concentrations were significantly affected only by the genotype (Table 3.2).

In our correlation analysis, we were interested in observing the 50 and 100 mM NaCl treatment correlations because we are using 75 mM NaCl treatment for our next grafting experiment which is expected to give effects intermediate to those two concentrations of this experiment (Chapter 4). Leaf Mg\(^{2+}\) and Mg\(^{2+}\): Na\(^+\) ratio were significantly correlated with shoot DW in the three NaCl treatments (Tables 3.5 and 3.6, data not shown for 150 mM NaCl). Leaf K\(^+\): Na\(^+\) ratio was highly significantly correlated with shoot DW at 100 and 150 mM NaCl (Table 3.6, data not
shown for 150 mM NaCl), whereas leaf Ca$^{2+}$: Na$^+$ ratio was significantly correlated with shoot DW at 50 and 100 mM NaCl (Tables 3.5 and 3.6). Leaf Ca$^{2+}$ and Na$^+$ were significantly negatively correlated at 150 mM NaCl ($P=0.04$, $r^2=0.528$, Fig. 3.10). The three elemental ratios in the leaves were significantly correlated with each other in all NaCl treatments (Tables 3.5 and 3.6). Root to shoot ratio was significantly correlated with leaf Na$^+$, K$^+$, and Mg$^{2+}$ at 50 mM NaCl (Table 3.5).

**Discussion**

*Solanum* species and genotypes vary in their response to salinity stress (Cuartero et al. 1999; Cuartero & Flower 1992), and that response also differs according to NaCl concentration and duration of exposure (Munns 2002; Estañ et al. 2005). Cultivated tomato plants are moderately sensitive to salinity, however, some accessions from wild species such as *S. pimpinellofolium* were found to be more tolerant to salinity (Cuartero et al. 1999; Cuartero et al. 2006; Maas 1993; Flowers & Flowers 2005). Cultivated tomato plants can tolerate ECs of up to 2.5 dS/m of the saturated soil extract without yield reduction; however, a reduction of 10% in yield occurs with every increase of 1 dS/m above this threshold. The EC of soils irrigated with fresh water and fertilized at normal rate usually lies in the range of 1.6 to 3.1 dS/m (Reviewed by Cuartero et al. 1999). Soils are defined as saline when the EC of the saturated soil extract is equal to or above 4 dS/m (Chinnusamy et al. 2005).

Tomatoes are mainly grown in arid and semiarid areas where salinity can be a significant challenge for tomato growth and yield (Cuartero et al. 1999). For example, more than 35% of agricultural lands in Egypt are affected with salinity that has an EC higher than 4 dS/m (~> 40 mM NaCl, Kotb et al. 2000). Grafting tomatoes onto salt-tolerant rootstocks could improve tomato tolerance to salinity under these conditions.

In this experiment, we screened 21 tomato genotypes for salinity tolerance under four NaCl treatments, 0, 50, 100, and 150 mM (EC of 1.11, 6.30, 8.06, and 8.93 dS/m, respectively). Genotypes differed in growth performance under salinity stress, particularly at 50 or 100 mM NaCl treatments. At 150 mM NaCl, those differences were much less noticeable. At 50 mM, some genotypes, e.g. ‘LA722’ and ‘MM’ had no significant growth reductions (~3.7 and 1%,
respectively) while other genotypes showed marked growth reduction (e.g. MAS, 46%). At 100 mM NaCl, genotypes such as ‘IM’, ‘AR’, and ‘MM’ showed minimal growth reductions compared to the other genotypes (e.g. ‘PHO’ and ‘LA1310’) that showed higher reductions. Because of the higher genotypic variation at 50 and 100 mM NaCl, we chose NaCl treatment of 75 mM in our next experiment (Chapter 4) intended to study the effects of using these rootstocks to improve salinity tolerance in tomato plants.

Response to salinity should be considered in the context of overall plant vigor

Many researchers have assessed plant tolerance to salinity by measuring the shoot weight or fruit yield in salinized plants relative to the non-salinized plants (Colla et al. 2006; Maas & Hoffman 1977; Munns et al. 2002; Negrão et al. 2017). We also found a range of salinity tolerance in our genotypes using the shoot reduction measurement in salinized plants relative to the control plants (Fig. 3.1).

Plant vigor and plant response to salinity are key criteria for selecting plants that are productive under salt stress. Other parameters that have been considered as indicators for plant tolerance to salinity (such as plant survival) may not necessarily imply yield stability under saline conditions (Albacete et al. 2014). It is agriculturally more desirable to have vigorous plants regardless of their tolerance level because farmers have more interest in genotypes that maintain vigor and yield under saline conditions. Although ‘Ironman’ in our experiment was reduced in shoot weight (especially at 100 and 150 mM), it maintained a more vigorous growth than other genotypes that were not reduced in shoot growth under all NaCl treatments.

Selecting a vigorous genotype, however, does not necessarily guarantee that plants will perform well under salinity conditions. For example, ‘SC’ was a relatively vigorous genotype under control conditions, but shoot DW was reduced by more than one third under 50 mM NaCl treatment.

The results of the present experiment showed that the root to shoot ratio increased with increasing NaCl concentrations in most genotypes (Fig. 3.3). High root to shoot ratio is frequently an indication of stress. The increase in root growth is believed to be a plant
adaptation mechanism to facilitate water and nutrient acquisition under salinity stress (Maggio, De Pascale, et al. 2007; Yu et al. 2012; Shiyab et al. 2013; Albacete et al. 2014; Albacete et al. 2008). Cuartero et al., 1999, have reported that despite the negative effects of salinity on the roots, root growth in tomato plants seems to be less affected by salinity than the shoot, hence the increased root to shoot ratio with salinity (Cuartero et al. 1999). In our experiment, root to shoot ratio was significantly negatively correlated with shoot DW at 100 mM NaCl, and genotypes varied in their responses at this NaCl concentration, which could have reflected different degrees of stress on the genotypes. This was supported by the significant negative correlation of root to shoot ratio with shoot dry weight. However, at 150 mM NaCl the stress may have been too severe for us to detect differences in shoot DW reduction or root growth among genotypes.

We also studied the correlation between root to shoot ratio and ion concentrations. Under 50 mM NaCl treatment, root to shoot ratio was positively correlated with leaf Na+, whereas it was negatively correlated with K+ and Mg2+ (Table 3.5), indicating that plants were able to maintain their root growth at this NaCl concentration. However, this relation was not significant at higher NaCl concentrations as more stress is related to both higher root to shoot ratio and to leaf Na+ concentration.

For this reason, genotypes screened in this experiment that showed strong plant vigor in addition to low shoot DW reduction were selected as rootstocks for the next experiment (Chapter 4) to examine their growth under salt stress conditions and to study their tolerance mechanisms.

Out of the 21 genotypes screened under the four salt concentrations, we chose 8 genotypes that had either minimal percent reduction in shoot DW or high vigor under salinity conditions (despite large reductions in shoot DW), to measure the concentrations of Na+, K+, Ca2+, and Mg2+ ions in the leaves and the roots. The aim of measuring the ions in these particular genotypes was to learn more about their mechanisms of tolerance and ion partitioning strategies under varying NaCl treatments before using them as rootstocks in our grafting experiment (Chapter 4).
Leaf Na\(^+\) does not explain genotypic variation in performance

The results of this experiment showed no relationship between Na\(^+\) concentration in the leaf and shoot DW (or percent reduction in shoot DW) in any of the salt treatments. For example, ‘LA722’ and ‘LA1630’, which showed no or mild shoot DW reduction under 50 mM NaCl treatment, had the highest leaf Na\(^+\) concentrations (~38 and 34 mg/g DW, respectively). On the other hand, ‘LA3120’ and ‘Im’, which also had mild reduction in shoot DW, showed the lowest leaf Na\(^+\) concentration (~20 and 19 mg/g DW, respectively) under 50 mM NaCl treatment. At 100 mM NaCl, shoot DW reduction of ‘Im’ plants was lower than most of the genotypes although its leaf Na\(^+\) concentrations were higher than most of the cultivated genotypes. Similar results were observed in other studies where no correlation was found between leaf Na\(^+\) and the vegetative growth in tomato or bread wheat plants grown in saline conditions (Dasgan et al. 2002; Genc et al. 2007).

Increased leaf K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\) ratios with Na\(^+\) are associated with greater shoot DW

Leaf K\(^+\): Na\(^+\) ratio was significantly correlated with Ca\(^{2+}\): Na\(^+\) and Mg\(^{2+}\): Na\(^+\) ratios, whereas leaf Na\(^+\) was negatively correlated with leaf Mg\(^{2+}\) and leaf K\(^+\) in all NaCl treatments (and with Ca\(^{2+}\) at 150 mM NaCl only, data not shown). Besides the toxicity effects of Na\(^+\) on plants, elevated Na\(^+\) causes nutritional imbalances (Rengasamy 2006). High Na\(^+\) impedes the absorption of nutrients such as K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\) by competing with them, and consequently lowers their absorption and distribution within the plant (Maggio, Raimondi, et al. 2007; Maggio, De Pascale, et al. 2007; Yu et al. 2012; Shiyab et al. 2013; Albacete et al. 2008; Albacete et al. 2014). Na\(^+\) in the medium makes it harder for the plants to discriminate between K\(^+\) and Na\(^+\) at K\(^+\) pathways, as both elements have similar hydrated ionic radii. High Na\(^+\) in the growing medium also causes displacement of Ca\(^{2+}\) from cell membranes, and reduces Ca\(^{2+}\) uptake and transport by the plant (Maggio, De Pascale, et al. 2007; Yu et al. 2012; Shiyab et al. 2013; Albacete et al. 2014; Albacete et al. 2008). Genotypes with higher K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\) in the leaves, and with higher cytosolic K\(^+\): Na\(^+\), Ca\(^{2+}\): Na\(^+\) ratios, have better tolerance to salt stress (Cuartero et al. 1999; Munns & Tester 2008; Munns et al. 2002; Shabala & Cuin 2008; Subbarao et al. 1990; Yu et al. 2012).
In our experiment, no relationship was found between Na\(^+\) and Ca\(^{2+}\) at 50 and 100 mM NaCl, whereas a negative correlation was found between Na\(^+\) and Ca\(^{2+}\) at 150 mM NaCl. As the addition of Na\(^+\) to the growing medium reduces Ca\(^{2+}\) activity, it is hard to conclude if the effects are due to the toxic Na\(^+\) consequences on plants or due to the low Ca\(^{2+}\) activity in the growing medium (Munns & Tester 2008). To minimize this problem, we adjusted calcium concentrations using MINTEQ software program (Munns & Tester 2008) to maintain Ca\(^{2+}\) activity in the hydroponic growing medium. It seems that all the genotypes lost their ability to restrict Na\(^+\) uptake at 150 mM NaCl, and consequently these plants had lower leaf Ca\(^{2+}\):Na\(^+\) ratio. Adjusting Ca\(^{2+}\) in the growth medium would not prevent toxic Na\(^+\) effects on the plants.

In our experiment, shoot DW was significantly correlated with Mg\(^{2+}\) and Ca\(^{2+}\) ratios with Na\(^+\), except at 150 mM where Ca\(^{2+}\):Na\(^+\) was not correlated with shoot DW. As discussed earlier, salt tolerant plants are more selective to K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\) over Na\(^+\), and consequently have better nutritional balance than sensitive plants. Our results are in agreement with those reported by Dasgan et al., 2002, where higher shoot K\(^+\):Na\(^+\) and Ca\(^{2+}\):Na\(^+\) ratios resulted in less shoot damage and higher salinity tolerance (Dasgan et al. 2002). In that study, however, no correlation was found between the ion concentrations or their ratios with the shoot DW.

**Wild genotypes accumulated more Na\(^+\) in the leaves than cultivated genotypes**

Sodium is not an essential nutrient for most glycophytes, and its presence in excess impairs plant growth (Maathuis 2013). Plants use three mechanisms of tolerance to cope with the detrimental effects of Na\(^+\). The first is osmotic tolerance which is related to water uptake and signals from the root to the shoot. Salt (Na\(^+\) and Cl\(^-\)) exclusion is the second mechanism, by which tolerant plants are able to exclude most of Na\(^+\) ions and maintain low Na\(^+\) concentrations in the leaves. The third strategy is the salt inclusion mechanism, where Na\(^+\) ions are harmlessly accumulated in the leaves by compartmentalizing them in the vacuoles (Tester & Davenport 2003; Munns & Tester 2008; Roy et al. 2014).

At 50 mM NaCl treatment, wild genotypes (LA722 and LA1630) showed no or minimal reduction in their shoot DW (-3.6 and 12%, respectively) compared to the average weight reduction of all genotypes (~21%). At 100 and 150 mM NaCl concentrations, percent shoot DW reductions were
comparable to other genotypes (~48% and 57% in LA1630; 46 and 63% in LA722, respectively) compared to the average reduction in all genotypes (~44 and 61%, respectively).

