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SENESCENCE OF NATIVE PERENNIAL WARM SEASON GRASSES.  
SENESCENCE ASSOCIATED SWITCHGRASS TRANSCRIPTOME

BY

MICHAELLONG TRAN

A thesis submitted in partial fulfillment of the requirements for the

Master of Science

Major in Biological Sciences

Specialization in Microbiology

South Dakota State University

2016

SENESCENCE OF NATIVE PERENNIAL WARM SEASON GRASSES.  
SENESCENCE ASSOCIATED SWITCHGRASS TRANSCRIPTOME

This thesis is approved as a creditable and independent investigation by a candidate for the Masters of Science in Biological Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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## ABBREVIATIONS

SG	switchgrass
PCG	prairie cordgrass
in	inch
ft	feet
wt	weight
nt	nucleotide
C	Celsius
9-13	September 13 <sup>th</sup>
9-27	September 27 <sup>th</sup>
JA	jasmonic acid

## ABSTRACT

SENESCENCE ASSOCIATED TRANSCRIPTOME OF NATIVE PERENNIAL  
WARM SEASON GRASSES

MICHAEL LONG TRAN

2016

Senescence of perennial crops enable continuous harvests after one sowing event. Perennials senesce at adapted rates of their native environments; however, early senescencing crops do not maximize the growing season as nutrient reallocation takes precedence. Chlorophyll degradation and nitrogen reallocation was observed to occur rapidly between mid to late September. Transcriptome analysis on early and late senescencing switchgrass cultivars reveals upregulation of starch metabolism, light reactions, Calvin-Benson Cycle, and anthocyanin synthesis in late senescencing switchgrass. Morphological variations between the two germplasms prolong the growing season of late senescencing switchgrass, maximizing yield. Expression of mRNA as senescence progresses and between the two genotypes reveals potential targets and genes of interest for crop breeding techniques to maximize the growing season of perennial crops, optimize nutrient reallocation, and enhance yield.



# CHAPTER 1

## Literature Review

### Introduction

Liquid fossil fuel reserves are anticipated to be depleted by 2050 (OPEC, 2007) while consumption is forecasted to increase to 2030 (EIA, 2007), (BP, 2007), (Shafiee & Topal, 2009). In order to meet demand, alternative replacement fuels are a necessity. The production of plant-based fuel, biofuel, uses current infrastructures in development and could be a direct replacement in the liquid fossil fuel niche. Many investments and resources have been dedicated in order to quench demand and fulfill the energy gap when reserves are exhausted by funding progress in developing energy independence.

Advancements in these fields, such as biofuel production, have shown that sustainable resources is not an idealistic dream, but achievable in the near future. In 2005, Brazil, one of the oldest biofuel producing countries, produced 4.6 billion gallons of ethanol using 7.6 million acres of sugarcane as raw materials (Budny & Sotero, 2007). Boasting an \$8 billion industry while achieving a net energy output of 8 to 10 with 1 unit of input, Brazil is the hallmark of biofuel progression and success in the early 2000s (Macedo Isaias, 2004). Global dependence on fossil fuels and the subsequent carbon emissions may be mitigated through the use of alternative energy. Usage of corn ethanol has shown a 19% reduction in greenhouse emissions as opposed to conventional petroleum (Wang, Hong, & Huo, 2007). Due to a developing biofuel industry, the demand for raw materials has taken the agriculture community by surprise. Although biofuel production has progressed much in infrastructure, current energy input to output efficiencies of corn-based biofuel is lacking. Current corn energy ratios show a range of efficiencies from an output energy

ratio of 1.25 (Sanderson & Adler, 2008) to 2.3 (USDA, 2010). Despite a positive net gain in energy, the increasing global demand for energy stresses the need for alternative, low input, high output biomass crops for fuel production. Through modern agricultural techniques combined with genetic methods, developing alternative crops would rely first on choosing suitable candidates and then maximizing yields. Perennial C4 grasses such as *Spartina pectinata* (prairie cordgrass, PCG) and *Panicum virgatum* (switchgrass, SG) show promise as biomass crops.

## C4 Photosynthesis

PCG and SG shown much promise as biomass from their innate characteristics, C4 photosynthetic capacity and perennial life cycle. Traditional C3 photosynthesis involves the intake of CO<sub>2</sub> gas by stomata and the initiation of the light independent reactions, the Calvin-Benson-Bassham (CBB) Cycle, by first converting CO<sub>2</sub> into 3-phosphoglycerate by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) (Calvin & Benson, 1948). RuBisCo doubles as both a carboxylase and oxygenase where ideally it would bind to CO<sub>2</sub> and proceed into the CBB Cycle. However, RuBisCo has a high affinity to O<sub>2</sub> gas as well. The binding of O<sub>2</sub> leads into the C2 Cycle, known as photorespiration, where the RuBisCo enzyme is diverted away from entering the CBB Cycle and energy must be inputted to regenerate RuBisCo. The binding to O<sub>2</sub> gas is undesirable as it reduces the efficiency of the CBB Cycle by hindering available RuBisCo as well as requiring additional energy inputs to regenerate RuBisCo (Sharkey, 1988). C4 photosynthesis minimizes photorespiration by forming phosphoenolpyruvate (PEP) from pyruvate and converting both PEP and CO<sub>2</sub> into a four

carbon organic acid, oxaloacetate, via PEP carboxylase. Unlike RuBisCo, PEP carboxylase has a high affinity with CO<sub>2</sub> and a low affinity for O<sub>2</sub>. Oxaloacetate is transferred from mesophyll cells to bundle sheath cell. Decarboxylation of oxaloacetate yields CO<sub>2</sub>, which may proceed into the CBB Cycle (Slack & Hatch, 1967). Although additional energy inputs are required for C4 photosynthesis, the mitigation of photorespiration has been shown to be evolutionary advantageous in grass populations.

## **Perennial Plant Advantage**

Perennial plants have a distinct advantage in multiple growth and harvest season from one sowing as opposed to annuals whose life cycle consists of sowing, growth, reproductive maturity, and overall organism death and biennials which undergo an annual-like life cycle over the course of two years. Multiple growing seasons from one sowing reduces inputs from tilling and fertilizers, reducing greenhouse gas emission (Adler, Grosso, & Parton, 2007). Perennials require an establishment period post germination from one to two years. Intricate root systems with carbohydrate reserves in rhizomes promote growth post establishment for multiple years (Steen & Larsson, 1986). The underlying principle behind annuals/biennials life cycles and perennials lies with their respective senescence phases which result in the differences in the number of reproductive cycles. Senescence is referred to a highly regulated process involving molecular, biochemical, and physiological changes that result in death (Nooden, 1988). Annuals and biennials are typically monocarpic plants, flowering plants that release seeds and terminate. Two senescence phases occur in monocarpic plants, one during vegetative growth and one during reproductive development. During vegetative development, there

is a reallocation of nutrients from older leaves to younger leaves as older leaves senesce. With reproductive development senescence in monocarpic plants, nutrients are reallocated and prioritized in seed formation and development while senescence occurs throughout the organism, culminating in death of the plant (Munne-Bosch, 2008). However, perennial plants, polycarpic plants, undergo reproductive phases at least twice before organismal senescence and death. Senescence of older leaves and the reallocation of nutrients to young leaves occurs during vegetative growth in perennials; however, senescence during reproductive development does not involve the whole plant. Nutrients are reallocated to both seeds and subterranean tissues, such as roots and rhizomes, while senescence of aerial tissues senesce (Munne-Bosch, 2007).

Taking advantage of perennial growth cycles and their subsequent harvests requires an intricate overview on the process and regulation of senescence in perennials. By further elaborating and evaluating potential senescence control mechanisms, increases in biomass yields may result from hybridizing early senescencing and late senescencing cultivars of C4 perennial grasses by extending growing seasons or optimizing strains for sowing locations.

## **Prairie Cordgrass**

PCG is a perennial C4 grass native to tallgrass prairies and wet soils (Weaver, 1968), (Pohl, 1954). It is a tall and coarse sod-forming grass that reproduces via rhizome propagation and seeds. PCG develops 4-10 mm thick and scaly rhizomes which are the basis for extensive rhizome networks within sods, and reach an aboveground height of 7-8 ft. during flowering while achieving a depth of 7-9 ft. (Weaver, 1968), (Stubbendieck,

1997), (Darke, 2004). The vastness of the rhizome network has been observed at 80 ft. lengthwise per square ft., with lateral branching reaching 2 ft. or greater. Within the first season of growth, germination occurs readily in wet soils as PCG may reach a height of 2.5 ft. Noted for its low maintenance, yet high biomass yield and high cellulose/lignin content, PCG is a potential candidate for biofuel production. Optimizing PCG lines for biomass production requires knowledge of available strains. However, little is known in the cultivation of PCG.

### **PCG Molecular Data**

A cytogeographical census of wild PCG across the Midwest and New England revealed variation in ploidy. PCG has a base chromosome number of 10 ( $x = 10$ ). Tetraploid PCG ( $2n = 4x = 40$ ) is abundant in the New England area. Tetraploid and octaploid ( $2n = 8x = 80$ ) lines were observed in the Midwest, while a hexaploid strain was found in Illinois (S. Kim, Rayburn, Parrish, & Lee, 2012). Cultivars of PCG cannot breed with different ploidy PCG. A comprehensive census of PCG phenotypes and ploidy is lacking, stunting breeding efforts. No reference genome is available. Insights in ploidy and genome size allows breeding to more efficient, diversity in traits, and development of hybrid.

### **Prairie Cordgrass Usage**

PCG is rated as poor to fair forage for cattle. Fair quality hay is produced with immature PCG (Stubbendieck, 1997). The U.S. Department of Transportation has authorized growth and maintenance of PCG in roadside clear zones, defined as the area

of the roadside border for errant vehicles (Harper-Lore, 1999). PCG usage in clear zones includes soil erosion prevention, growth inhibition of hazardous trees and shrubs, and remediation for areas the road imposes upon. PCG is salt tolerant enabling grass cover to recover saline habitats and growth for production in adverse soils (Anderson, Voigt, Kim, & Lee, 2015).

## **Switchgrass Background**

SG is a tall, coarse, sod-forming perennial C4 grass found natively in prairies, riverbanks, open ground, open woods, and even brackish marshland (Pohl, 1954), (Darke, 2004). Reproduction occurs via rhizome propagation, tillers, and seeds. Rhizomes are thick and scaly. A dominant grass that grows in intermediately aerated and moist soils (Ladd & Oberle, 2005). Aboveground biomass height may reach 6-10 ft. during flowering while a long, broad root system could reach depths of 9-11 ft. (Weaver, 1968). Growth begins in late spring with flowering occurring during July/August (Darke, 2004).

## **Switchgrass Molecular Data**

SG is characterized by two ecotypes, upland and lowland. Upland SG is commonly found north of 34°N latitude while lowland SG is predominantly found around 42°N latitude. Upland SG is commonly associated with drought tolerance while lowland SG is generally larger in size and height and flowers later. Divergence of the two ecotypes is thought to have occurred 0.8-1.0 million years ago.

The base chromosome number of SG is 9 ( $x=9$ ). Lowland SG is primarily tetraploid ( $2n = 4x = 36$ ) with rare octaploid varieties ( $2n = 8x = 72$ ). Upland SG are

usually tetraploid or octaploid at a 1:2-3 ratio. Rare hexaploids are found among mixed populations of tetraploids and octoploids. The occurrence of a hexaploid could be the result of the fusion of a gamete and an unreduced gamete.

SG is known to reproduce via cross pollination through wind with a gametophytic self-incompatibility system. A transition zone exists between upland and lowland SG populations. Upland and lowland SG can be crossed at the tetraploid level.

Despite difficulties in sequencing polyploid SG, a SG reference genome is available via [www.phytozone.org](http://www.phytozone.org).

### **Switchgrass Usage**

Rated good for livestock forage and fair for wildlife, SG has seen production as a forage crop (Stubbendieck, 1997). SG is authorized as a clear zone grass for soil erosion prevention while inhibiting growth of roadside hazardous plants (Harper-Lore, 1999). SG retains rigidity throughout winter, providing cover for wildlife. Minimal maintenance is required for SG upkeep, ideal for production or habitat rehabilitation (J. R. Johnson, Larson, & Brashier, 2007).

### **Current Problems**

Cultivable land is limited and production of biofuel crops should be thoroughly investigated before prioritization over food production. With little changes in dietary consumption, global agricultural area is estimated to increase to 5.4 billion ha by 2030 (Wirsenius, Azar, & Berndes, 2010). The carbon footprint of biofuels and the associated alternative crops has yet to be established. Energy returns need to be higher than fossil

energy input and current infrastructure will have to adapt to accommodate biofuel inputs someday. Land management, population sustainable levels of food production, biofuel production efficiency, and neutral carbon footprints are idealistic; however, these are issues that need to be addressed for a better future.

Despite being native grasses of the Midwest, PCG and SG cultivation is fairly recent as opposed to established crops such as corn and soybean. The lack of development for PCG and SG entails that a comprehensive survey of known varieties along with a breeding program would need to be developed for crop production. Optimization of PCG and SG involves balancing biomass output against resource inputs, accounting for growth potential in colder and warmer climates, and the crop quality in regards to biofuel chemical conversion.

