

# Understanding Genetic and Physiological Bases of Salt Tolerance in Almond Rootstocks

## Problem and Its significance

- One of the biggest challenges California almond (*Prunus* sp.) growers are facing is limited availability of fresh water.
- Due to the reduced availability of good quality water, the only current alternative water supplies are more saline groundwaters or degraded waters.
- The identification of genetic mechanisms regulating salt tolerance will be the key in developing genetic material tolerant to salt.
- Improving salt tolerance in almonds will not only improve yield but will provide incentives to make augmented use of alternative/degraded waters, which can sustain high almond cultivation.

## Objectives

- Evaluation of diverse rootstocks for tolerance to salinity of solutions of mixed salt composition, more representative of available waters than the typical NaCl solutions commonly utilized.
- Characterization of physiological and biochemical markers associated with salt tolerance and salt composition of irrigation water in almond rootstocks.
- Identification and characterization of genes involved in salinity tolerance in almond rootstocks.

## Experimental results

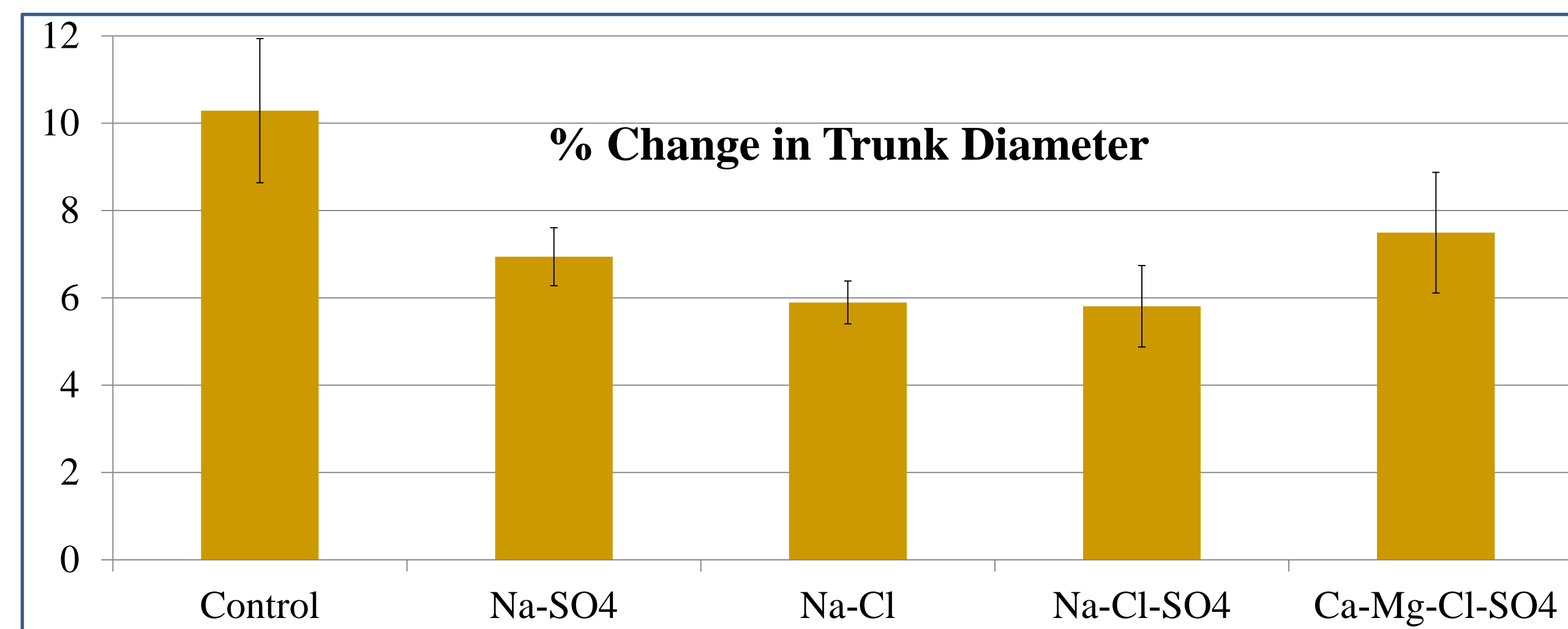
### 1. Evaluation of diverse rootstocks for tolerance to salinity of solutions of