Leaf Na\(^+\) concentrations were significantly different among the genotypes ($P=0.001$, Table 3.2). At 50, 100, and 150 mM NaCl, leaf Na\(^+\) concentrations in ‘LA722’ (~38, 61, 43 mg/g DW, respectively) and in ‘LA1630’ (~34, 43, 48 mg/g DW, respectively) were higher than the average leaf Na\(^+\) concentration in all genotypes (~25, 34, 37 mg/g DW, respectively). Earlier studies have reported that salt tolerance in most wild genotypes is associated with the halophytic character of accumulating higher Na\(^+\) concentrations compared to the cultivated genotypes (Bolarín et al. 1995; Cuartero & Flower 1992; Cuartero et al. 1999). Our results agree with earlier studies, since the wild genotypes ‘LA1630’ and ‘LA722’ had higher leaf Na\(^+\) concentrations than cultivated genotypes at 50 and 100 mM NaCl, however, ‘LA722’ plants had lower leaf Na\(^+\) accumulation at 150 mM than at 100 mM NaCl. It seems that the inclusion tolerance mechanism used by these wild plants remains effective up to certain Na\(^+\) level, and then it fails. These results suggest that ‘LA722’ and ‘LA1630’ have a greater level of tissue tolerance than the other studied genotypes.

‘LA1630’ and ‘LA722’ are both Solanum pimpinellifolium, which is closely related to the cultivated tomato species S. lycopersicon. Tomato accession ‘LA722’ has the reputation of being salt-tolerant according to its high survival rate under salinity stress conditions (Foolad & Chen 1999; Foolad 1999). Foolad and Chen, 1999, reported that about 80% of the 8-week-old plants of ‘LA722’ survived under very high salt stress treatment (700 mM NaCl +70 mM CaCl\(_2\)) for two weeks (Foolad & Chen 1999). Wild tomato accessions are considered to be a good source of salinity tolerance for breeding programs (Saranga et al. 1993; Foolad & Chen 1999; Foolad 1999). It is difficult to develop elite lines by transferring genes from wild species because of the large number of small-effect genes that are involved in salt tolerance and also because of the high cost involved in recovering the genetic background of the receptor genotype (Cuartero et al. 2006). In addition, lack of vigor and small fruit size makes it hard to use wild accessions in commercial production. Therefore, grafting scions with high productivity onto wild genotype
rootstocks might help in ameliorating salinity problems, while maintaining the desired fruit characteristics and yield.

‘AR’ regulates Na\(^+\) transport to the shoot under mild salinity conditions

‘AR’ had a smaller shoot DW reduction than the average of all genotypes at 50 mM NaCl treatments (~2.5% vs. 21%). This was reversed at higher NaCl treatments. AR had lower leaf Na\(^+\) accumulation than the average levels of all genotypes at 50 mM, and was even lower at higher NaCl concentrations (e.g. ~20 vs. 34 mg/g DW at 100 mM NaCl, Fig. 3.5) compared to the average. ‘AR’, however, had higher Na\(^+\) accumulation in roots than the average at both 50 mM and 100 mM NaCl treatments, and was similar to the average at 150 mM (Fig. 3.6). ‘AR’ had higher leaf Ca\(^{2+}\) and Mg\(^{2+}\) than the average levels of all genotypes at 100 and 150 mM. The lower levels of Na\(^+\) in the leaf can be explained as ‘AR’ is excluding most Na\(^+\) ions by sequestering them in the roots to limit their transport to the shoot, in addition to the higher selectivity to Ca\(^{2+}\) and Mg\(^{2+}\) over Na\(^+\) (Alfocea et al. 1993; Yetisir & Uygur 2010).

Genotypes chosen as potential rootstocks because of their performance under salinity

Genotypes were chosen for the grafting experiment (Chapter 3) because of their performance under NaCl treatments. Genotypes that maintained a high vigor or mild percent reduction in shoot DW were found to be potential candidates to be used as rootstocks. In addition to the above mentioned genotypes (LA1630, LA722, and AR), ‘Im’ was selected for our next grafting experiment because it was the most vigorous genotype in our study in all NaCl treatments (Fig. 3.2). It also had the lowest leaf Na\(^+\) concentration at 50 mM NaCl as compared to the average concentration in all genotypes (~19 vs. 25 mg/g DW).

‘RS’, on the other hand, was chosen as a rootstock because it showed a mild percent reduction in shoot DW at 50 mM NaCl as compared to the average percent reduction in all genotypes (~8.5% vs. 21%). In addition, it showed a better shoot DW than the average weight of other genotypes in all NaCl treatments (~18 g vs. 15 g at 50 mM, 13 g vs. 11g at 100 mM). ‘RS’ also had a lower leaf Na\(^+\) concentration as compared to the average leaf Na\(^+\) concentration of all genotypes at 50 and 100 mM NaCl treatments (Fig. 3.5).
‘LA3120’ was selected for the grafting experiment because it showed a vigorous growth at 50 mM NaCl as compared to the average shoot DW of all genotypes (~18 g vs. 15 g) although it did not maintain the better shoot DW at higher NaCl concentrations. ‘LA3120’ had lower Na\(^+\) accumulation in the leaf than the average concentration (Fig. 3.5). ‘LA3465’ had a very low percent reduction in DW (~4%) under 50 mM NaCl treatment. It also accumulated less Na\(^+\) in the leaf than the average leaf Na\(^+\) concentration at 50 and 100 mM NaCl (Fig 3.5).

In general, these cultivated genotypes excluded Na\(^+\) from the leaf; however, most genotypes lost their Na\(^+\) exclusion ability at higher NaCl treatments. In addition, most genotypes (except for ‘LA722’ and ‘LA1630’) had higher Na\(^+\) retention in the roots than in the shoot, which is a typical character of most cultivars of *S. lycopersicon* (Alfocea et al. 1993).

Understanding the performance of tomato plants under salinity conditions and the different tolerance mechanisms can greatly help in selecting rootstocks that can be used in grafting to improve plant tolerance to salinity. It is quite reasonable to evaluate salt tolerance by measuring plants growth or yield under salinized conditions as compared to plants under control conditions (Cheeseman 2013). If lack of growth reduction under salinity conditions was the only criteria for choosing tolerant genotypes, ‘LA722’ (*S. pimpinellofolium*) would be a good candidate because of its satisfactory performance under 50 mM NaCl. However, ‘LA722’ would not be used commercially because of lack of vigor and its small fruit size. Nevertheless, it might be a candidate for use as a rootstock for grafting for salinity tolerance. ‘Im’, on the other hand, would be the preferred farmers’ choice since it maintained a good vigor, especially under both 50 and 100 mM NaCl treatments, even though it showed a higher percent reduction in shoot DW compared to ‘LA722’. The results of this screening experiment show that ‘RS’, ‘Im’, ‘AR’, ‘LA3120’, and ‘LA1630’ genotypes could serve as potential rootstocks targeting improving tomato tolerance to salinity while maintaining the desired growth.
References


Yu, S., Wang, W., & Wang, B. (2012). Recent progress of salinity tolerance research in plants. *Russian
Table 3. Two-way Analysis of Variance (ANOVA), for the effect of genotype and NaCl treatment (0, 50, 100, 150 mM) on leaf and root ion concentrations. Also shown are means and standard errors for salt effects over all genotypes.

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Table 3. 3: Two-way Analysis of Variance (ANOVA), for the effect of genotype and NaCl treatment (0, 50, 100, and 150 mM) on leaf and root elemental ratios. Also shown are means and standard errors for salt effects over all genotypes.

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</tr>
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</tr>
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</tr>
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<td>Mean</td>
<td>SE</td>
</tr>
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<td>NA</td>
</tr>
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</tr>
<tr>
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<tr>
<td>150 mM NaCl</td>
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Table 3.4: Two-way Analysis of Variance (ANOVA), for the effect of genotype and NaCl treatment (0, 50, 100, and 150 mM) on the growth variables of the genotypes. Also shown are means and standard errors for salt effects over all genotypes.

<table>
<thead>
<tr>
<th></th>
<th>Shoot DW (g·plant⁻¹)</th>
<th>% reduction in shoot DW</th>
</tr>
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<td>df</td>
<td>F</td>
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<tr>
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<td>Rep</td>
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<td>61.62</td>
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<td>SE</td>
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<td>0 mM NaCl</td>
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<tr>
<td>50 mM NaCl</td>
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<td>100 mM NaCl</td>
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<td>150 mM NaCl</td>
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<table>
<thead>
<tr>
<th></th>
<th>Root: shoot ratio</th>
<th>Height change (cm/day)</th>
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</thead>
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<td>Treatment</td>
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<td>34.47</td>
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<tr>
<td>Genotype*treatment</td>
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<td>0.96</td>
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<td>Treatments</td>
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<td>SE</td>
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<td>50 mM NaCl</td>
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</tr>
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<td>100 mM NaCl</td>
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<td>150 mM NaCl</td>
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<td>0.03</td>
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Table 3.5: Correlation matrix for leaf ion concentration, growth and physiological traits of 8 genotypes grown under salts stress (50 mM NaCl).

<table>
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<tr>
<th>K$^+$</th>
<th>Ca$^{2+}$</th>
<th>Mg$^{2+}$</th>
<th>Na$^+$</th>
<th>K$^+$:Na$^+$</th>
<th>Ca$^{2+}$:Na$^+$</th>
<th>Mg$^{2+}$:Na$^+$</th>
<th>Percent DW Red</th>
<th>Shoot DW</th>
<th>Height (cm/d)</th>
<th>Root : Shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td>K$^+$</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>-0.06</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
<td>0.36</td>
<td>0.66**</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>-0.58**</td>
<td>-0.29</td>
<td>-0.67**</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K$^+$:Na$^+$</td>
<td>0.87***</td>
<td>0.06</td>
<td>0.55*</td>
<td>-0.83***</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ca$^{2+}$:Na$^+$</td>
<td>0.34</td>
<td>0.62**</td>
<td>0.83***</td>
<td>-0.87***</td>
<td>0.69**</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mg$^{2+}$:Na$^+$</td>
<td>0.43</td>
<td>0.48*</td>
<td>0.9***</td>
<td>-0.85***</td>
<td>0.74***</td>
<td>0.95***</td>
<td>1</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Percent DW Red</td>
<td>0.03</td>
<td>-0.55*</td>
<td>-0.11</td>
<td>0.06</td>
<td>0.07</td>
<td>-0.17</td>
<td>0</td>
<td>1</td>
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</tr>
<tr>
<td>Shoot DW</td>
<td>0.17</td>
<td>0.35</td>
<td>0.45*</td>
<td>-0.33</td>
<td>0.36</td>
<td>0.5*</td>
<td>0.51*</td>
<td>-0.28</td>
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<tr>
<td>Height (cm/d)</td>
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<td>-0.07</td>
<td>0.06</td>
<td>-0.12</td>
<td>0.42</td>
<td>0.1</td>
<td>0.13</td>
<td>0.1</td>
<td>0.34</td>
<td>1</td>
</tr>
<tr>
<td>Root: Shoot</td>
<td>-0.56*</td>
<td>-0.31</td>
<td>-0.46*</td>
<td>0.46*</td>
<td>-0.64**</td>
<td>-0.48*</td>
<td>-0.49*</td>
<td>0.01</td>
<td>-0.22</td>
<td>-0.49*</td>
</tr>
</tbody>
</table>

Significant differences (Pearson correlation, 1-tailed) are indicated: *, P <0.05; **, P <0.01; ***, <0.001 levels; Height (cm/day), height change/day; Percent DW Red, Percent reduction in shoot DW; Root: Shoot, root to shoot ratio
Table 3.6: Correlation matrix for leaf ion concentration, growth and physiological traits of 8 genotypes grown under salt stress (100 mM NaCl).

<table>
<thead>
<tr>
<th></th>
<th>K⁺</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>Na⁺</th>
<th>K⁺:Na⁺</th>
<th>Ca²⁺:Na⁺</th>
<th>Mg²⁺:Na⁺</th>
<th>Percent DW Red</th>
<th>Shoot DW</th>
<th>Height (cm/d)</th>
<th>Root: Shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁺</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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</tr>
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<td>-</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>0.63**</td>
<td>0.58**</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Na⁺</td>
<td>-0.59**</td>
<td>-0.12</td>
<td>-0.49*</td>
<td>1</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K⁺:Na⁺</td>
<td>0.85***</td>
<td>0.32</td>
<td>0.57**</td>
<td>-0.76***</td>
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<tr>
<td>Ca²⁺:Na⁺</td>
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<td>0.54*</td>
<td>0.54*</td>
<td>-0.81***</td>
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<td>Mg²⁺:Na⁺</td>
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<td>0.72***</td>
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<td>0.89***</td>
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<tr>
<td>Percent DW Red</td>
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<td>0.07</td>
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<td>-0.51*</td>
<td>-0.45*</td>
<td>-0.5*</td>
<td>1</td>
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</tr>
<tr>
<td>Shoot DW</td>
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<td>0.27</td>
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<td>0.68***</td>
<td>0.47*</td>
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<td>-0.56**</td>
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<td>Height (cm/d)</td>
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<tr>
<td>Root: Shoot</td>
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<td>-0.23</td>
<td>0.04</td>
<td>-0.12</td>
<td>-0.09</td>
<td>-0.14</td>
<td>0.24</td>
<td>-0.48*</td>
<td>-0.57**</td>
<td>1</td>
</tr>
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Significant differences (Pearson correlation, 1-tailed) are indicated: *, P < 0.05; **, P < 0.01; ***, < 0.001 levels; Height (cm/day), height change/day; Percent DW Red, Percent reduction in shoot DW; Root: Shoot, root to shoot ratio.
Table 3. 7: Correlation matrix for root ion concentration, growth and physiological traits of 8 genotypes grown under salts stress (100 mM NaCl).