The problems that society faces is vast and many; however, only with single steps could humanity progress in resolving them.

## **Recent Studies/Solutions**

### **Growth Potential**

Fertile farm lands are a limited global resource. As demand for agricultural goods clash with ranching, land use and management is critical in meeting the needs today and the future. Marginal lands, areas of land deemed poor for agriculture practices, are vast and plentiful; however, advancement in crop breeding and alternative crops may lead to viable crop production (Gopalakrishnan, Cristina Negri, & Snyder, 2011). Native grasses such as PCG and SG have shown sustainable growth in marginal lands such as wet and coarse soil environments of the Midwest. On the Eastern U.S., marginal lands include



marshy and coastal areas that provide a hostile environment for agriculture. Flooded lands of marshes are waterlogged and anaerobic while coastal lands are saline. PCG has been shown to grow in wet and coarse soils while varieties have adapted to the saline conditions of coastal lands (Anderson et al., 2015).

Post establishment, both PCG and SG require little maintenance and little fertilizer. During the vegetative growth stage, nitrogen is reallocated to the subterranean tissues, roots and rhizomes, in preparation for dormancy. Wayman et al. determined the potential savings of nitrogen reallocation to subterranean tissues to be  $\$9.1 \text{ ha}^{-1}$ ; however, the authors noted that delaying harvest for optimal nitrogen translocation may coincide with the onset of the killing frost, resulting in a much greater loss in biomass yield (Wayman, Bowden, & Mitchell, 2014).

### **Biomass Output**

As mentioned prior, both PCG and SG are perennial C4 grasses. Both traits are desirable in terms of yield and output. Perenniality allows multiple reproductive cycles and harvests from one sowing event (Adler et al., 2007) while C4 photosynthesis minimizes lost from photorespiration (Slack & Hatch, 1967). However, perennial plants have an overlying weakness, lack of consistent yields. Johnson et al. observed the biomass yields of multiple herbaceous and woody biomass crops over a five year period in order to determine consistency and variations. ‘Sunburst’ SG was shown to be the only cultivar of SG to establish and produce consistent biomass. ‘Aureomarginata’ and ‘Red River’ PCG cultivars were the most consistent, high yielding plants; however, yield variability occurred across test locations (G. A. Johnson, Wyse, & Sheaffer, 2013).

Further temporal biomass studies are much needed in order to identify cultivar potential in order to progress PCG and SG as biomass crops.

## **Energy Output and Carbon Footprint**

### ***Energy Output***

Much controversy occurs over crop use in biofuel production. The production and use of corn in ethanol production generates vitriol in the reallocation of crops outside foodstuff use. The efficiency of corn ethanol is often in question. The USDA 2008 Corn Ethanol Energy Balance study shown that for every 1 BTU of fossil fuel input, 1.9-2.3 BTU of energy is gained (USDA, 2010). A positive energy balance allows corn ethanol to be carbon neutral; however, the global energy needs have emphasized the need of higher energy outputs. The full biofuel output of all U.S. corn and soybean production would only meet 12% of current gasoline demands (Hill, Nelson, Tilman, Polasky, & Tiffany, 2006). The use of SG in biofuel production have promising results, showing a 1 BTU input to 5.4 BTU output (Schmer, Vogel, Mitchell, & Perrin, 2008). A 5.4 fold energy investment return further ensures global energy demands can be met.

### ***Carbon Footprint***

The environmental impact of SG and PCG production range from greenhouse gas (GHG) emissions, carbon sequestration, and nutrient runoff to name a few.

GHG emissions include CO<sub>2</sub>, methane gas, and nitrous oxide as the primary perpetrators in agriculture. CO<sub>2</sub> emissions occur primarily during combustion of biomass to generate energy and the use of fossil fuels in production. Methane gas emissions occur throughout

the use of fossil fuels and the application of fertilizers. Nitrous oxide occurs primarily through fertilizer usage.

### **Land Management and Bonuses**

PCG growth in saline environments and wet, coarse soils is accompanied with environmental remediation and restoration efforts. As a sod-forming grass that builds an extensive root network, PCG may be used to prevent soil erosion and restore wet prairies (Fraser & Kindscher, 2005). As a native grass of the U.S., it is not considered as a potential invasive plant; however, it may be used to combat the invasive *Phalaris arundinacea* in stormwater wetlands (Bonilla-Warford & Zedler, 2002).

Gamble et al. has shown that SG and PCG show promise in alley cropping systems. Both grasses established well in the alley between woody perennials (Gamble, Johnson, Sheaffer, Current, & Wyse, 2014). Alley cropping further utilizes agricultural land while maintaining benefits such as reducing abiotic stresses of temperature and wind and increases water conservation (Jose, Gillespie, & Pallardy, 2004), (Quinkenstein et al., 2009).

### **Crop Quality in Biofuel Production**

The use of biomass in order to produce biofuel is known as second generation technology, in particular lignocellulosic biofuels. The first generation being biofuel produced from oils and sugars. Second generation biofuel production involves alternative reactions to produce hydrocarbon fuel. Through either thermochemical or biochemical processing, biofuel is produced. One common thermochemical process involves the

formation of syngas from gasified biomass. This syngas is subjected to the Fisher Tropsch method (Naik, Goud, Rout, & Dalai, 2010). Ratios of H and C are critical in achieving efficiency in the conversion from syngas to liquid biofuel. Quality of raw materials used heavily influences the rate of reactions as well of compounds produced. As such, Moutsoglou has modeled syngas H<sub>2</sub> and CO composition of PCG and SG. In identifying their chemical profiles, the variables in the Fischer Tropsch method may be controlled, optimizing liquid biofuel formation (Moutsoglou, 2012). In the production in cellulosic biofuels via biochemical processes, separation of components is a high priority. Simplistic composition, such as high lignin content, low nitrogen and simple sugars are preferred in order to proceed into fermentation smoothly. Nitrogen and simple sugars hinder enzymatic activity of cellulases. Biomass crops grown on marginal lands with low nutritional value are preferred in biofuel production. The perennial nature of PCG and SG includes the reallocation of nutrients to the root system prior to and during senescence. By time the killing frost occurs, aboveground biomass has senesce with low to no nutritional value, yet high in lignin content. Cybulska et al. shown that SG has higher lignin purity compared to PCG and corn stover, facilitating biochemical conversion (Cybulska, Brudecki, Rosentrater, Julson, & Lei, 2012).

## **Senescence**

Nooden (1988) described senescence as a highly regulated process involving molecular, biochemical, and physiological changes that results in death. Senescence is a broad term to cover the complex process with many factors involved. Factors promoting senescence range from abiotic stresses such as water, light, temperature, salinity, and

nutrient deficiency to biotic markers of meristem indeterminacy, reproductive stages, plant age, pathogen infection, and hormonal response (Munne-Bosch, 2008). Plant senescence is typically associated with physiological changes such as leaf yellowing and aerial tissue death; however, molecular and biochemical changes during senescence is relatively unknown in perennials and PCG and SG are no exceptions.

Water is an essential ingredient to life, influencing a wide range of biochemical reactions. Both the abundance and lack of water cause deviations to plant growth, development, and senescence.

In periods of drought, leaf senescence of older leaves is seen as a survival mechanism. While photosynthetic capacity is reduced, so is water loss and remobilization of nutrients occurs from leaves to nutrient sinks in an attempt to weather the drought. Absciscic acid (ABA) and cytokinins have been observed to regulate sugar reallocation in rice (*Oryza sativa*) during water stress (Yang, Zhang, Wang, Zhu, & Liu, 2002). In *Sorghum bicolor*, drought-induced leaf senescence is linked to a deviation in the carbon: nitrogen ratio (Chen, Wang, Xiong, Cao, & Deng, 2015). As drought causes the remobilization of sugars and nitrogen, an imbalance occurs further aggravating leaf senescence drought response.

Periods of flooding and waterlogging induce early senescence. Waterlogged plants suffer from lowered cell permeability and root respiration, causing roots to be prone to early senescence (Patwardhan, Nieber, & Moore, 1988). Waterlogging decreases root respiration and the overall uptake of oxygen, severely limiting growth. The lack of oxygen causes a cascade of detrimental effects on a plant. In rape seedlings, photosynthetic genes were downregulated while early senescence genes were upregulated

(Lee et al., 2014). In wheat (*Triticum aestivum*), waterlogging induces early leaf senescence (Araki, Hamada, Hossain, & Takahashi, 2012).

### **Light, Temperature, and Senescence**

Light of varying light intensities and photoperiod duration regulate the onset of senescence. Low intensities and the absence of light results in a dark-induced senescence. Arabidopsis under dark conditions synthesizes ethylene in response (Ueda & Kusaba, 2015).

Photoperiod length and temperature are often associated with senescence by affecting flowering. Arabidopsis flowering is thought to be dependent on regulatory pathways signaled by photoperiod changes and vernalization, known as the process which a plant gains the capacity to flower post exposure to periods of cold temperature (Reeves & Coupland, 2000).

### **Nutrition and Senescence**

Nutrition influences senescence in multiple methods. Inadequate adsorption of nutrients such as nitrogen and phosphorus affects growth and proper development. Transitions from growth stages to flowering stage may be delayed accordingly. Nutrient-dependent source-sink communication has been proposed as a senescence regulating factor (Thomas, 2013). Removal of the shoot above a senescencing leaf in *Nicotiana rustica* and cytokinin treatment causes a greening of the leaf (Zavaleta-Mancera, Franklin, Ougham, Thomas, & Scott, 1999). High sugar levels to nitrogen content has

been shown to initial leaf senescence in *Arabidopsis* (Wingler, Purdy, MacLean, & Pourtau, 2006).

### **Plant Age, Developmental Stage, and Senescence**

Aging and senescencing are commonly linked traits among a majority of higher life. Many theories have reasoned the evolution of senescence is associated due to mortality and fertility factors in relation to aging (Hamilton, 1966). However, range of variability with age and senescence is vast and species-dependent (Caswell, 1978). In some plant species, Caswell and Salguero-Gomez demonstrated plant age and development stages pressure the senescence process (Caswell & Salguero-Gomez, 2013). Stage-dependent senescence may not only be species-specific, but rather genetic-specific. Upland and lowland switchgrass varieties vastly differ in senescence period as well as metabolism. Longer growing seasons of southern cultivars of switchgrass demonstrate a longer period of growth and development and senescence much later than northern, shorter growing seasoned cultivars (Palmer et al., 2014).

### **Hormonal Response and Senescence**

Multiple phytohormones, ABA, cytokinin, ethylene, strigolactone, jasmonic acid, and salicylic acid, are linked in regulating senescence. These phytohormones are generally downstream of signaling pathways such as environmental responses and cues. As previously mentioned, ABA and cytokinins remobilize sugars in rice as a response during drought. The translocation of sugars further disrupts the carbon: nitrogen balance enhancing the senescence response.

Ethylene synthesis is stimulated in dark-induced senescence. Ethylene is implicated in fruit ripening, leaf abscission, and flower senescence (Reid & Wu, 1992) while functioning in the inhibition of auxin (Burg, 1968). The phytohormone strigolactone is noted to enhance leaf senescence progress when coupled with ethylene in *Arabidopsis* (Ueda & Kusaba, 2015).

Cytokinins promote cell division, but also serve to inhibit senescence (Gan & Amasino, 1995). Cellular levels of cytokinin would need to be low in order to shift the balance to cellular senescence.

External application of jasmonic acid on *Arabidopsis* facilitate early leaf senescence. Jasmonic acid pathways increase in expression during leaf senescence (He, Fukushige, Hildebrand, & Gan, 2002).

The onset of drought-induced leaf senescence in common sage (*Salvia officinalis*) has shown an accumulation of salicylic acid (Abreu & Munné-Bosch, 2008).



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## CHAPTER 2

### Abstract

Senescence of perennial biofuel crops is a major obstacle in limiting yield quantity and quality. Perennial senescence refers to the death of above ground biomass and reallocation of nutrients to subterranean tissues prior to dormancy. Early senescence of crops limits optimal use of the growing season while late senescence becomes detrimental to crop viability next growing cycle. Transcriptome level analysis of early and late senescencing genotypes of switchgrass over the progression of senescence reveals pathway differences accounting for early senescence initiation and for prolonging the growing season. Notable upregulated pathways for prolonged growth and delayed senescence are focused around light reactions, the Calvin-Benson Cycle, and starch metabolic pathways. Phenylpropanoids are up- and down-regulated in the late senescencing genotype with an accumulation of anthocyanin. A late accumulation of anthocyanin may indicate a delayed cold response in the late genotype. These pathways are excellent candidates for investigation for yield enhancement for future applications in crop breeding in respect to senescencing perennials.