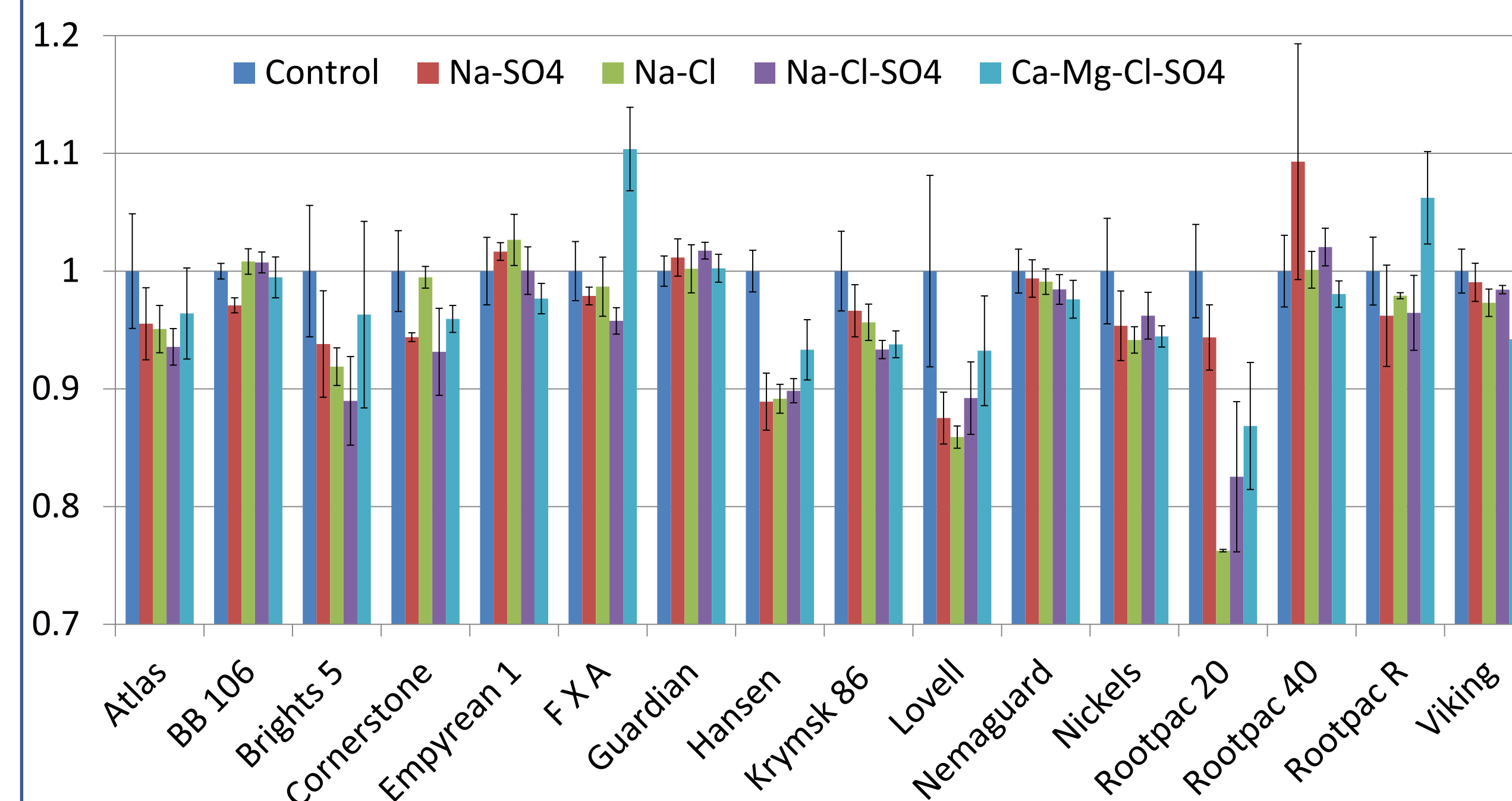
**Figure 1.** Experimental setup showing 16 rootstock genotypes rearranged in 15 blocks. Students are taking data on these plants.

