Understanding Genetic and Physiological Bases of Salt Tolerance in Almond Rootstocks

Project No.: **17-HORT26.Sandhu (COC)**

Project Leader: Devinder Sandhu
Research Geneticist
USDA-ARS, US Salinity Lab
450 W Big Springs Rd.
Riverside, CA 92507
(951) 369-4832
Devinder.Sandhu@ars.usda.gov

Project Cooperators and Personnel:
Donald Suarez, USDA-ARS
Jorge Ferreira, USDA-ARS
Amita Kaundal, USDA-ARS US Salinity Lab

Objectives:

1. Evaluation of almond rootstocks to determine their tolerance response to a range of salt concentrations.
2. Characterizing different almond genotypes based on different components of salt tolerance mechanism.
3. Study global changes in the gene expression profiles under normal versus salt stress conditions in almond rootstocks.

Interpretive Summary:

Salinity is one of the most important abiotic stresses that adversely affect plant growth and productivity globally. To tackle this complex problem, it is important to link the biochemical and physiological responses with the underlying genetic mechanisms. In an effort to understand underlying genetic and biochemical mechanisms for the salt tolerance process, we have evaluated 16 commercial almond rootstocks for salt tolerance. To determine importance of specific ions toxicities we applied 5 different treatments of irrigation water that included control (T1), sodium sulfate-based solution (T2), sodium chloride-based solution (T3), sodium dominant water with mixed anions (chloride and sulfate) (T4), and calcium and magnesium dominant water with mixed anions (chloride and sulfate) (T5).

We studied the effect of different treatments on trunk diameter, survival rate, physiological and biochemical traits. The survival rate and the change in trunk diameter analyses suggested that mostly Na and, to a lesser extent, Cl concentration in irrigation water are the most critical ion toxicities for almond rootstocks. Photosynthesis showed the highest correlation with change in trunk diameter, followed by correlations with stomatal conductance and chlorophyll content. Different rootstocks were characterized based on their ability to store Na and Cl in their leaf tissues. For the most part, ion analysis was correlated with trunk diameter and survival rate assessments. Currently, we are testing toxicity limits of 15 different rootstocks under four salinity levels.

The expression analysis of 10 genes known to play important roles in salt tolerance revealed that treatments where Na and Cl were the main ions in irrigation water (T3 and T4) led to induction of most genes, suggesting importance of both the chloride and sodium toxicities during salt stress in almonds. The *HKT1* and *AKT1* genes displayed the highest upregulation (expression) in salinity treatments in roots and *NHX1*, *SOS3* and *AKT1* were highly upregulated in salinity treatments in leaves. In addition to the 10 genes used for the expression analysis in our recent analysis, we have included 14 additional genes for our current trial, which will help us characterize rootstocks based on the genetic makeup.

Based on our recent finding we are conducting RNA sequencing (RNAseq) analysis on one most tolerant and one most sensitive rootstock to study global changes in gene expression between salinity and control treatments, root and leaves, and salt-tolerant and salt-sensitive rootstocks. The differentially expressed genes will be studied to develop a link between the predicted function and their functional relevance to the physiological or the biochemical mechanisms involved in salt tolerance. The expression analyses and the RNAseq experiment will aid us in characterizing the salt tolerance mechanism in almonds into different component traits. Combining different components of the salt tolerance mechanism may lead to the development of superior salt tolerant rootstocks. Improving salt tolerance in almond rootstocks will not only improve crop yield, but also will provide incentives to expand the use of alternative/degraded waters that will allow almonds to be cultivated in new lands and at lower costs for irrigation.

Materials and Methods:

Experimental set up and salt treatments:

The experiment is being conducted at United States Salinity Laboratory (USDA-ARS) in Riverside, CA. Non-grafted plants of 16 different rootstocks (Atlas, BB106, Bright's 5, Cornerstone, Empyrean 1, Flordaguard c Alnem (F x A), Guardian, Hansen, Krymsk 86, Lovell, Nemaguard, Nickels, Rootpac 20, Rootpac 40, Rootpac R, and Viking) were obtained from various nurseries and transplanted into 1.5-gallon pots containing sandy loam soil.

Experiment was set up in a randomized complete block design with 16 genotypes, 3 replications, 3 plants per replication (one plant per pot) and 5 treatments of saline water (total 720 trees). Plants were allocated into fifteen different blocks, each containing combinations of all genotypes, and 3 replications. Blocks with different treatments were color-coded. The five different treatments were as follows:

1. Treatment 1: Non-saline control {Na^+ 1.65 meq L^{-1}, K^+ 6.5 meq L^{-1}, PO_4^{3-} 1.5 meq L^{-1}, Mg^{2+} 1.3 meq L^{-1}, SO_4^{2-} 1.5 meq L^{-1}, Cl^- 1.5 meq L^{-1}, NO_3^- 5 meq L^{-1} and micronutrients}
2. Treatment 2: mixed cations (Ca^{2+} = 1.25Mg^{2+} = .25 Na^+) with predominantly sulfate (Cl^- = 0.2 SO^{2-}_4) {Na^+ 18 meq L^{-1}, Ca^{2+} 4.5 meq L^{-1}, K^+ 6.5 meq L^{-1}, PO_4^{3-} 1.5 meq L^{-1}, Mg^{2+} 3.6 meq L^{-1}, SO_4^{2-} 22 meq L^{-1}, Cl^- 4.4 meq L^{-1}, NO_3^- 5 meq L^{-1} and micronutrients}
3. Treatment 3: mixed cations (Ca^{2+} = 1.25Mg^{2+} = .25 Na^+) with predominantly chloride (SO^{2-}_4 = 0.2 Cl^-) {Na^+ 15.5 meq L^{-1}, Ca^{2+} 3.8 meq L^{-1}, K^+ 6.5 meq L^{-1}, PO_4^{3-} 1.5 meq L^{-1}, Mg^{2+} 3.1 meq L^{-1}, SO_4^{2-} 3.8 meq L^{-1}, Cl^- 19 meq L^{-1}, NO_3^- 5 meq L^{-1} and micronutrients}
4. Treatment 4: mixed anions SO_4-Cl (SO^{2-}_4=Cl^-), predominantly Sodium (Ca^{2+} = 1.25Mg^{2+} = .25 Na^+) {Na^+ 17 meq L^{-1}, Ca^{2+} 4.25 meq L^{-1}, K^+ 6.5 meq L^{-1}, PO_4^{3-} 1.5 meq L^{-1}, Mg^{2+} 3.4 *meq L^{-1}, SO_4^{2-} 12.32 meq L^{-1}, Cl^- 12.32 meq L^{-1}, NO_3^- 5 meq L^{-1} and micronutrients}*

5. Treatment 5: mixed anions SO^{2-}_4-Cl^- (SO^{2-}_4=Cl^-), predominantly Ca^{2+} and Mg^{2+}. (Ca^{2+} = 1.25 Mg^{2+} = 5 Na^+) {Na^+ 2.75 meq L^{-1}, Ca^{2+} 13.5 meq L^{-1}, K^+ 6.5 meq L^{-1}, PO_4^{3-} 1.5 meq L^{-1}, Mg^{2+} 10.8 meq L^{-1}, SO_4^{2-} 13.5 meq L^{-1}, Cl^- 13.5 meq L^{-1}, NO_3^- 5 meq L^{-1} and micronutrients}

These mixtures represent a range of natural saline water compositions, including municipal water as control. Water concentrations of the nutrients NPK were constant in all treatments. Treatment 1 was the control treatment with irrigation water salinity with Electrical Conductivity (EC) of 1.36 dS m^{-1} (municipal water+nutrients) and salinity of Treatments 2 through 5 was maintained at 3.0 dS m^{-1}. The pH of the different treatments was tested to make sure they were between 7.3 and 7.6. Each plant was irrigated with 500 ml of treatment solution once a day.

