Salinity tolerance in *P. hallii*

By Sonali Singh

Introduction

Why salinity tolerance?

- Salt stress occurs when excessive salts in soil solution cause inhibition of plant growth or plant death

  - Poor irrigation/ drainage systems

  - Previous exposure to seawater

  - High evaporation rates

- High salt levels in soil affect approximately **20%** of irrigated lands across the world

- In the future, more land will need to be used to support increased food and biofuel production

Panicum hallii

- Genetic model for the biofuel producing species, *P. virgatum*
  - Diploid vs. tetraploid/ octoploid

- *P. hallii var. hallii* (HAL) is typically found in drier/ inland climates
  - Central Texas, Coastal, Sympatry

- *P. hallii var. filipes* (FIL) is typically found in more coastal climates
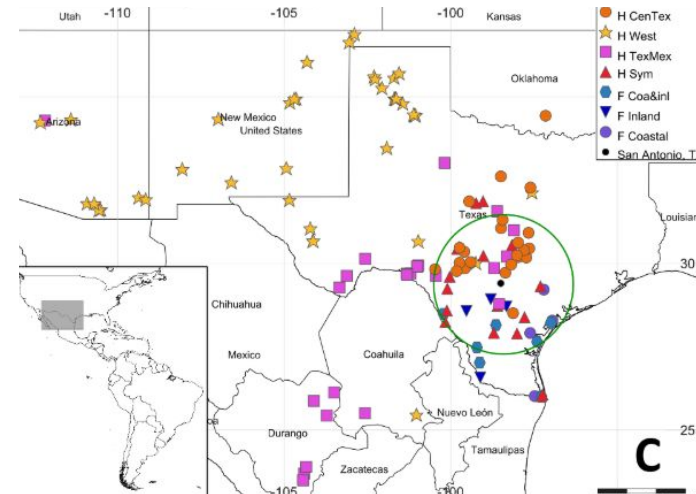
In the past

- Tobacco plants

    - Upregulation of a specific gene or set of genes, allowing them to combat abiotic stresses

    - Positive responses to genetic manipulation aiming to increase salt tolerance

- ABA-deficient Arabidopsis mutants

    - ABA improved the ability for plants to tolerate abiotic stresses

    - Downregulation of genes that limited ABA

WE SOUGHT TO TEST AND ANSWER THE FOLLOWING

research questions

1. Are there differences in responses to increased salinity within HAL?

2. Are there differences in responses to increased salinity between HAL and FIL?

3. Is there a difference when QTL mapping HAL and FIL to two reference genomes?

Methods

Experimental Setup

1. Germinated 40 seeds from 24 distinct varieties of HAL for 2 weeks in greenhouse

2. Transplanted ~20 seeds from 6 varieties of HAL and watered every other day for 3 weeks, after which 6 plants from 3 distinct HAL varieties remained

3. Transplanted 24 additional FIL plants to expand experiment

4. All plants were grown for 1 more week, being watered every other day

5. Began 7 day experimental period of control, 150mM, 200mM, and 250mM salt treatments using 42 total plants (24 FIL and 18 HAL)

6. Plants were watered every other day, with the first day consisting of half the treatment concentration to allow for acclimation

	CONTROL	
F	F	F
F	F	F
H (A)	H (A)	H (A)
H (P)	H (P)	H (P)
H (W)	H (W)	H (W)

CONTROL

	150 mM	
F	F	F
F	F	F
H (A)	H (A)	H (A)
H (P)	H (P)	H (P)
H (W)	H (W)	H (W)