## Introduction

Plant senescence is a poorly understood process that greatly influences crop yield. It plays a pivotal role in food security as well as a limiting constraint in biofuel research. Senescence is described as a highly regulated process involving molecular, biochemical, and physiological changes that result in death (Nooden, 1988). However, the death is generally isolated to organ systems in perennial plant models. Perennials generally establish themselves without propagating within the first years of germination. Subsequent growing seasons include the development of seeds. One advantage of perenniality is that multiple harvests may be achieved for one establishment period. The establishment of crops generally involves heavy investments in nutrients such as fertilization for nitrogen and phosphate needs. Multiple seasons of crop sowing involves financial losses in nutrient input and detrimental effects on the environment from nitrogen runoff (Wherley et al., 2015) (Adler, Grosso, & Parton, 2007). Utilizing perennial systems minimizes startup costs; however, senescence among these plants generally vary due to native growing season lengths.

Switchgrass (SG) and prairie cordgrass (PCG), a native, warm season, perennial grass, have genotypes adapted to higher latitudes senescencing earlier in the year than lower latitude genotypes due to an earlier onset of killing frost (Palmer et al., 2014). The molecular differences among different latitude adjusted cultivars are poorly understood. In order to maximize biomass yields for perennials by fully utilizing the growing season, current SG breeding programs need to take advantage of the genetic differences accounting for earlier or later onset of senescence.

## **Objectives**

The objectives of this study were:

1. To document the phenotypes exhibited by early and late senescencing genotypes of SG and PCG
2. To generate transcriptome data of early senescencing and late senescencing
3. To identify senescence associated differentially expressed transcripts in SG

## **Materials and Methods**

### **Plant Material**

Field established genotypes of SG and PCG were grown over several years. SG cultivars were chosen from a native cv Sunburst population. Of the SG population, two genotypes, early and late senescencing, were identified while early and late senescencing populations of PCG refers to North Dakotan and South Dakotan genotypes, respectively. Early senescencing SG and PCG is characterized by heading 1-2 weeks earlier than late senescencing genotypes and reaching full senescence 2-3 weeks earlier in autumn. Leaf and crown samples and measurement data of early and late senescencing were collected from late August to early October every 3-4 days. Determination of pre- and post-senescence states was determined through chlorophyll readings on a handheld chlorophyll meter (CCM-200 by Opti-Sciences), moisture content, and nitrogen content. Three repetitions of leaf, crown, and rhizome tissues were isolated from early and late senescencing genotypes and flash frozen by liquid nitrogen submersion and stored in -80°C.

### **Collection for biomass determination**

Three repetitions of 1 ft. by 1 ft. cross section of above ground biomass was collected for each PCG senescence group while five tillers of SG were collected. Three repetitions of below ground biomass of PCG was collected by coring a 3 in. diameter, 6 in. deep cylinder of soil of PCG sod. Soil was brushed off. Samples were measured for fresh weight and then placed in a 90° C chamber for 2 days. After drying the samples, dry weight was measured and moisture content was calculated.



### **Determination of chlorophyll content**

Chlorophyll content was measured using CCM-200 chlorophyll meter. Ten chlorophyll measurements were taken for each senescence genotype of SG and PCG every 3 to 4 days for twelve sampling dates from late August to early October.

### **Moisture Content Determination**

Moisture content was determined for above- and below-ground biomass. Samples were first weighed to determine fresh weight, incubated at 100°C for 24-36 hours, and weighed to determine dry weight. Percent moisture was calculated as:

$$\% \text{Moisture} = \frac{\text{Fresh wt} - \text{Dry wt}}{\text{Fresh wt}}$$

### **Nitrogen Determination**

Leaf, crown, and rhizome samples were dried overnight and grounded into a fine powder. Samples were sent to Agvise Laboratories (Benson, MN) for nitrogen content determination via combustion.

### **RNA Extraction**

RNA was extracted via the TriZol Reagent protocol by Life Technologies (Carlsbad, CA). Samples were extracted and RNA integrity was determined by gel electrophoresis on a 1% agarose gel. A NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA) was used to test RNA samples of purity and concentration.

### **RNASeq Library Preparation and Illumina Sequencing**

Library construction and sequencing was performed by the University of Illinois-Urbana Champaign Sequencing Facilities. RNAseq Library construction was prepared using Illumina's TruSeq Stranded RNAseq Sample Prep Kit (San Diego, CA). The

libraries were pooled in equimolar concentration, quantitated by qPCR and sequenced on four lanes for 161 cycles from one end of the fragments on an Illumina HiSeq2500 (San Diego, CA) using a HiSeq SBS sequencing kit version 4. Fastq files were generated and demultiplexed with the bcl2fastq v1.8.4 Conversion Software (Illumina, San Diego, CA). Total read length are 160 nt.

### **Genomic Mapping and Identification of Differentially Expressed Transcripts**

The reads were trimmed and mapped on CLC Genomic Workbench 7.5. RNASeq and statistical analyses were performed on CLC Genomic Workbench 7.5. Parameters used include: maximum number of mismatches of 2, minimum length fraction of 0.8, minimum similarity fraction of 0.9, and an unspecific match limit of 10. Transcript expression was calculated by reads per kilobase of exon model per million mapped reads (RPKM). MapMan 3.5.1R2 was used to map differentially expressed transcripts over Arabidopsis metabolic pathways.

## **Results**

### **Moisture Content**

Moisture content of above ground biomass of early senescencing SG began to decrease after the 5<sup>th</sup> collection interval (September 23, Figure 1). Late senescencing SG began decreasing in moisture content after the 8<sup>th</sup> interval (October 5, Figure 2). On the 7<sup>th</sup> interval (October 2), rainfall accounted for an increase in moisture. Above ground biomass of early senescencing PCG began decreasing after the 5<sup>th</sup> collection (Figure 3) while late senescencing PCG decreased slightly after the 4<sup>th</sup> collection (September 18, Figure 4). Figure 5 shows a slight increase in moisture content in below ground biomass of early senescencing PCG as opposed to a steady moisture content of late senescencing PCG (Figure 6).

### **Chlorophyll Content**

Chlorophyll content decreased over time in both SG and PCG (Figures 7, 8, 9, 10). However, Figure 9 shows early senescencing PCG consistently has lower chlorophyll content as opposed to the late senescencing genotype. Figures 7 and 8 shows a sharp decrease in SG chlorophyll after the 6<sup>th</sup> collection (September 21).

### **Nitrogen Concentration**

Leaf nitrogen decreased as senescence progresses in both SG and PCG. Early senescencing SG showed the steepest decline over 15 days while the others are uniform in decline (Figure 11). Figure 12 shows nitrogen content of crown samples. Early senescencing SG showed a sharp increase, inverse of rate of nitrogen loss in its leaves while late senescencing SG was generally constant. Both genotypes of PCG was observed to steadily increase in nitrogen. SG rhizome nitrogen content (Figure 13) of

both genotypes sharply increased after the 2<sup>nd</sup> week of September while late senescencing PCG gradually increased in rhizome nitrogen accumulation. Early senescencing PCG appeared to have reallocated nitrogen stores to its rhizomes prior to sampling.

### **Differentially Expressed Transcripts and Expression Mapping**

Average reads per sample was around 40 million reads (Table 1).

#### **Temporal Expression**

Pathway analysis in MapMan across sampling points span two weeks. The first sampling date, representing an early/pre-senescence state was September 13 (9-13). The second sampling date, representing a late/post-senescence state was September 27 (9-27). Differences in expression as senescence progresses in early senescencing SG leaves are reflected in Figure 14 and for late senescencing leaves in Figure 15. Crown expression is likewise expressed for early senescencing SG in Figure 16 and late senescencing SG in Figure 17.

#### **Cross Genotype Expression**

The expression variation between the two genotypes is shown in Figure 18 and 19 for leaf samples on 9-13 and 9-27, respectively. Figures 20 and 21 show crown expression between the genotypes on 9-13 and 9-27, respectively.

### **Transcript Expression as Senescence Progresses**

#### ***Photosynthetic Pathways***

Mapped differentially expressed transcripts to photosynthetic pathways of early senescencing SG leaves show consistent downregulation of light reactions, the Calvin-Benson cycle, and photorespiration as senescence progressed (Figure 14 and 22). Late senescencing SG leaves reveals the opposite, upregulated transcripts across

photosynthetic pathways (Figure 15 and 23). When comparing early and late senescencing genotypes on 9-13 and 9-27, late senescencing SG leaves show consistent upregulation of photosynthetic pathways on both days (Figure 18, 19, 24, and 25).

### ***Jasmonic Acid Synthesis***

Differentially expressed transcripts over the course of collection shows that jasmonic acid (JA) synthesis was downregulated in early senescencing SG leaves and crowns (Figure 26 and 27). Figure 28 shows differentially expressed transcripts for late senescencing SG leaves with the downregulation of lipoxygenase (an oxidoreductase), decreasing conversion of 13(S)-hydroperoxylinolenic acid from linolenic acid. However, the lipoxygenase is upregulated in the crowns while downregulation of allene oxide cyclase, mediating the conversion of 13, 13(S)-epoxylinolenic acid to 12-oxo-PDA (Figure 29). When contrasting early and late senescencing SG on 9-13 and 9-27, late senescencing SG shows an overall higher expression for JA synthesis (Figures 30-33).

### ***Nucleotide Synthesis***

Although few differentially expressed transcripts were mapped in nucleotide synthesis, transcripts from early senescencing SG leaves and crowns reveal downregulation in the pathway (Figures 34 and 35). Late senescencing SG leaves show a mixed result between 9-13 and 9-27, with slight upregulation in nucleotide synthesis (Figure 36). However, late senescencing SG crowns downregulated nucleotide synthesis (Figure 37). When contrasted on 9-13 and 9-27, the late senescencing genotype was more active in nucleotide synthesis than its counterpart (Figures 38-41).

### ***Secondary Metabolism***

Secondary metabolism covers a wide array of metabolites. Differentially expressed transcripts of early senescencing SG leaves and crowns show a general downregulation of pathways involved with secondary metabolites (Figure 42 and 43). When observing transcript expression in late senescencing SG leaves, expression was relatively mixed, with upregulation in the Non MVA pathway and flavonoid synthesis (Figure 44). Late senescencing SG crowns, however, appeared to have a general downregulating trend occurring (Figure 45). Contrasting the genotypes on 9-13 reveals the late senescencing genotype was skewed towards higher expression values than the early senescencing genotype in both leaves and crowns (Figure 46 and 47). On 9-27, late senescencing SG leaves showed higher expression in most secondary metabolism pathways (Figure 48) while the crowns show higher expression of phenylpropanoids as well as lignin and lignan pathways with lower expression of dihydroflavonols (Figure 49).

### ***Pentose Phosphate Pathway***

Transcripts involved in the pentose phosphate pathway are shown to be downregulated in early senescencing SG leaves on 9-27 when compared against 9-13 (Figure 50) while the inverse is observed for late senescencing SG leaves (Figure 51). When contrasting the leaves of the two genotypes on 9-13, no differentially expressed transcripts are identified; however, on 9-27, late senescencing SG leaves showed a higher expression of transcripts between along the conversion from glucose-6-phosphate to ribose-5-P (Figure 52).

## Discussion

### Moisture, Chlorophyll, and Nitrogen Content

During senescence, perennials reallocate nutrients from above ground biomass, leaves and shoots, to below ground biomass, crowns and rhizomes. To determine senescence status, moisture content was used as a marker of senescence progression. Leaf samples show a decline as senescence progresses (Figures 1-4). Moisture content of crowns of both SG genotypes remain consistent through the sampling period (Figure 5-6). Wayman et al. determined SG belowground biomass displays no seasonal changes as opposed to aboveground biomass (Wayman, Bowden, & Mitchell, 2014). Crowns should be preparing to enter dormancy and are expected to remain metabolically static.

Leaf de-greening is commonly associated as the early phenotypic change for senescence, indicating rapid chlorophyll degradation (Nooden, 1988) and could be utilized as a marker to determine senescence initiation and progression. PCG and SG chlorophyll content gradually decreases as much of it is expected to be broken down and reallocated to subterranean tissues in preparation for the winter season (Figure 7-10). Late senescencing PCG is shown to retain more chlorophyll in later dates, indicative of its longer growing season; however, no significant difference was observed between SG genotypes.

As chlorophyll and proteins are broken down and nutrients reallocated, nitrogen distribution across organs would show the migration of nutrients during senescence. In both genotypes of PCG and SG, leaf nitrogen content is shown to decrease as senescence progresses (Figure 11) while crown nitrogen content generally increases (Figure 12). However, nitrogen content in crown of late senescencing SG appears to remain static,

although leaf nitrogen content is on the decline. Late senescencing SG may be prioritizing nitrogen storage in rhizomes rather than crown during sampling. Nitrogen content of rhizomes (Figure 13) shows SG rapidly increased in nitrogen content as senescence progresses. SG grown in Pennsylvania and Nebraska demonstrated similar nitrogen concentrations, gradual reduction in aboveground biomass and an increase of nitrogen in belowground biomass (Wayman et al., 2014). SG's ability to store and conserve more nitrogen for dormancy and the next growing season reduces the dependency on fertilizers (Reynolds, Walker, & Kirchner, 2000) and may further serve as a biologically marker for senescence initiation and progression.