To test the toxicity tolerance limits of different rootstocks, in the second experiment we are using four salinity levels of irrigation waters that include control, EC 2.0 dS m^{-1}, EC 2.5 dS m^{-1} and EC 3.0 dS m^{-1}. One-year-old non-grafted plants from 15 different rootstocks were transplanted into 2-gallon pots in the field. Each treatment consists of three plants and is replicated three times in a randomized design (15 rootstocks x 4 treatments x 3 replications x 3 plants/replication = 540 plants). After 8 weeks of treatment, leaf samples will be taken to determine tissue ionic composition. Salinity of soil will also be determined at the end of the treatment period to precisely quantify the actual salinity level in the containers.

Trunk diameter and ion analysis:

At the beginning of treatment, trunk diameter was recorded 10 cm above the soil level using a Vernier caliper. The second reading for the trunk diameter was recorded after ten months of salt treatment to calculate the change. Leaf samples were collected 8 weeks after the initiation of salt treatments to determine tissue ion composition. Tissue samples were dried, digested in a Milestone Ethos EZ microwave digestion system and analyzed with a Perkin Elmer Optima ICP OES for macro and micro minor elements. Chloride content was determined using a Labconco chloridometer. Statistical analysis was performed with the SAS software package.

For the second experiment, the first trunk diameter readings were taken before initiating the treatment and second reading will be taken in the Spring of 2019 when the rates of growth will be compared. The survival rate of different rootstocks will also be recorded at the same time.

Physiological and biochemical analysis:

Photosynthetic parameters and stomatal conductance were measured with a Li- Cor (Li-Cor Biosciences) 8 weeks from the initiation of the salt treatments. Also, leaf samples were collected, frozen in liquid nitrogen, and lyophiolized for analyses of total phenolics, hydrophilic antioxidant capacity, and proline.

Primer design for expression analyses:

Almond genes involved in different mechanisms leading to salt tolerance were selected based on functional conservation with the genes identified in Arabidopsis (Munns and Tester, 2008; Gupta and Huang, 2014; Sandhu et al., 2018). These gene sequences were used in Basic Local Alignment Search Tool (BLAST) analyses to identify corresponding sequences from the peach genome (Verde et al., 2013). For each gene, the sequence with the highest homology

was used, and intron/exon boundaries were identified. At least one PCR primer out of each pair was designed from two exons flanking an intron.

Expression analyses:
Tissue samples were taken 24 hours after the initiation of salt treatment for RNA isolation. Young leaf and root samples were harvested from 720 plants (16 genotypes x 3 plants per genotype x 3 replications x 5 salt treatments). Samples from 3 plants for each genotype were pooled. Samples were frozen immediately in liquid Nitrogen and RNA was extracted using Spectrum™ Plant Total RNA kit (Sigma, St. Louis, MO). To remove contaminating DNA, RNA was treated with DNase I am following manufacturer's instructions (Thermo Scientific, Waltham, MA, USA). The qRT-PCR amplification was carried out in a BioRad CFX96 System using iTaq™ Universal SYBR® Green One-Step Kit (Bio-Rad Laboratories, Hercules, CA, USA). Reactions for qRT-PCR were performed in 10 µl volume that contained 100 ng total RNA, 0.125 µl iScript™ Reverse Transcriptase, 0.75 µM of each of the primers and 5 µl of 2x one-step SYBR® Green Reaction mix. The PCR program was as follows: 50 °C for 10 min, 95 °C for 1 min, then 40 cycles of 95 °C denaturation for 10 s, 57 °C annealing for 30 s, and 68 °C extension for 30 s (Sandhu et al., 2017). For normalization of expression in different plates, four samples were used as inter-plate controls. The peach EF2 and Ubiqutin genes were used as reference genes for the qRT-PCR analyses (Tong et al., 2009). The cycle threshold values of each gene to the reference gene were used to calculate the relative expression and differentially expressed genes were identified. For the quality control, the melt curve analysis was used to test the amplification specificity by ramping the temperature to 95 °C for 10 s, then back to 65 °C for 5 s, followed by incremental increases of 0.5 °C/cycle up to 95 °C.

RNAseq analysis:
To study the global changes in gene expression, we selected rootstocks based on our recent results from trial 1. The most-tolerant rootstock (Rootpac 40) and the most-sensitive rootstock (Nemaguard) were selected. Three biological replications of both rootstocks were used for our RNAseq experiment and samples will be taken from the leaves and the roots. Our approach involves total RNA preparation from the roots and the leaves of the normal and salt stressed plants. cDNA libraries will be prepared for each sample with a unique index to facilitate pooling for several samples in a single lane for sequencing. Sequencing will be done at the University of California Riverside Genomics facility using the Illumina Hiseq 2000 platform. Sequences will be aligned with the peach genome (Verde et al., 2013). Bioinformatic analysis will be performed with the support of UC Riverside Bioinformatics facility. We expect to find a large number of genes that may be differentially expressed between normal and salt- stress conditions. We will identify the top ten genes with extreme differences between normal and salt-stress conditions and validate those genes using qRT-PCR. The differentially expressed genes will be studied to develop a link between the predicted function and their functional relevance to the physiological or the biochemical mechanisms involved in salt tolerance (growth, Na uptake and translocation, Cl uptake, etc.).

With these experiments and approaches we will generate information on transcription patterns of specific genes that are already known to play important roles in salt stress in model plants and some novel genes that may be specific to the genus *Prunus*.

Results and Discussion:

Objective 1. Evaluation of almond rootstocks to determine their tolerance response to a range of salt concentrations.

