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Genetic resources for the improvement of switchgrass (*Panicum virgatum* L.) for biomass and forage

Gautam Sarath, Nathan A. Palmer, Aaron J. Saathoff, Robert B. Mitchell, Serge Edme, Erin D. Scully, Scott E. Sattler
United States Department of Agriculture-Agricultural Research Service, Lincoln, USA
Corresponding author e-mail: Gautam.Sarath@ars.usda.gov

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Introduction

Switchgrass (*Panicum virgatum* L.) is an important forage and biomass species for many parts of the USA. Switchgrass can be of several ploidies. Octoploid cultivars are most often used in forage and conservation settings, while the tetraploid cultivars are mostly targeted for bioenergy end-uses, due to their higher biomass yields. Switchgrass populations also occur as upland and lowland ecotypes, and constitute different heterotic groups. Switchgrass is mostly an obligate outcrosser resulting in substantial genotypic and phenotypic variation within populations. In the last ~15 years, significant resources have been dedicated to both breeding and understanding the genomic makeup of this plant, with a focus on bioenergy. This investment has resulted in the development of elite lines as well as a considerable increase in available genetic, physiological, and biomass-related information. The United States Department of Agriculture-Agricultural Research Service has been a major player in these developments (Mitchell and Schmer, 2012; Vogel *et al*., 2011).

With significant improvements in DNA-sequencing technologies (High Throughput Sequencing, HTS), it has become possible to undertake large-scale analysis of both the genomic and functional genomic components of switchgrass. One such undertaking by the United States Department of Energy-Joint Genomics Institute has provided a draft assembly and annotation of the switchgrass genome (www.phytozome.org). This remarkable resource has permitted a complete utilization of HTS to analyze gene expression using RNA-Seq and related bioinformatic pipelines. Large-scale studies that are performed using field-grown plants and populations with well-characterized phenotypic traits, it increases the likelihood of discovering molecular events that underpin phenomena of interest. Even though lowland tetraploid cultivars have higher biomass yields than upland tetraploid cultivars, they can suffer significant winter-kill in more northern locations (Central Great Plains of the USA). Winter-kill is associated with the loss of rhizomes and other perenniating structures resulting in a complete or partial loss of tillering ability in the following seasons. Partial attrition of tiller production serves to limit new rhizome growth in successive years. One or more cycles of winter kill will ultimately kill the plant. We are trying to understand the cellular metabolism associated with the onset of rhizome dormancy and to connect the links between tiller/leaf senescence and rhizome metabolism using field grown plants from diverse populations, HTS and RNA-Seq.

Materials and Methods

Plants comprising upland *cv* Summer, lowland cv Kanlow, a population derived from cv Kanlow for early maturity (Kanlow EM) and Kanlow x Summer hybrids (KxS) were raised in a greenhouse from seeds and individual seedlings planted at sward densities in small replicated field plots. Flag leaves and rhizomes were harvested at discrete points during the second and third growing seasons, once plants were fully established. Flag leaves were collected only from *cv* Summer. Rhizomes were harvested from all accessions at spring green-up, late vegetative, heading, anthesis, hard-seed set stages, and after the first killing frost. All tissues were cleaned and flash frozen in liquid nitrogen. Materials were stored at -80 °C until used. All materials were cryogenically ground and aliquots used for RNA and metabolite extractions. cDNA libraries were sequenced on an Illumina HiSeq2000 instrument. Polar metabolites were analyzed by gas chromatography-mass spectrometry.

Results and Discussion

RNA-Seq analysis has allowed us to document the overall changes in the transcriptome during the progression of flag leaf development, and document transcriptional changes that trigger leaf senescence. By comparing changes in leaf chlorophyll levels and the expression of genes for chlorophyll biosynthesis and degradation, a four -phase molecular roadmap for switchgrass flag leaf ontogeny was developed. Genes associated with early leaf development were up-regulated in phase 1. In Phase 2, leaves had increased expression of genes for chlorophyll biosynthesis, and those needed