## **Differentially Expressed Transcripts**

### **Metabolism Overview**

Metabolism of early senescencing leaves of SG declined as senescence progresses. As nutrients are reallocated before the killing frost, SG was abruptly interrupting leaf function in preparation for dormancy. More strikingly is the difference in expression between metabolism pathways in late senescencing SG over time. Transcript expression of amino acid and light reaction pathways were upregulated and the Calvin-Benson Cycle was continuing. The lack of an organ-wide down regulation in metabolism illustrates the longer growing season of the more active late senescencing SG genotype.

Crown metabolism in early senescencing SG (Figure 16) revealed few down regulated pathways and few upregulated pathways. Notably were the down regulation of starch and sucrose degradation pathways. Crowns of early senescencing SG may be slowing down for entry into dormancy. In contrast, late senescencing SG crowns reveal a



mix of up- and down regulated pathways. Figure 17 shows the up-regulation of starch synthesis and down regulation of starch degradation. On par with a longer growing season, late senescencing SG was more metabolically active than its early senescencing counterpart.

Contrasting between the early and late senescencing genotypes show the variation in active pathways in early and late senescence. On 9-13, Figure 18 shows that late senescencing SG leaves were more metabolically active in most pathways than early senescencing leaves. Light reactions were well distinguished to be active in late senescencing SG leaves. The expression difference on 9-27, about 2 weeks after, displayed a starker contrast. Figure 19 shows that leaves of late senescencing SG was much more metabolically active than the early senescencing genotype. Figure 14 shows that early senescencing SG leaves would be much less active at 9-27 than 9-13; however, leaves of late senescencing SG stand pose to have enhanced yields as growth continues late in the season.

Expression of crown transcripts show late senescencing SG was more metabolically active both earlier in the senescence period (Figure 20) as well as later on (Figure 21). On 9-27, late senescencing SG crowns were more active in cell wall synthesis and starch metabolism.

### **Photosynthetic Pathways**

Rapid chlorophyll degradation is indicative of senescence initiation and progression. On the phenotypic level, general de-greening of leaf tissues are most predominant. A rapid reduction in chlorophyll would have multiple effects downstream in light reactions, the Calvin-Benson cycle, and photorespiration. Figure 22 shows that

transcript expression for early senescencing SG leaves decreased on 9-27 when compared to 9-13. Conversely, late senescencing SG leaves showed the upregulation of photosynthetic pathways on 9-27 when compared to 9-13 (Figure 23). Comparing genotypes reveal that transcript expression was higher as a whole in late senescencing SG leaves as oppose to the early senescencing genotype (Figures 24 and 25).

### **Jasmonic Acid Synthesis**

JA synthesis transcripts were downregulated on 9-27 when compared to 9-13 in both leaves and crowns. Although jasmonic acid has been shown to promote de-greening in *Arabidopsis* through regulation of chlorophyll degradation genes (Zhu et al., 2015), transcript expression of JA synthesis decreased from 9-13 to 9-27 in early senescencing leaves and crowns. A mixed result of up and down regulated transcripts was observed for late senescencing leaves and crowns when comparing 9-13 to 9-27. Potentially, JA mediated senescence initiated prior to 9-13 in the early genotype while remaining relatively active in the late genotype. The interaction JA has in senescence is not completely known as associated genes show mixed regulation during *Arabidopsis* leaf senescence (He, Fukushige, Hildebrand, & Gan, 2002) (Kim, Chang, & Tucker, 2015). When both genotypes were compared each at 9-13 and 9-27, the late genotype was observed to have higher transcription expression than the early genotype indicating the late genotype was more active in JA synthesis.

### **Nucleotide Synthesis**

Nucleotide synthesis may serve as a general indicator of growth and cell replication. Leaves of the early genotype displayed a general decline in differentially expressed transcripts on 9-27 versus 9-13 while the late genotype over the same interval

revealed a few number of differentially expressed transcripts that are both up and down regulated. Crowns of the early genotype showed two downregulated transcripts when comparing 9-27 to 9-13, few points to indicate whether nucleotide synthesis was dynamically changing over the collection dates. Likewise, crowns of the late genotype have few hits mapped, displaying downregulated transcripts over the same sampling dates. Comparing the genotypes on 9-13 show a few hits indicating higher transcript expression in the late genotype in both leaves and crowns. The lack of differentially expressed transcript hits to nucleotide synthesis may indicate that nucleotide synthesis is generally static or a lack of mapping coverage. However, comparing the genotypes on 9-27, the late genotype displayed multiple mapped upregulated transcripts, indicating that lack of coverage is not an issue. While crowns remained static in nucleotide synthesis in leaves in both genotypes, the early genotype decreased transcript expression while the late genotype was slightly upregulated going from 9-13 to 9-27.

### **Secondary Metabolism**

In leaves and crowns, the early genotype displayed a general downregulation trend of differentially expressed transcripts on 9-27 when comparing to 9-13. Synthesis of secondary metabolites declined over the progression of senescence. The late genotype followed a similar declining trend in crowns, however, leaves displayed a mix result of up and down regulation. As the late genotype is expected to initiate and complete senescence later in the season, a mixture of up and down regulated transcripts may be an indicator of easing secondary metabolite synthesis later on. On 9-27, when compared to early leaves, the late genotype displayed upregulation of multiple pathways.

Phenylpropanoids are commonly associated with stress response pathways. Phenylpropanoids, particularly anthocyanins, may accumulate in response to low temperature (Dixon & Paiva, 1995). Differentially expressed transcripts of the late genotype leaves showed that anthocyanin synthesis transcripts are upregulated later in the season. An accumulation of anthocyanin later in the season for the late genotype may represent a delayed response to cold that is not observed in the early genotype. If the late genotype delays anthocyanin cold response, other cold acclimation responses may be likewise delayed.

### **Pentose Phosphate Pathway**

Early senescencing SG leaves showed downregulation of pentose phosphate pathway transcripts later in the season while the inverse is observed for the late genotype. However, on 9-13, no differentially expressed transcripts were mapped between early and late genotypes indicating similarity in expression. On 9-27, the late genotype displayed upregulation of transcripts from glucose-6-phosphate to ribose-5-P. Paudel et al. investigated the proteome of early and late senescencing prairie cordgrass genotypes where RuBisCo regeneration associated proteins, transketolase and sedoheptulose-1, 7-bisphatase, were upregulated in the late prairie cordgrass genotype (Paudel et al., 2016) (Jones, Ougham, Thomas, & Waaland, 2012) (Lefebvre et al., 2005). Late senescencing SG exhibited upregulation of a putative transketolase and sedoheptulose-1, 7-bisphatase when compared to the early genotype on 9-27. Regeneration of RuBisCo is key to maintaining light reactions and is the limiting factor in those pathways. RuBisCo synthesis and degradation is believed to occur independently, where when synthesis is in occurring, breakdown pathways are not upregulated and vice versa (Thomas, 2013). The

upregulation of RuBisCo regeneration transcripts would entail continuing growth of vegetative tissues in late senescencing SG much later in the growing season than the early genotype.

## Conclusion

Plant senescence is a complex process involving a network of biochemical, morphological, and physiological changes leading toward death of living cells, tissues, or whole organism (Nooden, 1988). Late senescencing genotypes of PCG and SG displayed a delay in biochemical and physiological changes compared to early senescencing counterparts. The transcriptome of SG revealed that the late senescencing genotype retains an active metabolism longer in senescence.

Factors that may influence the upregulation of many transcripts in the late senescencing genotype include any known senescence triggering stimuli such as temperature, light, and nutrient deficiency among many biotic and abiotic elements (Munne-Bosch, 2008).

This study represents an initial characterization of senescence associated transcriptome of SG. Transcript expression was shown to be upregulated in many pathways in the late senescencing genotype while downregulated in the early senescencing genotype. The associated up and downregulated transcripts may lead to protein and metabolite investigations to elucidate potential gene targets to enhance future breeding programs for yield and efficiency.

## Figures

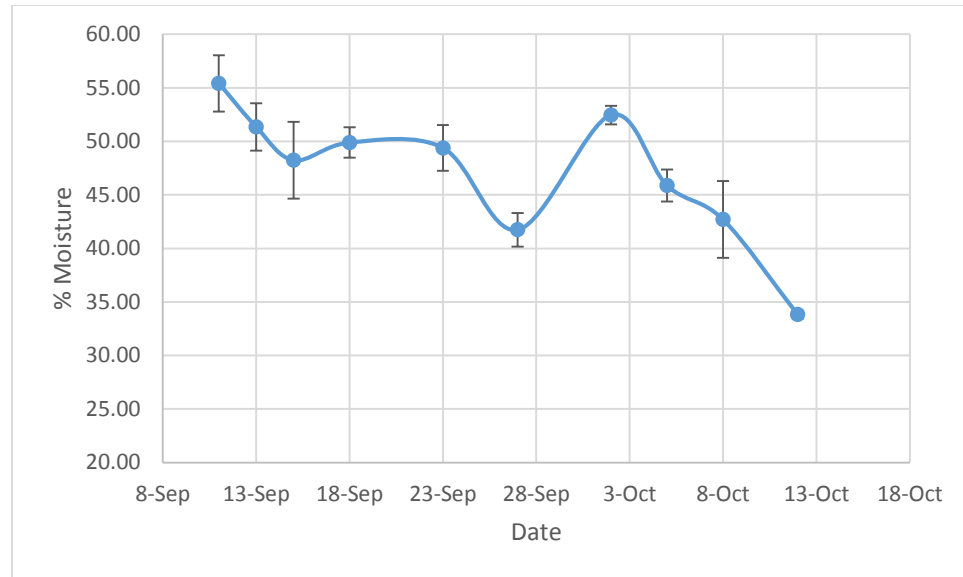


Figure 1 Moisture content of early senescencing switchgrass. Data collection period of September 11 to October 12.

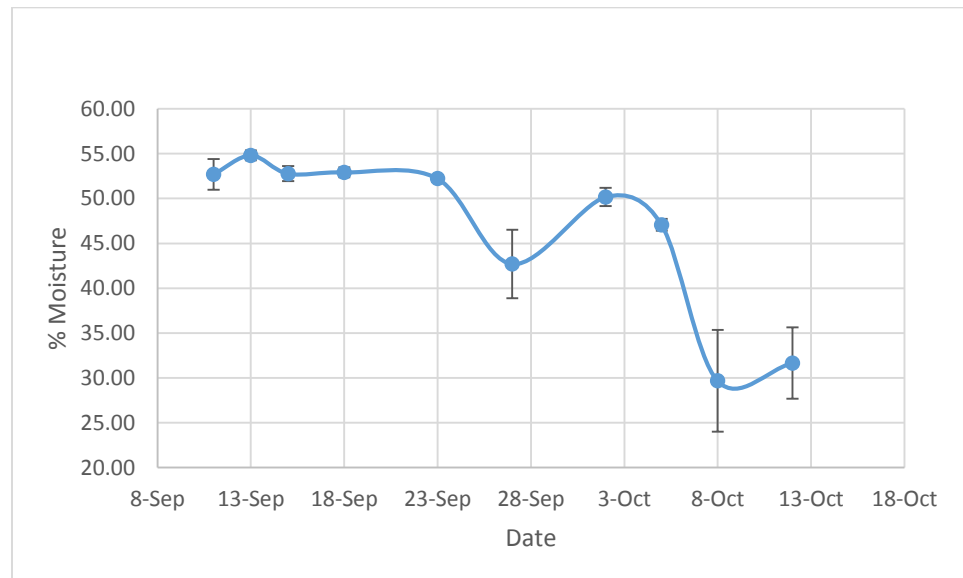
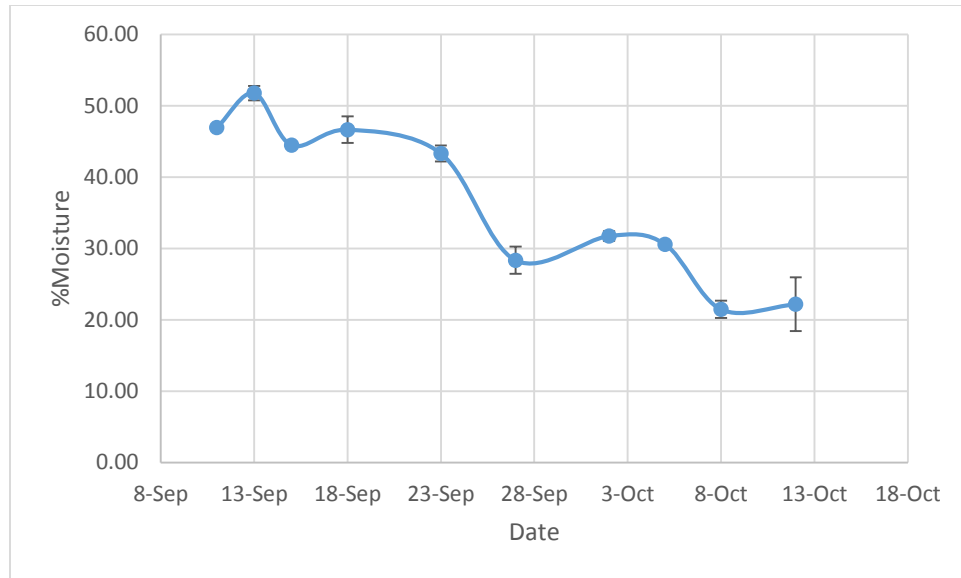
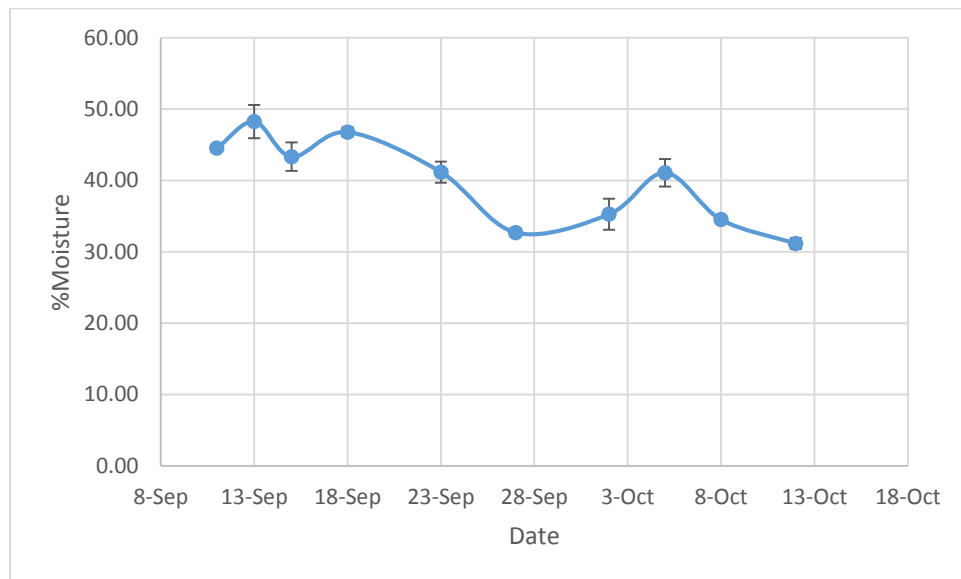