In trial 1, we have analyzed sixteen (non-grafted) rootstocks in mixed salt compositions. Different irrigation water treatments included control (T1), Na-SO_4 based irrigation water (T2), Na-Cl based irrigation water (T3), Na-Cl-SO_4 based irrigation water (T4) and Ca-Mg-Cl-SO_4 based irrigation water (T5). Before initiating salinity treatments, trunk diameter was measured for all the plants in July 2017. Trunk diameter was again measured in May 2018 (**Figures 1, 2 and 3**). Percent change in trunk diameter was calculated. As expected, T1 showed the highest change in trunk diameter followed by T5 and T2 (**Figure 2**). The T3 and T4 treatments displayed the lowest increase in trunk diameter. A smaller increase in all three Na based treatments (T2, T3 and T4) indicated importance of Na^+ toxicity in almonds. A smaller increase in trunk diameter in T3 (Na-Cl based treatment) versus T2 (Na-SO_4 based treatment) suggests importance of both Na^+ and Cl^- toxicities during salt stress (**Figure 2**).

Figure 1. Experimental setup for the salt tolerance experiment on 16 almond rootstocks. Students measured trunk diameter before the application of salt treatment.

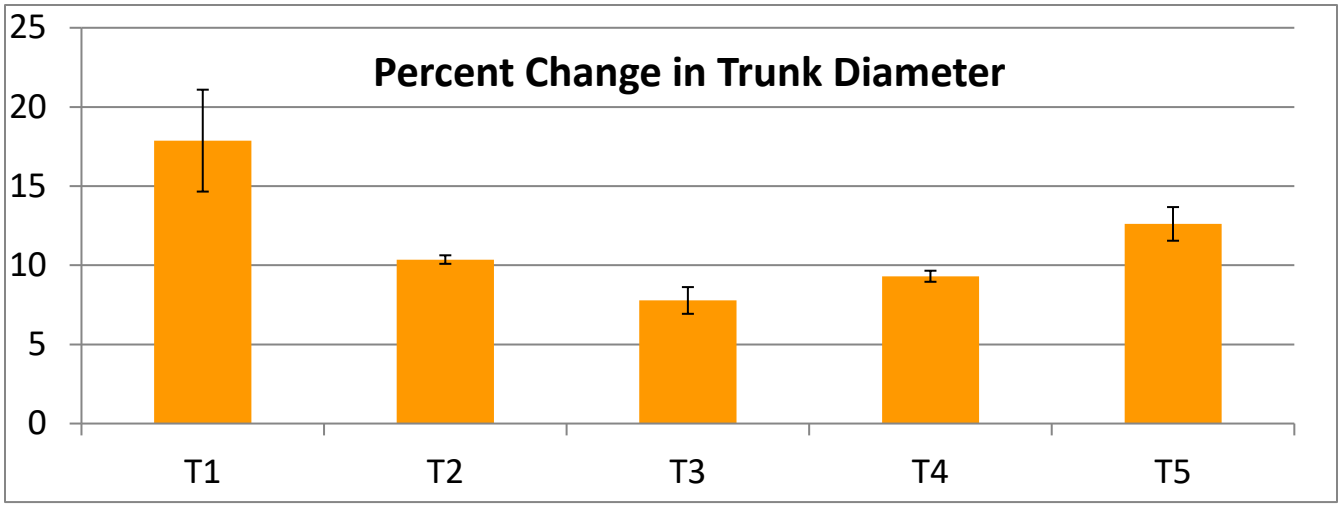

Figure 2. Percent change in trunk diameter 10 months after the initiation of the salt treatments. T1, Control; T2, Na-SO4 based irrigation water; T3, Na-Cl based irrigation water; T4, Na-Cl-SO_4 based irrigation water; T5, Ca-Mg-Cl-SO_4 based irrigation water. Error bars represent standard error.

Out of 16 tested rootstocks, Rootpac 40 displayed 1.09-fold, 1.00-fold, 1.02-fold and 0.98-fold relative increase in trunk diameter in T2, T3, T4 and T5 as compared to the control treatment (T1) (**Figure 3**). Other rootstocks that performed well in salinity treatments include Empyrean 1, BB106, Cornerstone, Viking and F x A (**Figure 3**). The rootstocks that performed poorly in salinity treatments were Rootpac 20, Guardian, Lovell and Krymsk 86.

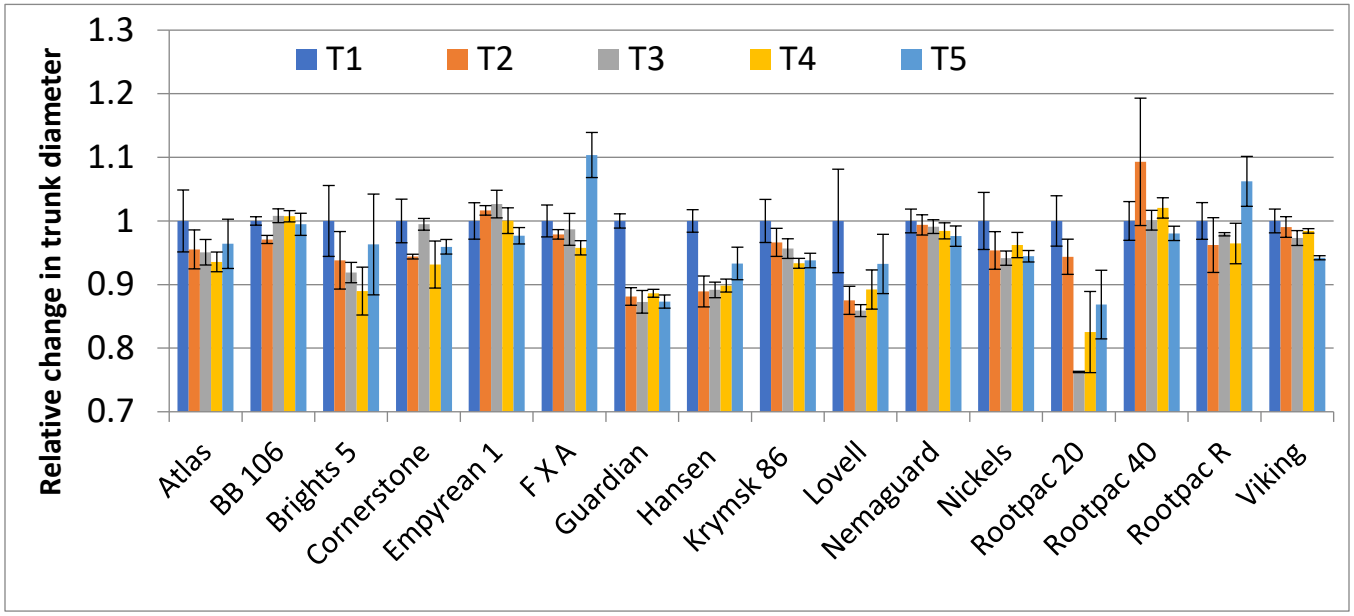

Figure 3. Relative trunk diameters of 16 almond rootstocks treated with 5 irrigation water treatments. T1, Control; T2, Na-SO_4 based irrigation water; T3, Na-Cl based irrigation water; T4, Na-Cl-SO_4 based irrigation water; T5, Ca-Mg-Cl-SO_4 based irrigation water. Error bars represent standard error

