Gene expression of CYP72A enzymes in corn in response to combinations of stressors

Presented by: Netree Pandya

Introduction

- The consumption of grain accounts for more than 50% of the world daily caloric intake
- Various problems that arise in the fields are due to biotic and abiotic stresses such as: pests, drought, heat and salinity
    - price spikes and food shortages
- Tolerance to a combination of different stresses vs. single abiotic and biotics stresses
- Plants respond to abiotic stresses by changing their gene expression levels
    - acclimate to extreme environments in order to sustain their growth and productivity
- The CYP family genes encode Cytochrome P450 enzymes that help regulate biochemical responses in maize plants
- This project aims to determine the relationship between CYP72A28 and CYP72A349 gene expression in maize plants under stress.

Background

- CYP72A349 mutants actually showed no difference in caterpillar stress response after isolating mutants lacking CYP72A349 activity (Chauhan, 2018)
    - Another gene compensating for the absence of CYP72A349 or biotic stress works in the combination with abiotic stress response
- What is the role of CYP72A genes, specifically CYP72A349 and CYP72A28, during the acclimation process under biotic and abiotic stresses?
- RT-PCR
- Hypothesis: CYP72A28 and CYP72A349 are important for environmental stress responses by being induced by a combination of abiotic and biotic stresses.

Experimental Design

Overall Question: Whether 72A28 is being upregulated in the absence of 72A349 using specific primers ordered for RT-PCR (A28+A349)

- First step: Establish PCR conditions that are able to amplify each of the genes (A28 and A349) separately

- Second step: Perform RNA extraction that are exposed to different stresses to address the induction of genes in wild-type and mutant plants

- Third step: Perform RT-PCR to see changes in gene expression for each of the genes (A28 and A349) under drought and heat stress