150 mM

	200 mM	
F	F	F
F	F	F

200 mM

	250 mM	
F	F	F
F	F	F

250 mM

- Set number of FIL and HAL in each, randomly assigned to a tray

- Randomized within trays every other day

- Tray position randomized every other day

Traits Measured

Above Ground Biomass

   a.   Cut plants above root and measured in grams

Chlorophyll (A, B and Total)

   a.   Measured fresh leaf weight in 5mL test tube

   b.   Added 3 mL of 80% acetone and stored in dark for 24 hours

   c.   Ran in spectrophotometer

Leaf Water Potential

   a.   Measured second-youngest fully grown leaf in pressure bomb

Relative Water Content

   a.   Measured fresh weight of one cut leaf in 5mL test tube

   b.   Added 1 mL of water to tube and soaked for 24 hours before measuring turgid weight

   c.   Dried leaves for 72 hours and measured dry weight

Osmotic Potential

   a.   Froze one cut leaf for 72 hours

   b.   Centrifuged to extract sap

   c.   Measured sap in osmometer

Results & Interpretations

SIGNIFICANT DIFFERENCES BETWEEN
Salt Levels

TRAIT	SIGNIFICANT	IN FAVOR OF
Biomass	Yes	Control
Chlorophyll	No	-
Water Potential	Yes	Control
RWC	Yes	Control
Osmotic Potential	Yes	Control

SIGNIFICANT DIFFERENCES BETWEEN
HAL vs. FIL

TRAIT	SIGNIFICANT	IN FAVOR OF
Biomass	No	-
Chlorophyll	No	-
Water Potential	Yes	HAL
RWC	Yes	FIL
Osmotic Potential	No	-

Overall Chlorophyll
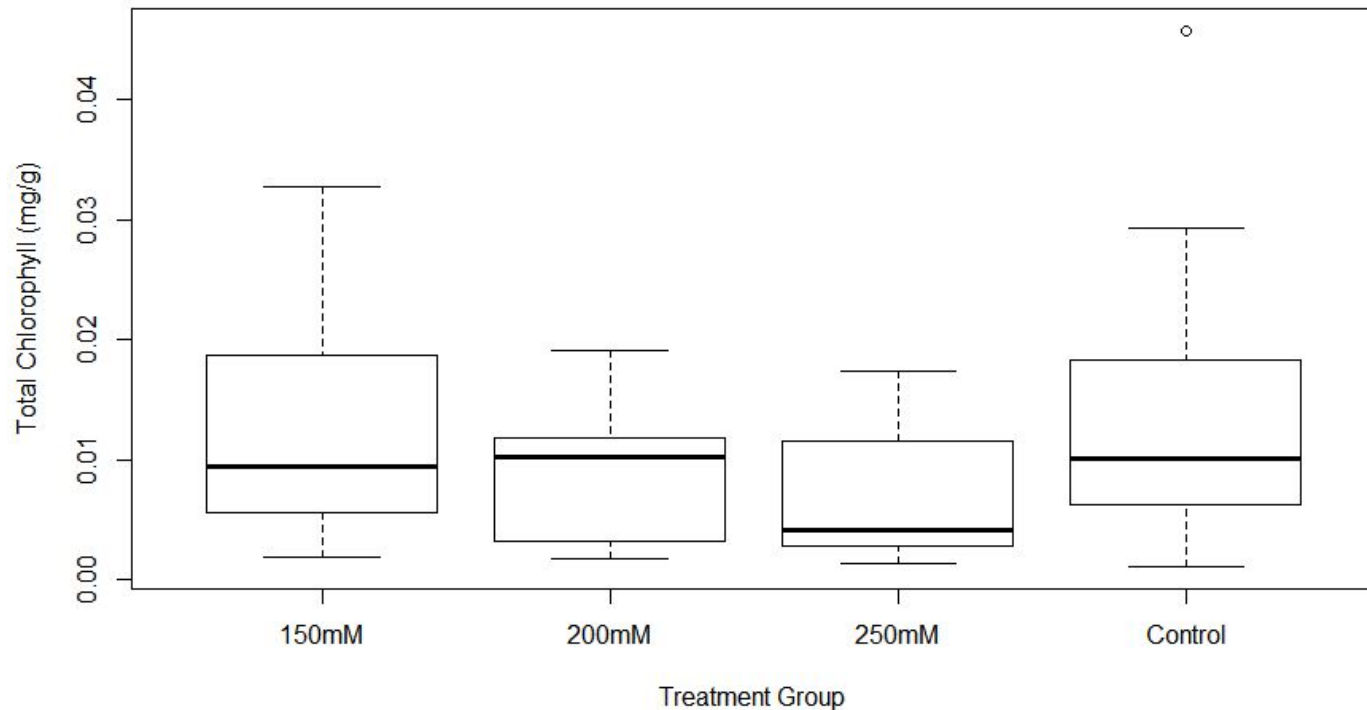
150 p-value = 0.5639
200 p-value = 0.2416
250 p-value = 0.08658