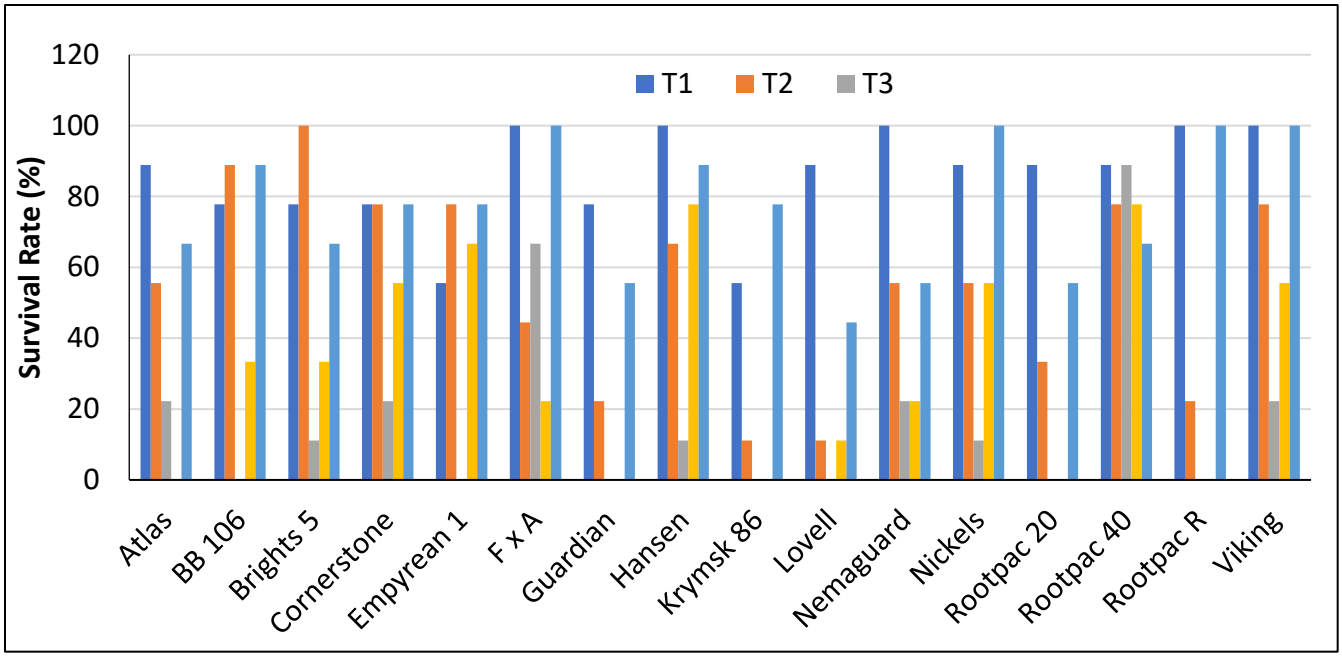

Figure 4. Survival rates of 16 almond rootstocks treated with 5 irrigation water treatments. T1, Control; T2, Na-SO_4 based irrigation water; T3, Na-Cl based irrigation water; T4, Na-Cl-SO_4 based irrigation water; T5, Ca-Mg-Cl-SO_4 based irrigation water.

Ten months after the initiation of salt treatments, plants were analyzed for survival rates and the toxicity symptoms on leaves. T3 was the harshest treatment for the plants with the lowest average survival rate (17.4 %) followed by T4 (31.9%), T2 (54.9%), T5 (76.4%) and T1 (85.4%) (**Figure 4).** Rootpac 40 displayed very high overall average survival rate (77.8%) for four salinity treatments, followed by Viking (63.9%), Hansen (61.1%), Cornerstone (58.3%), F x A (58.3%), Empyrean 1 (55.6%), and Nickels (55.6%). On the other hand, Lovell (16.7%), Guardian (19.4%), and Rootpac 20 (22.2%) had low average survival rates for the four salinity treatments. While many of the rootstocks were dead in T3, Rootpac 40 had minimal toxicity symptoms on the leaves (**Figure 5**).

Figure 5. Physical appearances of 16 almond rootstocks treated with 5 irrigation water treatments for 11 months. Three plants in a row belong to the same rootstock. The closes three plants belong to Rootpac 40.

Ion analysis was done for digested leaf sample for Na, Cl, K, Ca, Mg, P, S, B, Cu, Fe, Mn, Mo and Zn. Of the 16 rootstocks, Rootpac 40 stored least amount of Na^+ in the leaves in the control treatment and Nemaguard stored the most (**Figure 6**). In all other 4 salinity treatments, among all rootstocks, Rootpac 40 stored least amount of Na^+. Other rootstocks that stored low amount of Na^+ include Cornerstorne, Empyrean 1, Viking, Atlas, Nickels, BB 106 and Brights 5.

In general, Empyrean 1 stored the least amount of Cl^- in the salinity treatments, followed by Nickels and BB 106 (**Figure 7**). Interestingly, Rootpac 40 was also among the rootstocks that stored low amount of Cl^- in their leaves.

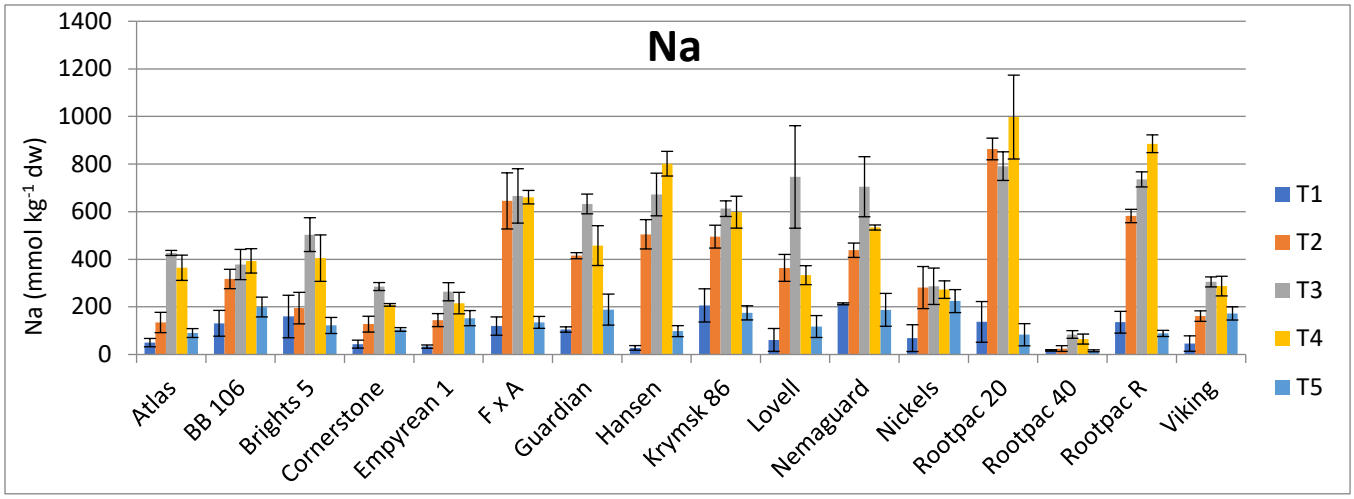

Figure 6. Shoot Na concentrations of 16 almond rootstocks treated with 5 irrigation water treatments. Error bars represent standard errors of three biological replicates.