Experimental Design

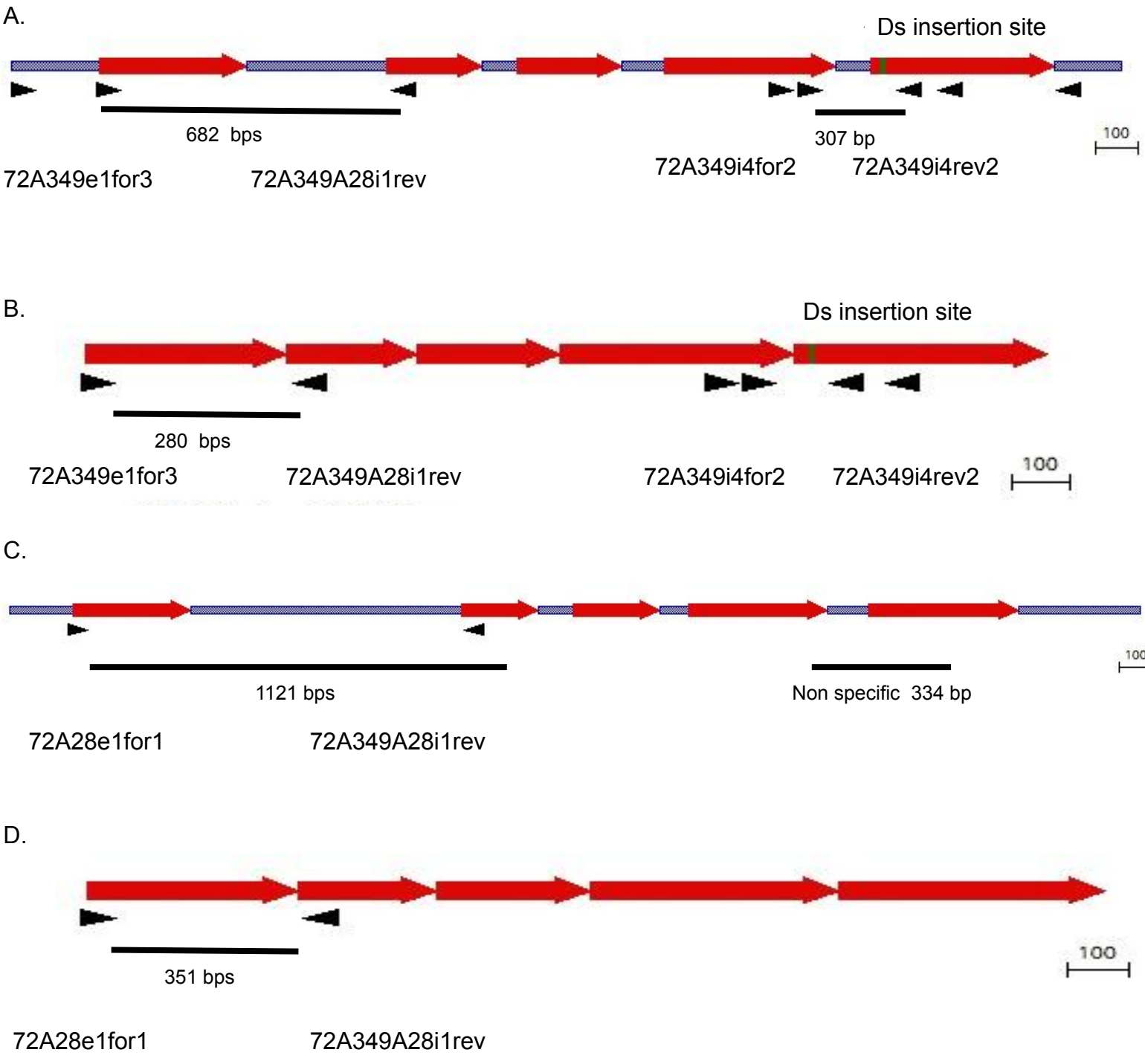
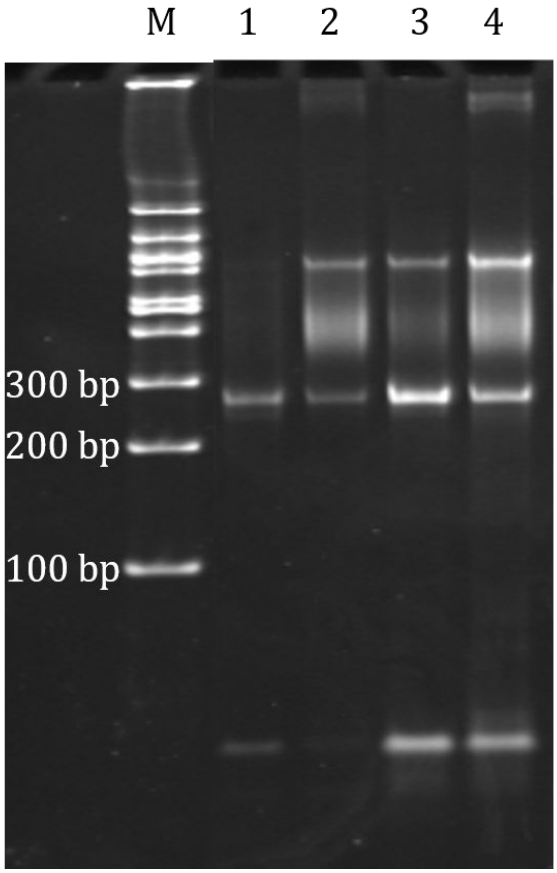


Figure 1. Gene map to visualize PCR products. The exons (red) and introns (blue) are shown including the base pairs that are specific to the gene. The scale is out of 100.

How does PCR work?



