Iowa State University
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Iowa State Honors Program
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Sequencing Lipoate-Protein Ligase for Association Studies with Photosynthetic Traits Under Cold Stress in Sorghum bicolor

Introduction

Cold tolerance is a valued trait in sorghum breeding in the northern hemisphere as it allows for the possibility of increased biomass due to a longer growing season. A genome-wide association study, was conducted to locate chromosomal regions associated with photosynthetic capacity under cold stress. The chromosomal regions found contained several candidate genes, one being *lipoate-protein ligase*. *Lipoate-protein ligase* adds lipoate groups to enzymes that function in photorespiration. *LPL* is hypothesized to be associated with photosynthetic traits related to cold stress.

Objectives

- Repeat previously failed sequencing reactions to add novel allele data into diversity panel.
- Run an association study using TASSEL to see if markers in the gene are associated with photosynthetic capacity under cold stress.

Materials and Methods

- Performed primer optimization using a temperature gradient
- Utilized PCR to amplify missing accessions
- Gel electrophoresis of PCR product
- Cleanup of PCR product for Sanger sequencing
- Sanger sequencing with Big Dye 3.1
- Precipitation and cleanup of Sequencing reaction
- Sequencing using ABI 3730 platform
- Quality control and sequence alignment using Sequencher
- Association study using TASSEL

Results

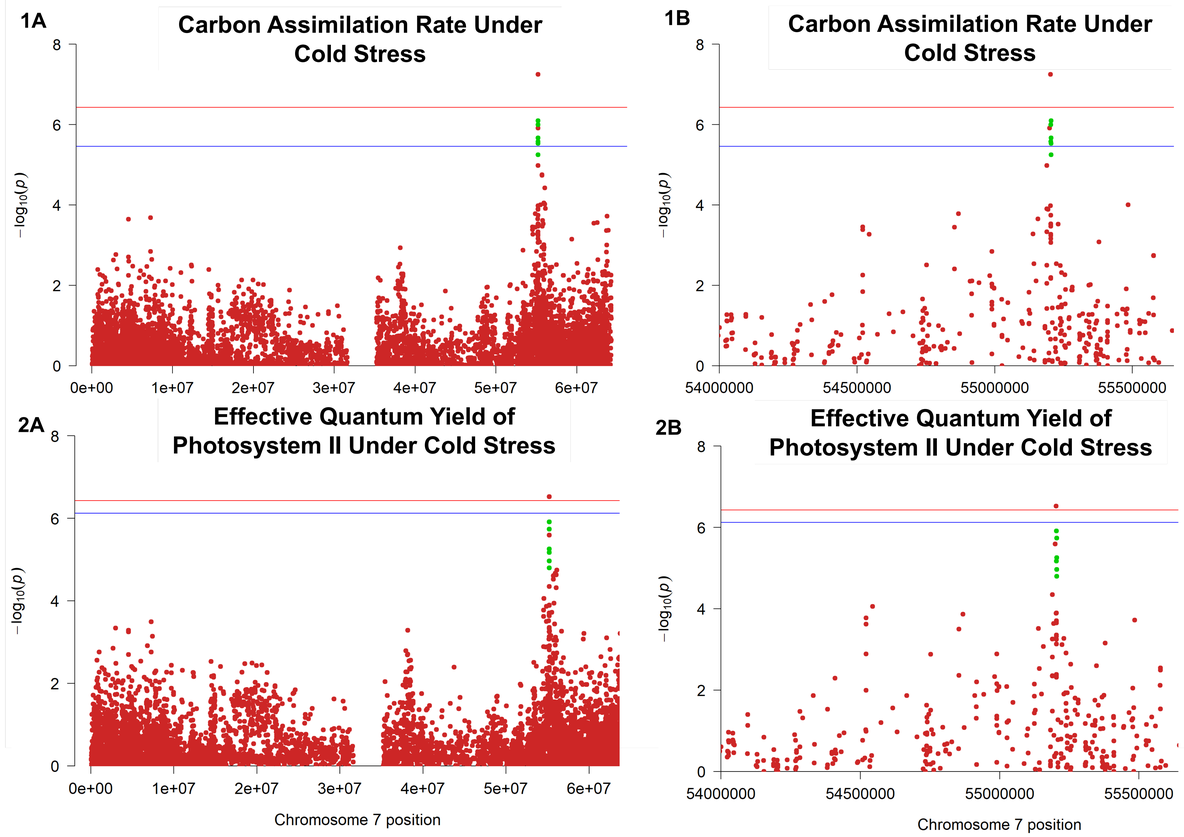

Figure 1 A and B. Manhattan plots depicting the significance of marker-trait association for carbon assimilation rate under cold stress. The blue line indicates a False Discovery Rate (FDR) of 0.05. The red line is the Bonferroni correction. Novel allele data was added at markers indicated with a green dot. A. Marker results for chromosome 7. B. Close up on the results for the region containing newly sequenced markers.

Figure 2 A and B. Manhattan plots depicting the significance of marker-trait association for the effective quantum yield of photosystem II. The blue line indicates a FDR of 0.05. The red line is the Bonferroni correction. Novel allele data was added at markers indicated with a green dot. A. Marker results for chromosome 7. B. Close up on the results for the region containing newly sequenced markers.

Conclusions

- P-values above the Bonferroni line and FDR line suggest that the polymorphisms are associated with the target trait.
- Newly sequenced markers were significantly associated with the carbon assimilation rate under cold stress given an FDR of 0.05.
- There is increased significance in the markers towards the end of the *LPL* gene.

Future Goals

- Once the diversity panel of 304 accessions is completed, another association study will be run with the goal of locating a functional polymorphism associated with the trait. Experiments will then be conducted analyzing the trait-marker association via cloning and selection.

References

Ortiz, D., Hu, J. & Fernandez, M. G. S. Genetic architecture of photosynthesis in Sorghum bicolor under non-stress and cold stress conditions. *Journal of Experimental Botany* **68,** 4545–4557 (2017)

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