Targeted Late Flowering Time and Forage Quality Association Study within a Segregating Orchardgrass Population.

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INTRODUCTION

As plants mature and transition from vegetative to reproductive stages, yield continues to increase but quality decreases (See figure below). To maximize both yield and forage quality, we ideally harvest just prior to flowering (Between red lines).

Commonly grown together for forage, Orchardgrass (Dactylis glomerata L.) and alfalfa (Medicago sativa) vary in heading dates, even within each species. However, no current commercially available orchardgrass cultivars in northern Utah flowers near the time of locally grown alfalfa cultivars. Generally, Orchardgrass flowers prior to alfalfa, causing growers to choose between optimizing plant species for forage. If we delay orchardgrass flowering time to align with alfalfa flowering time we can maximize both plants’ forage yield and quality. In hopes of achieving this, five genes of interest from previous studies have been identified as: FLOWERING TIME (FT), VERNALIZATION 2 (VRN2), VERNALIZATION 1 (VRN1), CONSTANS (CO1), and GIGANTEA (GI). We are currently collecting data in preparation for a genetic association study between these genes and phenotypic data from late flowering, forage orchardgrass (tetraploid) population.

MATERIALS AND METHODS

• Based on previous studies- FT, CO, VRN1, VRN2, and GI-like genes are targeted for PacBio sequencing
• We have designed primers for each gene using a diploid orchardgrass reference genome (using Geneious) and tested each primer on the parent population
• We grew 94 half-sib families in a randomized block design with four replications giving us 372 plots. Each plot consists of 10 plants. Two locations: Bothwell, UT (flood irrigated) and Providence, UT (sprinkler irrigated)
• Field data collected
  • Heading Date (50% of plants per plot had the first floret exposed from boot)
  • Plot yield (three harvests a year)
  • Dry Matter content ($\frac{\text{Weight}_\text{Dry Matter}}{\text{Weight}_\text{Fresh}}$)
  • Tissue sample from each plant (for DNA extraction)
• Heading date and dry matter yield were both analyzed using analysis of variance to assess location and blocking effects, and least significant differences were calculated between half-sib families.
• GBS will be used to analyze population structure
• Genotype association, 4x variant detection, and regression analysis will be performed using CLC workbench, R, and Geneious

RESULTS

Figure 1 shows a PCA we performed on GBS data from the 94 parents of our half-sib population (the parent population was created from two years of open pollination between three diverse varieties). The 94 parents separate into six diverse genetic groupings. The blue circles identify more maternal like groups while the red circles identify the more heterogenous individuals.

Figure 2 shows the heading date distribution of the 94 half-sib families. True at both locations, an analysis of variance suggests that there is a significant difference ($p$-value $<$ 0.001) in heading date between at least two of the half-sib families. The population shows a normal distribution at both locations.

• For both locations a least significant difference test shows significant difference of heading dates between the half-sib families for the top 30% (earliest heading dates), and bottom 30% (latest heading dates).
• A very strong correlation (0.85) exists between heading dates in the two locations, meaning an earlier heading date in Bothwell would most likely mean an early heading date in Providence.
• 79, 80, and 82 are the earliest heading half-sib families in both locations while half-sib families 5, 52 and 55 are three of the five latest heading dates in both locations.

Figure 3 shows dry matter yield distribution of the 94 half-sib families. For both locations an analysis of variance indicated that there were differences among half-sib families for dry-matter yield, yet no patterns emerged.

CONCLUSION

For a successful association study, both genetic and phenotypic variation needs to be captured within the mapping population. Our population, both genetically and phenotypically, shows variation and, no reason to doubt, a very successful genetic association study will lead us to molecular markers for use in selecting for late flowering in orchardgrass. Please watch for further results from this study as we finish this years forage harvests. We would love to hear/discuss any thoughts and answer any questions you many have. Email Megan at megan.getz@aggiemail.usu.edu.