Figure 2 Moisture content of late senescencing switchgrass. Data collection period of September 11 to October 12.

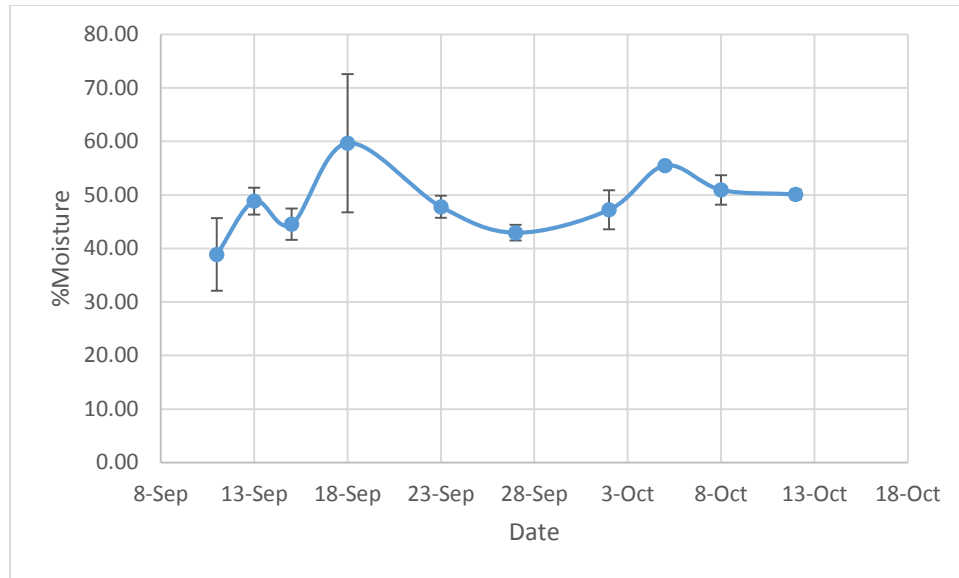


*Figure 3 Moisture content of above ground biomass of early senescencing prairie cordgrass. Data collection period of September 11 to October 12.*

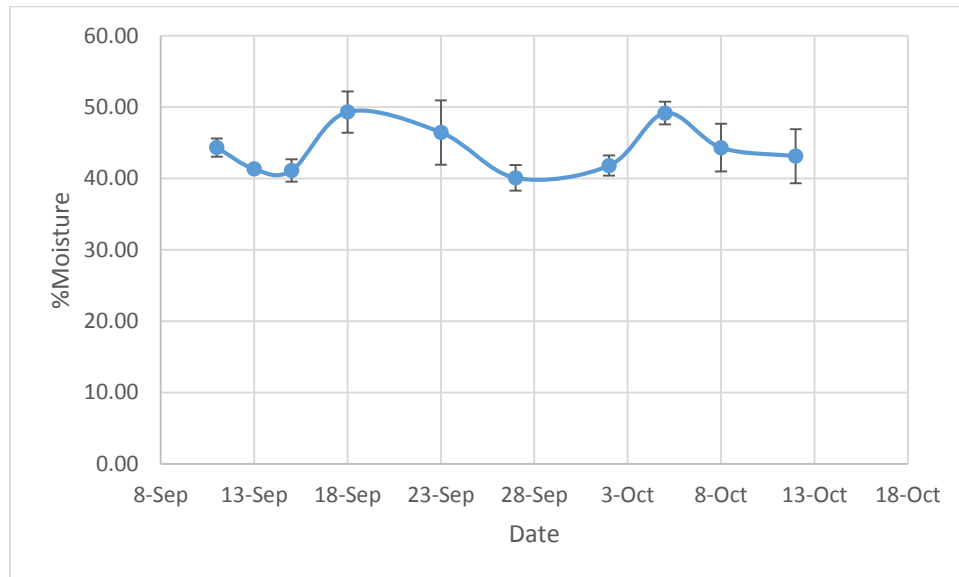


*Figure 4 Moisture content of above ground biomass of late senescencing prairie cordgrass. Data collection period of September 11 to October 12.*





*Figure 5 Moisture content of below ground biomass of early senescencing prairie cordgrass. Data collection period of September 11 to October 12.*



*Figure 6 Moisture content of below ground biomass of late senescencing prairie cordgrass. Data collection period of September 11 to October 12.*

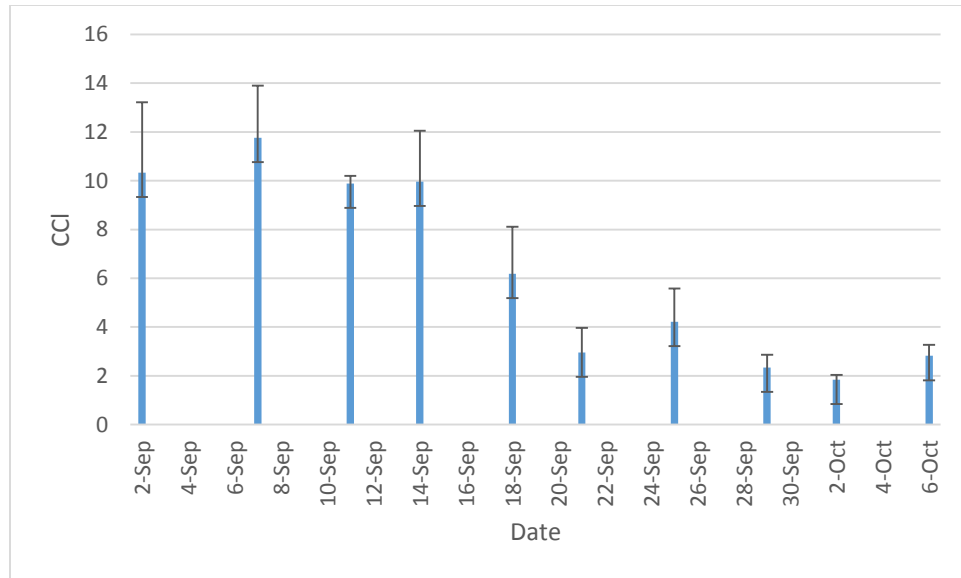


Figure 7 Chlorophyll content of early senescencing switchgrass. Data collection period of September 2 to October 6.

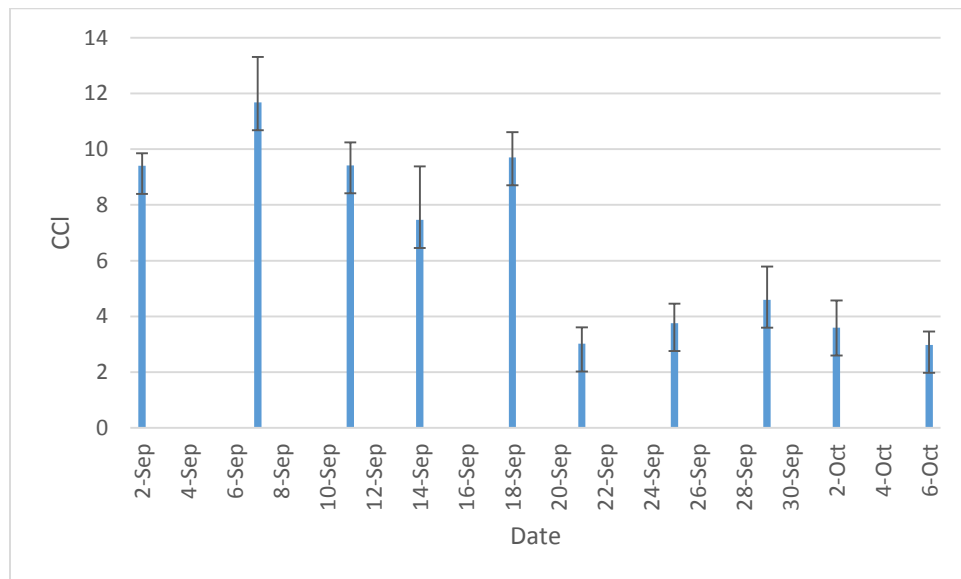


Figure 8 Chlorophyll content of late senescencing switchgrass. Data collection period of September 2 to October 6.

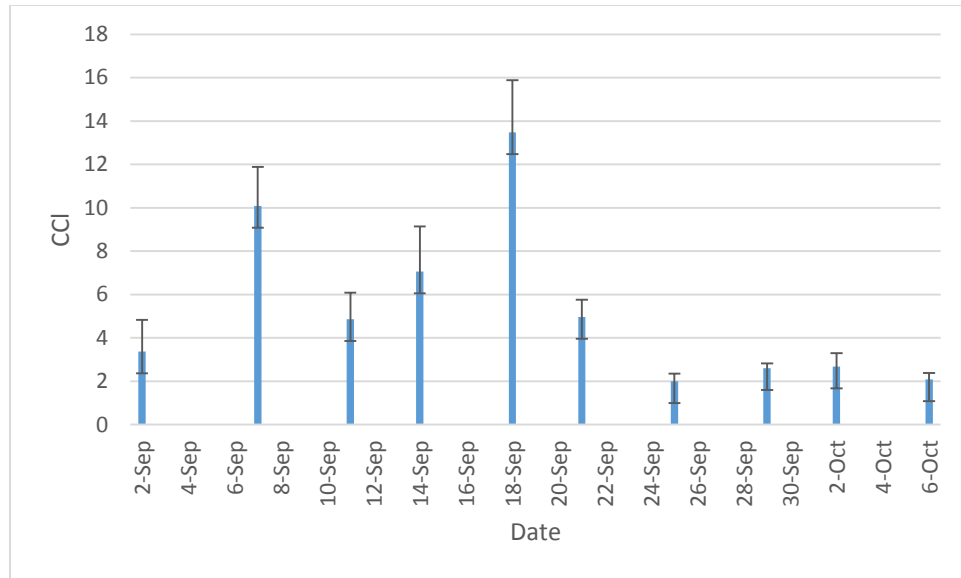


Figure 9 Chlorophyll content of early senescencing prairie cordgrass. Data collection period of September 2 to October 6.

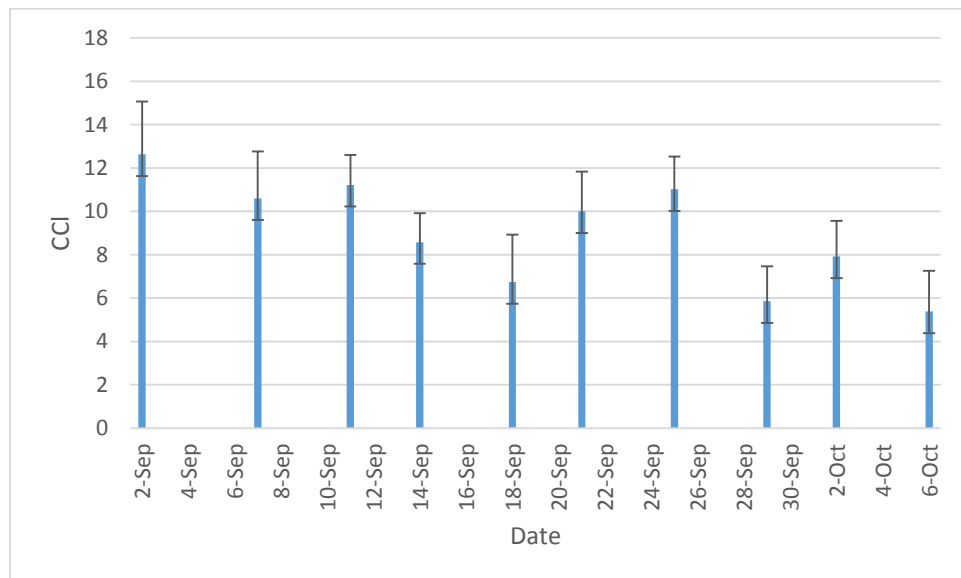


Figure 10 Chlorophyll content of late senescencing prairie cordgrass. Data collection period of September 2 to October 6.

