



# The Genetics of Salt Stress Tolerance in Arabidopsis

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## Abstract

Rising soil salinity levels are emerging as a significant challenge due to factors such as irrigation practices, climate change-induced sea level rise, soil degradation, and anthropogenic factors such as road salting. Investigating salt tolerance mechanisms in plants is crucial for agricultural sustainability, as high soil salinity inhibits crop growth and reduces yield, threatening global food security. We wondered if certain genes within the plant *Arabidopsis thaliana* could be linked to higher salt tolerance as understanding the genetic and physiological basis of salt tolerance in an extensively studied plant could guide the development of resilient crop varieties capable of thriving in saline environments and may have implications for drought resistance. Using two *Arabidopsis thaliana* ecotypes, Columbia (Col) and Landsberg (Ler), we compared the effects of water without a stressor (control) and water with NaCl treatment (variable) as well as comparing the results of the ecotypes with each other. Our results showed the following statistical differences: Water retention in the Col control vs Ler control, with Col control retaining more water than Ler control ( $p=3.34 \text{ E-}10$ ); Water retention in Col control vs Col treatment, with Col control retaining more water than Col treatment ( $p=1.33 \text{ E-}11$ ); Water retention in Ler control vs Ler treatment, with Ler control retaining more water than Ler treatment ( $p=2.14 \text{ E-}11$ ); Water retention in Col treatment vs Ler treatment, with Col treatment retaining more water than Ler treatment ( $p=0.0053$ ). The results indicate that there are genes present within *Arabidopsis thaliana* ecotypes that could lead to water retention when in the presence of soil salinity stress. Following the parent ecotypes, recombinant inbred lines (RILs) were used to further study the correlation in genes to water retention. Fifteen RILs were studied, and an ANOVA was run. With the RIL dataset, a QTL map was generated. Future research involves a more in-depth look of these gene regions in both ecotypes.

## Parentals

### Methodology:

The statistical difference for each comparison was determined by running a two tailed t-test with unequal variance. If a p value was calculated to be less than 0.05 it was determined that the difference was statistically significant.

### Data and Results:

Is there a statistical difference between water retention in the controls? Yes, Col retains more water than Le.

$p = 3.34584\text{E-}10$

Is there a statistical difference between water retention in the Col control vs Col NaCl?

Yes, Col control retains more water than Col treated with a NaCl solution.

$p = 1.333\text{E-}11$

Is there a statistical difference between water retention in the Ler control vs Ler NaCl?

Yes, Ler control retains more water than Ler treated with a NaCl solution.

$p = 2.14\text{E-}08$

Is there a statistical difference between water retention in the Col NaCl vs Ler NaCl?

Yes, Col treated with a NaCl solution retains more water than Ler treated with a NaCl solution.