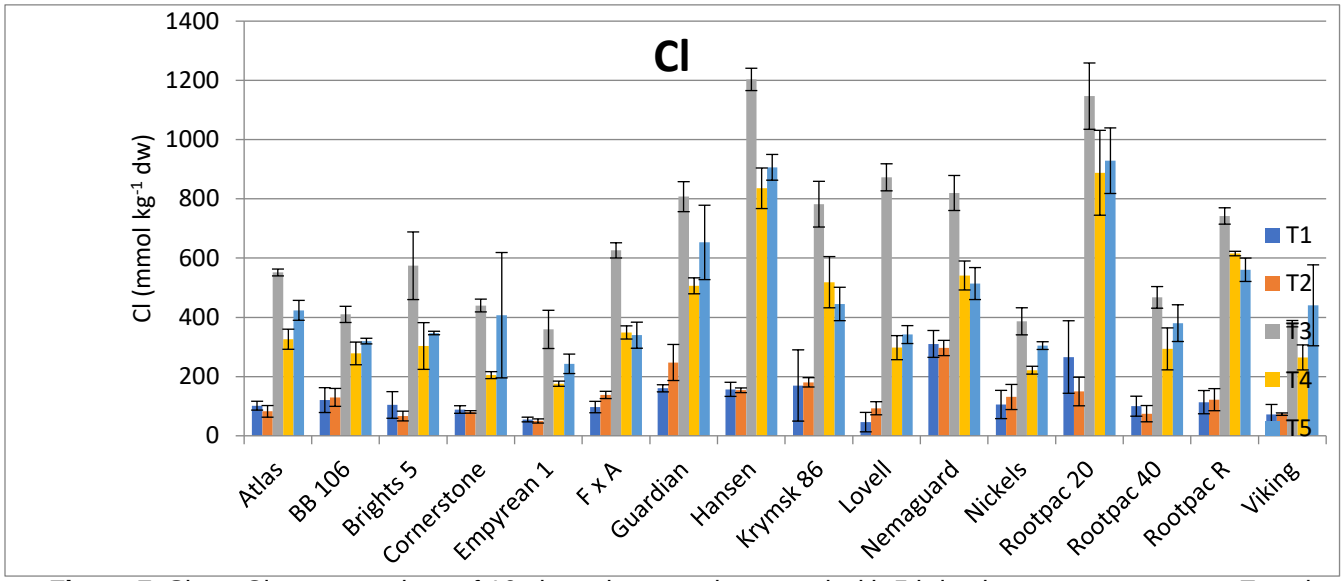

Figure 7. Shoot Cl concentrations of 16 almond rootstocks treated with 5 irrigation water treatments. Error bars represent standard errors of three biological replicates.

In most rootstocks, the total K content in the leaves was reduced in all four salinity treatments as compared to the control (**Figure 8**). However, Rootpac 40 displayed clear increase in K content in T3 (Na-Cl based irrigation water) and T5 (Ca-Mg-Cl-SO_4 based irrigation water) and maintained K levels in T4 (Na-Cl-SO_4 based irrigation water) as compared to the control (**Figure 8**). Reduction in K^+ content in salinity treatments was consistent with many other plant species and can be explained as some protein channels play important role in transport of both Na^+ and K^+.

Different rootstocks showed variation in tissue Ca concentrations in different treatments T2 to T4 did not show much change in tissue Ca concentrations as compared to T1 in most genotypes (**Figure 9**). However, T5, which contained high amount of Ca in irrigation water displayed high tissue Ca as compared to T1 in most genotypes. In many plant species the

ability to maintain high Ca content in plant tissue under salinity is associated with salinity tolerance. However, in almond rootstocks we did not observe any association between tissue Ca concentration and salinity tolerance.

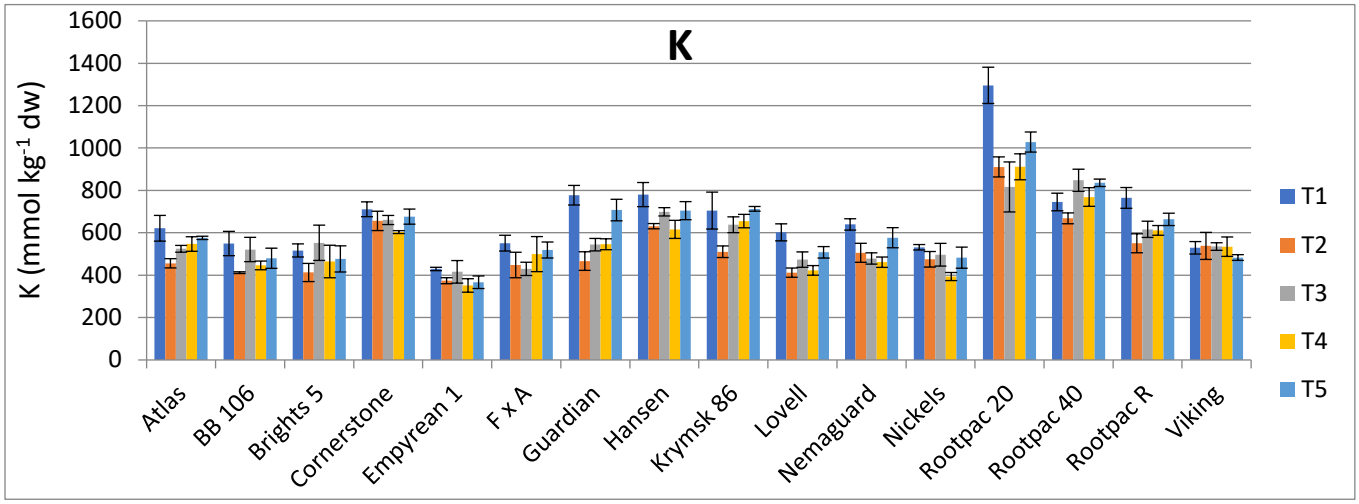

Figure 8. Shoot K concentrations of 16 almond rootstocks treated with 5 irrigation water treatments. Error bars represent standard errors of three biological replicates.