<table>
<thead>
<tr>
<th></th>
<th>K⁺</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>Na⁺</th>
<th>K⁺:Na⁺</th>
<th>Ca²⁺:Na⁺</th>
<th>Mg²⁺:Na⁺</th>
<th>Percent DW Red</th>
<th>Shoot DW</th>
<th>Height (cm/d)</th>
<th>Root : Shoot</th>
</tr>
</thead>
<tbody>
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<td>K⁺</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>Na⁺</td>
<td>0.54*</td>
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<td>-0.09</td>
<td>1</td>
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<td>-</td>
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<td>0.93***</td>
<td>-0.44</td>
<td>0.11</td>
<td>0.94***</td>
<td>1</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Percent DW Red</td>
<td>0.49*</td>
<td>0.07</td>
<td>0.08</td>
<td>0.12</td>
<td>0.5*</td>
<td>0.04</td>
<td>0.03</td>
<td>1</td>
<td>-</td>
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</tr>
<tr>
<td>Shoot DW</td>
<td>-0.54*</td>
<td>-0.27</td>
<td>-0.35</td>
<td>-0.39</td>
<td>-0.28</td>
<td>-0.14</td>
<td>-0.18</td>
<td>-0.56**</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Height (cm/d)</td>
<td>0.22</td>
<td>0.03</td>
<td>0.09</td>
<td>-0.1</td>
<td>0.26</td>
<td>0.08</td>
<td>0.11</td>
<td>-0.06</td>
<td>0.4</td>
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<tr>
<td>Root: Shoot</td>
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<td>0.28</td>
<td>-0.04</td>
<td>-0.1</td>
<td>-0.16</td>
<td>0.24</td>
<td>-0.48*</td>
<td>-0.57**</td>
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Significant differences (Pearson correlation, 1-tailed) are indicated: *, P < 0.05; **, P < 0.01; ***, <0.001 levels; Height (cm/day), height change/day; Percent DW Red, Percent reduction in shoot DW; Root: Shoot, root to shoot ratio
Fig 3. 1: Reduction of shoot dry weight in tomato genotypes grown for 16 days in the presence of 50, 100, or 150 mM NaCl compared to control plants of the same genotype. Data are means of three plants. The black dashed line represents the overall average for genotypes grown under 50 mM NaCl and the red dashed line represents the overall average at 100 mM NaCl. Percent reduction was calculated for each block, and then the average percent reduction in shoot DW was calculated.
Fig 3. 2: Scatterplots of the shoot dry weight (g) plotted for control plants versus salt-treated plants grown with 50 mM NaCl (A), 100 mM NaCl (B), 150 mM NaCl (C).

Fig 3. 3: Root to shoot ratio in tomato genotypes grown for 16 days in control, 50, 100, or 150 mM NaCl. Data are means of three plants ±SE. The black dashed line represents the overall average for genotypes grown under 50 mM NaCl and the red dashed line represents the overall average at 100 mM NaCl.
Fig 3.4: Plant height change between 7 and 16 days of salt treatment in control, 50, 100, or 150 mM NaCl. Data are means of three plants ±SE.
Fig 3. 5: Na⁺ concentration in the 4th leaf of eight tomato genotypes grown for 16 days in the presence of 50, 100, or 150 mM NaCl. Data are means of three plants ±SE. The black dashed line represents the overall average for genotypes grown under 50 mM NaCl and the red dashed line represents the overall average at 100 mM NaCl.

Fig 3. 6: Na⁺ concentration in the roots of salt-treated tomato genotypes grown for 16 days in the presence of 50, 100, or 150 mM NaCl. Data are means of three plants ±SE. The black dashed lines represent the overall average for genotypes grown under 50 mM NaCl and the red dashed line represents the overall average at 100 mM NaCl.
Fig 3. 7: Relationship between percent reduction in shoot DW and leaf Na\(^+\) concentrations in genotypes treated with 50, 100, or 150 mM NaCl after 16 days of salt treatment.

Fig 3. 8: Relationship between leaf K\(^+\) and Na\(^+\) concentrations in genotypes treated with 100 mM NaCl after 16 days of salt treatment.
Fig 3. 9: Relationship between leaf $K^+$ and $Na^+$ concentrations in genotypes treated with 150 mM NaCl after 16 days of salt treatment.

Fig 3. 10: Relationship between leaf $Ca^{2+}$ and $Na^+$ concentrations in genotypes treated with 150 mM concentrations of NaCl after 16 days of salt treatment.
CHAPTER 4
Examining the Use of Grafting for Improving Salinity Tolerance in Tomato Plants

Introduction

Soil salinity is one of the major agricultural causes of yield reductions in agriculture (Cabot et al. 2014). Salt ions such as Na$^+$, Cl$^-$, SO$_4^{2-}$, Ca$^{2+}$, Mg$^{2+}$, K$^+$, and CO$_3^{2-}$ accumulate in the soil above the level plants can tolerate, which consequently reduces plant growth and production (Rengasamy 2010). Saline soil covers approximately 397 million hectares (3.1%) of the world’s total land area (Setia et al. 2013, FAO, 2005). Salts are primarily found in arid and semi-arid areas although they could also be found in every climatic zone of the world (Rengasamy 2006; Rengasamy 2010).

Not surprisingly, most plants get exposed to Na$^+$ in soil at some stage of their life. For most plants, Na$^+$ is not required for their growth and reproduction (Maathuis 2013). However, in halophytes such as *Atriplex*, small concentrations are required for growth. Na$^+$ in small concentrations has a role in the osmoregulation and enzyme activation functions of plant cells. However, higher Na$^+$ concentrations are toxic to different degrees depending on the plant species. The salt concentration in soils varies greatly from low to intermediate to extreme levels (Maathuis 2013).

Plants go through two major phases when they are exposed to salt stress. One is the osmotic phase, which causes early growth reduction and water uptake inhibition by plants. The other phase is the ionic phase, when salts accumulate over time and cause ion toxicity or ion imbalance in the plants. Thus, it is important to differentiate between the osmotic and toxic effect of salt when studying salinity stress (Munns and Tester 2008; Munns 2002; Rengasamy 2010).

The main aim of research done on salinity tolerance is to develop plants that can maintain their growth and productivity under saline conditions compared to their growth in non-saline
conditions (Roy et al. 2014). Conventional breeding programs have not been efficient for the selection of salt-tolerant genotypes because of the large number of small-effect genes involved in salinity tolerance (Cuartero et al. 2006; Roy et al. 2014; Schwarz et al. 2010).

Using genetic transformation through the transfer of one or more genes for salt exclusion, osmotic, or tissue tolerance is of a great significance and has led to claims of salt-tolerance improvement (Ashraf and Akram 2009; Roy et al. 2014). However, due to the complexity of the trait, its possible negative side effects and lack of public acceptance to GM crops, this task might be challenging (Colla et al. 2010; Roy et al. 2014). Therefore, using other technologies might be more useful immediately.

Grafting is one of techniques that could be used to reduce the negative effects of salinity on plant growth and yield (Colla et al. 2010; Estañ et al. 2005; Martinez-Rodriguez et al. 2008; Santa-Cruz et al. 2002). This could be achieved by using salt-tolerant rootstocks that are capable of ameliorating the damage caused by salinity on the shoot (Colla et al. 2010; Estañ et al. 2005; Gioia et al. 2013; Martinez-Rodriguez et al. 2008; Santa-Cruz et al. 2002). The rootstock is the lower part of the grafted plant, and regulates the uptake and translocation of water, ions and nutrients from the soil or the growing media. The scion, however, is the upper portion, which uses and transports whatever it receives from the rootstock. Research studies are conducted to test and prove the effectiveness of grafting in improving salinity tolerance in plants (Asins et al. 2010; Estañ et al. 2005; Gioia et al. 2013; Martinez-Rodriguez et al. 2008; Santa-Cruz et al. 2002).

The first experiment on tomato grafting for salinity tolerance by Santa-Cruz et al., 2001, tested the use of ‘Moneymaker’ (M) as a scion when grafted onto ‘Pera’ (P). The authors in this study considered ‘Moneymaker’ as a salt-sensitive cultivar and ‘Pera’ as a salt-tolerant one, without mentioning their salt response characteristics. Generally, M/P had higher yield and higher leaf Na⁺ and Cl⁻ concentrations than M/M plants when treated with 50 mM NaCl. This tolerance mechanism was also accompanied with higher K⁺ uptake to the leaf. Therefore, the authors suggested that ‘Pera’, with its includer mechanism, seemed to use inorganic ions to contribute
to the osmotic potential of the leaf. From the energetic view point, using this strategy to increase plant salt-tolerance is less expensive than salt exclusion.

Another study by Santa-Cruz et al., 2002, showed the importance of taking the salt tolerance mechanism of the scion into account when choosing cultivars for grafting. The authors observed a positive effect of the rootstock ‘Kyndia’ when the salt includer ‘UC-82B’ scion was used, whereas a small or no difference was found when the excluder ‘Moneymaker’ scion was used. Consequently, the authors concluded that rootstock ‘Kyndia’ was more able to regulate ion uptake when the includer ‘UC-82B’ was used as scion. The authors also believe that the type of growth has an effect on the capacity of the plants to accumulate salts (Santa-Cruz et al. 2002).

A more detailed study was conducted to investigate the effects of using rootstocks with different tolerance mechanisms on the scion’s growth (Martinez-Rodriguez et al. 2008). Plants of M/M treated with 150 mM NaCl for 27 days had lower shoot weight and higher leaf Na⁺ concentrations than M/ Radja (a salt excluder) or M/P (includer) rootstocks. On the other hand, roots of M/M had significantly lower Na⁺ concentration than those of M/R or M/P. The results suggest that ‘Moneymaker’ has lower ability to store Na⁺ in its own roots. However, in this study, the authors tested two genotypes only in comparison to M/M, and one of the genotypes ‘Radja’ was an excluder. They could have found combinations with lower Na⁺ accumulation in the roots than M/M if they have used more genotypes in grafting.

‘Pera’ rootstock used its ‘includer’ salt-tolerance mechanism when plants were exposed to mild salt (50 mM for 35 days). This pattern, however, was reversed when M/P were treated with increasing salt levels up to 225 mM for 14- 27 days, as Na⁺ continued to increase in M/M but not in M/P. Interestingly, this study proved that plants change their mechanism of tolerance depending on the stress level and length of the salt treatment (Martinez-Rodriguez et al. 2008).

A few studies have examined ion concentrations in the xylem sap of the tomato stems under NaCl treatments (Albacete et al. 2008, 2009; Shabala et al. 2010). Albacete et al., 2009, found that under 75 mM NaCl treatment, the less productive tomato graft combinations (in terms of leaf biomass and chlorophyll fluorescence) had higher leaf xylem Na⁺ concentrations than in the
tolerant combinations. Productivity was positively correlated with leaf xylem K\(^+\) concentration and Na\(^+\): K\(^+\) ratio (Albacete et al. 2009). In another study, shoot xylem sap Na\(^+\) and K\(^+\) concentration were higher in tolerant barely varieties than in the sensitive ones. The authors concluded that Na\(^+\) exclusion from the xylem sap did not have a role in the tolerance to salinity in barley (Shabala et al. 2010). Almeidat et al. 2014, measured Na\(^+\) and K\(^+\) concentrations in the xylem sap of S. pennellii and S. lycopersicum tomato cultivars grown under 5 and 25 mM NaCl treatments. Na\(^+\) concentration in the shoot xylem sap was higher in S. pennellii than in S. lycopersicum. Also, Na\(^+\): K\(^+\) ratio was significantly higher in S. pennellii. Under 75 mM NaCl treatment, there was a significantly higher expression of HKT1;2 gene in the stems and roots of S. lycopersicum, but was not significantly increased in S. pennellii. The authors found that the expression of HKT1;2 in S. lycopersicum was correlated with lower Na\(^+\) accumulation in the leaves.

In another study on Arabidopsis, Sunarpi et al. 2005, measured Na\(^+\) concentrations in the xylem sap of the inflorescence stem. The authors reported that AtHKT1 was selectively removing Na\(^+\) from the xylem vessels to xylem parenchyma cells. HKT was found to reduce Na\(^+\) concentrations in the xylem sap and leaves to protect the leaves from NaCl stress. In the absence of HKT in the athkt1 mutant plants, K\(^+\) concentrations were slightly reduced in the xylem sap and the shoot, whereas Na\(^+\) content was increased. So, HKT seemed to play a role in removing Na\(^+\) from the xylem stream while loading K\(^+\) into the xylem. Although many studies were conducted on different plant species, no previous studies were conducted to measure Na\(^+\) concentrations in the sap of the stems of the grafted tomato plants. Therefore, in addition to investigating the role of using different rootstocks in grafting for improving tomato tolerance to salinity, we were also interested in measuring Na\(^+\) concentrations in the xylem sap of the grafted (whether below or above the graft union) and the non-grafted tomato plants.

In this study, we will use tomato genotypes (AR, RS, LA1630, LA3120, LA722, Im, and LA3465) that were found to be salt-tolerant in our screening experiment. Those genotypes will be used as rootstocks for ‘MM’ scions and will be treated with 75 mM NaCl. We hypothesize that using
salt-tolerant and vigorous genotypes as rootstocks would improve the tolerance and growth of the scion when treated with 75 mM NaCl.

Materials and Methods

Experiment 1: Grafting for better tolerance to salinity

The overall goal of the experiments is to

1. Examine if the use of vigorous rootstocks will improve the growth performance of the scion under salt stress.
2. Test how grafting of ‘Moneymaker’ onto different salt-tolerant rootstocks (whether wild or cultivated) can affect the growth and ion concentrations in the leaf and the roots.