$p = 0.0053$

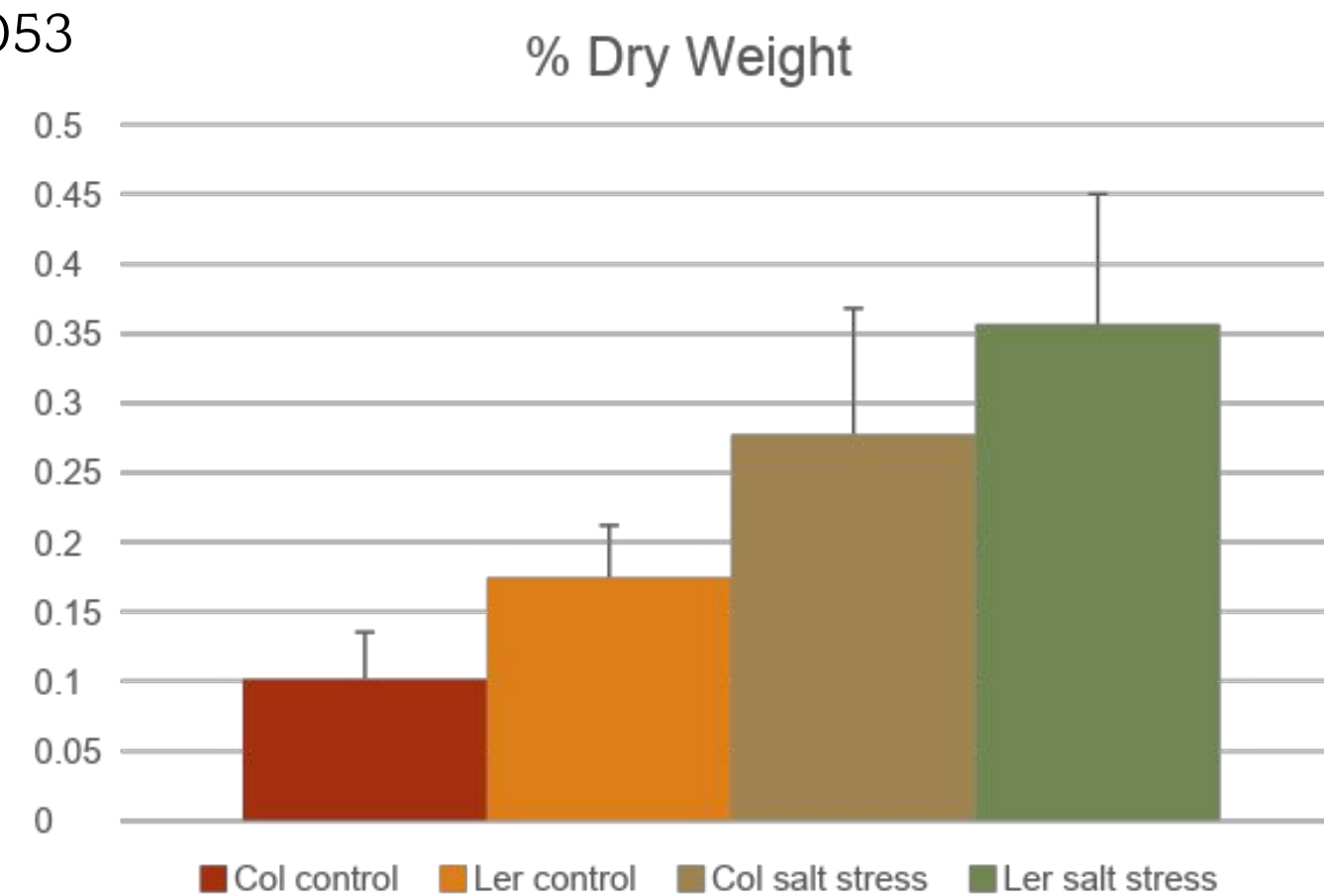


Fig. 1: Parental water retention comparison experiment results. For each parental, a control group of with regular water treatment and a experimental group treatment of salt stress via salt water was run. The dry weight percentage for each plant in the groups was calculated, and averaged. With statistical analysis, it was found that there was a statistically significant difference between Columbia and Landsberg water retentions, with Columbia retaining more.

## Recombinant Inbred Lines



Figure 2 *Arabidopsis thaliana* in the growth chambers  
Plants were grown in a growth chamber under 24 hour lighting at 22C. Watering was 4 times per week (M, W, F, S). After 3 weeks of growth, the experimental population recieved 50ml of 400mM NaCl, while the control recieved 50ml of tap water, for 7 days (4 waterings). Plants were the allowed to recover for 4 days, then wet weight (cut at the rosette) and dry weight (after 1 day out) were taken.