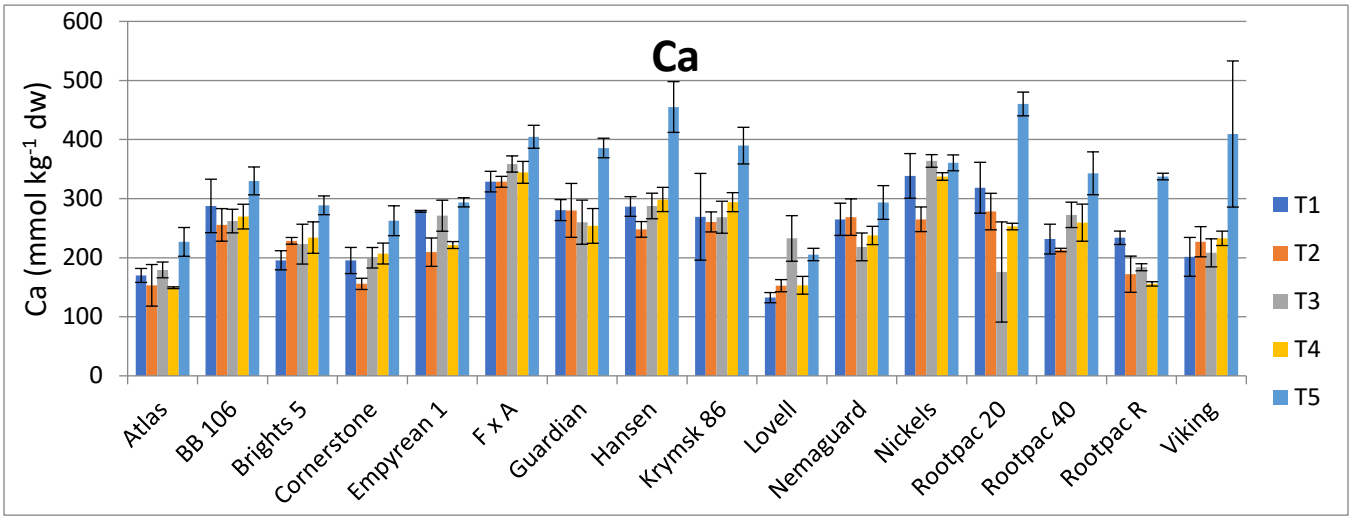

Figure 9. Shoot Ca concentrations of 16 almond rootstocks treated with 5 irrigation water treatments. Error bars represent standard errors of three biological replicates.

The comparison of 16 different almond rootstocks for tolerance to salinity of various solutions of different salt compositions showed that Na and to a lesser extent Cl concentration in irrigation water are the most critical ion toxicities for almond rootstocks. This investigation demonstrated that both Na and Cl are critical for salt stress in almonds. Our previous work showed that Na toxicity is known to play important role during salt stress in salt tolerant crops such as alfalfa (Cornacchione and Suarez, 2017; Sandhu et al., 2017); however Cl toxicity plays a major role in salt-sensitive crops like strawberries and avocado (Suarez and Grieve,

2013). For some plants, such as fava beans, concentrations of both Na and Cl ions are critical during salt stress (Tavakkoli et al., 2010).

In our current trial, we are testing the toxicity limits of the respective ions in 15 different almond rootstocks. Rootstocks were transplanted into pots in early spring and maintained with essential nutrition until the start of the salinity treatments. We are using four salinity levels of irrigation waters that include control, EC 2.0, EC 2.5 and EC 3.0. Measurement were taken for the trunk diameter before initiation of the treatments. Salt tolerance of different rootstocks will be determined by studying association among change in trunk diameter, tissue ion accumulation and genetic/biochemical markers.

Objective 2. Characterizing different almond genotypes based on different components of salt tolerance mechanism.

The analyses of physiological parameters for 16 almond rootstocks treated with five treatments of irrigation water suggested that there were significant differences among treatments and among rootstocks ($P<0.001$) for the net photosynthetic rate (*Pn*), leaf stomatal conductance (*gs*) and *SPAD* chlorophyll content. T3 showed maximum reduction for *Pn*, *gs* and *SPAD* as compared to the control (T1) (**Figure 10**). On the other hand, T5 had least reduction in *Pn* and *SPAD* as compared to the control and T4 exhibited least reduction for *gs*.

Pn had the highest correlation ($R^2 = 0.88$) with trunk diameter, followed by *gs* ($R^2 = 0.63$), then *SPAD* ($R^2 = 0.88$). High correlation between *Pn* and the trunk diameter suggests that *Pn* is an important physiological parameter and can be used an indicator of the salinity stress (**Figure 10A**).

Comparison of different rootstocks in T3 revealed that Nickels, Empyrean 1, Hansen and Brights 5 had high *Pn* values as compared to the other rootstocks (**Figure 11A**). Nemaguard was the worst performer for *Pn* in T3. Hansen performed well in all four salinity treatments (T2 – T5) (**Figure 11A**). Nickels, Emyprean 1 and Hansen were also the top three performers for *gs* in T3 suggesting that observations for *Pn* and *gs* were somewhat consistent for different rootstocks (**Figure 11B**). For the *SPAD* chorophyll content Hansen, Brights 5, Cornerstone, BB106 and Nickels were good performers in T3 (**Figure 11C**). Hansen was the best performer for *SPAD* readings among all four salinity treatments.

Leaf samples were taken to determine total phenolics, hydrophilic antioxidant capacity and proline. All three parameters have been evaluated. We are currently analyzing data to study association between these biochemical markers and trunk diameter.

Expression analysis was carried out for a set of 10 genes selected for their involvement in salt stress. These include genes known to be associated with Na^+ efflux from root to soil (SOS1, SOS2 and SOS3), genes involved in sequestration of Na^+ in vacuoles (NHX1, NHX2 and AVP1), genes important for retrieving Na^+ from xylem (*HKT1* and *AKT1*), and genes involved in signal transduction during salt stress (SAL1 and SERF1). The expression analyses for 10 genes revealed that Treatment 3 and Treatment 4 both led to induction of majority of salt associated genes during salt stress, suggesting importance of both the chloride and sodium toxicities during salt stress in almonds. Both *HKT1* and *AKT1* genes displayed the highest upregulation in salinity treatments in roots (**Figure 12**). On the other hand, *NHX1*, *SOS3,* and *AKT1* were highly upregulated in salinity treatments in leaves (**Figure 13**).

Our recent results suggested that we need to analyze a larger set of genes to characterize different rootstocks based on components of the salt tolerance mechanism. In addition to the 10 genes used for the expression analysis in our recent analysis, we have included 14 additional genes for our current trial. Thus, evaluation of the expression of these candidate genes will help us in characterizing genotypes based on different components of the salt tolerance mechanism. This information will be extremely valuable to almond breeders and geneticists in making crosses and combining different components of salt tolerance mechanism into a single genotype that may result in development of a highly tolerant rootstock to salt.