Plant material and growing conditions

In this experiment, eight tomato genotypes including ‘Ironman’, ‘Resistar’, ‘Arnold’, ‘Moneymaker’, ‘LA3120’, ‘LA3465’, ‘LA722’ and ‘LA1630’ were used as rootstocks in grafting to be tested for their ability to confer salinity tolerance upon ‘Moneymaker’ used as a scion (Table 4.1). ‘Moneymaker’ is one of the oldest heirloom tomatoes in the world. It is an indeterminate type tomato cultivar with medium-sized, high quality red fruits. Seeds of “Moneymaker”, ‘Resistar’, ‘Ironman’ and ‘Arnold’ were treated, whereas those from the Tomato Genetics Resource Center were not treated.

Table 4.1: Genotypes used in grafting in the experiment.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Type</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arnold ‘AR’</td>
<td>Hybrid rootstock</td>
<td>Syngenta Seeds, Boise, Idaho, USA.</td>
</tr>
<tr>
<td>Ironman ‘Im’</td>
<td>Hybrid rootstock</td>
<td>Syngenta Seed company (Minnetonka, MN, USA).</td>
</tr>
<tr>
<td>Resistar ‘RS’</td>
<td>Hybrid rootstock</td>
<td>Hazera seed company (Coconut Creek, FL, USA).</td>
</tr>
<tr>
<td>LA3120</td>
<td>S. lycopersicum</td>
<td>Tomato Genetics Resource Center (Davis, CA, USA).</td>
</tr>
<tr>
<td>LA3465(Walker)</td>
<td>S. lycopersicum var. cerasiforme</td>
<td>Tomato Genetics Resource Center (Davis, CA, USA).</td>
</tr>
<tr>
<td>LA722</td>
<td>S. pimpinellifolium</td>
<td>Tomato Genetics Resource Center (Davis, CA, USA).</td>
</tr>
<tr>
<td>LA1630</td>
<td>S. pimpinellifolium</td>
<td>Tomato Genetics Resource Center (Davis, CA, USA).</td>
</tr>
<tr>
<td>Moneymaker ‘MM’</td>
<td>S. lycopersicum</td>
<td>Johnny’s Seeds (Winslow, Maine, USA).</td>
</tr>
</tbody>
</table>
To sterilize the seeds and to increase seed germination percentage, seeds were surface sterilized with 0.5% NaOCl solution for 10 minutes, followed by three washes with sterile distilled water and sown in rockwool cubes (3.81 cm wide). Sowing times of the scion and rootstocks were staggered over two weeks to provide a range of stem diameters to match the scions with the rootstocks when grafting. Seeds of the scion were sown 5 days before the rootstocks, at the same time as the rootstocks and one week after the rootstocks were sown to make sure the stems of the scions and rootstocks were the same diameter. The cubes were kept in a germination chamber until seedlings emerged. Seedlings were irrigated with deionized water during the germination period.

The experiment was conducted under controlled conditions in the greenhouse facility at the Pennsylvania State University, University Park, PA, USA (40° 85’’ N, 77° 82’’ W). Natural light was supplemented from 0800 to 2000 h with 110 μmol photons m⁻² s⁻¹ from 400 W metal halide bulbs (Energy Technics, York, PA, USA).

**Grafting process**

Grafting was performed using the cleft grafting method when seedlings had 3-4 true leaves (4-5 week-old seedlings) (Wang 2011). The scion, ‘Moneymaker’ (MM) was either grafted onto roots derived from a different plant of the same genotype, or onto ‘Resistar’ (M/RS), ‘Arnold’ (M/AR), ‘Ironman’ (M/Im), ‘LA722’ (M/LA722), ‘LA3120’ (M/LA3120) or ‘LA1630’ (M/LA1630).

**Healing conditions**

Grafted plants were kept in plastic cell inserts under completely dark conditions in the healing chamber for 7 days until the graft union was healed. The healing chamber is constructed with PVC pipes covered with one layer of black plastic. The healing chamber’s bench was covered with a capillary mat that is constantly saturated with water to keep constant humidity. Grafted plants were moved to another chamber covered with a transparent plastic layer for one more week. The plastic was gradually opened in the following three days by rolling it up to acclimate the plants to the low humidity. Plants were exposed to higher light intensity to gradually expose
them to light. Grafted plants were watered with half-strength Hoagland’s nutrient solution (Hoagland and Aaron, 1950). Two weeks after grafting, plants were moved to the greenhouse.

**NaCl treatments**

In the greenhouse, grafted seedlings were placed in plastic containers (4 plants per each container) containing 25 l of half-strength Hoagland's nutrient solution. NaCl treatments started two weeks after moving the plants to the greenhouse, when most of the plants had 3-4 leaves and when the grafted plants were well-established. Increments of 25 mM NaCl were added until a final salt concentration of 75 mM was reached to avoid salt shock. To avoid NaCl-induced Ca\(^{2+}\) deficiencies, CaCl\(_2\) was added to the tanks containing NaCl. The additional Ca\(^{2+}\) was required to maintain the activity of Ca\(^{2+}\) the same as in the control (~1.4 mM), calculated using Minteq software, version 3.1. Control plants were grown in tanks filled with half-strength Hoagland's nutrient solution only. Thereafter, plants were grown with or without NaCl for 25 more days. Constant aeration was provided by submerged bubblers attached to the aeration pumps. Solutions were replaced every week. The electrical conductivity (EC) and the pH were recorded every day using hand held EC-pH meter. EC value was ~7.65 dS/m, and pH was maintained from 5.9 to 6.1. Tanks were rinsed with bleach solution weekly to keep them sanitized and free from disease infection.

Measurements included: Leaf and root ion concentrations (Na\(^+\), K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\)), shoot fresh and dry weights (at harvest), root fresh and dry weights (at harvest), root to shoot ratio on a DW basis, leaf relative water content (RWC), and leaf stomatal conductance (gs). RWC was calculated as follows: leaf fresh weight-leaf dry weight/leaf saturated weight-leaf dry weight (Penella et al. 2014). Leaf stomatal conductance (SC) was assessed by measuring the abaxial stomatal conductance between 9 and 11 am using a diffusion SC-1 porometer (Decagon, Pullman, WA, USA) before NaCl treatment, after 1, 2, 3 days of NaCl treatment, and then weekly until harvest.
Cation analysis

We chose the fifth leaf for ion analysis because it emerged after NaCl treatment. Most of the plants had 3-4 leaves at the time of NaCl treatment. At the end of the experiment, the fifth leaf was rinsed in 500 ml of double-deionized water for 5 seconds, while roots were quickly rinsed in 10 mM CaCl$_2$ for 10 seconds to maintain cell integrity and to remove apoplastic Na$^+$. Leaf number 5 and the roots were dried in separate paper bags for ion analysis. Samples of roots and the leaves were oven-dried at 65 °C for 48 h and powdered in a grinder. Dry powdered samples (0.5 g) were digested in a mixture of H$_2$SO$_4$–salicylic acid–H$_2$O$_2$–selenium. Na$^+$, K$^+$, Ca$^{2+}$ and Mg$^{2+}$ were assessed using an atomic absorption spectrometer (Perkin-Elmer 1100, USA) at the plant analysis lab of the Pennsylvania State University. The K$^+$:Na$^+$, Ca$^{2+}$:Na$^+$, and Mg$^{2+}$:Na$^+$ ratios were calculated.

Experimental design

The experiment was conducted in a randomized complete block design. Scion and rootstock combinations were randomized in the containers in each block. Block is the replication in time. Duration of 7 days was spaced between blocks. The experiment was repeated three times and consisted of three plants per each combination per each treatment.

Experiment 2: Sap ion concentrations

The aim of the experiment was to study Na$^+$ and K$^+$ concentrations in the xylem sap below and above the graft union to assess the effect of grafting on ion transport from the roots to the shoot. In this study, five salt-tolerant tomato rootstocks, selected based on the results from experiment 1, were used to measure the concentrations of Na$^+$ and K$^+$ in the tissue sap above and below the graft union under 75 mM NaCl treatment. Cultivated tomato genotypes included *S. lycopersicum* hybrids ‘Arnold’ and ‘Moneymaker’, *S. lycopersicum*, ‘Resistar’, and *S. lycopersicum*, ‘LA3120’ and the wild tomato genotype *S. pimpinellifolium*, ‘LA1630’. ‘Resistar’, ‘Arnold’, ‘LA3120’, and ‘LA1630’ were used as rootstocks, whereas ‘Moneymaker’ was used as a scion. We followed the same procedure with seed sterilizing, planting, and growing conditions as in experiment 1. The experiment was carried out in a greenhouse located at The
The grafting process was the same as in experiment 1. The scion, ‘Moneymaker’ was either grafted onto roots derived from a different plant from the same genotype, or onto ‘Resistar’ (M/RS), ‘Arnold’ (M/AR), ‘LA3120’ (M/LA3120) or ‘LA1630’ (M/LA1630).

The experiment consisted of three blocks. Each block involved three plants per treatment per combination for shoot weight measurements. We performed tissue sap analysis on 4 plants per combination per treatment. However, in some genotypes, we had less than 4 sap samples because we could not get enough sap for analysis. Self-grafted and non-grafted (NG) plants were used as controls. Non-grafted plants were planted 7 days after rootstock and scion plants that were intended for grafting in order to match the developmental stage when starting NaCl treatment. Healing conditions for grafted plants were the same as in experiment 1. Plant growth conditions and treatments after moving grafted plants to the greenhouse were also as in experiment 1, but plants were grown, with or without NaCl for 10 days.

**Sap Na\(^+\) and K\(^+\) concentrations**

According to the technique described by Adem et al., 2014, the freeze-thaw method was used to measure sap ion concentrations in the below-graft and above-graft segments (Adem et al. 2014). In this technique, we cut stem segments (~2-3 cm) below the graft union (~2 mm above the rockwool cube) and ~2 cm above the graft union. The shoot above the collected segment was removed and used for shoot weight measurements. The stem segments were kept on dry ice while collecting samples in the greenhouse, then stored immediately in the freezer at -17°C. The stored stem segments were then thawed and hand-squeezed in 10-ml plastic syringes to extract the sap. The squeezed sap (~80 μL) was then diluted in 2% HNO\(_3\) solution for a total volume of 10 ml solution. Thereafter, sap Na\(^+\) and K\(^+\) concentrations were measured with an atomic absorption spectrometer (Perkin-Elmer 1100, USA) at the plant analysis lab of the Pennsylvania State University.
Experimental design

The experiment was conducted in a randomized complete block design. Plants were randomized in each hydroponic container (8 plants/container). Block was the replication in time. Duration of 1 day was spaced between blocks.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA). Differences between graft combinations and treatments were assessed by Tukey test at $P = 0.05$. Statistical analysis was accomplished using the statistical package in “Rstudio” computer software Version 0.98.490 (2009-2013 RStudio, Inc). The ggplot package was used to analyse the data, to plot the figures, and to place error bars on the graphics.

Results

In our previous screening experiment, we found that most of the differential responses among genotypes, whether growth reductions or ionic concentrations, were more evident under 50 or 100 mM NaCl. Therefore, a concentration of 75 mM NaCl was chosen in the grafting experiment to study the rootstock effects on scion performance under salt stress. Also, mild (25 mM) or moderate (50 mM) NaCl concentrations were shown to not have evident effects on the scion in other studies (Estañ et al. 2005; He et al. 2009).

Seven tomato genotypes were used to form eight different graft combinations. All grafting combinations had the same scion (Moneymaker), while rootstock varied. Rootstock genotypes used in this experiment were chosen for having small reductions in shoot DW and/or high vigor under NaCl treatments in our screening experiment. Plant growth parameters, ion concentrations, relative water content (RWC %) and stomatal conductance (SC) were measured to examine the effect of using those rootstocks on shoot growth and the physiological mechanisms associated with salinity tolerance.
Plant Growth

In the absence of salt, M/Im achieved the highest shoot DW (Fig. 4.1). Self-grafted plants (M/M) did not show significant differences in their shoot DW compared to the hetero-grafts (Fig. 4.1). In salinized plants, the percentage of shoot DW reduction varied among graft combinations, ranging from ~4% in M/RS to ~40% M/Im (Fig. 4.3). The absolute shoot DW, however, was not significantly different among graft combinations at harvest time (Fig. 4.1). Genotypes on or above the blue line on figure 4.2 were not reduced in shoot DW, whereas those under the blue line were reduced in shoot DW under NaCl treatment relative to their weight under control conditions. M/AR was the most vigorous combination, followed by M/Im under NaCl treatment, whereas M/LA3120 had the lowest weight under NaCl treatment (Fig. 4.2).

Root to shoot ratio showed no differences among the graft combinations under control conditions (Table 4.2). Root to shoot ratio was higher in salinized plants compared to the control plants (Fig. 4.4). Plants of M/RS showed the highest root to shoot ratio (0.133), while M/LA3465 had the lowest ratio (0.087).

Ion concentration and partitioning

In salt-treated plants, leaves of M/LA1630 had the highest Na\(^+\) concentration (32.32 mg/g DW), whereas M/LA3120 had the lowest concentration (16.26 mg/g DW, Fig. 4.5). Except for M/LA1630 compared with M/LA3120, no significant differences in leaf Na\(^+\) concentrations were found among the graft combinations ($P=0.078$, Fig. 4.5, Table 4.2). Self-grafted plants showed significantly higher Na\(^+\) concentrations in their roots compared to those in M/RS and M/LA3465 (Table 4.2). Other graft combinations had comparable root Na\(^+\) concentrations ($P<0.05$, Fig. 4.5).