### Experimental Setup

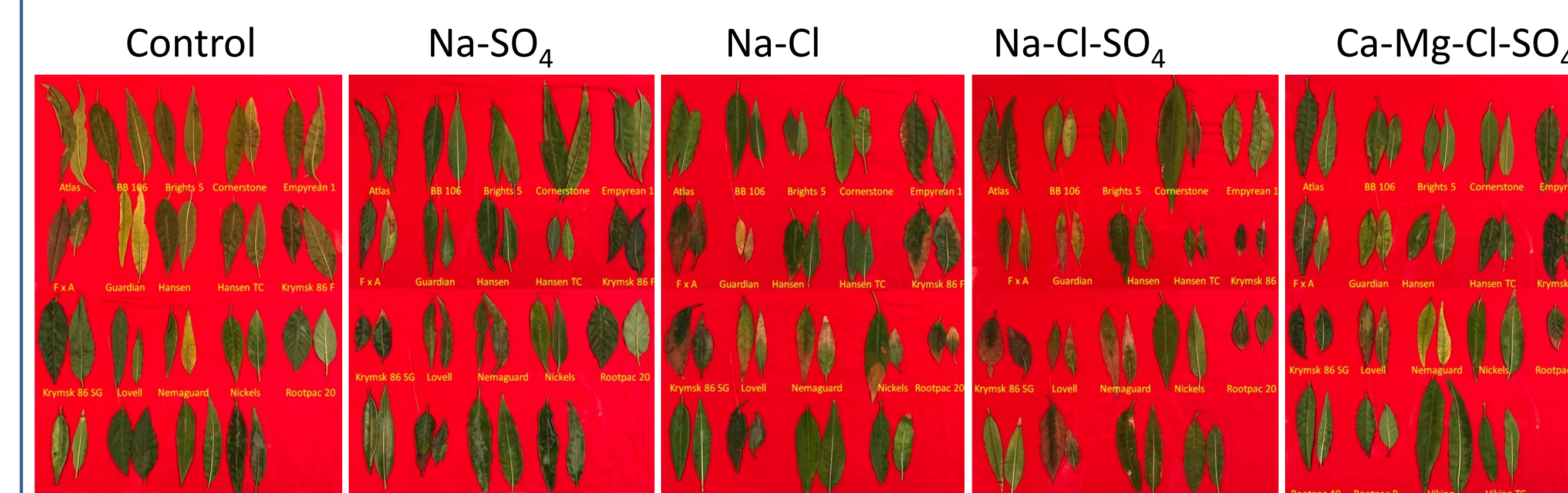
- Experiment was set up in a randomized complete block design
- Non-grafted plants of 16 different rootstocks
- 3 replications
- 3 plants per replication (one plant per pot)
- 5 treatments of water (irrigation water composition) with total 720 trees.
- 15 blocks, each containing combinations of genotypes and replications
- Five different treatments of mixed irrigation solutions used.
  - 1) control
  - 2) a mixed cation composition with Na<sup>+</sup> and SO<sub>4</sub><sup>2-</sup> as the predominant ions
  - 3) a mixed cation composition with Na<sup>+</sup> and Cl<sup>-</sup> as the predominant ions
  - 4) a mixed SO<sub>4</sub><sup>2-</sup>:Cl<sup>-</sup> anion composition with Na<sup>+</sup> as predominant cation and
  - 5) a mixed SO<sub>4</sub><sup>2-</sup>:Cl<sup>-</sup> anion composition with Ca<sup>2+</sup> and Mg<sup>2+</sup> as predominant cations
- Samples were screened at moderately low salinity levels (EC =3 dS/m).



**Figure 2.** Percent change in trunk diameter in different salt treatments. Plants were allowed to grow in different treatments for six months and differences in trunk diameters were calculated. Na<sup>+</sup> plays most important role in ion toxicity followed by Cl<sup>-</sup>.