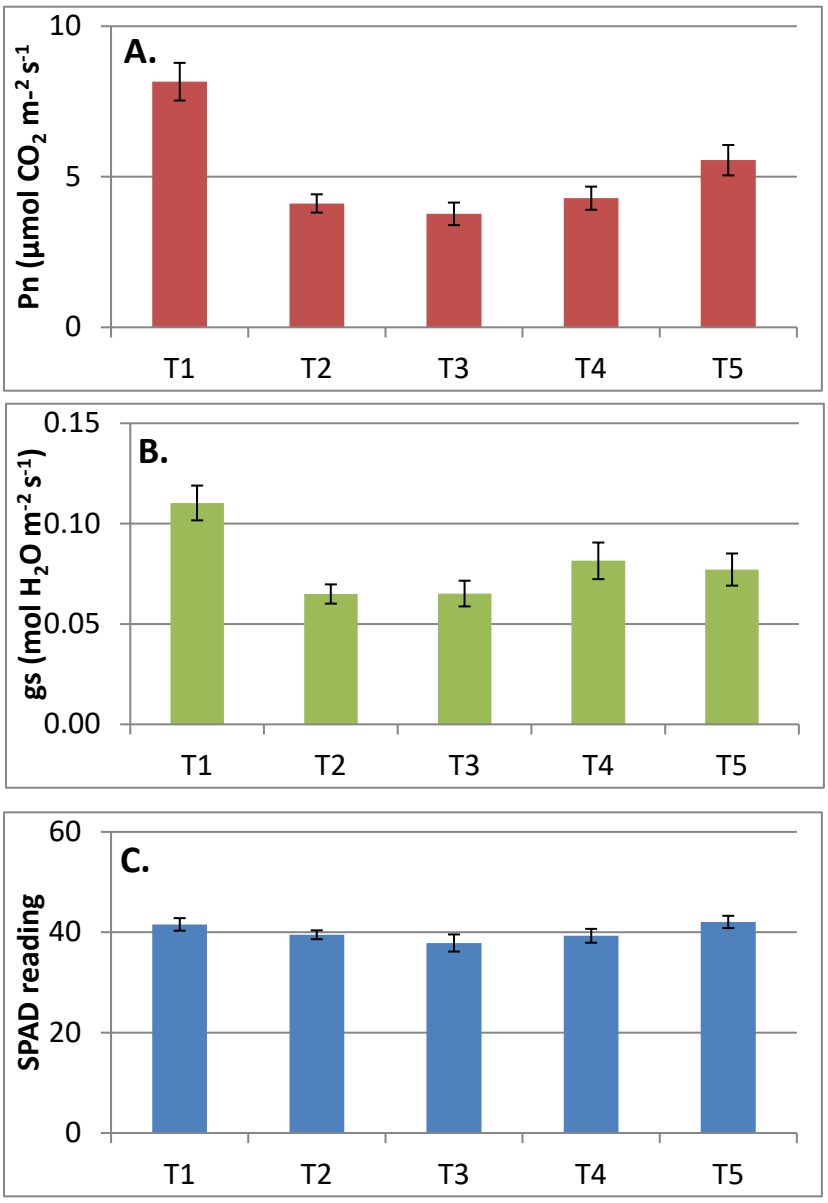

Figure 10. Physiological measurements in almond rootstocks under different salt treatments. Data for all the rootstocks was pooled for each treatment. A, Net photosynthesis (*Pn*); B, stomatal conductance (*gs*); C, *SPAD* reading for Chlorophyll content. Error bars represent standard error.

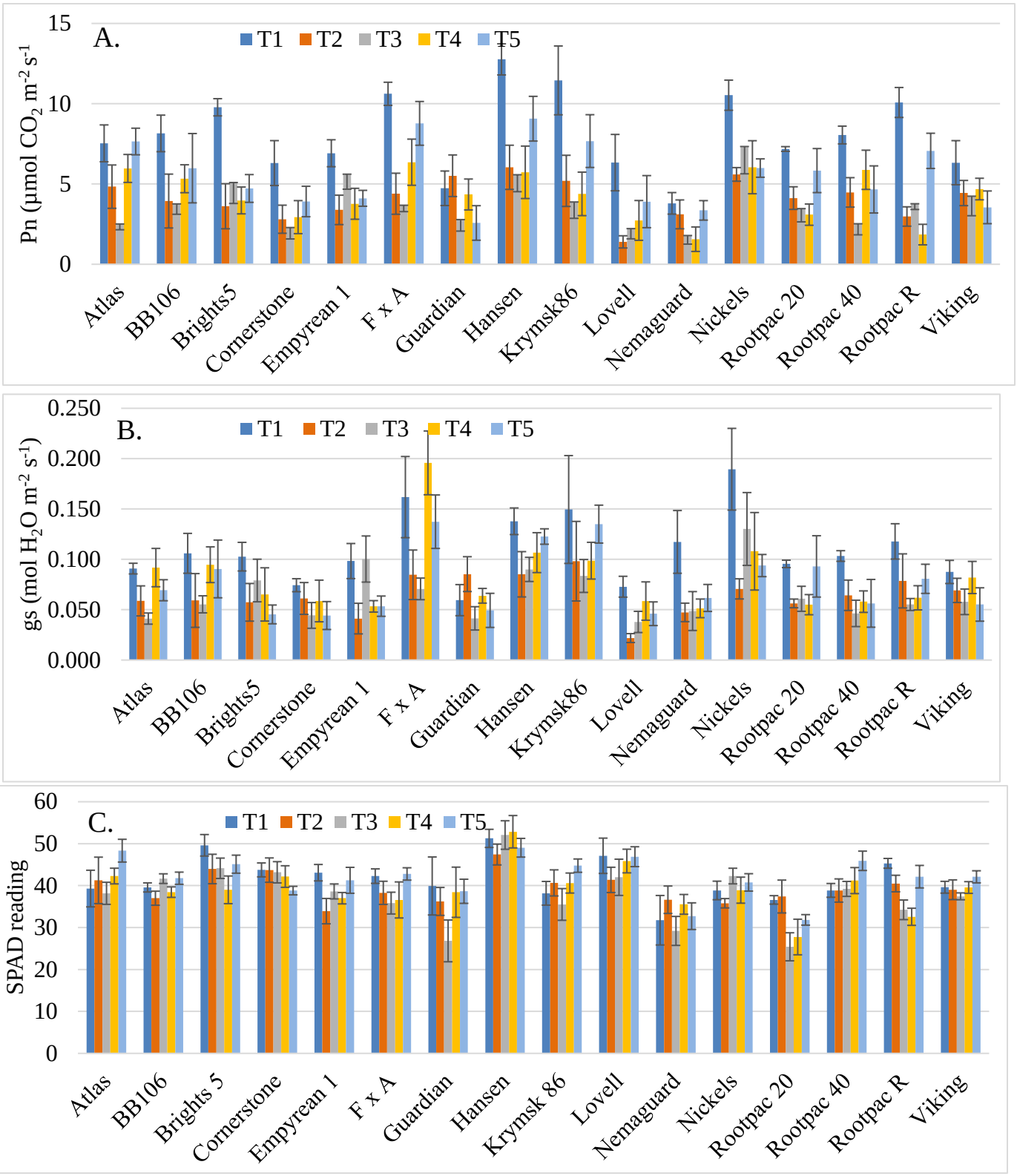

Figure 11. Physiological parameters of 16 almond rootstocks in response to five salt treatments. A, Net photosynthesis (*Pn*); B, stomatal conductance (*gs*); C, *SPAD* reading for Chlorophyll content. Error bars represent standard error.