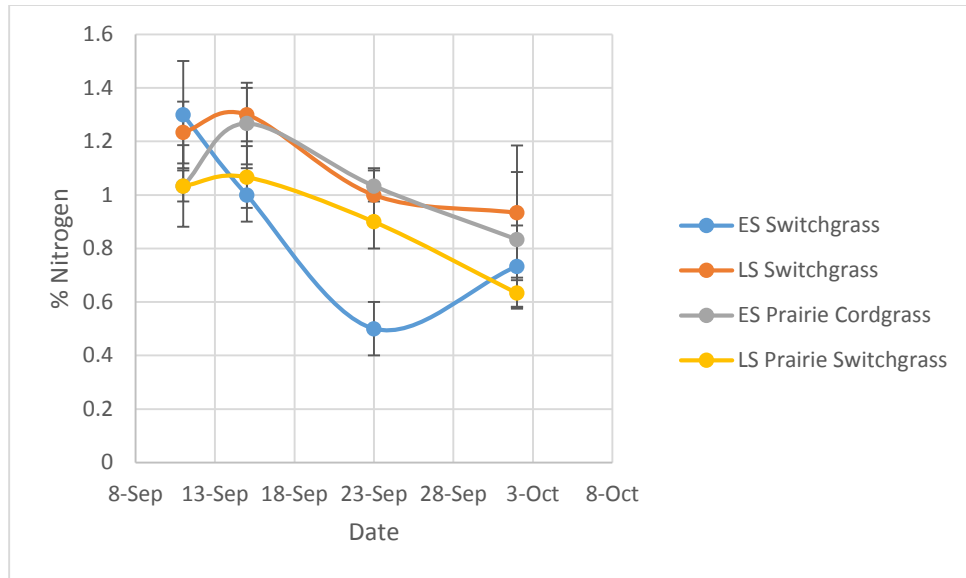


Figure 11 Average nitrogen content of leaves from early (ES) and late (LS) senescencing genotypes of switchgrass and prairie cordgrass. Data spans from September 11 to October 2.

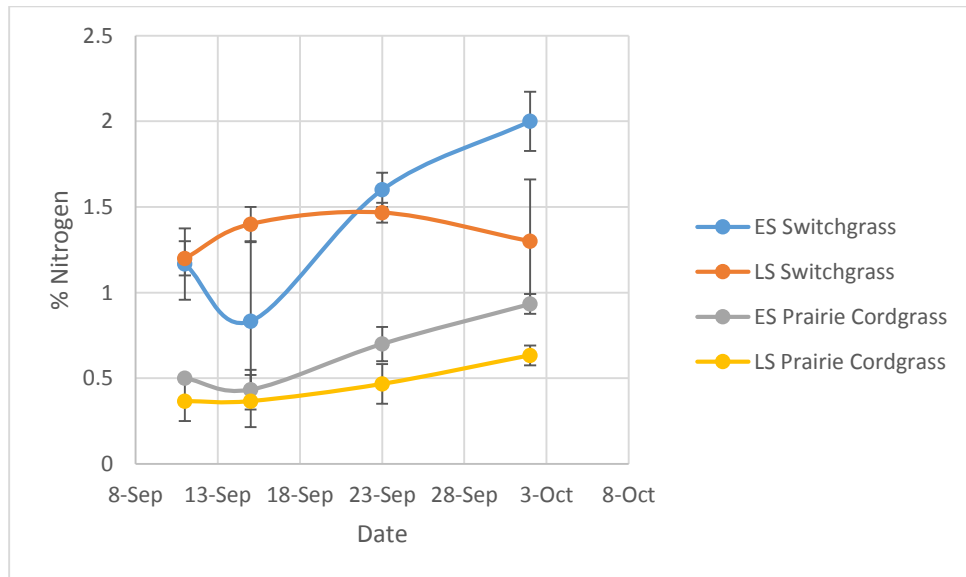


Figure 12 Average nitrogen content of crown from early (ES) and late (LS) senescencing genotypes of switchgrass and prairie cordgrass. Data spans from September 11 to October 2.

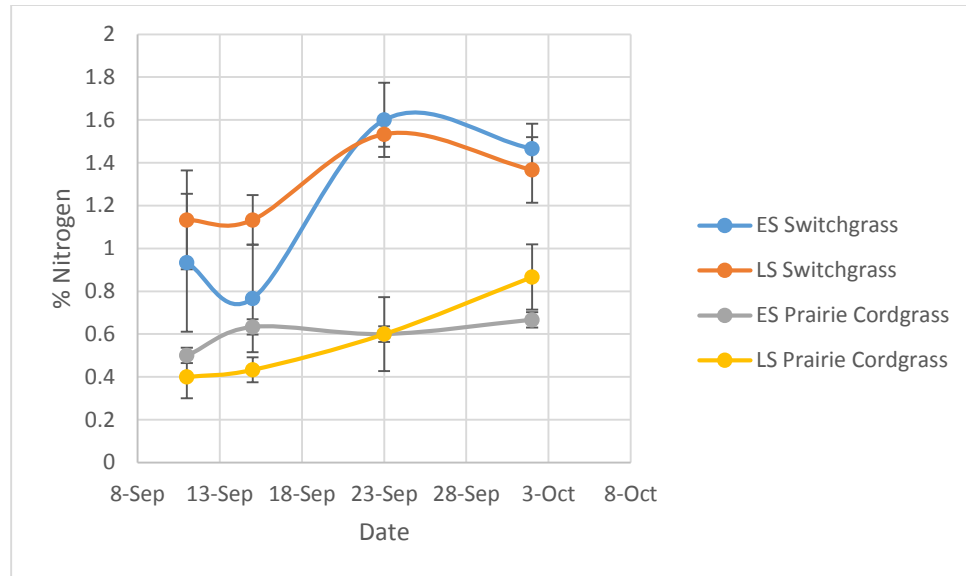


Figure 13 Average nitrogen content of rhizomes from early (ES) and late (LS) senescencing genotypes of switchgrass and prairie cordgrass. Data spans from September 11 to October 2.

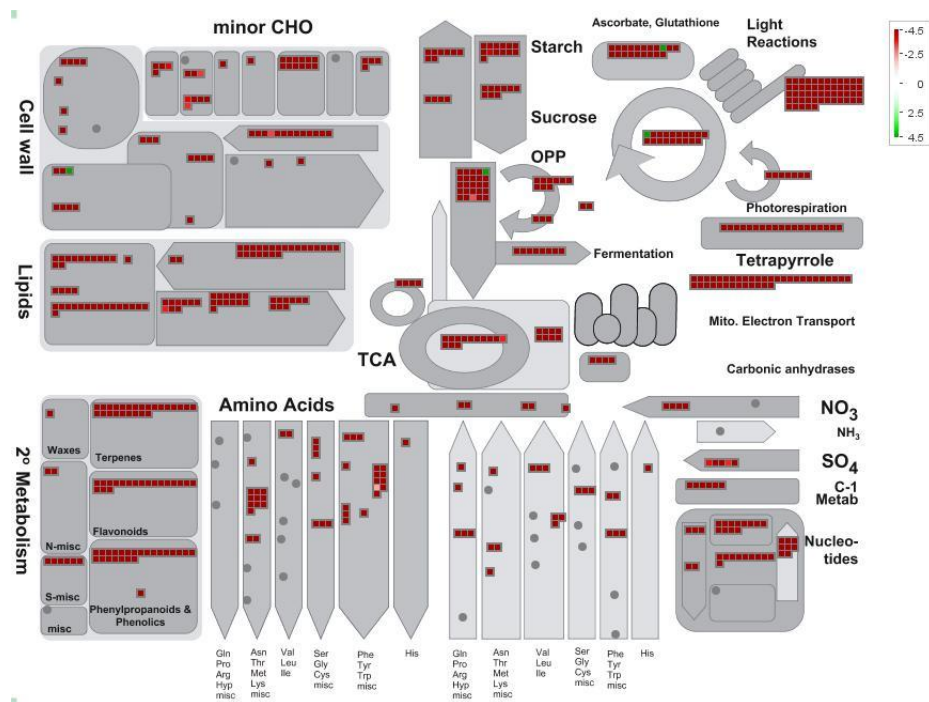


Figure 14 Metabolism overview of differentially expressed transcripts of leaf mRNA of early senescencing switchgrass. Green indicates upregulation of transcripts on 9-27 when compared to 9-13. Red indicates downregulation of transcripts on 9-27 when compared to 9-13.

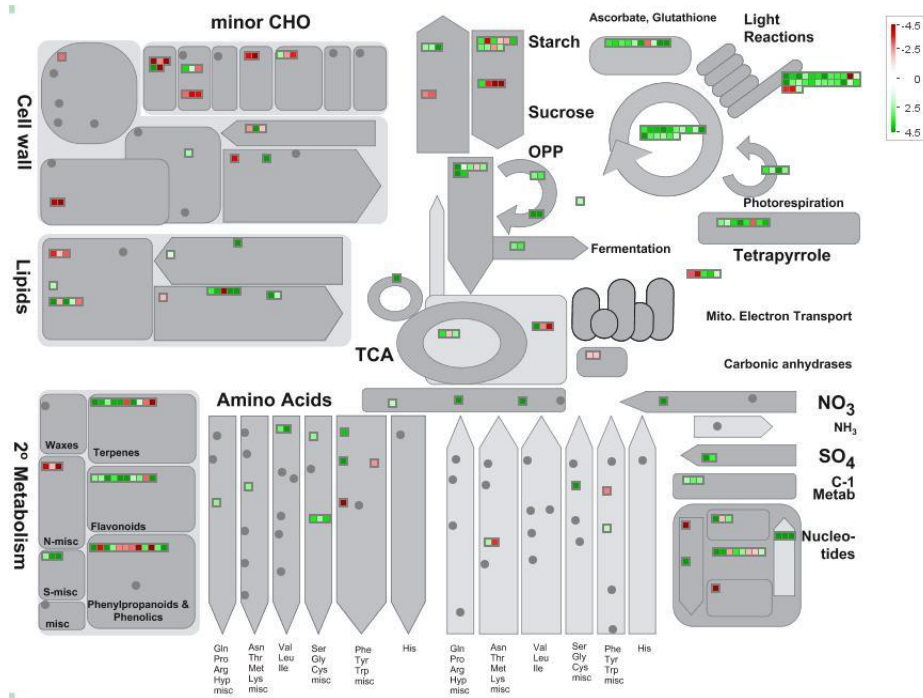


Figure 15 Metabolism overview of differentially expressed transcripts of late senescencing switchgrass leaves. Green indicates upregulation of transcripts on 9-27 when compared to 9-13. Red indicates downregulation of transcripts on 9-27 when compared to 9-13.

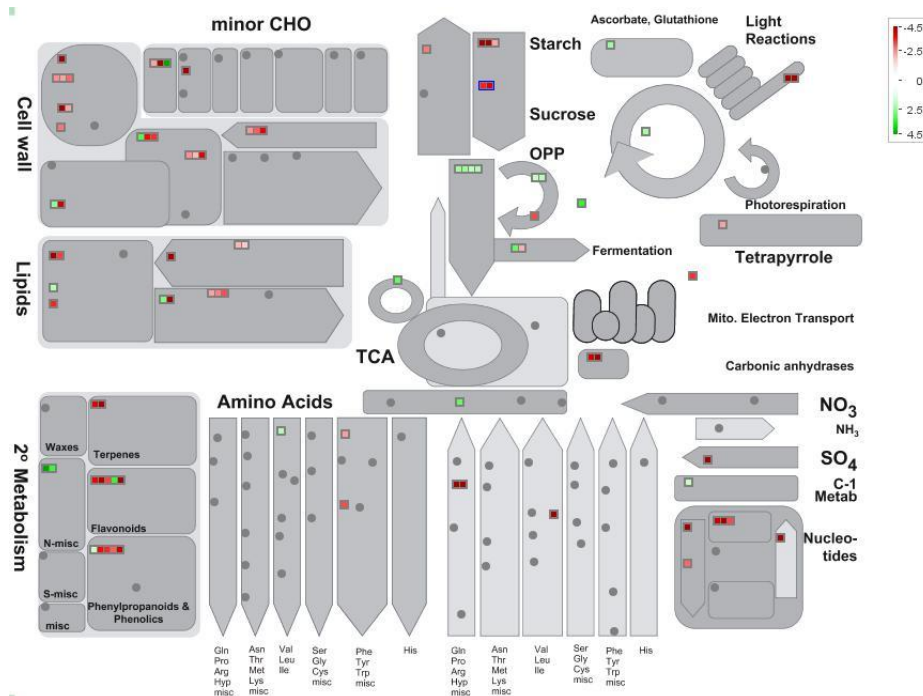


Figure 16 Metabolism overview of differentially expressed transcripts of early senescencing switchgrass crowns. Green indicates upregulation of transcripts on 9-27 when compared to 9-13. Red indicates downregulation of transcripts on 9-27 when compared to 9-13.