for full leaf function. Phase 3 coincided with the most active phase for leaf C and N assimilation. Phase 4 was associated with the onset of senescence, as observed by declining leaf chlorophyll content, a significant up-regulation in transcripts coding for enzymes involved with chlorophyll degradation, and a large number of senescence-associated genes (Figure 1). Of considerable interest were switchgrass NAC transcription factors with significantly higher expression in senescing flag leaves. Two of these transcription factors were closely related to a wheat NAC gene that impacts mineral remobilization. The third switchgrass NAC factor was orthologous to an Arabidopsis gene with a known role in leaf senescence. Other genes coding for nitrogen and mineral utilization, including ureide, ammonium, nitrate, and molybdenum transporters, shared similar expression profiles with the three NAC transcription factors and could direct targets of these NACs (Palmer *et al*., 2014a). Results from the flag-leaf RNA-Seq analysis in conjunction with preliminary RNA-Seq analyses to document transcriptional changes in senescing rhizomes have provided clues that could signal the onset of senescence in the rhizomes. When rhizomes from contrasting populations were analyzed by RNA-Seq at a time when aerial senescence had occurred in the upland cultivar (cv Summer) but not in the lowland cultivar (cv Kanlow), significant differences were documented in their metabolism. Rhizomes from cv Kanlow appeared to be in a growth mode whereas rhizomes from cv Summer were entering dormancy (Palmer *et al*., 2014b). Growth processes were essentially arrested in summer rhizomes, but not in Kanlow. Furthermore, transcriptional signatures indicated that metabolism was diverted towards the breakdown of primary substrates to produce acetyl CoA to support rhizome energy needs (Figure 2) in summer rhizomes transitioning to dormancy (Palmer *et al*., 2014b). These and other related studies have provided a detailed map of the changes that occur to the transcriptomes over the course of a growing cycle and have illuminated metabolism that occurs in dormant rhizomes. Much of the metabolism in dormant rhizomes appears to be dependent on starch and nitrogen accumulated during the growing season, and from materials remobilized during aerial senescence. Also, metabolism is significantly redirected away from active aerobic respiration through mitochondria towards more substrate-level regeneration of ATP and reducing equivalents. All of these and related data currently under analysis will be discussed in relation to switchgrass and other warm-season, temperate grasses.

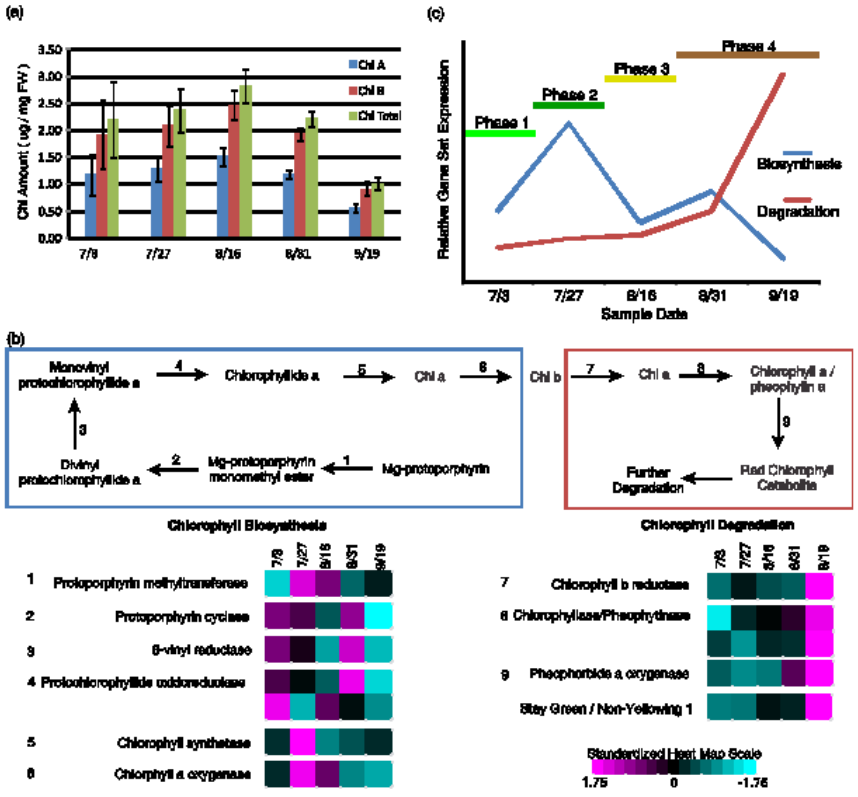

Fig 1: Road map of switchgrass flag leaf development. (a) Total leaf chlorophyll content. (b) Expression profiles of genes coding for proteins involved in chlorophyll biosynthesis and degradation. (c) Phases in gene regulation.