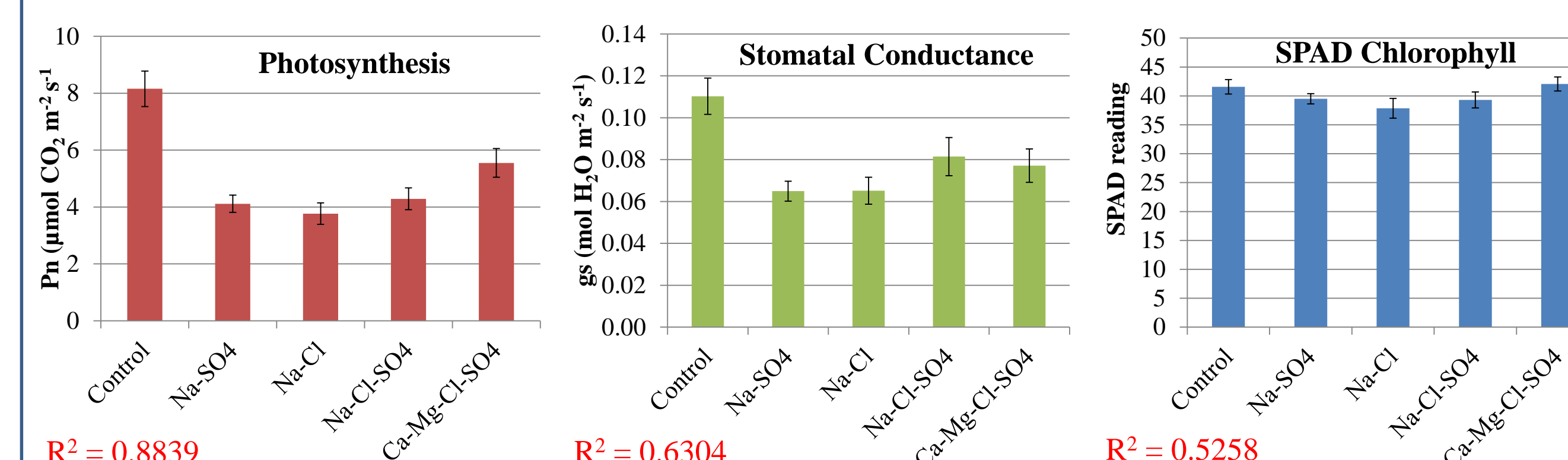


**Figure 3.** Relative change in trunk diameter in 16 almond rootstocks under different salt treatments. Relative change was calculated by comparing each treatment with the control



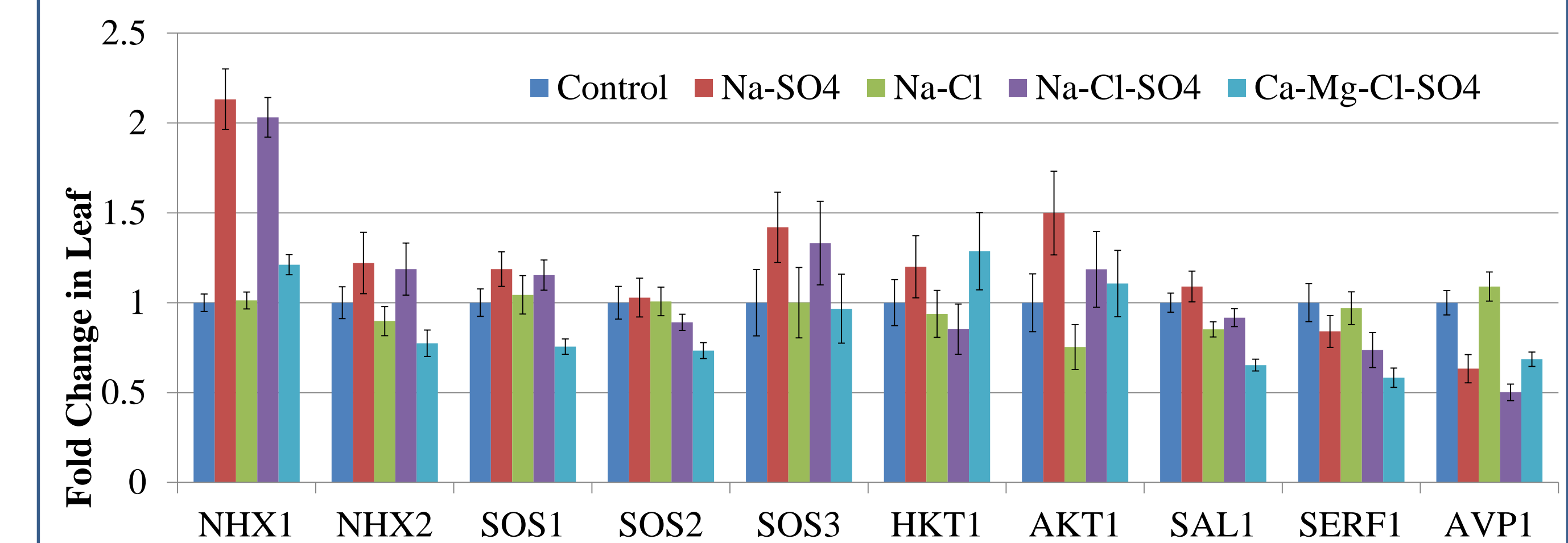
**Figure 4.** Phenotypic effects of different salt treatments on the leaves of almond rootstocks. The treatments that were high in Na-Cl or Na-Cl-SO<sub>4</sub> displayed most severe symptoms.