Overall Water Potential
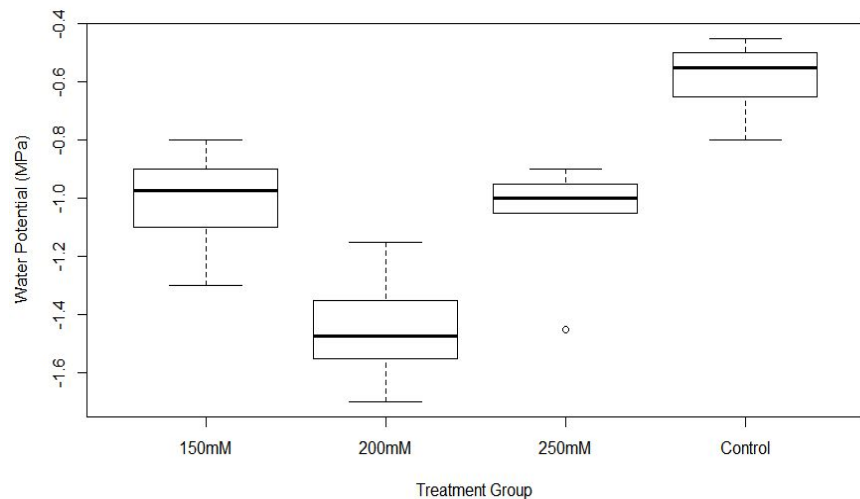
Overall Shoot Mass