Since Na\(^+\) content of leaves and roots can affect plant growth, linear regression analysis was performed between leaf and root ion concentrations and percent reduction in shoot DW within the 75 mM NaCl treatment. No significant relationship was found between the percent reduction in shoot DW and leaf Na\(^+\) concentration ($r^2=0.004$, $P=0.879$, data not shown), or
between percent reduction in shoot DW and root Na\(^+\) concentration \((r^2=0.143, P=0.356, \text{data not shown})\). Plants of M/RS (that were not reduced in shoot DW with salinity) had comparable leaf Na\(^+\) concentrations to the other graft combinations (Fig. 4.5). Plants of M/LA1630 (that showed \(~12.5\%\) reduction in shoot DW with salinity) had the highest leaf Na\(^+\) concentration.

M/RS showed the highest leaf K\(^+\) concentration (43 mg/g DW) while M/LA1630 plants showed the lowest K\(^+\) concentrations (18.5 mg/g DW, Fig. 4.6). M/LA1630 plants showed a two-fold greater K\(^+\) concentration in the roots compared to the leaves. However, no significant genotypic differences in root K\(^+\) concentrations were noted among the studied graft combinations (Fig. 4.6, Table 4.2). Also, no correlation was found between shoot DW and leaf K\(^+\) concentration (Table 4.3).

Leaf K\(^+\): Na\(^+\) ratio was the highest in M/LA3120 plants and the lowest in M/LA1630 plants. No significant differences were noted in leaf K\(^+\): Na\(^+\) ratio among the other graft combinations (Fig. 4.7). There was an inverse linear relationship \((r^2=0.802; P=0.003)\) between Na\(^+\) and K\(^+\) concentration in the leaves (Fig. 4.8). No significant relationship was found between Na\(^+\) and K\(^+\) concentrations in the roots \((r^2=0.099; P=0.448, \text{Table 4.4, data not shown})\).

Leaves of M/AR had the highest Ca\(^{2+}\) concentrations while those of M/LA1630 had the lowest concentrations (Fig. 4.9). In contrast, Ca\(^{2+}\) concentrations in the roots were not significantly different among graft combinations (Fig. 4.9). The ratio of Ca\(^{2+}\): Na\(^+\) in the leaf was significantly different among grafting combinations \((P=<0.05)\); however, it was not significantly different in the roots (Fig. 4.10). The highest Ca\(^{2+}\): Na\(^+\) ratio was found in the leaf of M/LA3120, and the smallest ratio was found in the leaf of M/LA1630 (Fig. 4.10). There was an inverse relationship between root Ca\(^{2+}\) concentration and percent reduction in shoot DW \((r^2=0.572; P=0.023, \text{Fig. 4.11, Table 4.4})\). On the other hand, there was no significant relationship between leaf Ca\(^{2+}\) concentrations and percent reduction in shoot DW (Table 4.3, data not shown).

M/LA1630 and M/LA722 had lower root Mg\(^{2+}\) concentrations as compared to the self-grafted plants or the other combinations (Fig. 4.12). In all graft combinations, Ca\(^{2+}\) concentrations were much higher in the leaf than in the roots, whereas the opposite was noticed with Mg\(^{2+}\) concentrations (Fig. 4.9 and 4.12). Leaf Mg\(^{2+}\): Na\(^+\) ratio was the lowest in the wild genotype.
LA1630, followed by LA722, whereas LA3120 had the highest ratio ($P<0.001$, Fig. 4.13). Root K$^+$ and Mg$^{2+}$ concentrations were significantly negatively correlated with shoot DW (Table 4.4). Also, root K$^+$, Ca$^{2+}$, and Mg$^{2+}$ ratios with Na$^+$ were negatively correlated with shoot DW (Table 4.4).

**Water relations and leaf gas exchange parameters**

Plant water relations were assessed in the present study by measuring leaf RWC. Under control conditions, leaf RWC was relatively similar in all combinations, except for M/RS and M/LA3120 that were significantly different (Fig. 4.14). No significant genotypic differences were found among salinized plants (Table 4.2).

Gas exchange was assessed by measuring leaf stomatal conductance. Salt treatment decreased stomatal conductance in most genotypes, most evidently after the third day of salt treatment (Fig. 4.15). There were significant genotypic differences in stomatal conductance at harvest week (Table 4.2, ANOVA analysis not shown for the rest of the time points). Under salinity conditions, ‘Moneymaker’ grafted onto ‘LA3120’ had a stable stomatal conductance in most of the weeks. Also, M/LA3120 had a very low reduction in stomatal conductance throughout the growing period, until the last week when the reduction increased to 27%. Plants of M/LA1630 also suffered comparatively low reduction in stomatal conductance, and were not different from controls at 3 weeks. On the other hand, stomatal conductance of M/LA3465 dropped to ~310 mmol/m$^2$/s at 3 weeks. Percent reduction in stomatal conductance was higher in plants of M/M than most of the combinations especially at harvest week (Fig. 4.15).

**Sap Na$^+$ and K$^+$ analysis**

‘RS’, ‘LA1630’, and ‘AR’ were chosen as they had small shoot reductions and/or high vigor as in ‘AR’. ‘LA1630’ was also chosen because it showed an inclusion mechanism of tolerance, whereas ‘LA3120’ was chosen because it showed an exclusion mechanism of tolerance. After 10 days of NaCl treatment, tissue sap samples were collected from plant stems at ~2 cm above and below the graft union to determine whether sap Na$^+$ and K$^+$ concentrations change across the graft union.
The results showed that sap Na$^+$ concentrations were different among the selected genotypes (P<0.001, Table 4.5). Sap position, whether from grafted (above or below) or non-grafted stem segments, had a significant effect on Na$^+$ concentration (Table 4.5). Overall, stem segments from non-grafted plants had higher sap Na$^+$ concentrations (11.8 mg/L) than those in the above-graft segments (7.3 mg/L) of the grafted plants (Fig. 4.17). Also, average Na$^+$ concentration in the above-graft union was lower (7.3 mg/L) than that in the below-graft union (9.5 mg/L, Fig. 4.17). There was a significant interaction of genotype and position on sap Na$^+$ concentrations (Table 4.5).

The segment position had a significant effect on Na$^+$ concentrations in ‘LA1630’ and ‘AR’ (Table 4.6). Grafted and non-grafted ‘LA1630’ had much higher sap Na$^+$ concentration than other rootstocks (Fig. 4.17). M/LA1630 and M/AR had higher sap Na$^+$ concentration in the below-graft segments (36% and 33% higher Na$^+$, respectively) than at the above-graft segments. In M/M, sap Na$^+$ concentrations were quite similar above and below the graft union (Table 4.6).

Although not significant, non-grafted plants had higher sap Na$^+$ concentrations than grafted plants. There was no significant sap position effect on Na$^+$ concentration in M/RS or M/LA3120 (Table 4.6, Fig. 4.17).

Genotypes varied significantly in their sap K$^+$ concentrations (P<0.001, Table 4.5). There was a significant interaction of genotype and position on sap K$^+$ concentrations (Table 4.5). Sap K$^+$: Na$^+$ ratio was significantly different among genotypes (P<0.001, Table 4.5). The ratio was higher in the above-graft segments than in the below-graft segments, and also higher in grafted than non-grafted plants (Fig. 4.19). The wild genotype ‘LA1630’ had lower sap K$^+$ concentrations than other rootstocks and the lowest sap K$^+$: Na$^+$ ratios under 75 mM NaCl, whereas ‘LA3120’ above-graft segments had the highest sap K$^+$: Na$^+$ ratios in the experiment (Fig. 4.19).

Shoot DW was measured after 10 days of NaCl treatment to examine the differences in the size of the grafted and non-grafted plants (Fig. 4.20). Our results showed that, except for ‘AR’ and ‘RS’, shoot DW was quite similar in grafted and non-grafted genotypes. Non-grafted ‘AR’ had significantly higher shoot DW than grafted ‘AR’ under 0 and 75 mM NaCl treatment (Fig. 4.20 and 4.21). However, non-grafted ‘AR’ and ‘RS’ grew bigger than the 7-day older grafted M/AR.
and M/RS plants (Fig. 4.20 and 4.21). Plants grafted onto ‘LA1630’ had the lowest salt-induced reduction in shoot DW (12.3%). Percent reduction in shoot DW of M/LA3120 was also lower than M/M (Fig. 4.16). Percent reduction of M/AR was slightly higher than M/M. However, M/RS was more reduced in shoot weight (26.5%) than M/M (Fig. 4.16).

Discussion

Grafting has made essential contributions to agriculture for asexual propagation (Kumari et al. 2015), and in vigor control and the amelioration of root diseases in fruit trees. Grafting has also improved disease resistance, growth, and productivity in vegetable crops (Davis et al. 2008; Gioia et al. 2013). Scientists believe that grafting can be beneficial for improving salinity tolerance. Unlike traditional breeding, grafting can combine salt-susceptible scions that have desired yield and fruit quality characteristics with vigorous, salt-tolerant rootstocks that are capable of enhancing salinity tolerance of the scions (Gioia et al. 2013). Therefore, we are examining whether salt tolerance mechanism and vigor of the rootstock could affect the scion's growth performance. Thus, ‘Moneymaker’ with its excluder character (Cuartero et al. 1992; Martinez-Rodriguez et al. 2008) was grafted onto 7 different rootstocks. Self-grafted ‘Moneymaker’ plants (M/M) were used as controls.

The vigor of the rootstock affects the overall plant growth

All rootstock genotypes used in this experiment were chosen from the previous screening experiment for having small reductions in shoot DW and/or high vigor under NaCl treatments. Rootstock clearly had an effect on the growth and vigor of the scion in the absence of salt. In our screening experiment (Chapter 3), ‘Im’ was the most vigorous genotype under control and NaCl treatments, and that was the reason for its choice as a potential rootstock. In this experiment, plants grafted onto ‘Im’ had the highest shoot DW under control conditions. On the other hand, M/LA1630 plants had the lowest shoot weight under control conditions (Fig. 4.2). The less vigorous ‘LA1630’ rootstock did not seem to improve the absolute shoot growth of the scion as compared to M/M. Vigor of the rootstock seems to affect overall plant growth under control conditions.
Our results demonstrated that salt tolerance was improved by grafting onto salt-tolerant rootstocks as measured by shoot DW reduction. In the present experiment, plants of M/RS, M/AR, and M/LA1630 were the combinations that showed less shoot DW reduction than M/M plants, whereas M/LA3120, M/LA3465, M/LA722, and M/Im had greater growth reductions than M/M under 75 mM NaCl treatment (Fig. 4.3). M/AR had the highest shoot DW, partly because ‘AR’ is a vigorous rootstock that was developed by seed companies for the purpose of grafting for improving crop productivity. In addition, M/AR had less percent reduction in shoot DW (17%) than most other genotypes under salinity stress (Fig. 4.3).

In the screening experiment, ‘Im’ was the most vigorous genotype under all salinity treatments. In the grafting experiment, shoot DW of M/Im was reduced by ~40% but despite that, ‘Im’ was the second most vigorous combination after M/AR in the presence of 75 mM NaCl. Both M/AR and M/Im were more vigorous than M/M (Fig. 4.2). On the other hand, M/RS showed no major reduction in shoot DW as compared to M/M under NaCl treatment, but they showed comparable vigor to M/M (Fig. 4.2 and 4.3).

For plants grown under salt stress conditions, the ultimate goal is to maintain plant growth or minimize the growth reduction of the elite genotypes. Even though they suffer higher degree of stress-induced growth reduction, vigorous genotypes may still result in a good shoot DW compared to the less vigorous plants with lower stress-induced growth reduction. For this reason, the former may be preferred over the less vigorous genotypes that have smaller reductions in growth with salinity treatment. In our experiment, the positive effect of using vigorous rootstocks on overall plant growth was observed when ‘AR’ and ‘Im’ were used as rootstocks.

**Reduction in shoot DW under salinity stress is not caused by the osmotic effect of salinity**

To determine how salinity stress affects the water content of grafted tomatoes, RWC was measured in all grafting combinations. All combinations had comparable RWC (Fig. 4.14), which means that plants were able to recover their water uptake under salinity stress, and that the reductions in shoot DW under salt stress were not results of the osmotic effects of salinity.
Similar results were also found in other studies where rootstock did not have a significant effect on RWC (Estañ et al. 2005; Huang et al. 2009).

The first phase of growth reduction in plants grown under saline conditions is due to the osmotic effect on water uptake caused by NaCl in the growing medium. Leaf cells lose their turgor when there is a sudden increase in soil salinity; however, cells regain their turgor within hours due to the leaf osmotic adjustment. Over the next few days, leaf appearance and elongation rates are slow. Consequently the size of the leaves become smaller because of the reduction in cell division and elongation (Munns and Tester 2008). This phase is usually rapid and happens early after the transient change in salinity levels in the growing medium. In this phase, growth reduction is probably regulated by hormonal signals from the roots.

The second ionic phase occurs later when excessive amounts of salt ions enter the plants (Munns 2002). In our experiment, plants appeared to maintain their osmotic adjustment and the water uptake in this ionic phase as Na\(^+\) in the leaves did not affect leaf RWC after 25 days of 75 mM NaCl treatment.