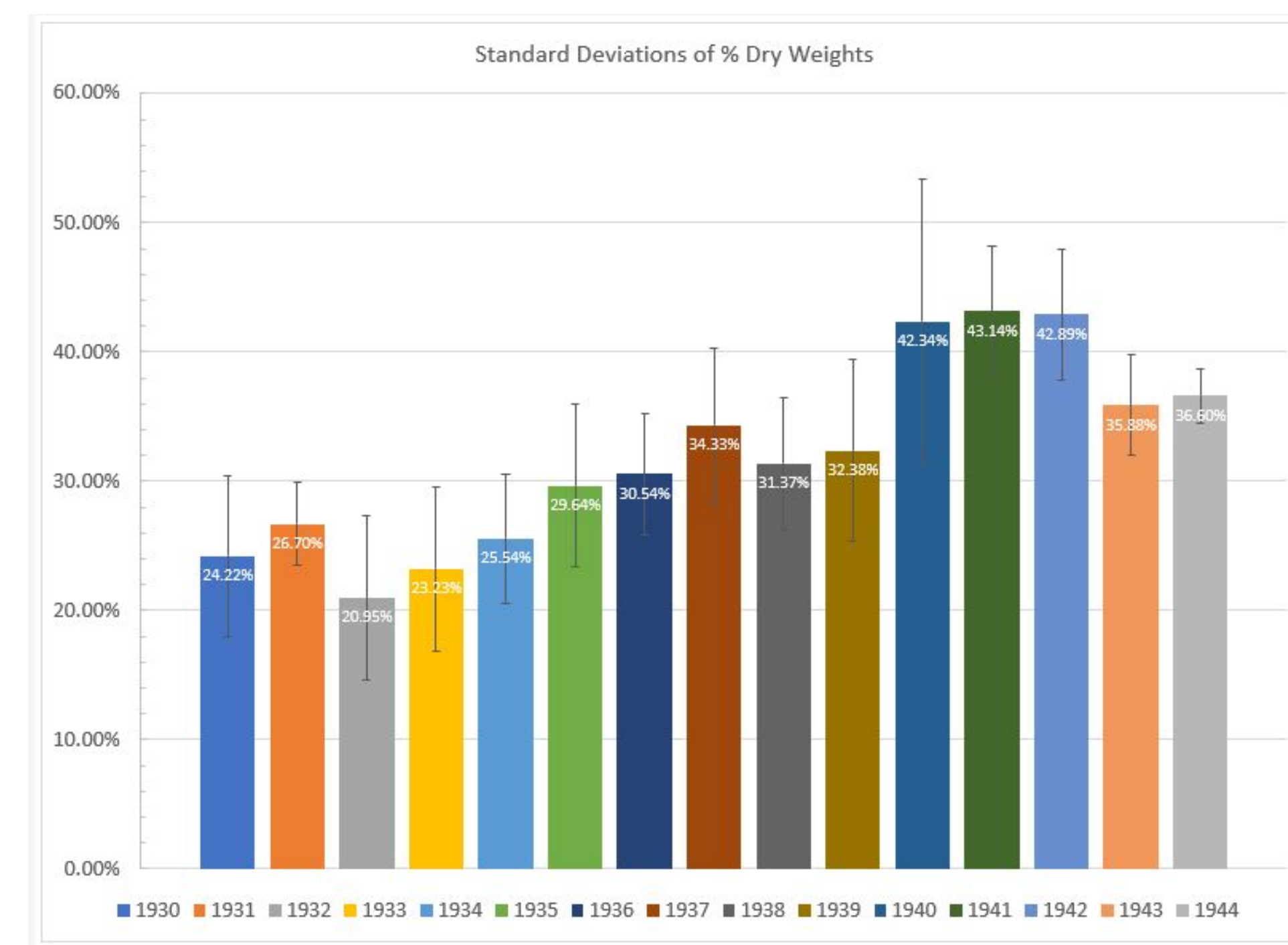


Figure 3: Dry weight for *Arabidopsis thaliana* inbred lines. Two pots were planted for each inbred line with one pot receiving tap water and the other salt water. The percentage dry weight was calculated for each inbred line and a standard deviation was added. It was found that different inbred lines did in fact have different salt tolerance ability with lines 1940, 1941, and 1942 having the highest percent dry weight.

Wet Weight - Dry Weight = Water Retention of Plant

## QTL Mapping

### Methodology:

To assess biomass accumulation under drought conditions in *Arabidopsis thaliana*, both fresh (wet) and dry weights of plants were measured following a controlled drought treatment. Plants were grown in a controlled environment chamber until reaching the desired developmental stage, after which salt water was added or water was withheld to induce water stress. After a set amount of time, wet weights were collected immediately via plant removal from pots, followed by a drying period to obtain dry weights. The difference between wet and dry weights was calculated to determine water retention. Biomass data was statistically analyzed to evaluate the impact of drought treatment on plant growth. Subsequently, Quantitative Trait Loci (QTL) analysis was performed to identify genomic regions associated with drought tolerance traits, linking phenotypic observations with underlying genetic mechanisms. The different phenotypes and genetic strains of *Arabidopsis thaliana* were mapped using QTL in order to compare the collected drought stress data with actual genetic sequence.

### Data and Results:

The different recombinant inbred lines that we graphed showed a correlation between the genetic sequences of the different phenotypes and the drought tolerance data that we collected (See Figure 4). As seen within the graph, there were many peaks that indicate linked markers in the genetic sequence to our drought tolerance characteristic. The best supported QTL appear to be the peaks that rise about 1.25 lod. More research will have to be done in order to determine whether the phenotypic differences are primarily due to a few loci with fairly large effects, or to many loci, each with minute effects.