150 p-value: 1.246e-08
200 p-value: 2.525e-5
250 p-value: 0.001196
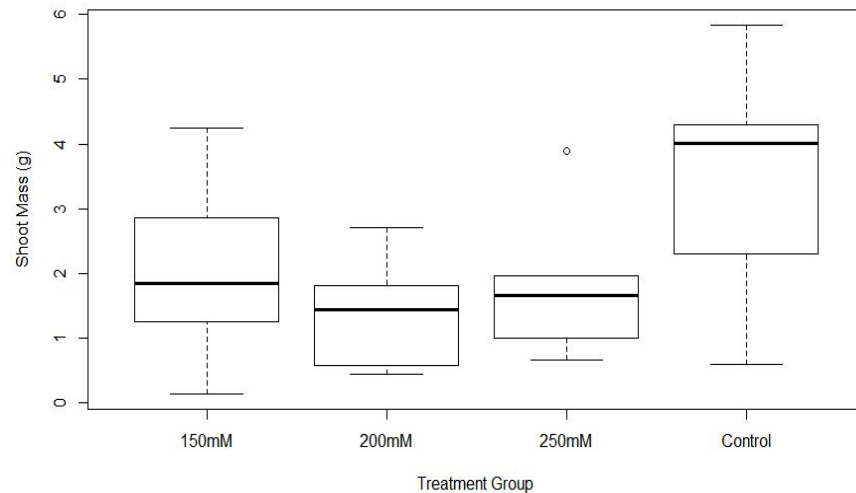
150 p-value: 0.01046
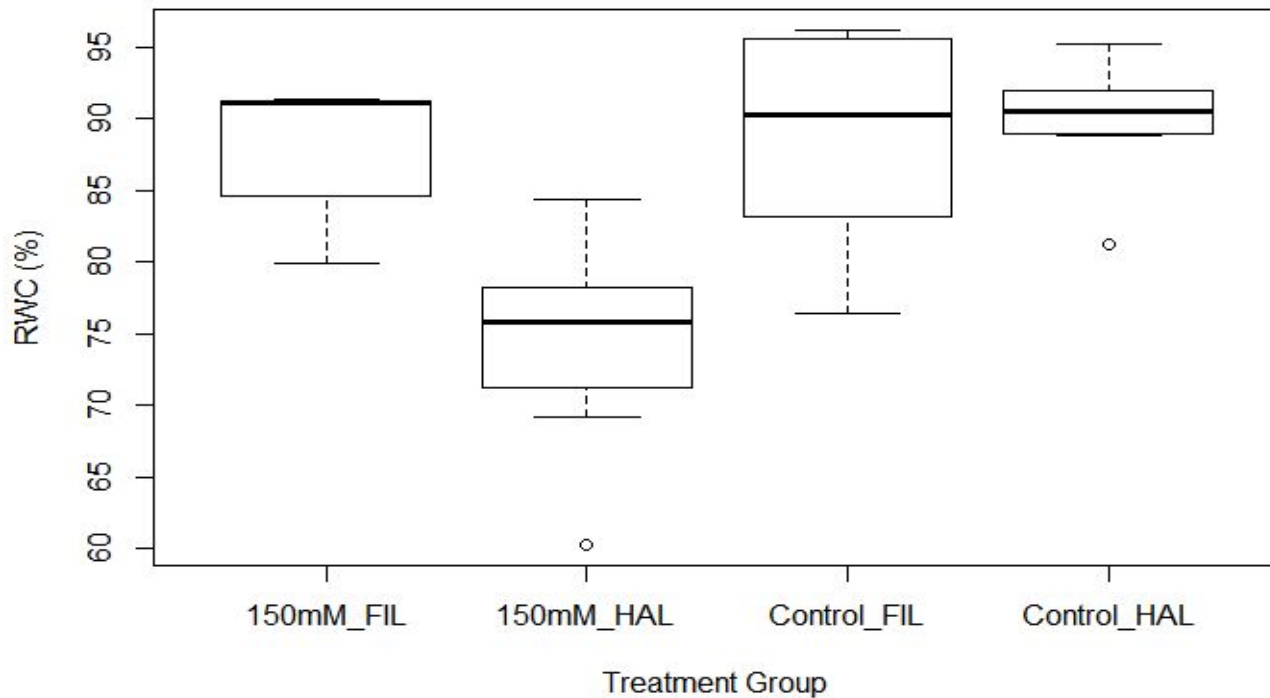200 p-value: 0.001465
250 p-value: 0.01976