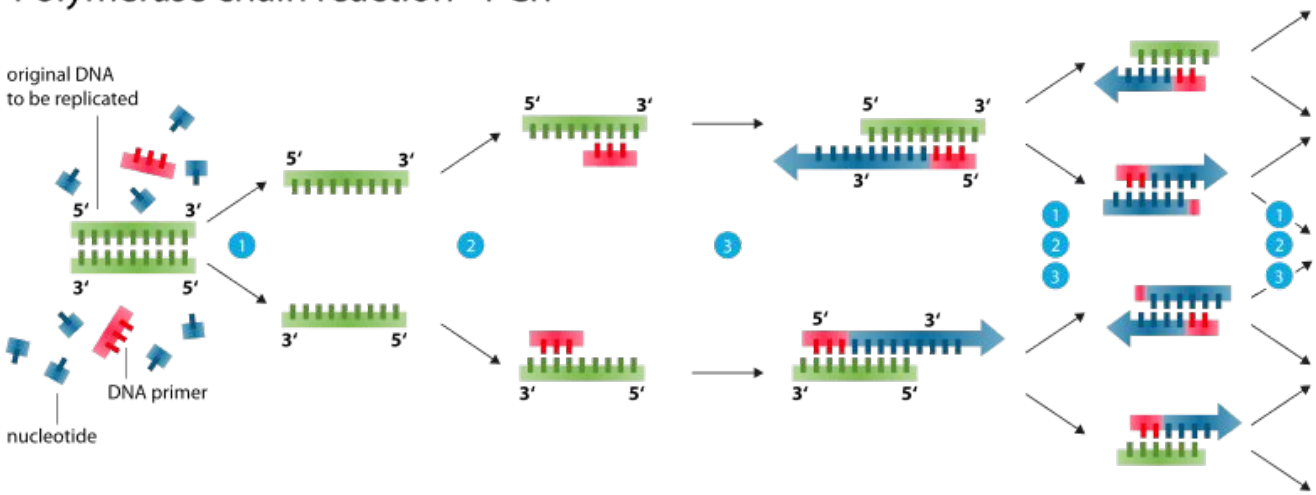
Non-specific
Large PCR product

← Expected size ~259

1=10uM primer, 10cycles
2=10uM primer, 20cycles
3=100uM primer, 10cycles
4=100uM primer, 20cycles

← Unused primer

Polymerase chain reaction - PCR



1 **Denaturation** at 94-96°C
2 **Annealing** at ~68°C
3 **Elongation** at ca. 72 °C

Results: PCR Optimization

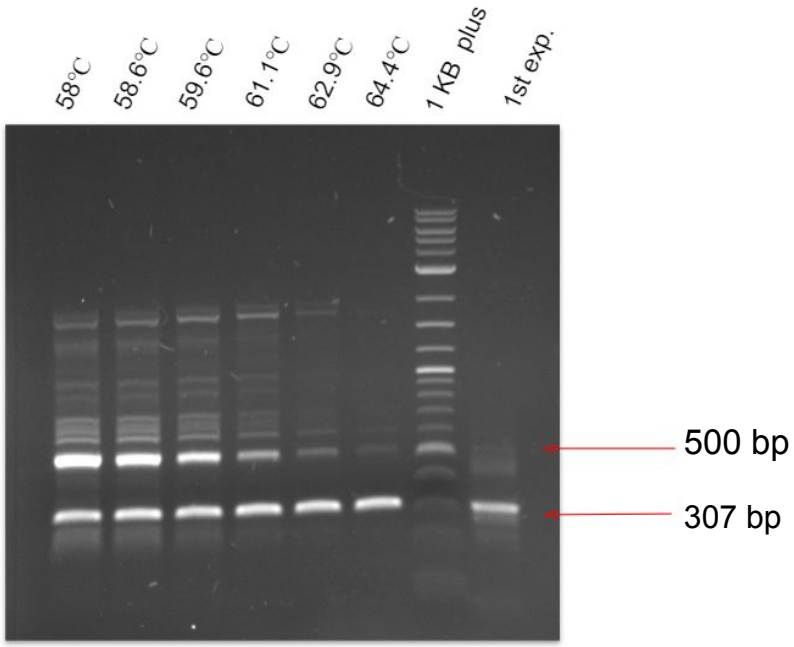


Figure 2. PCR to test whether 72A349 primers are able to amplify genes using Long Amp enzyme and temperature gradient.

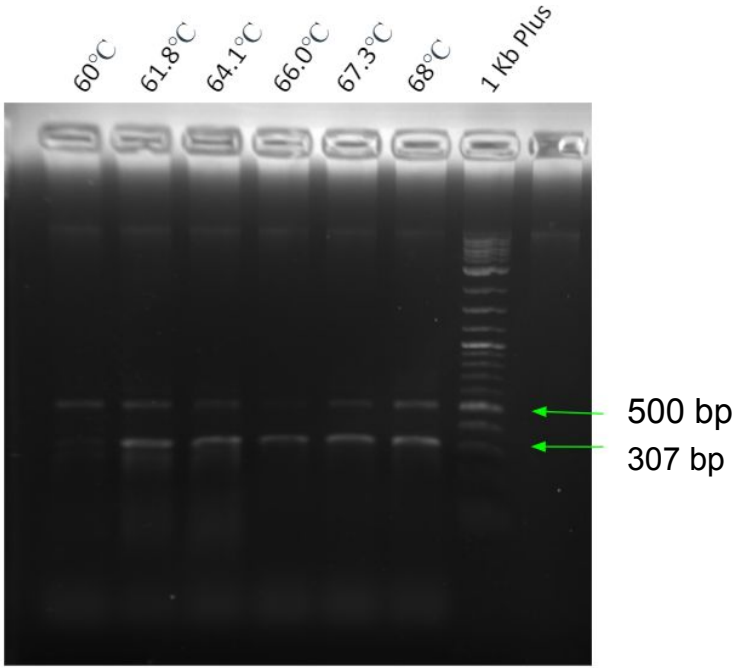


Figure 3. PCR repeats previous experiment with modifications using Phusion enzyme and higher temperature gradient.