### 2. Characterization of physiological and biochemical markers associated with salt tolerance and salt composition of irrigation water in almond rootstocks

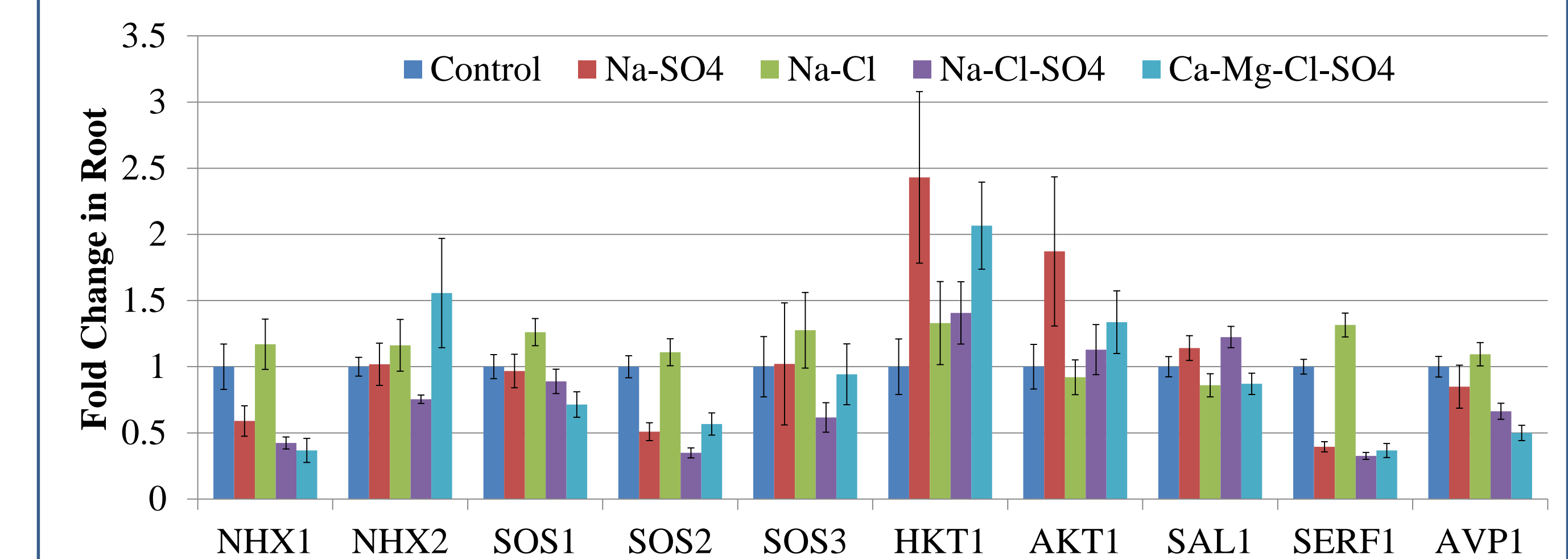


**Figure 5.** Physiological measurements in almond rootstocks under different salt treatments. Correlation between each of the parameters and trunk diameter is represented as R<sup>2</sup>. Photosynthesis showed highest correlation with change in trunk diameter. Stomatal conductance and SPAD chlorophyll also presented significant correlation with trunk diameter.