HAL vs. FIL Water Potential
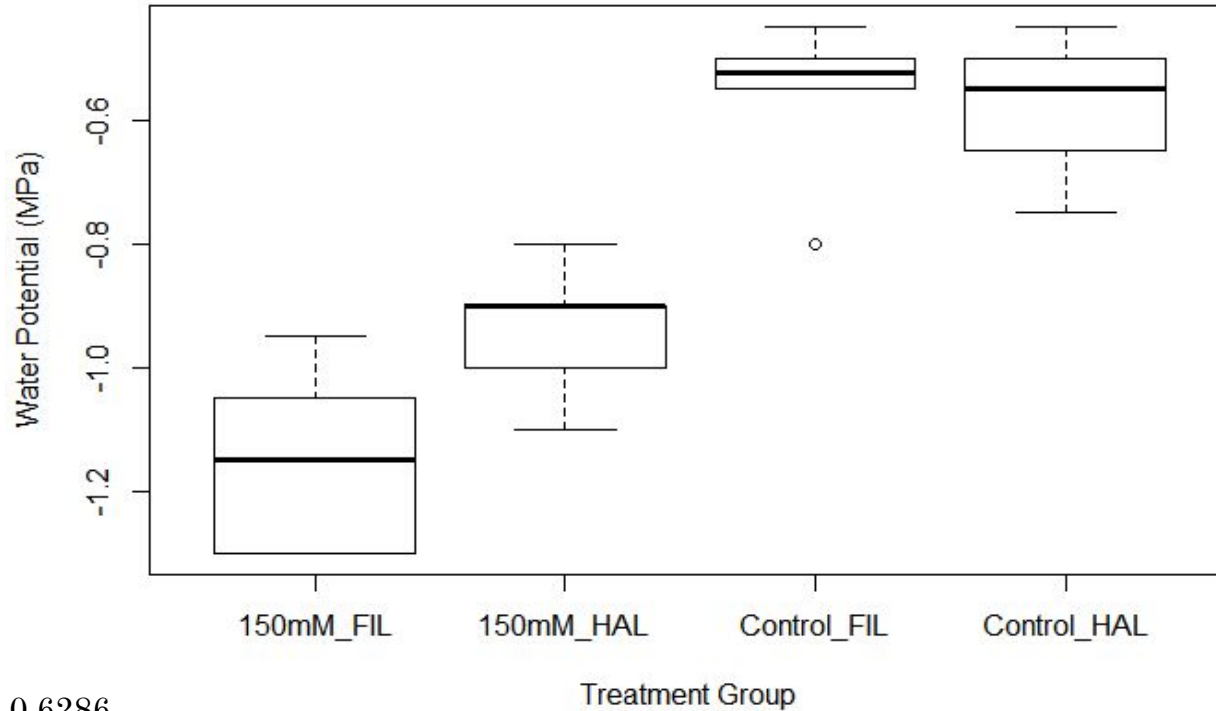
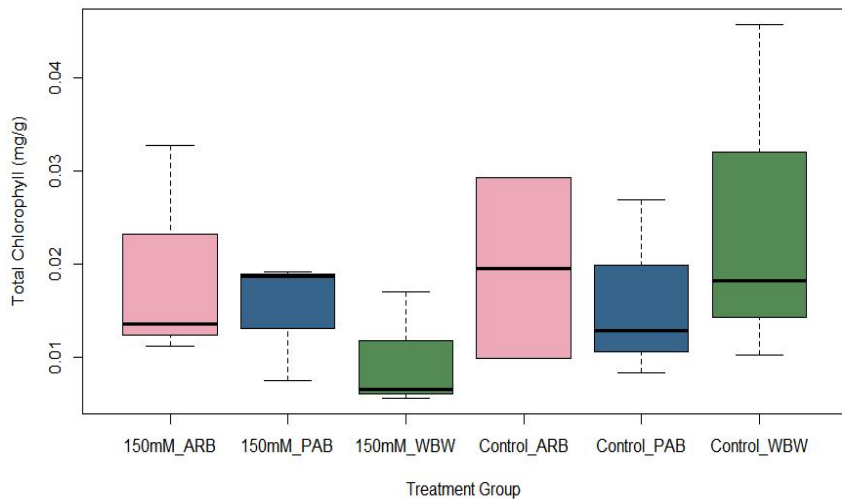Control p-value: 0.6286
150 p-value: 0.03037
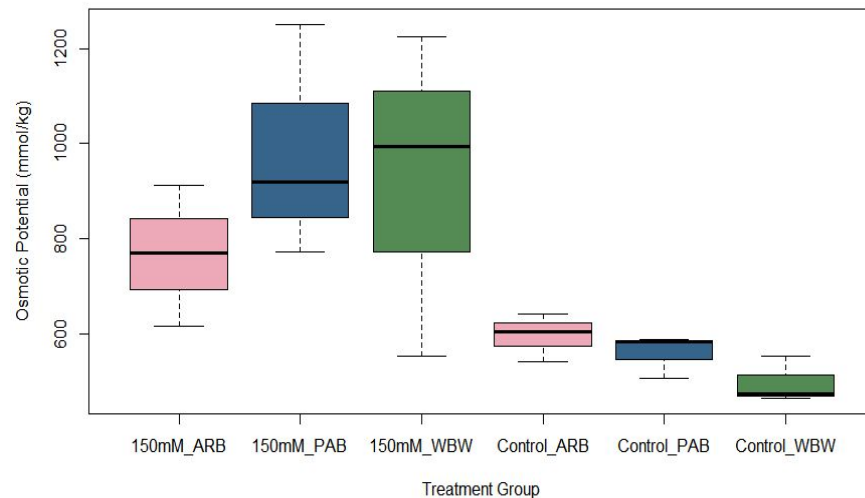
HAL vs. FIL RWC