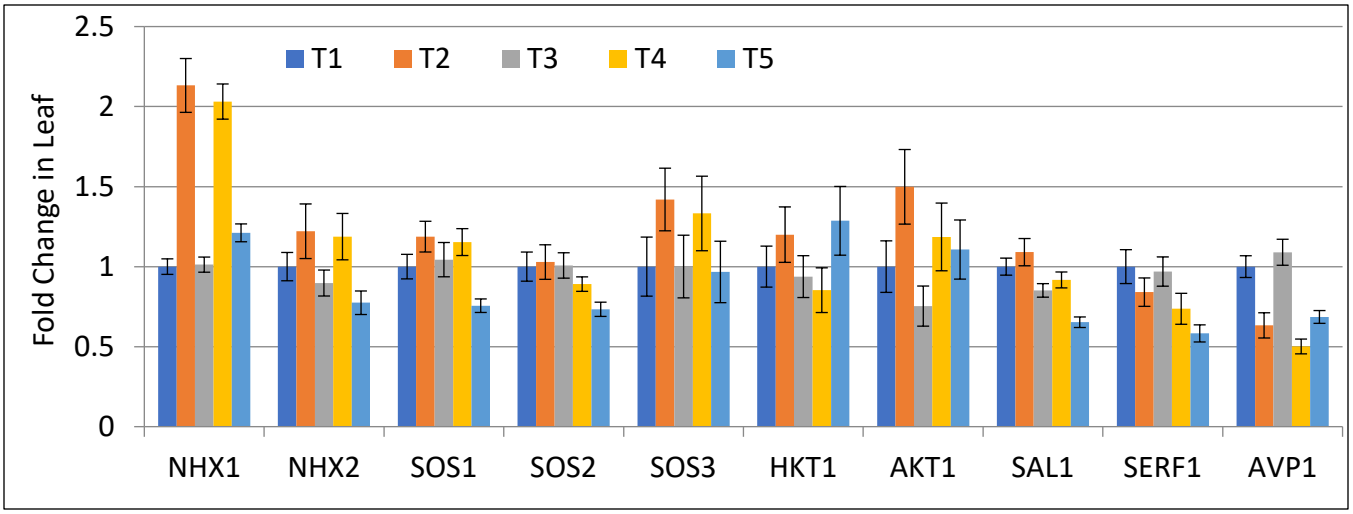

Figure 12. Expression of genes involved in the salt tolerance mechanism in roots of 16 almond rootstocks under different saline treatments. Error bars represent standard error

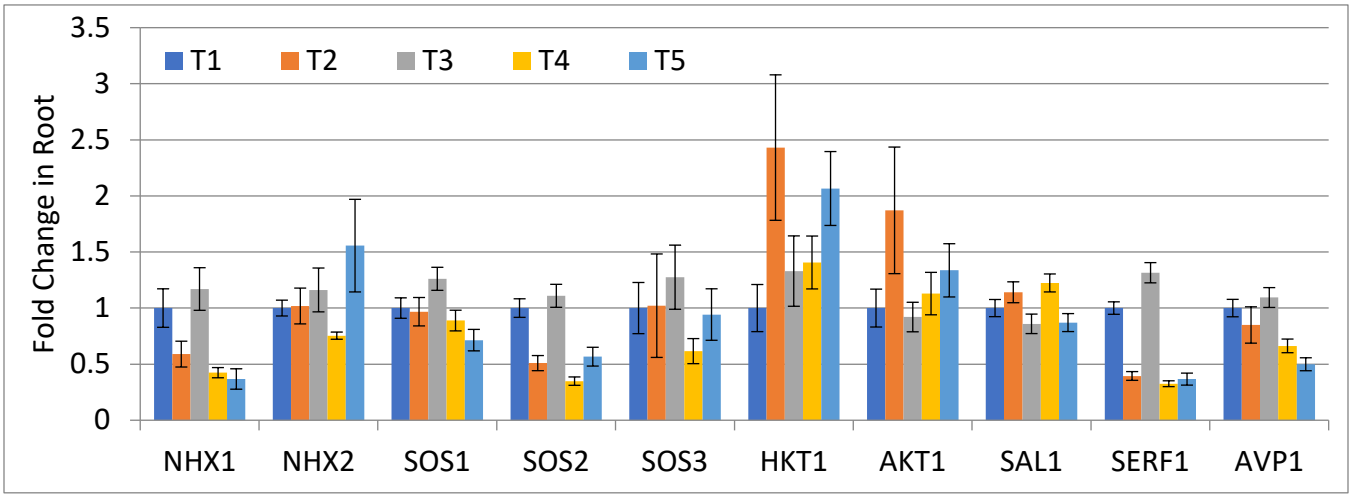

Figure 13. Expression of genes involved in the salt tolerance mechanism in leaves of16 almond rootstocks under different saline treatments. Error bars represent standard error.

<u>Objective 3. Study global changes in the gene expression profiles under normal versus salt stress conditions in almond rootstocks.</u>

Based on our recent findings, we have selected a salt-tolerant rootstock (Rootpac 40) and a salt sensitive rootstock (Nemaguard) for our RNA sequencing (RNAseq) analysis. RNAseq analysis will help us identify differentially-expressed genes between salinity and control treatments, between root and leaves and between salt-tolerant and salt-sensitive rootstock. We are currently growing three replications of each of the salt-tolerant and the salt-sensitive genotypes. RNA samples will be taken in mid-August 2018 and cDNA libraries will be prepared. RNA sequencing will be accomplished by December 2018. The differentially expressed genes will be studied to develop a link between the predicted function and their functional relevance to the physiological or the biochemical mechanisms involved in salt tolerance (growth, Na uptake and translocation, Cl uptake etc.).

Research Effort Recent Publications:

The results of this work will be published in a peer-reviewed scientific journal such as Plant Science. The focus of this article will be on establishing the most critical ions for tissue toxicity during salt stress in almond rootstocks. In addition, we will link genetic and biochemical determinants with physiological and morphometric components of the salt tolerance mechanism expressed by the almond rootstocks. Our results will be available to breeders and farmers who will benefit of the knowledge generated on salinity tolerance of these almond rootstocks. This knowledge will allow farmers to grow the crop on rootstocks that will tolerate higher salt concentrations present in recycled waters. Recycled waters are cheaper than fresh water and will come as an advantage in times of water shortage, as the one faced by Southern California growers in the past 6 years.

Acknowledgments:

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