Measurement of daily stomatal conductance is a good assessment of plant growth rate (Munns and Tester 2008). Reduction in stomatal conductance is a mechanism to reduce water loss through the stomata. In our experiment, M/LA3120 had a consistently low percent reduction in stomatal conductance compared to the other combinations except for the first and last weeks. ‘LA3120’ seemed capable of maintaining its water status while excluding most of Na\(^+\) from the shoot.

The percent reduction in stomatal conductance in M/LA1630 was lower than the average of all genotypes (except for day 2). The peak level of percent reduction in stomatal conductance was reached in week 1 then started to decrease to reach zero level at harvest. This suggests that although M/LA1630 had high Na\(^+\) concentrations in the leaf, it was able to maintain its water status and gas exchange activities. M/Im had a similar reduction in stomatal conductance to the average reduction of all genotypes except for day 1 (9% vs. 4 %) and day 3 (32% vs. 23%). M/M plants had higher percent reduction in stomatal conductance than most of the combinations at
most of the time points. These results suggest that grafting onto different rootstocks resulted in better stomatal conductance in hetero-grafts than in self-grafts.

**Cultivated genotypes as rootstocks are better at maintaining higher selectivity for K\textsuperscript{+} and Ca\textsuperscript{+2} over Na\textsuperscript{+} than M/M**

In the leaves, K\textsuperscript{+} : Na\textsuperscript{+} ratios were higher in ‘Moneymaker’ grafted onto cultivated rootstocks (RS, AR, LA3120, and LA3465) than in M/M plants. In the roots, M/M also had the lowest root K\textsuperscript{+} : Na\textsuperscript{+} ratios among all studied rootstocks (0.98 of M/M vs. 1.30 of the average of all genotypes). Similarly, sap K\textsuperscript{+} : Na\textsuperscript{+} ratio (whether above or below-graft union) was higher in ‘Moneymaker’ grafted onto cultivated rootstocks than in M/M. This suggests that grafted plants were better than self-grafts at maintaining higher selectivity for K\textsuperscript{+} over Na\textsuperscript{+} at the roots.

An inverse relationship was found between leaf K\textsuperscript{+} and Na\textsuperscript{+} in plants treated with 75 mM NaCl ($P<0.01$, Fig. 4.8). Such relationship between Na\textsuperscript{+} and K\textsuperscript{+} concentrations is a common response to the effect of increasing Na\textsuperscript{+} in the growing medium (Maggio, Raimondi, et al. 2007; Zhu et al. 2008). One of the parameters of tolerance to salt is related to the regulation of ion homeostasis and the differential ion selectivity that results in less Na\textsuperscript{+} accumulations (Penella et al. 2015; Shabala and Cuin 2008). Because of the similarity in the physicochemical properties of K\textsuperscript{+} and Na\textsuperscript{+} ions, the latter usually competes with K\textsuperscript{+} at the binding sites at the plasma membrane for important metabolic reactions in the cytoplasm such as regulating enzyme activities and protein synthesis. The concentration of K\textsuperscript{+} relative to Na\textsuperscript{+} in the cytosol is thought to contribute to salinity tolerance of the plants grown under NaCl stress conditions (Munns and Tester 2008; Santa-Cruz et al. 2002; Saranga et al. 1993; Shabala and Cuin 2008). Lower leaf Na\textsuperscript{+}: K\textsuperscript{+} ratio was associated with less salinity effects on plants grafted onto roots other than theirs, whereas self-grafted plants had three fold increase of leaf Na\textsuperscript{+}:K\textsuperscript{+} ratio (Santa-Cruz et al. 2002).

**Rootstock plays a dominant role in controlling salt concentrations in the scion**

The different scion/rootstock combinations differed in their ion regulation mechanisms which resulted in different growth performances among combinations. ‘Moneymaker’ is known to have a salt-excluder character (Cuartero et al. 1992; Martinez-Rodriguez et al. 2008). Cuartero et al., 1992, found that leaf Na\textsuperscript{+} concentrations in ‘Moneymaker’ were the lowest among the
other cultivated and wild genotypes under 40% artificial seawater salinity treatment (Cuartero et al. 1992). The authors, however, did not measure root Na⁺ concentration in their experiment.

In our experiment, M/M plants had the highest concentration of Na⁺ in the roots among the studied combinations and less Na⁺ was accumulated in the leaf. This confirms that M/M is excluding Na⁺ from the shoot as a mechanism of tolerance (regulating Na⁺ transport to the shoot), while retaining it in the root. Leaf Na⁺ concentrations in the other graft combinations were not significantly different from each other (Fig. 4.5).

Plants grafted onto ‘LA3120’ had the lowest leaf Na⁺ concentration among the studied genotypes, and showed much higher Na⁺ concentrations in the root than in the leaf. The low concentration of Na⁺ in the leaf in M/LA3120 can be explained as retention of Na⁺ in the ‘LA3120’ rootstock and/or exclusion of Na⁺ and limitation of Na⁺ transport to the shoot by the ‘LA3120’ rootstock. In addition, M/LA3120 had the highest leaf K⁺, Ca²⁺, and Mg²⁺ ratios with Na⁺ of all genotypes indicating that ‘LA3120’ has a higher selectivity for K⁺, Ca²⁺, and Mg²⁺ over Na⁺. This may have contributed to the very low Na⁺ concentrations in the leaf (Fig. 4.5). However, root K⁺: Na⁺ ratio of M/LA3120 was comparable to most other genotypes. In accordance with these findings, the results of our sap experiment showed that ‘LA3120’ had the lowest sap Na⁺ concentration in the above graft segment, which was also lower than Na⁺ concentration in its below graft segment. This suggests that ‘LA3120’ rootstock is taking up Na⁺ into the root system but loading less Na⁺ into the xylem to the shoot or taking it back out before it gets to the leaves.

Sap K⁺: Na⁺ ratio was the highest in the above segment in all studied grafted and non-grafted plants but low in the below graft segment. These results may indicate that ‘LA3120’ is more selective to K⁺ at the transport system through xylem.

‘AR’ rootstock improved the growth of M/AR compared to the self-grafted plants under NaCl treatment. M/AR was the most vigorous combination in this experiment as it had the highest shoot DW under NaCl treatment. M/AR also showed slightly less percent reduction in shoot DW than M/M plants (~17% vs. 23%). Leaf Na⁺ concentrations, however, were comparable in M/AR and M/M (~25.5 mg/g DW) while root Na concentration in M/AR was only slightly less than that in M/M (Fig. 4.5). Leaf Ca²⁺ in M/AR was higher than the average of all genotypes, whereas leaf
Mg\(^{2+}\) concentration was also similar to the average. These results may suggest that the general vigor of ‘AR’ seems to be the main reason for its performance under NaCl treatment. However, high root to shoot ratio and the high Ca\(^{2+}\) in the leaf may contribute to the salinity tolerance of this combination. Results of the sap experiment have shown that sap collected from different stem segment positions had a significant effect on Na\(^+\) concentration (Table 4.5). M/AR had lower sap Na\(^+\) the above the graft segment than below it, but comparable to its concentration in the above graft segments in other cultivated rootstocks. Also, M/AR had higher sap K\(^+\): Na\(^+\) ratio in the above graft segment than in the below graft segment. This again suggests that M/AR had a higher selectivity for K\(^+\) over Na\(^+\) compared to most other graft combinations.

In our grafting experiment, although M/Im had the highest reduction in shoot DW among all salinized graft combinations (Fig. 4.3), it was the second most vigorous combination after M/AR. Leaf and root Na\(^+\), K\(^+\): Na\(^+\) ratio, and Ca\(^+\): Na\(^+\) ratio were quite similar in M/Im and in M/M. So, vigor of ‘Im’ also seems to be the main factor affecting the differences in the growth between M/M and M/Im.

In a study by Martinez-Rodriguez et al., 2008, ‘Moneymaker’ was grafted onto an excluder cultivar (cv. Radja) or onto an includer rootstock (cv. Pera). Under 50 mM NaCl for 35 days, ‘Moneymaker’ grafted onto ‘Radja’ had much lower leaf Na\(^+\) concentration than self-grafted plants, whereas ‘Moneymaker’ plants grafted onto ‘Pera’ had more leaf Na\(^+\) concentration than self-grafted plants (Martinez-Rodriguez et al. 2008). In another study, the authors used two S. lycopersicon scions ‘Moneymaker’ and ‘UC-82B’ (which has an includer strategy) and one commercial hybrid tomato used as a rootstock (cv. Kyndia). ‘Moneymaker’ scions grafted onto ‘Kyndia’ had slightly lower leaf Na\(^+\) under 50 and 100 mM NaCl than self-grafted plants (Santa-Cruz et al. 2002). Our results showed that grafting ‘Moneymaker’ onto different rootstocks resulted in different leaf and root Na\(^+\) concentrations. Grafting ‘Moneymaker’ (an excluder) onto the excluder rootstock ‘LA3120’ resulted in the lowest Na\(^+\) concentrations in the leaf of all combinations. Also, grafting onto ‘RS’ and ‘LA3465’ resulted in lower Na\(^+\) concentrations in the leaf than M/M. However, M/AR and M/Im had comparable leaf Na\(^+\) concentrations as in M/M.
Plants grafted onto wild rootstocks accumulated higher rates of Na\(^+\) in the leaf

In the screening experiment, leaves of wild genotypes ‘LA1630’ and ‘LA722’ had the highest Na\(^+\) concentrations in all NaCl treatments. Grafting ‘Moneymaker’ on these wild rootstocks in this experiment also resulted in the highest leaf Na\(^+\) concentrations, with ‘LA1630’ producing the highest leaf Na\(^+\) concentrations among all genotypes. The inclusion tolerance mechanism of the wild rootstock ‘LA1630’ was maintained, even when an excluder scion was grafted onto it, since leaf Na\(^+\) concentrations continued to be higher in M/LA1630 than in M/M or in any other graft combination.

In our experiment, the high Na\(^+\) concentrations in the leaves of M/LA1630 combined with the small reduction in shoot DW (Fig. 4.3 and 4.5) indicated a high tissue tolerance to NaCl in this combination. Adjustment of the cell osmotic potential is one of the essential factors affecting plant tolerance to salinity (Shabala and Cuin 2008). The increased vacuolar Na\(^+\) concentrations in the leaves require an increase in the osmotic pressure of the cytosol to maintain cell turgor and water uptake (Munns and Tester 2008; Tester and Davenport 2003). This could be achieved by the increase in osmotica in the cell, either by the use of the inorganic solutes (cheaper) or the synthesis of compatible solutes, which are more expensive from an energetic viewpoint (Munns and Tester 2008; Shabala and Cuin 2008; Shabala et al. 2010; Tester and Davenport 2003). In our experiment, K\(^+\) concentration was very low in the leaves of M/LA1630, while its leaf Na\(^+\) concentration was quite high. It is likely that increased leaf Na\(^+\) concentration in the grafted plants was associated with synthesis of compatible solutes for the osmotic adjustment as well as to organize biochemical processes in the cell (Estañ et al. 2005; Munns and Tester 2008; Roy et al. 2014; Tester and Davenport 2003). M/LA1630 was one of the combinations that showed low reduction in stomatal conductance under salinity conditions. After the first week of salt treatment, percent reduction in stomatal conductance started to decrease, reaching no reduction by harvest week. These results suggest that plants were able to recover from NaCl stress after the first week of salt treatment. This also suggests that plants were able to maintain their water status even though M/LA1630 had the highest leaf Na\(^+\) concentration.
In agreement with the results of the grafting experiment, ‘LA1630’ had the highest concentrations of sap Na⁺ in the below graft and above graft segments and in the non-grafted plants (Fig. 4.17). Na⁺ concentration in its above graft segment of ‘LA1630’ was lower than that in the below graft segment (~13 vs. 20 mg/L, respectively). These concentrations were higher than the average Na⁺ concentrations in the above and the below graft segments of all genotypes (7.3 and 10.6 mg/L, respectively). These findings suggest that M/LA1630 retains high Na⁺ in the below graft sap segment. However, it showed higher K⁺: Na⁺ ratio in the above graft segment, but the ratio was low compared to that in all the other combinations. This can point to the improved plant selectivity to K⁺ over Na⁺ in the above- than the below-graft union. In the previous grafting experiment, salinized M/LA1630 plants (75 mM NaCl for 25 days) had lower leaf Na⁺ concentrations than those found in the non-grafted ‘LA1630’ plants in the screening experiment (50 mM NaCl for 16 days). These findings support the role of grafting in reducing Na⁺ transport to the shoot, even with the salt includer ‘LA1630’. In addition, the lower K⁺: Na⁺ ratio in the above graft union segment than all the other graft combinations supports the tissue tolerance mechanism of this rootstock.

**An inverse relationship found between the percent reduction in shoot DW and root to shoot ratio**

Root to shoot ratio was not different among the grafting combinations in the control treatment. In the salinized plants, M/RS had the highest root to shoot ratio, followed by M/LA1630, and M/AR. Interestingly, percent reduction in shoot DW was also noted in the same order as for the root to shoot ratio, with M/RS having the lowest reduction, followed by M/LA1630, then M/AR. The correlation matrix showed a significant inverse relationship between the percent reduction in shoot DW and the root to shoot ratio ($r^2=-0.48$). These three combinations had less reduction in shoot DW than other combinations, so despite the high root to shoot ratio, they maintain shoot growth as well. This suggests that they are not curtailing shoot growth to balance source and sink. Interestingly, M/AR is more vigorous than M/LA1630 and M/RS, but still maintained a high root to shoot ratio. Perhaps the dilution of Na⁺ in the vigorous M/AR could have helped in minimizing transport of Na⁺ to the shoot or postponing the toxic effects of Na⁺. These genotypes might also have better ability to develop new roots that
extend and elongate for better water and nutrients acquisition than the other genotypes under salinity.