Control p-value: 0.6736
150 p-value: 0.02256

Variation Within HAL

HAL Total Chlorophyll

HAL Osmotic Potential

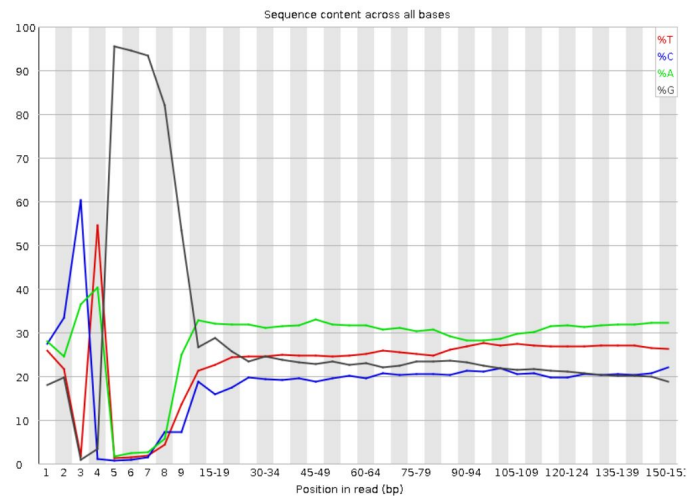# Bioinformatics

Coding Setup

1. Bunzip the files

    a. Converting from binary format (BAM) to a readable format (SAM)

2. Quality report

    a. Quality filter to trim primer sequences

3. Mapping

    a. Unspliced mapping using BWA

4. Filter mapping results

    a. Trim out low quality regions

5. Analysis

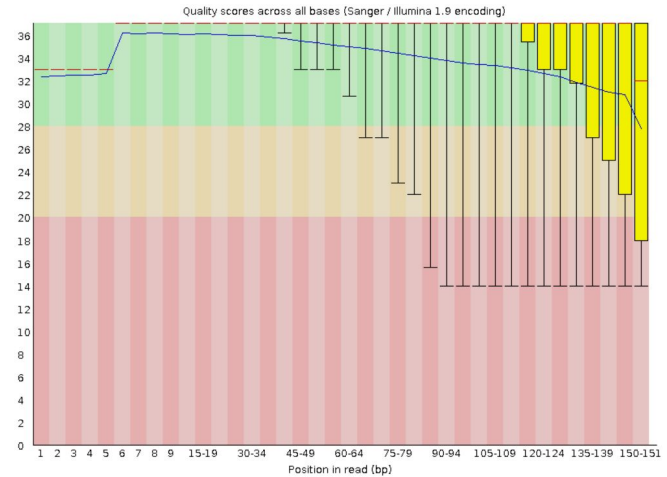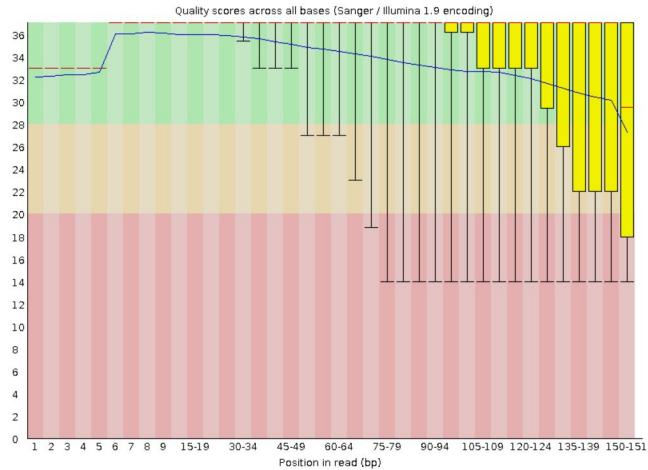   a. Convert SAM back to BAM and get a matching percentage
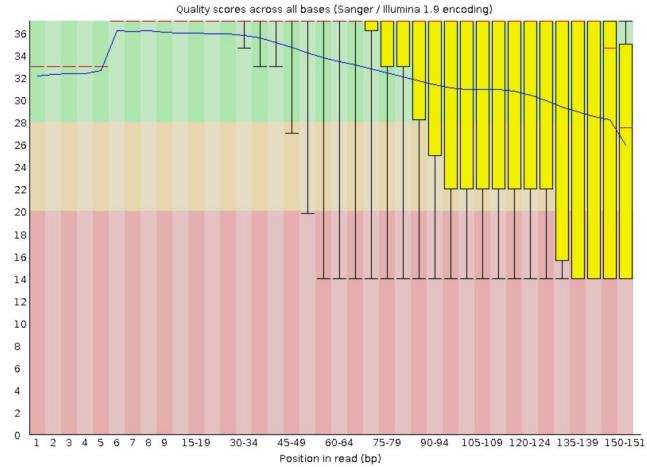
Quality Trimming