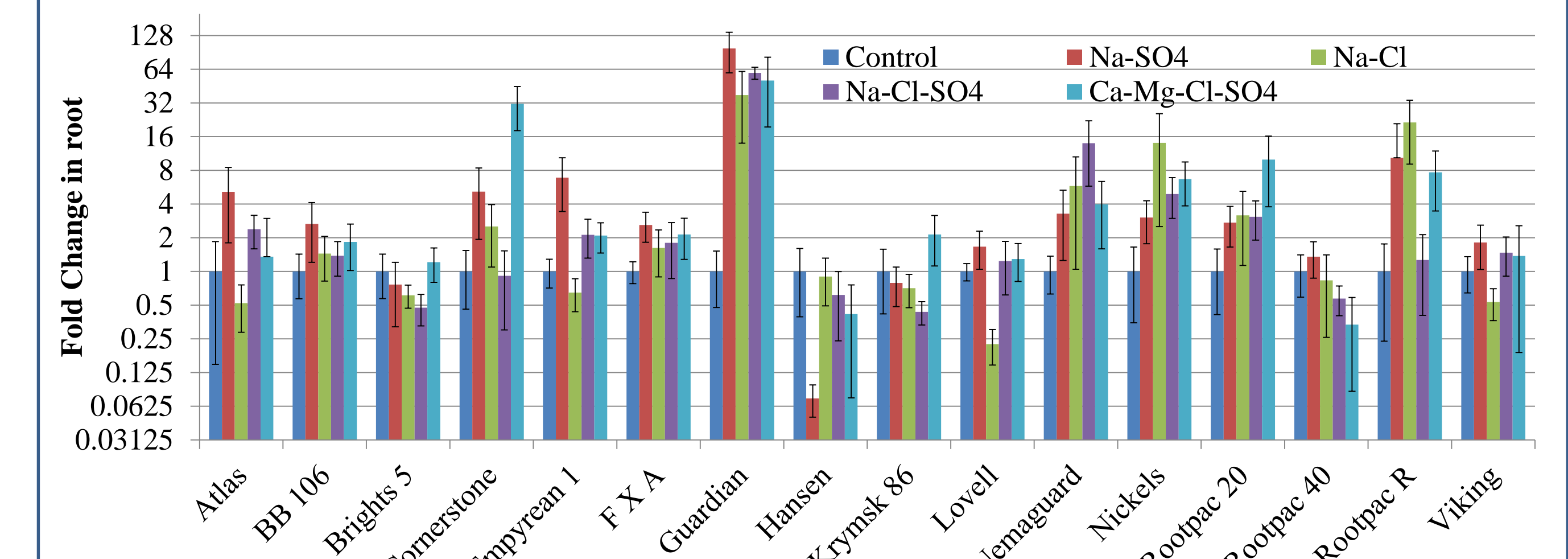
### 3. Identification and characterization of genes involved in salinity tolerance in almond rootstocks



**Figure 6.** Expression analysis of salt related genes in almond leaves. Fold change calculations were done by comparing expression of each treatment with the control. NHX1, SOS3 and AKT1 were significantly upregulated in at least of the salt treatments.



**Figure 7.** Expression analysis of salt related genes in almond roots. Fold change calculations were done by comparing expression of each treatment with the control. HKT1 and AKT1 were significantly upregulated in multiple salt treatments.



**Figure 8.** Expression analysis of *HKT1* in the roots of 16 almond rootstocks under 5 salt treatments. Fold change calculations were done by comparing expression of each treatment with the control.

## Conclusions

- There was maximum reduction in trunk diameter when irrigation water was high in Na<sup>+</sup> and Cl<sup>-</sup> suggesting that mostly Na<sup>+</sup> and to a lesser extent Cl<sup>-</sup> concentrations 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.
- *NHX1*, *SOS3* and *AKT1* were highly upregulated (expression) in salinity treatments in leaves
- *HKT1* and *AKT1* showed the highest upregulation in salinity treatments in roots

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