**Most graft combinations had lower sap Na\(^+\) in the above-graft than in the below-graft segment.**

Our results suggest that grafting onto salt-tolerant rootstocks was beneficial in decreasing Na\(^+\) transport in the sap compared to the non-grafted plants. Our results are in agreement with those reported in a study by Fernández-García et al., 2002, where the non-grafted tomato plants also had higher Na\(^+\) concentrations in the xylem sap than grafted plants (Fernández-García et al. 2002). The authors did not measure ion concentrations above or below the graft union segments but suggested that graft union might be limiting Na\(^+\) and Cl\(^-\) transport.

To our knowledge, no earlier research was designed to examine whether Na\(^+\) and K\(^+\) concentrations in the sap would change as the sap moves through the graft union under NaCl treatments. Our sap experiment has shown that plants of M/M had similar sap Na\(^+\) concentrations above and below the graft union, and those concentrations were not different than those in the non-grafted plants (Table 4.6). This indicates that the graft union itself did not act as a barrier to Na\(^+\) transport in the xylem.

Interestingly, stem position significantly affected Na\(^+\) concentrations in sap of ‘LA1630’ and ‘AR’ (Table 4.6). M/LA1630 and M/AR showed higher sap Na\(^+\) concentrations in the below-graft segment compared to the above-graft segment. However, there was no significant sample position effect on M/RS and M/LA3120 (Table 4.6). Hetero-grafts might be retaining Na\(^+\) in the below-graft segment while limiting its transport to the shoot, resulting in the higher concentration of Na\(^+\) ions below the graft union as compared to its concentration above it. However, self-grafts did not seem to perform the same way in that matter.

Significant position effects were also found for K\(^+\): Na\(^+\) ratio in all the genotypes, and only at \(P=0.06\) for ‘LA3120’ (Table 4.6). Sap of the above segments of all graft combinations had higher K\(^+\): Na\(^+\) ratio than those in the below segment. The change in ratio in the two positions could be because Na\(^+\) is selectively removed from the xylem in the stem, and to a greater extent in hetero-grafts.
Our results suggest that hetero-grafts and non-grafts seem to affect Na$^+$ loading and unloading from the xylem in a different way than that of the self-grafts. Since self-grafted plants had similar Na$^+$ concentrations in the above and below graft segments, it is unlikely that the graft union per se would form a barrier to ion transport. However, due to the different K$^+$: Na$^+$ ratios above and below the graft union, K$^+$ might exchange with Na$^+$ along the xylem stream. Na$^+$ could be selectively removed from the xylem stream, whereas K$^+$ might be loaded into the above-graft xylem stream. These results suggest that the choice of the scion/rootstocks and their interactions are involved in the regulation of ion transport from the rootstock to the scion and vice versa. The genetic interaction of the different genotypes of the rootstock and scion may affect ion transport through the xylem and cycling of ions in and out of the xylem. Ion selectivity can also change depending on the rootstock used, and consequently some ions will be facilitated while others might be limited or retained in the below-graft union. This could be achieved through regulation of certain ion transport proteins at the stem portion of the rootstock that can limit Na$^+$ transport to the above graft segment under salt stress conditions. The HKT and SOS families are involved in Na$^+$ and K$^+$ hemostasis in the xylem sap. HKT proteins are able to retrieve Na$^+$ from the xylem to avoid Na$^+$ accumulation in the shoot (Almeida et al. 2013; Munns and Tester 2008). Hormonal factors could also be involved in the regulation of ion concentrations in the above and below graft segments. In a study by Albacete et al., 2009, K$^+$ concentration in the leaf xylem sap was found to strongly correlate with xylem zeatin concentration of salinized grafted tomato plants (Albacete et al. 2009). The hormonal regulation can change depending on the rootstock used.

Recent studies have investigated the role of the graft union in response to stress conditions including changes in ion binding and ion transport proteins (Muneer et al. 2015, 2016). In tomato plants grown for two weeks after grafting under temperature stress (high/low, day/night), Muneer et al., 2016, found an increased number of proteins related to the transport/binding of ions in the vascular connections of the graft union (Muneer et al. 2016). The authors also found stress/defense responsive proteins in the graft union that resulted in activation of the antioxidant mechanisms in the stressed plants. Similar results were found in another study on watermelon grafted onto bottle-gourd rootstocks under different light
intensities (Muneer et al. 2015). Although those studies were conducted on two-week-old plants after grafting, the results suggest that ion transport/binding proteins may have a role in regulating the transport of Na\textsuperscript{+} and K\textsuperscript{+} ions through the graft union under salt stress conditions, which can contribute to limiting Na\textsuperscript{+} transport to the sap above the graft union while maintaining a higher K\textsuperscript{+} selectivity to the above segment. It would be valuable to know if the response of the ion transport binding proteins at the graft union will continue to have a role after the two-week period of graft union healing.

The rootstock/scion relationship is complex and not fully understood. Research should be conducted to study ion transport proteins in the xylem and at the graft union for further clarification of the mechanisms associated with salt tolerance in the grafted plants. More studies are needed to examine how the genetic interaction in a heterograft, affects Na\textsuperscript{+} loading and unloading from the xylem in comparison with self-grafts.

This work has demonstrated the useful benefits of grafting for improving salinity tolerance in plants. Rootstock clearly played a key role in controlling the mechanism of tolerance in the grafted plants. Cultivated ‘LA3120’ acted as an excluder and limited Na\textsuperscript{+} transport to the shoot, whereas wild ‘LA1630’ resulted in more Na\textsuperscript{+} accumulation in the leaves of ‘Moneymaker’. Sap Na\textsuperscript{+} concentration was lower in the above graft segment than in the below graft segment in some scion/rootstock combinations. This finding also support that grafting onto tolerant rootstock is helpful in limiting Na\textsuperscript{+} transport to the shoot. Vigorous rootstocks were useful in maintaining the growth of the scion under salinity stress conditions.
References


http://www.fao.org/ag/agl/agll/spush


### Tables

Table 4. 2: One-way Analysis of Variance (ANOVA) for the effect of genotype on ion concentrations and ratios, growth variables, and relative water content (RWC).

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<thead>
<tr>
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<th>K⁺ in Leaf (mg/g DW)</th>
<th>Ca²⁺ in Leaf (mg/g DW)</th>
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<th>Ca²⁺ in Root (mg/g DW)</th>
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Table 4.3: Correlation matrix for leaf ion concentration, growth and physiological traits of 8 genotypes grown under salts stress (75 mM NaCl).

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<th>SC</th>
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<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>Na⁺</th>
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<td>-0.34</td>
<td>0.19</td>
<td>0.24</td>
<td>0.37</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Na⁺</td>
<td>0.33</td>
<td>0.1</td>
<td>0.1</td>
<td>0.12</td>
<td>-0.05</td>
<td>-0.75***</td>
<td>0.08</td>
<td>-0.39</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K⁺:Na⁺</td>
<td>-0.35</td>
<td>-0.32</td>
<td>-0.01</td>
<td>-0.09</td>
<td>-0.11</td>
<td>0.93***</td>
<td>-0.31</td>
<td>0.24</td>
<td>-0.82***</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ca²⁺:Na⁺</td>
<td>-0.22</td>
<td>-0.06</td>
<td>-0.13</td>
<td>-0.11</td>
<td>0.08</td>
<td>0.71***</td>
<td>0.13</td>
<td>0.48*</td>
<td>-0.91***</td>
<td>0.83***</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Mg²⁺:Na⁺</td>
<td>-0.26</td>
<td>-0.05</td>
<td>-0.31</td>
<td>-0.27</td>
<td>0.09</td>
<td>0.67***</td>
<td>-0.01</td>
<td>0.72***</td>
<td>0.85***</td>
<td>0.79***</td>
<td>0.91***</td>
<td>1</td>
</tr>
</tbody>
</table>

Significant differences (Pearson correlation, 1-tailed) are indicated: *, P <0.05; **, P <0.01; ***, <0.001 levels. SDW, shoot DW; SC, Stomatal Conductance after 25 days of NaCl treatment; Root:shoot; Root to shoot DW Ratio, % SDW Red, Percent reduction in SDW
Table 4.4: Correlation matrix for root ion concentration, growth and physiological traits of 8 genotypes grown under salt stress (75 mM NaCl).

<table>
<thead>
<tr>
<th>SDW</th>
<th>K⁺</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>Na⁺</th>
<th>K⁺:Na⁺</th>
<th>Ca²⁺:Na⁺</th>
<th>Mg²⁺:Na⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDW</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K⁺</td>
<td>-0.49*</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>-0.4</td>
<td>0.03</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>-0.44*</td>
<td>0.05</td>
<td>0.89***</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Na⁺</td>
<td>0.33</td>
<td>-0.06</td>
<td>-0.2</td>
<td>-0.04</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K⁺:Na⁺</td>
<td>-0.59**</td>
<td>0.87***</td>
<td>0.14</td>
<td>0.07</td>
<td>-0.53**</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Ca²⁺:Na⁺</td>
<td>-0.47*</td>
<td>0.03</td>
<td>0.85***</td>
<td>0.68***</td>
<td>-0.67***</td>
<td>0.37</td>
<td>1</td>
</tr>
<tr>
<td>Mg²⁺:Na⁺</td>
<td>-0.54**</td>
<td>0.05</td>
<td>0.84***</td>
<td>0.83***</td>
<td>-0.58**</td>
<td>0.34</td>
<td>0.94***</td>
</tr>
</tbody>
</table>

Significant differences (Pearson correlation, 1-tailed) are indicated: *, P < 0.05; **, P < 0.01; ***, < 0.001 levels. SDW, shoot DW.

Table 4.5: Two-way Analysis of Variance (ANOVA), for the effect of genotype (grafted or non-grafted) and position under (0 and 75 mM NaCl) on sap ion concentrations. The three positions were above-graft segments, below-graft segments, and non-grafted stem segments.

<table>
<thead>
<tr>
<th></th>
<th>Sap Na⁺ concentration (NaCl treatment)</th>
<th>Sap K⁺ concentration (control treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Df</td>
<td>F</td>
</tr>
<tr>
<td>Genotype</td>
<td>4</td>
<td>63.90</td>
</tr>
<tr>
<td>Position</td>
<td>2</td>
<td>18.20</td>
</tr>
<tr>
<td>Rep</td>
<td>3</td>
<td>2.23</td>
</tr>
<tr>
<td>Genotype*position</td>
<td>8</td>
<td>2.51</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Sap K⁺:Na⁺ ratio</th>
<th>Sap K⁺ concentration (control treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
</tr>
<tr>
<td>Genotype</td>
<td>4</td>
<td>29.71</td>
</tr>
<tr>
<td>Position</td>
<td>2</td>
<td>29.43</td>
</tr>
<tr>
<td>Rep</td>
<td>3</td>
<td>1.54</td>
</tr>
<tr>
<td>Genotype*position</td>
<td>8</td>
<td>0.72</td>
</tr>
</tbody>
</table>
Table 4.6: One-way Analysis of Variance (ANOVA), for the effect on sap ion concentrations on the sap position in genotypes grown under 75 mM NaCl.

<table>
<thead>
<tr>
<th>Genotype and factor</th>
<th>Sap Na⁺</th>
<th>Sap K⁺</th>
<th>Sap K⁺:Na⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Position</strong></td>
<td>df</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>LA1630</td>
<td>2</td>
<td>16.34</td>
<td>0.024</td>
</tr>
<tr>
<td>Rep</td>
<td>3</td>
<td>2.27</td>
<td>0.260</td>
</tr>
<tr>
<td><strong>AR</strong></td>
<td>df</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Position</td>
<td>2</td>
<td>62.56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rep</td>
<td>3</td>
<td>1.42</td>
<td>0.288</td>
</tr>
<tr>
<td><strong>LA3120</strong></td>
<td>df</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Position</td>
<td>2</td>
<td>4.03</td>
<td>0.09</td>
</tr>
<tr>
<td>Rep</td>
<td>3</td>
<td>2.72</td>
<td>0.154</td>
</tr>
<tr>
<td><strong>MM</strong></td>
<td>df</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Position</td>
<td>2</td>
<td>2.54</td>
<td>0.194</td>
</tr>
<tr>
<td>Rep</td>
<td>3</td>
<td>1.79</td>
<td>0.288</td>
</tr>
<tr>
<td><strong>RS</strong></td>
<td>df</td>
<td>F</td>
<td>P</td>
</tr>
<tr>
<td>Position</td>
<td>2</td>
<td>2.41</td>
<td>0.185</td>
</tr>
<tr>
<td>Rep</td>
<td>3</td>
<td>9.65</td>
<td>0.27</td>
</tr>
</tbody>
</table>
Fig. 4.1: Shoot dry weight of Moneymaker (M) grafted onto different rootstocks in 75 mM NaCl-treated versus control plants grown for 25 days on half-strength Hoagland’s solution. Data are means of nine plants ±SE. Different upper case letters indicate significant genotypic differences under control conditions. Different lower case letters indicate significant genotypic differences under salinity conditions. The blue dashed line represents the overall average for genotypes grown under 75 mM NaCl.
Fig. 4. 2: Scatterplot of the shoot dry weight (g) plotted for control plants vs salt-treated plants (75 mM NaCl). Genotypes on blue line had no change in shoot DW with NaCl treatment.