AS DONE IN STEP 2
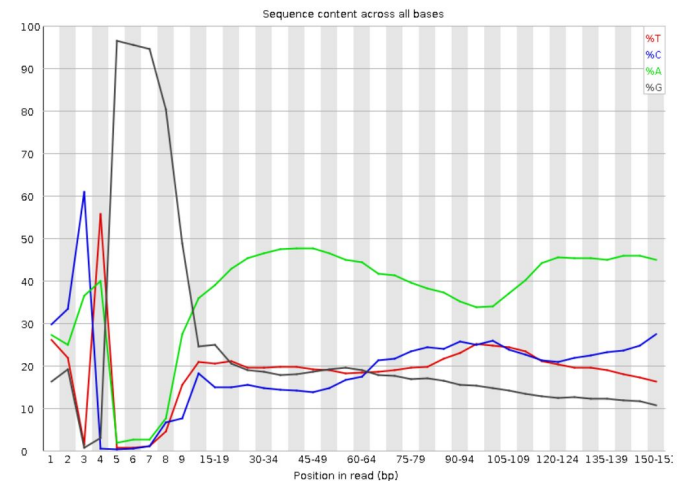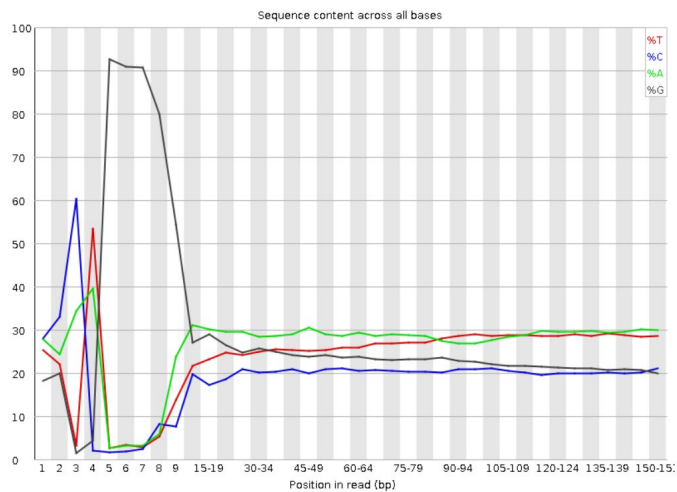
Quality Trimming

AS DONE IN STEP 4

Conclusions & Future Directions

Looking back

Are there differences in responses to increased salinity within HAL?

Yes, but our treatment size was not large enough to look into these statistically.

Are there differences in responses to increased salinity between HAL and FIL?

Overall, FIL reacts better to salt stress, with the exception of water potential.

Is there a difference when QTL mapping HAL and FIL to two reference genomes?

There is a reference bias when mapping HAL and FIL to the same genome.

Future Directions

- Repeat HAL vs. FIL experiment in greenhouse, collect RNA, and do RNAseq on those specific plants to correlate phenotypic and genomic expression

- Add on another variable of environment and grow both HAL and FIL in their natural environment and the other variety's environment

- Extend the length of the treatment to measure long term effects under more subtle conditions

References

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water deficits in leaves. Aust. J. Biol. Sci. 15:413–428.

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