Results: New Primers

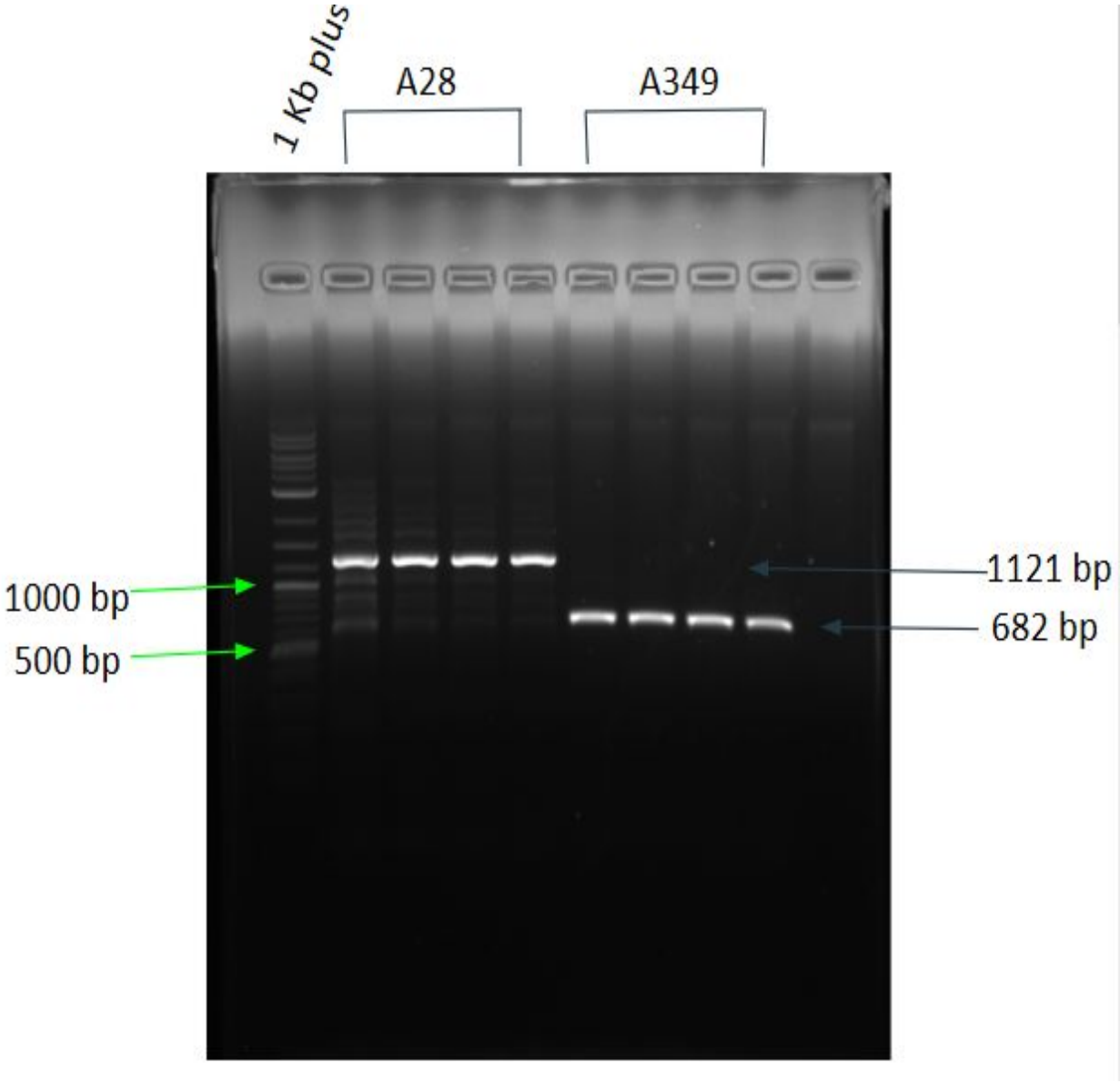


Figure 4. Gel picture for amplifying a single gene with 72A349 and 72A28 primers using a gradient of temperatures. The temperature gradients are 64.1 ℃, 66.6 ℃, 67.3 ℃, 68 ℃.

