

Comparison of stress genes in switchgrass (*Panicum virgatum* L.) genotypes under drought conditions

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1. Introduction

Switchgrass (*Panicum virgatum* L.) is being examined as a viable source for biomass feedstock. It is a native, warm-season perennial that belongs to the grass family (Poaceae). Because it is able to grow on marginal lands with minimum to no fertilizer requirements, and few obvious pest and disease issues, this plant could have great success as a novel agronomically produced crop. Since water is required for biological functions of all organisms, drought can be an abiotic stress affecting any crops within the agricultural field. Because of this, breeding of more drought-tolerant cultivars is an ongoing endeavor. Genes being expressed under such abiotic stresses have been identified in model organisms as well as agriculturally important crops. In this study we measured the expression of known drought-related genes; *rd22*, *rab18*, and *dreb2a*. The three genes were compared between the two different switchgrass genotypes; Alamo and Cave-In-Rock. The plants were grown under normal water conditions (control) and drought induced conditions (treated), in order to analyze the variation between expression.

2. Objectives

- Grow and apply drought and non-drought treatments to Alamo and Cave-In-Rock genotypes.
- Compare expression levels of the selected genes under drought stress between the two genotypes.

3. Materials and Methods

- **Plant Materials-** Alamo and Cave-In-Rock were grown from seed in a DSU greenhouse for approximately three months. Alamo is a lowland ecotype derived from Texas and Cave-In-rock is an upland ecotype from Missouri.
- **Methods-** Field capacity was determined using a percolation method, and control plants received a full watering of 355mL when soil moisture fell below 20% VWC (volumetric water content), while drought plants received half (177mL) when soil moisture fell below 6%.
- Leaves were harvested and stored in -80°C until RNA isolation.
- RNA was isolated using Total RNA isolation kit by Machery-Nagel (Bethlehem, PA)
- cDNA was synthesized using cDNA synthesis kit by NEB Protoscript (Ipswich, MA)
- qPCR was conducted using 7500 Real-time PCR System and SYBR Green Master Mix by Applied Biosystems (Grand Island, NY)

Gene name	Gene function	Sequences
<i>rd22</i> (responds to dehydration)	Produces LEA protein under dehydration, salt stress and exogenous ABA	Forward 5' ACCGTCCAGTTCACCAACAC 3' Reverse 5' AGGACTCTCTCGCCCTTG 3'
<i>rab18</i> (responds to abscisic acid)	Dehydrin expressed under low temperatures and dehydration. Helps regulate transports over cell membranes.	Forward 5' TCACACTGCACACACAGGTG 3' Reverse 5' AGAAGGGCATCATGGACAAG 3'
<i>dreb2a</i> (dehydration response element binding)	Transcription factor induced by dehydration and salinity stress.	Forward 5' TCTTGCAAGCAACCTCTG 3' Reverse 5' GTGCCTTCACAGTGTGAA 3'
<i>ap13ctg11779</i>	Protein involved in chromatin segregation (used as endogenous control for qPCR)	Forward 5' AAGAGTTGAGGAAGCGCAA 3' Reverse 5' ATGGATCCTGCTTATGGCCG 3'

Table 1: Genes being measured, their functions, and primer sequences.



Figure 1: Drought treated Alamo (left) adjacent to control Alamo (right).

4. Results



Figure 2: Isolated RNA

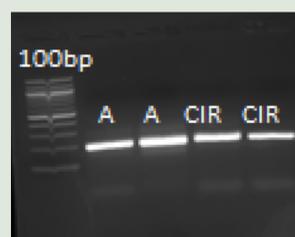


Figure 3: PCR of cDNA



Figure 4: Agarose gel of qPCR reactions

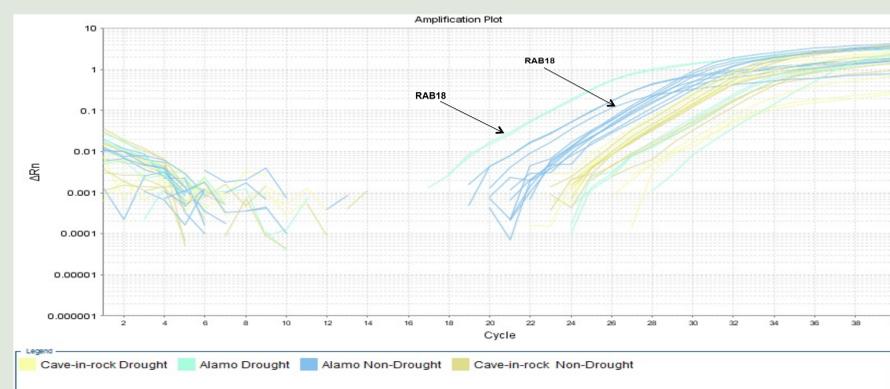


Figure 5: Amplification plot of qPCR procedure

5. Future Directions/Discussion

- Perform more biological replicates of the qPCR procedure
- Cultivate and test different genotypes in the same manner, and with different abiotic and biotic stresses and genes.

Drought response is a multigenic trait; the increase of a single gene's mRNA expression is not a direct correlation for tolerance, but can be used for a better understanding of the switchgrass germplasm for future purposes such as traditional breeding or gene transformation.

6. References

D. Aimar et.al, "Drought effects on early developmental stages of *Panicum virgatum* L: cultivar differences" *Biomass and Bioenergy*, 2014

7. Acknowledgments

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