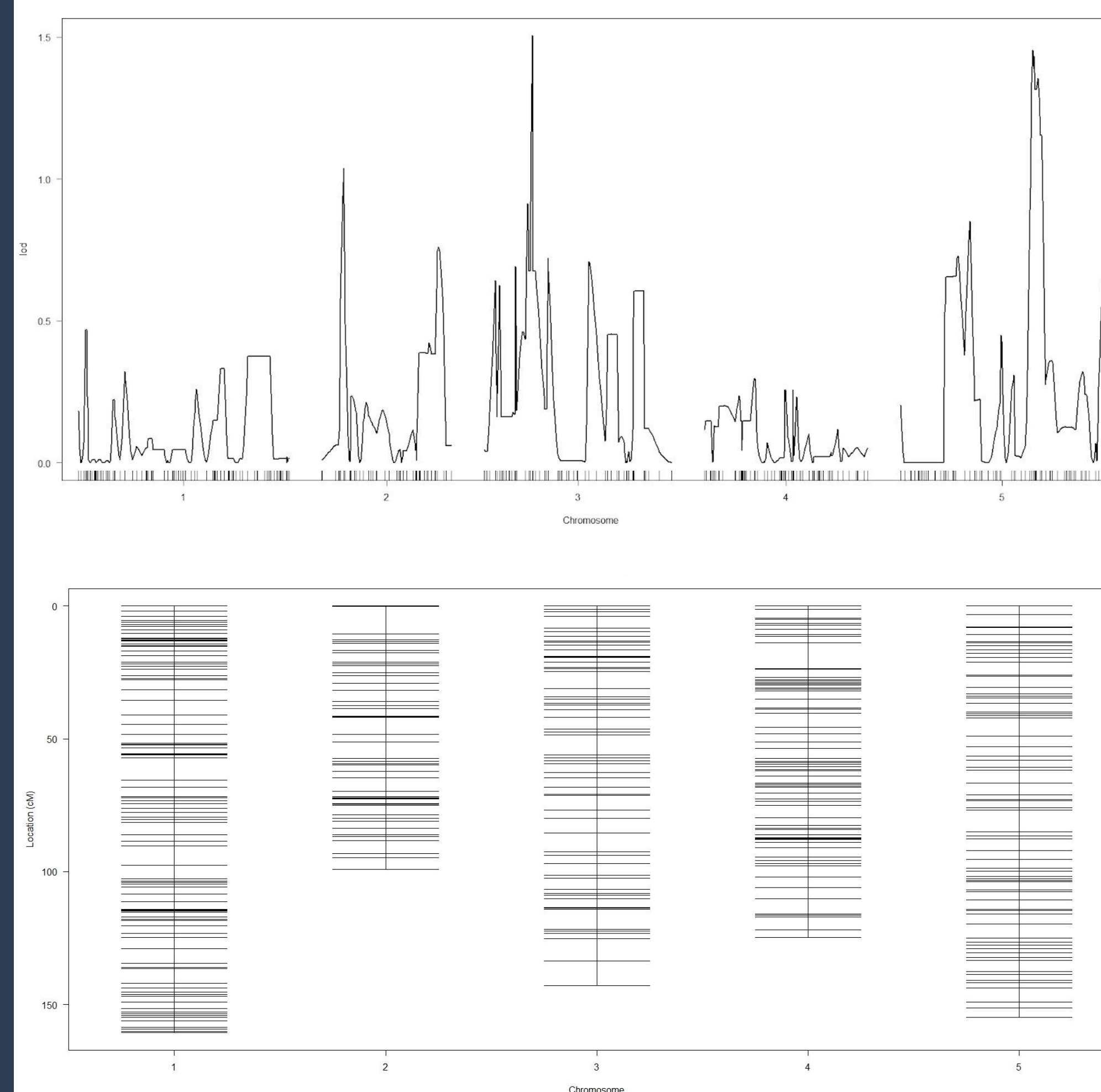


Figure 4: Quantitative trait loci (QTL) analysis reveals the genetic basis of drought tolerance in *Arabidopsis thaliana*. We examined the probability that a marker or an interval between two markers is associated with a QTL affecting the trait. The results of such an analysis are presented as a plot of the test statistic against the chromosomal map position. Identified genomic regions highlight key genetic markers, shedding light on the molecular mechanisms underlying this adaptation in the plant species.

## Conclusion

- There was statistical significance that Colombia *Arabidopsis thaliana* retains more water than Landsberg *Arabidopsis thaliana* when receiving both tap water treatment and the salt water treatment.
- There is also statistical significance that the control plants receiving tap water retain water better than the experimental plants receiving salt water.
- The recombinant inbred lines displayed transgressive segregation: phenotypes in some of the recombinant inbred lines were more extreme than either parental control.
- Our initial map shows several peaks worth further investigation

## Future Directions

- The observation of salt/drought resistance and sensitivity in two *Arabidopsis* ecotypes raises the possibility that more extreme phenotypes exist in the species. There are more than 1000 different ecotypes collected from a variety of habitats around the world available in academic seed banks. It may be possible to identify even more resistant ecotypes for further investigation.
- This study used salt tolerance as a proxy for drought tolerance. This idea needs to be more thoroughly investigated, with Col and Ler parents subjected to true drought conditions to see if they mimic the salt tolerance studies.
- This is a preliminary map constructed using less than 20 recombinant inbred lines. To have confidence and gain resolution in our map, more recombinant inbred line accessions will be tested.
- We can then map the genes to see at which specific loci the trait for salt tolerance lies. Going forward we could dive into figuring out possible candidate genes.
- Once we are confident in our map, identified QTLs will need to be further investigated to identify candidate genes for testing.

## Swanson Lab:

We would like to thank Dr. Swanson for his mentorship during this project and following projects, and Valparaiso University for the opportunity to do this research.



Left to right: Lilli Goss-Peirce, Jodie Brasseur, Dr. Robert Swanson, and Alyssa Suprenant