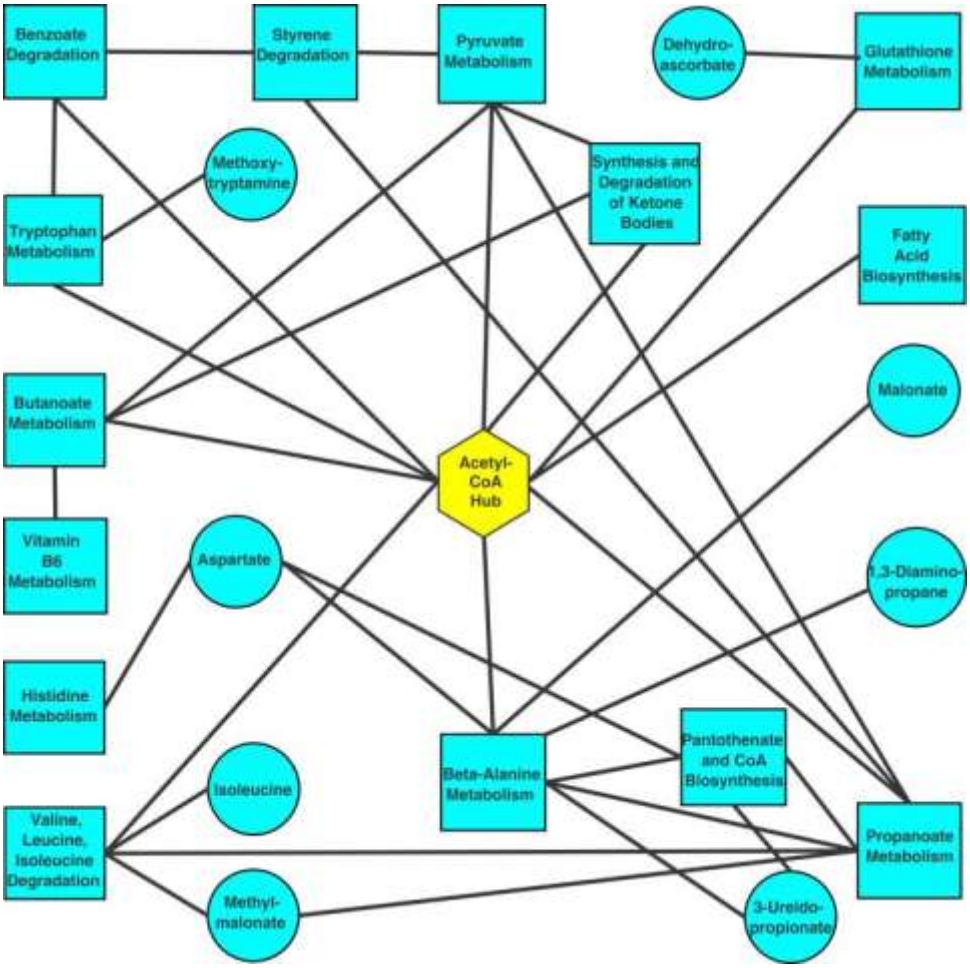

Fig 2: Acetyl-CoA appears to be a central hub connecting diverse pathways upregulated in crowns and rhizomes of the cultivar summer.

Conclusion

The massive rearrangement in rhizome metabolism from growth during the summer months to dormancy over the winter months appears to be a driver for rhizome health. Either loss of photosynthate or remobilized carbon/nitrogen would certainly impact rhizome metabolism over the winter months. A timely entry and exit from dormancy appears to also provide rhizomes with the resources required to maintain core metabolism during winter and all the needed winter-hardening mechanisms to assure perenniality (Sarath et al. 2014). These and related genetic data are being incorporated into our current breeding programs.

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