Results: Control Primer

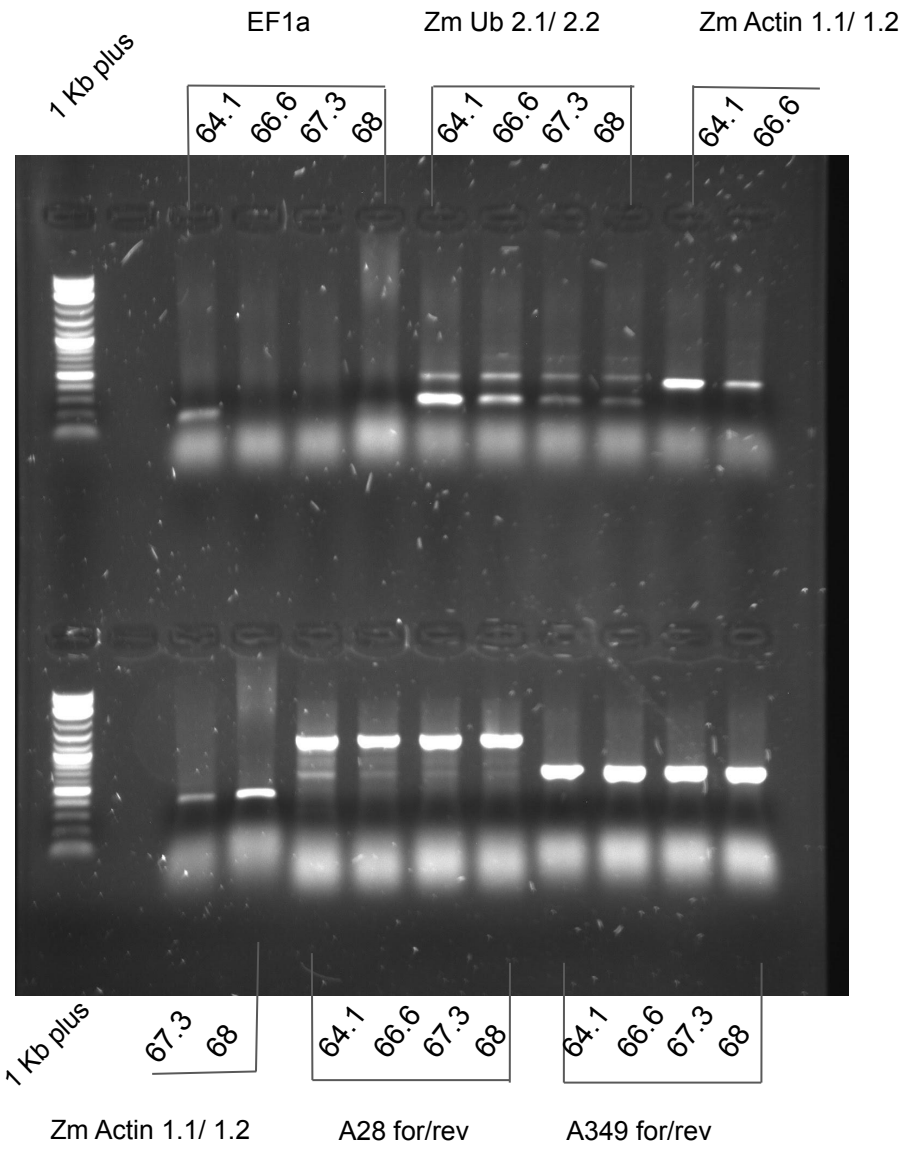


Figure 6. PCR to find correct control primers, and to test if 72A28 and 72A349 genes are able to amplify with the new DNA (W22-1) using a temperature gradient for each.