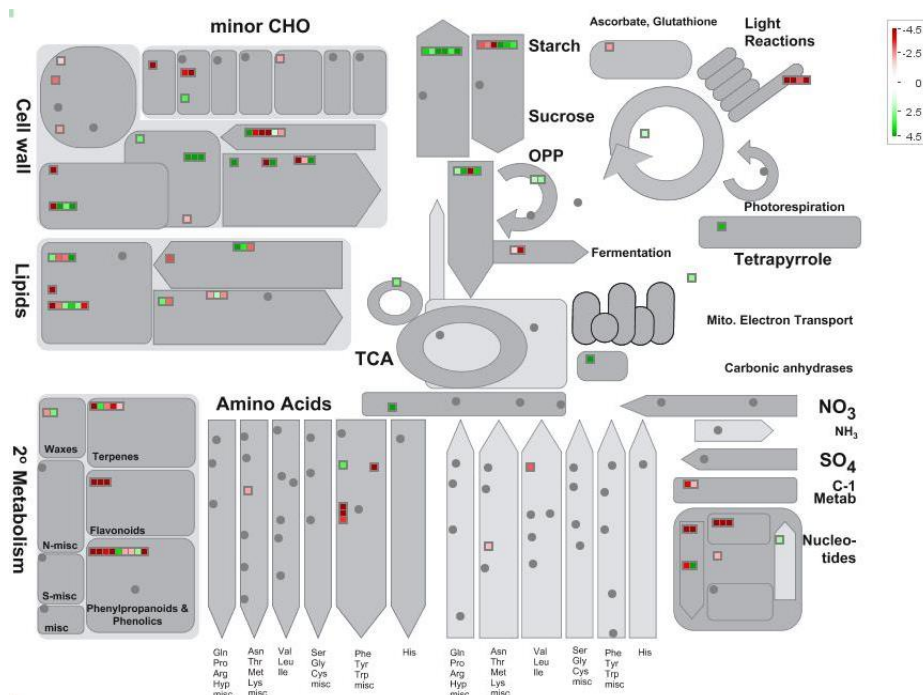


Figure 17 Metabolism overview of differentially expressed transcripts of late senescencing switchgrass crowns. Green indicates upregulation of transcripts on 9-27 when compared to 9-13. Red indicates downregulation of transcripts on 9-27 when compared to 9-13.

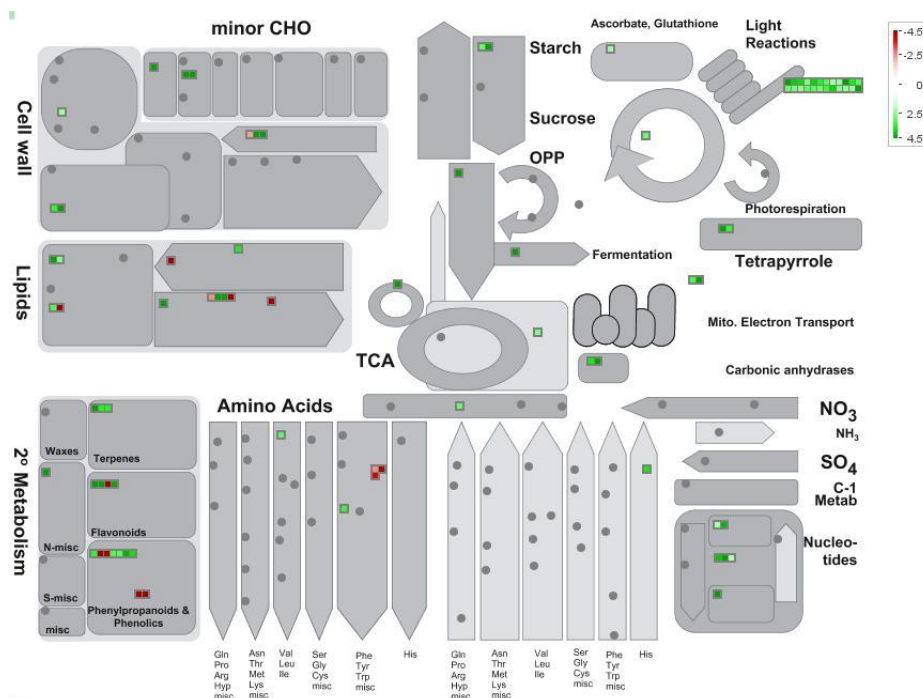


Figure 18 Metabolism overview of differentially expressed transcripts between early and late senescencing switchgrass leaves on 9-13. Green indicates upregulation of transcripts of the late genotype when compared to the early genotype. Red indicates downregulation of transcripts of the late genotype when compared to the early genotype.

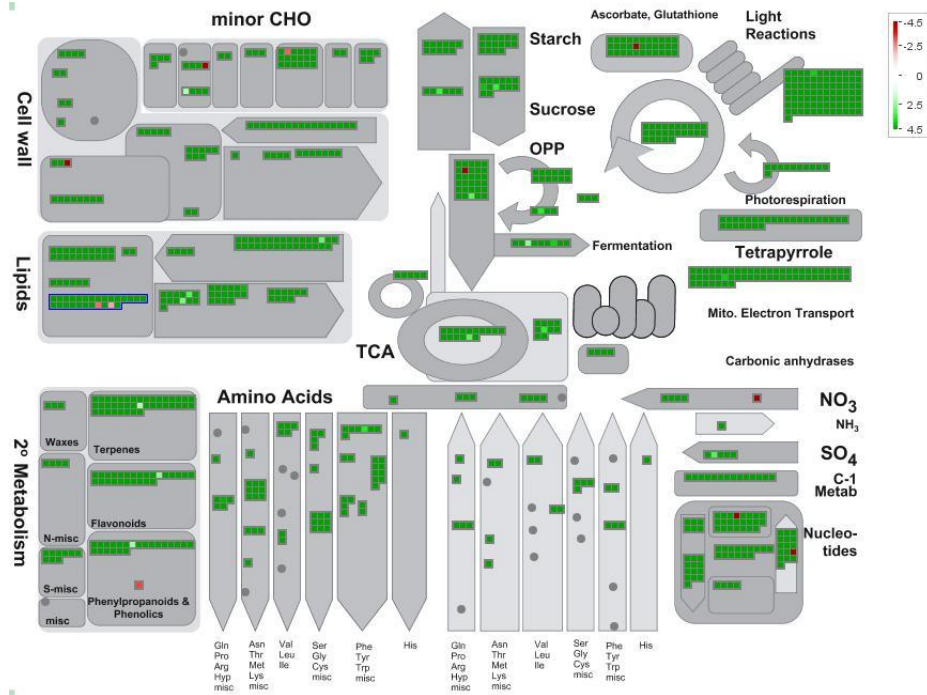


Figure 19 Metabolism overview of differentially expressed transcripts between early and late senescencing switchgrass leaves on 9-27. Green indicates upregulation of transcripts of the late genotype when compared to the early genotype. Red indicates downregulation of transcripts of the late genotype when compared to the early genotype.

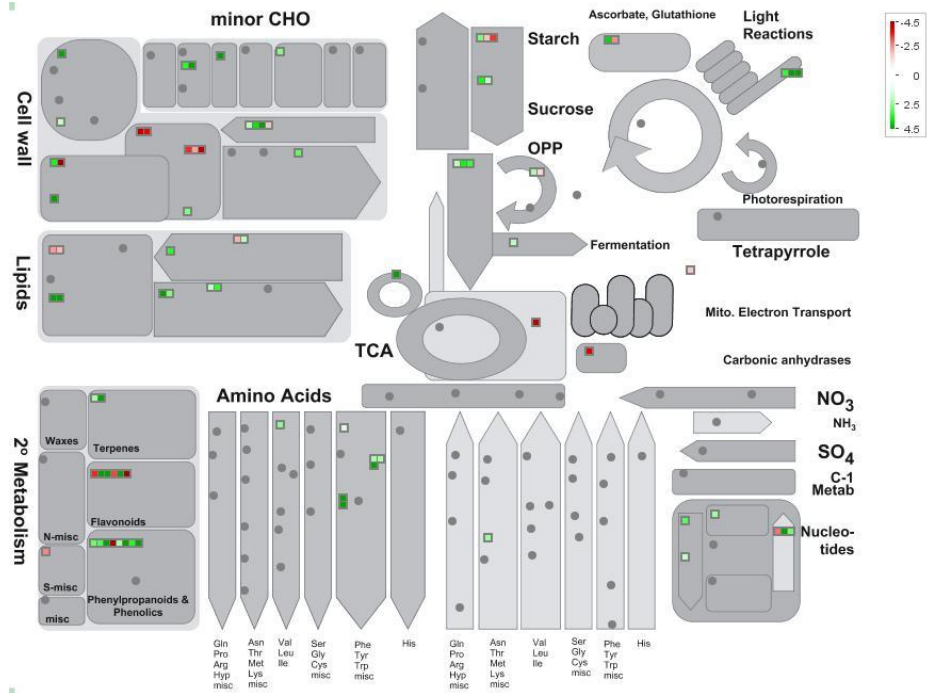


Figure 20 Metabolism overview of differentially expressed transcripts between early and late senescencing switchgrass crowns on 9-13. Green indicates upregulation of transcripts of the late genotype when compared to the early genotype. Red indicates downregulation of transcripts of the late genotype when compared to the early genotype.



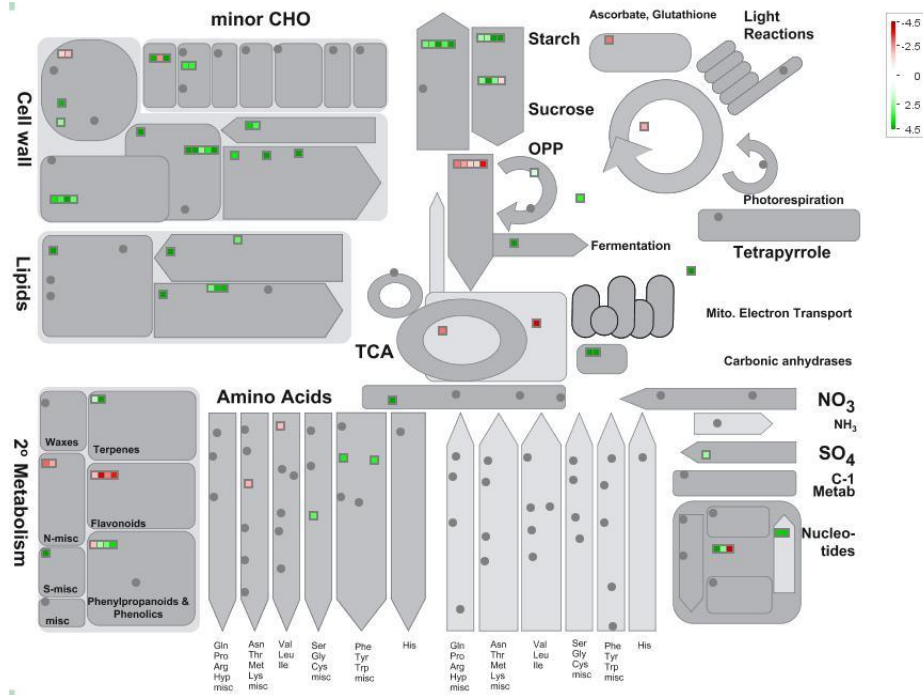


Figure 21 Metabolism overview of differentially expressed transcripts between early and late senescencing switchgrass crowns on 9-27. Green indicates upregulation of transcripts of the late genotype when compared to the early genotype. Red indicates downregulation of transcripts of the late genotype when compared to the early genotype.