Fig. 4. 3: Percent reduction of shoot dry weight in Moneymaker (M) scions grafted onto different rootstocks in 75 mM NaCl-treated relative to control tomato plants grown for 25 days on half-strength Hoagland’s solution. Data are means of nine plants.
Fig. 4.4: Root to shoot ratio of Moneymaker (M) scions grafted onto different rootstocks in 75 mM NaCl-treated versus control plants grown for 25 days on half-strength Hoagland’s solution. Data are means of nine plants ±SE. Different upper case letters indicate significant genotypic differences under control conditions. Different lower case letters indicate significant genotypic differences under salinity conditions.

Fig. 4.5: Na⁺ concentration (mg/g DW) in the 5th leaf and roots of the different graft combinations in 75 mM NaCl -treated plants grown for 25 days on half-strength Hoagland’s solution. Data are means of nine plants ±SE. Different upper case letters indicate significant genotypic differences under control conditions. Different lower case letters indicate significant genotypic differences under salinity conditions.
Fig. 4.6: $K^+$ concentration (mg/g DW) in the 5th leaf and roots of the different graft combinations in the presence of 75 mM NaCl. Data are means of nine plants ±SE. Different upper case letters indicate significant genotypic differences under control conditions. Different lower case letters indicate significant genotypic differences under salinity conditions.

Fig. 4.7: $K^+$/Na$^+$ ratio in the 5th leaf and roots of the different graft combinations in the presence of 75 mM NaCl. Data are means of nine plants ±SE. Different upper case letters indicate significant genotypic differences under control conditions. Different lower case letters indicate significant genotypic differences under salinity conditions.
Fig. 4.8: Relationship between leaf Na\(^+\) and leaf K\(^+\) concentrations (mg/g DW) in all graft combinations after 25 days of growth in 75 mM NaCl.

Fig. 4.9: Ca\(^{2+}\) concentration (mg/g DW) in the 5th leaf and roots of the different graft combinations in the presence of 75 mM NaCl. Data are means of nine plants ±SE. Different upper case letters indicate significant genotypic differences under control conditions. Different lower case letters indicate significant genotypic differences under salinity conditions.
Fig. 4. 10: Ca\(^{2+}\): Na\(^+\) ratio in the 5th leaf and roots of the different graft combinations in the presence of 75mM NaCl. Data are means of nine plants ±SE. Different upper case letters indicate significant genotypic effect under control conditions. Different lower case letters indicate significant genotypic effect under salinity conditions.

Fig. 4. 11: Relationship between root Ca\(^{2+}\) and percent reduction in shoot dry weight in all graft combinations after 25 days of growth in 75 mM NaCl.
Fig. 4.12: Mg$^{2+}$ concentration (mg/g DW) in the leaf and roots of the different graft combinations in the presence of 75 mM NaCl. Data are means of nine plants ±SE. Different upper case letters indicate significant genotypic effect under control conditions. Different lower case letters indicate significant genotypic effect under salinity conditions.

Fig. 4.13: Mg$^{2+}$/Na$^{+}$ ratio in the leaf and roots of the different graft combinations in the presence of 75 mM NaCl. Data are means of nine plants ±SE. Different upper case letters indicate significant genotypic effect under control conditions. Different lower case letters indicate significant genotypic effect under salinity conditions.
Fig. 4.14: Leaf relative water content (%) of Moneymaker (M) scions grafted onto different rootstocks in 75 mM NaCl-treated versus control plants grown for 25 days on half-strength Hoagland’s solution. Data are means of nine plants ±SE.

Fig 4.15: Percent reduction in stomatal conductance over time in the different graft combinations in 75 mM NaCl-treated plants relative to control during three weeks of 75 mM NaCl treatment. Data are means of nine plants ±SE.
Fig 4. 16: Percent reduction of shoot dry weight in Moneymaker (M) scions grafted onto different rootstocks in 75 mM NaCl-treated relative to control tomato plants grown for 10 days on half-strength Hoagland’s nutrient solution. Data are means of nine plants.

Fig 4. 17: Stem xylem sap Na⁺ concentrations (mg/L) above the graft union (A), below the graft union (B), and in stem segments of non-grafted (NG) plants. ‘MM’ was grafted onto ‘AR’, ‘LA1630’, ‘LA3120’, ‘MM’ (self-grafts), or RS in plants grown for 10 days in the presence of 75 mM NaCl. The blue dashed line represents the overall average for genotypes grown under 75 mM NaCl.
Fig 4. 18: Stem xylem sap K⁺ concentrations (mg/L) above the graft union (A), below the graft union (B), and in the stem segments of non-grafted (NG) plants. ‘MM’ was grafted onto ‘AR’, ‘LA1630’, ‘LA3120’, ‘MM’ (self-grafts), or ‘RS’ in plants grown for 10 days in the 75 mM NaCl and control treatment.
Fig 4. 19: Stem xylem sap K⁺:Na⁺ ratio above the graft-union (A), below the graft union (B), and in the stems of non-grafted (NG) plants of ‘MM’ grafted onto ‘AR’, ‘LA1630’, ‘LA3120’, ‘MM’ (self-grafts), or ‘RS’ in plants grown for 10 days in the presence of 75 mM NaCl.

Fig 4. 20: Shoot dry weight in grafted (G) and non-grafted (NG) plants grown for 10 days in 75 mM NaCl treatment. Data are means of nine plants ±SE.
Fig 4. 21: Shoot dry weight in grafted (G) and non-grafted (NG) plants grown for 10 days in the control treatment. Data are means of nine plants ±SE.
CHAPTER 5
OVERALL CONCLUSIONS/SUMMARY

Plant grafting could be a tool to immediately improve tomato tolerance to salinity. This is best achieved by using salt-tolerant rootstocks, and scions with the desired fruit yield and quality characteristics that are compatible with the rootstock. In this research project, we were interested in studying the effects of using salt-tolerant and vigorous rootstocks on the growth and tolerance of the scion. In the initial experiment, 66 tomato graft combinations were evaluated for their survival and growth performance. Different graft combinations showed different survival rates at 3-weeks post grafting and at harvest. ‘MX’, ‘TA’, and ‘AR’ rootstocks and ‘RL’ scion resulted in the highest survival rates in the experiment, whereas ‘MT’ scion showed the lowest rates compared to other scions. It was noted that success of grafting should be evaluated throughout a long-term assessment because some graft combinations (e.g. PR/TA and PHO/MT) had initial 100% survival rates then decreased at harvest to 80% and 73.3%, respectively. Also, ‘PR’ scion grafted onto ‘AR’, ‘RCK’, or ‘PR’ initially gave lower number of leaves at 3-weeks post grafting than most combinations then developed more leaves later in the experiment. This is because some of the graft combinations had some delay in the development after grafting but then changed their performance later in the experiment. All surviving plants had comparable shoot dry weight at harvest.

In the second experiment, 21 tomato genotypes (hybrid, wild, and cultivated) were screened for salinity tolerance. The aim of the experiment was to choose salt-tolerant genotypes to use them in grafting. Our results showed that different genotypes varied in their growth performance under salinity stress, particularly at 50 and 100 mM NaCl treatments. Under 50 mM NaCl treatment, some genotypes (including LA722, MM, RCK, and AR) showed a lower percent reduction in shoot DW than the average. At 100 mM NaCl, ‘Im’, ‘AR’, ‘MM’, and ‘LA1697’ had lower percent reduction in shoot DW than the average. Figure 5.1 illustrates the distribution of the 21 genotypes under 50, 100, and 150 mM NaCl treatments in the figure.
adapted from Maas, 1993, showing the divisions for classifying crop tolerance to salinity (Maas 1993). Plant response to salinity was found to be related to the overall vigor of the plants. Although ‘Im’ showed a considerable shoot weight reduction with higher salinity, it continued to have a better vigor than other genotypes that showed no shoot weight reduction such as ‘LA722’. Root to shoot ratio significantly increased with increasing NaCl concentration in most of the genotypes.

Fig 5.1: Divisions for classifying crop tolerance to salinity; adapted from Maas, 1993. Red line indicates the distribution of our 21 tomato genotypes under 50, 100, and 150 mM NaCl treatments of our experiment.

Genotypes that had the least growth reduction and/or high vigor were used for Na⁺, K⁺, and Ca²⁺ analysis in the leaf and roots to test their tolerance mechanisms. Under 50 mM NaCl, ‘LA3120’ and ‘Im’, which showed mild reduction in shoot DW, had the lowest leaf Na⁺ concentration among the genotypes. ‘AR’ had lower leaf Na⁺ accumulation than the average levels of all genotypes at 50 mM, and was even lower at higher NaCl concentrations. Wild lines ‘LA722’ and ‘LA1630’, however, had the highest leaf Na⁺ concentration at all NaCl treatments.
Shoot DW was significantly correlated with Mg$^{2+}$: Na$^+$ and Ca$^{2+}$: Na$^+$ ratios, except at 150 mM where Ca$^{2+}$: Na$^+$ was not correlated with shoot DW.

From the screening experiment, genotypes that showed low reduction or high vigor were chosen as potential rootstocks (AR, RS, LA3120, LA1630, LA722, LA3465, and Im), while ‘Moneymaker’ was used as a scion in grafting for salinity tolerance experiments. The resulting eight graft combinations were treated with 0 and 75 mM NaCl. The aims of the experiment were to test how grafting onto different salt-tolerant rootstocks (whether wild or cultivated) affects plants’ growth performance and ion concentrations in the leaf and roots. We also aimed at examining the use of vigorous rootstocks on the growth of the scion under NaCl stress conditions. We found that M/AR had the highest shoot DW under 75 mM NaCl treatment. The percent reduction in shoot DW of M/AR was only 17%, which was lower than most of the combinations under NaCl stress. M/Im was the second most vigorous combination after M/AR. Therefore, the use of vigorous rootstocks such as ‘AR’ and ‘Im’ had improved overall plant growth.

Rootstock clearly played a key role in controlling Na$^+$ concentration in the leaf. ‘Moneymaker’ plants grafted onto ‘LA3120’ had the lowest leaf Na$^+$ concentration, and showed much higher Na$^+$ concentration in the roots. In addition, M/LA3120 had the highest leaf K$^+$, Ca$^{2+}$, and Mg$^{2+}$ ratios with Na$^+$ among all genotypes, indicating that ‘LA3120’ has higher selectivity for K$^+$, Ca$^{2+}$, and Mg$^{2+}$ over Na$^+$. Therefore, ‘LA3120’ excluded Na$^+$ from the shoot, by retaining most of Na$^+$ in the root and/or excluding Na$^+$ from the root. Both mechanisms would limit Na$^+$ transport from the root to the shoot. On the other hand, ‘Moneymaker’ grafted onto ‘LA1630’ had the highest leaf Na$^+$ concentration, with less Na$^+$ accumulation in the root. K$^+$ concentration was very low in the leaves of M/LA1630. ‘LA1630’ used the inclusion mechanism of tolerance by transporting and accumulating Na$^+$ in the leaf. We found that grafting onto cultivated rootstocks (RS, AR, LA3120, and LA3465) improved the selectivity for Ca$^{2+}$ and K$^+$ over Na$^+$ compared to M/M plants. In the salinized plants, M/RS had the highest root to shoot ratio, followed by M/LA1630, and M/AR. These three combinations had less percent reduction in
shoot DW compared to other combinations. So, despite the high root to shoot ratio, those three graft combinations managed to maintain their shoot growth.

Four graft combinations (M/AR, M/RS, M/LA3120, and M/LA1630) were used for sap Na\(^+\) and K\(^+\) analysis. Na\(^+\) concentrations in the sap collected from non-grafted plants and grafted plants (whether from above or below the graft union segments) varied significantly. Sap Na\(^+\) concentrations were higher in all non-grafted stem samples than in the above-graft samples. M/LA1630 and M/AR showed higher sap Na\(^+\) concentrations in the below-graft samples than the above-graft samples. Generally, grafted and non-grafted ‘LA1630’ had the highest sap Na\(^+\) concentrations, whereas ‘LA3120’ had the lowest concentrations in the above-graft sample and in non-grafted stem samples. M/M plants had similar Na\(^+\) concentrations in sap above and below the graft union. Significant position effects were also found for K\(^+\): Na\(^+\) ratio in all the genotypes (except for LA3120, \(P=0.06\)). In all scion-rootstock combinations, sap from above the graft union segment had a higher K\(^+\): Na\(^+\) ratio than that from the below graft union segment. Our results suggest that grafting onto salt-tolerant rootstocks was beneficial at decreasing Na\(^+\) transport in the xylem sap. It also improved K\(^+\) transport in the sap compared to non-grafted plants. Na\(^+\) could be selectively removed from the xylem in the stem. This was more often noted in hetero-grafts than in self-grafts or non-grafted plants.

This study has helped in learning more about the useful benefits of grafting onto salt-tolerant rootstocks for improving tomato tolerance to salinity. It also helped understanding the different tolerance mechanisms associated with grafting onto certain rootstocks. We identified salt-tolerant tomato rootstocks that could be used in salinity research. More research work is still needed to study the anatomy of the graft-union in the different graft-combinations under NaCl stress conditions. Future studies are also needed to examine how the genetic interaction in a hetero-graft affects Na\(^+\) loading and unloading in a different way from self-grafts.
References

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PUBLICATION