Results: RT-PCR

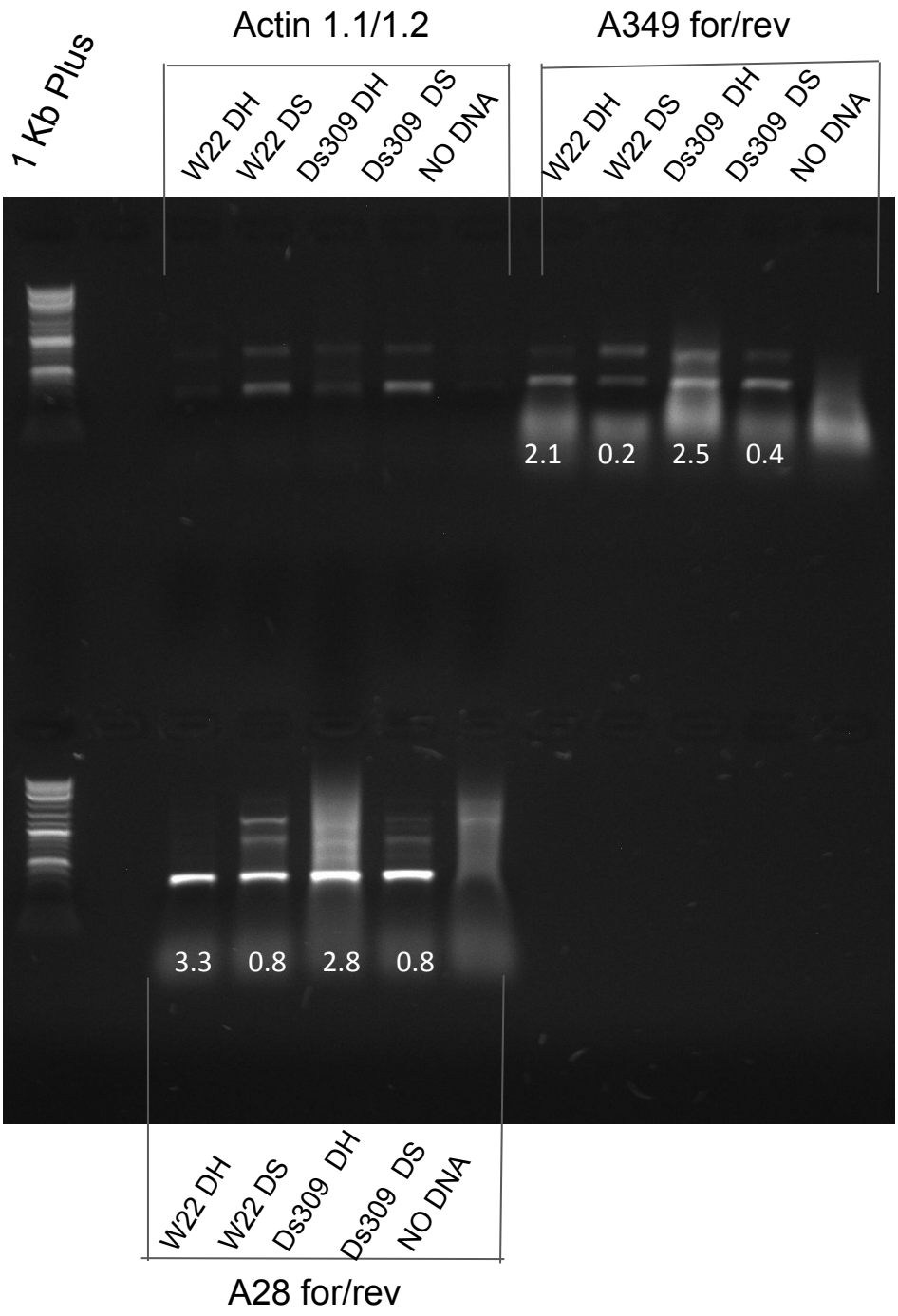


Figure 9. Gel picture for testing cDNA with gene specific primers for A349 and A28 along with control primer, Actin. The numbers under the bands indicate the band intensity values normalized to Actin (quantified with Image J).

Conclusion

- Heat stress is inducing expression levels for both A28 and A349 relative to Actin more than salt stress
- A28 can be functioning in a similar way to A349 since it is being induced by heat stress in a similar way to A349
- It does appear A28 is being induced by heat stress in a similar way to A349 is, but I do not see an upregulation of A28 when A349 is mutated. But, it is possible that A28 is being regulated in a similar way to A349
- Next step:
    - Redo RT-PCR with gene specific primers for A28 and A349 with the control primer, Actin to see to get rid of any nonspecific bands that had appeared
    - Use Image J again to reassure the expression levels for each of the samples since it was only done once
    - Test abiotic AND biotic stress by isolating more RNA from Wild-type corn to test gene expression

Acknowledgements

Thank you for your contributions:

- Parika Chauhan: PCR conditions and mutant isolation
- Grace Sandel: Wild-type DNA
- Ruha Reddy and Krina Patel: Plant material for stressed plants