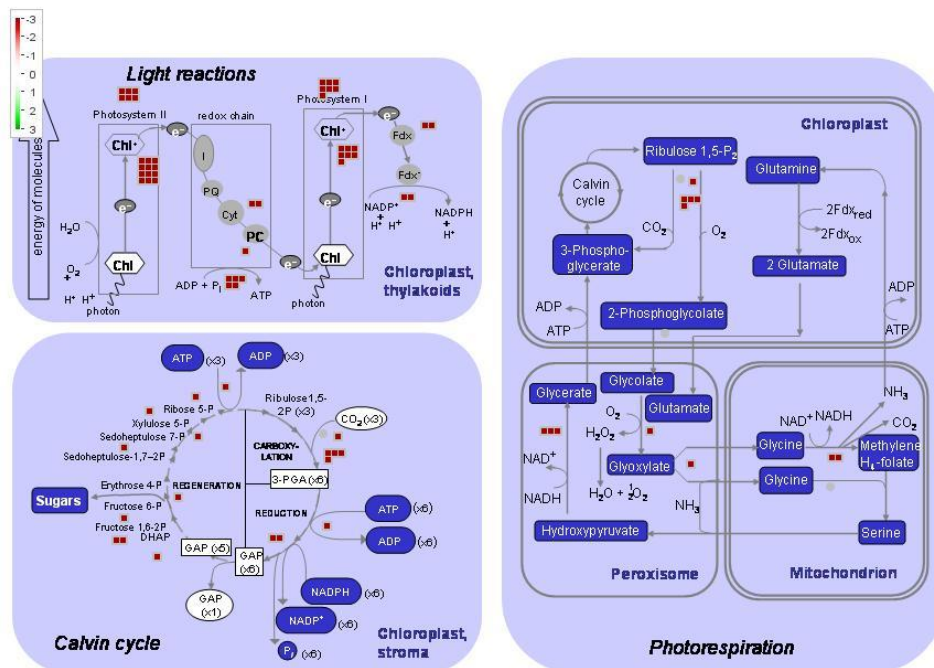


Figure 22 Photosynthetic pathway overview of differentially expressed transcripts of early senescencing switchgrass leaves. Green indicates upregulation of transcripts on 9-27 when compared to 9-13. Red indicates downregulation of transcripts on 9-27 when compared to 9-13.

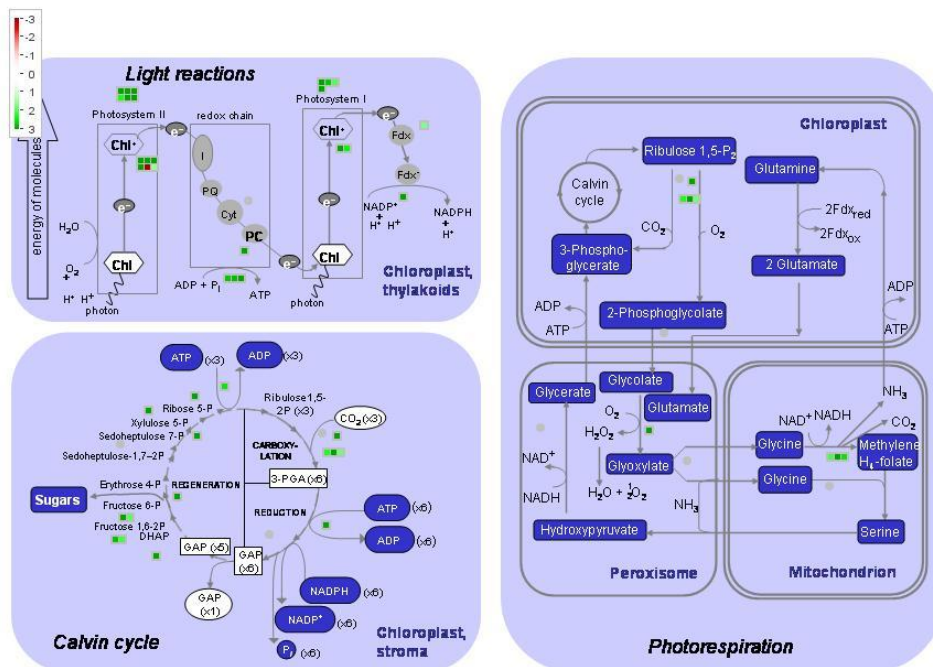


Figure 23 Photosynthetic pathway overview of differentially expressed transcripts of late senescencing switchgrass leaves. Green indicates upregulation of transcripts on 9-27 when compared to 9-13. Red indicates downregulation of transcripts on 9-27 when compared to 9-13.

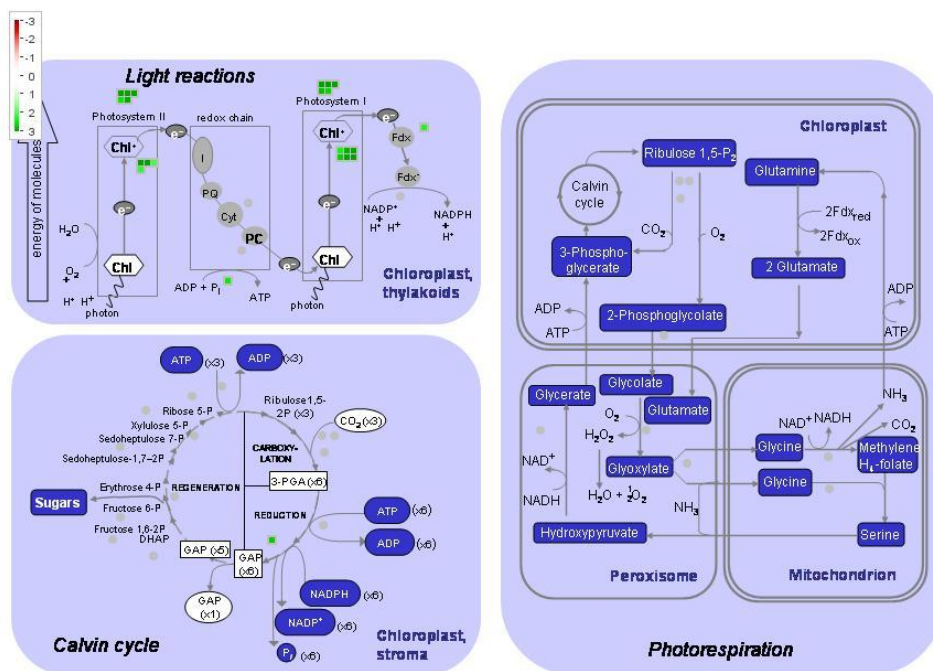


Figure 24 Photosynthetic pathway overview of differentially expressed transcripts between early and late senescencing switchgrass leaves on 9-13. Green indicates upregulation of transcripts of the late genotype when compared to the early genotype. Red indicates downregulation of transcripts of the late genotype when compared to the early genotype.

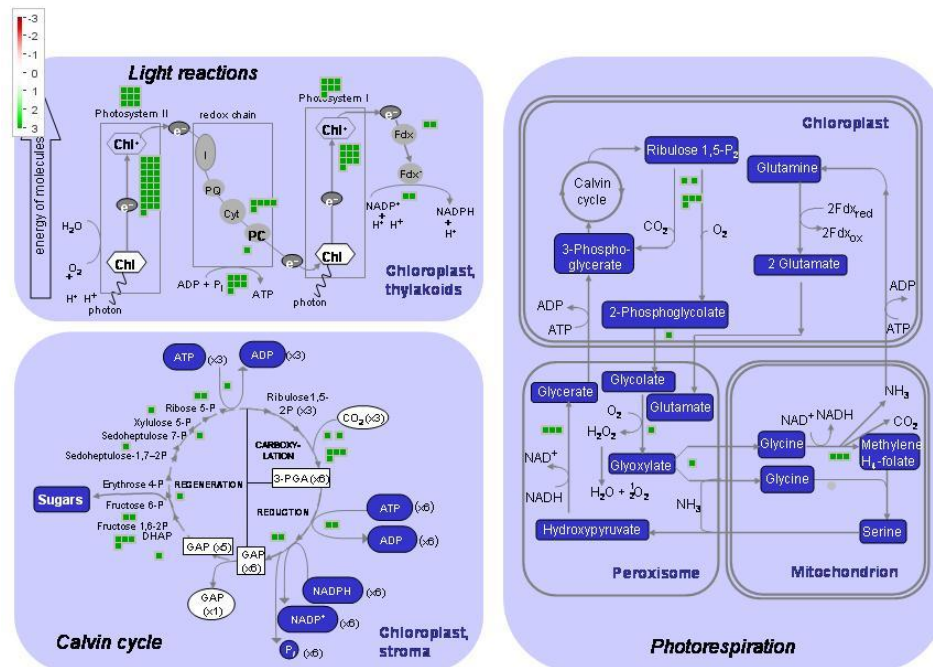


Figure 25 Photosynthetic pathway overview of differentially expressed transcripts between early and late senescencing switchgrass leaves on 9-27. Green indicates upregulation of transcripts of the late genotype when compared to the early genotype. Red indicates downregulation of transcripts of the late genotype when compared to the early genotype.

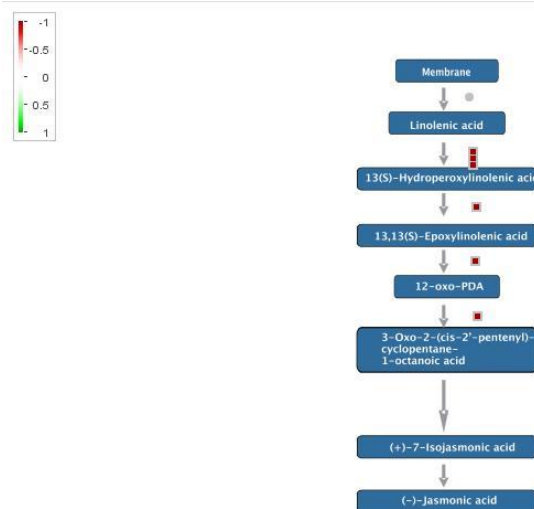


Figure 26 Jasmonic acid synthesis overview of differentially expressed transcripts of early senescencing switchgrass leaves. Green indicates upregulation of transcripts on 9-27 when compared to 9-13. Red indicates downregulation of transcripts on 9-27 when compared to 9-13.

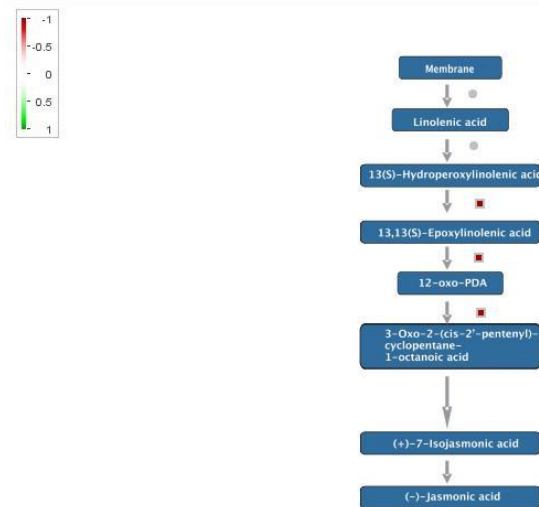


Figure 27 Jasmonic acid synthesis overview of differentially expressed transcripts of early senescencing switchgrass crowns. Green indicates upregulation of transcripts on 9-27 when compared to 9-13. Red indicates downregulation of transcripts on 9-27 when compared to 9-13.

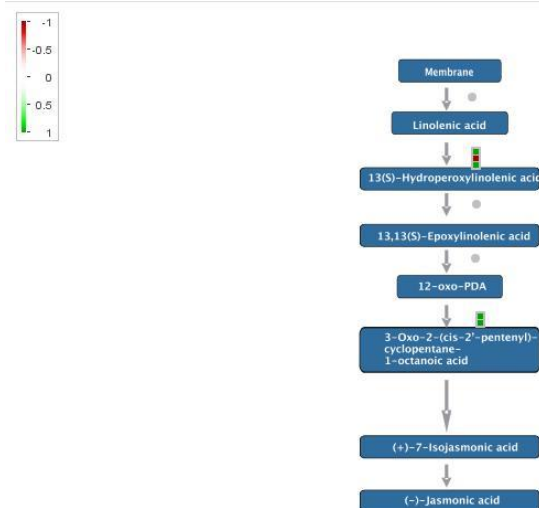


Figure 28 Jasmonic acid synthesis overview of differentially expressed transcripts of late senescencing switchgrass leaves. Green indicates upregulation of transcripts on 9-27 when compared to 9-13. Red indicates downregulation of transcripts on 9-27 when compared to 9-13.

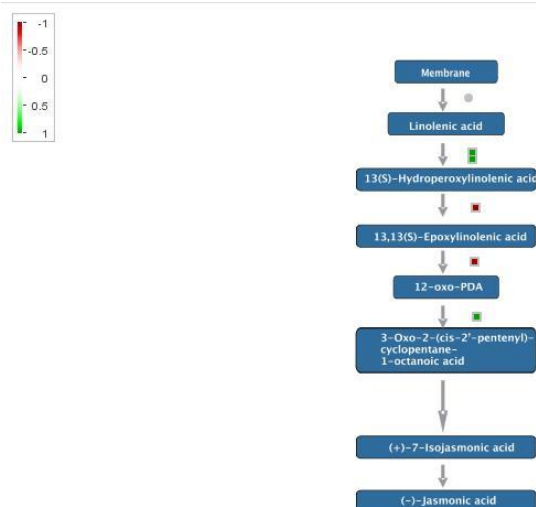


Figure 29 Jasmonic acid synthesis overview of differentially expressed transcripts of late senescencing switchgrass crowns. Green indicates upregulation of transcripts on 9-27 when compared to 9-13. Red indicates downregulation of transcripts on 9-27 when compared to 9-13.

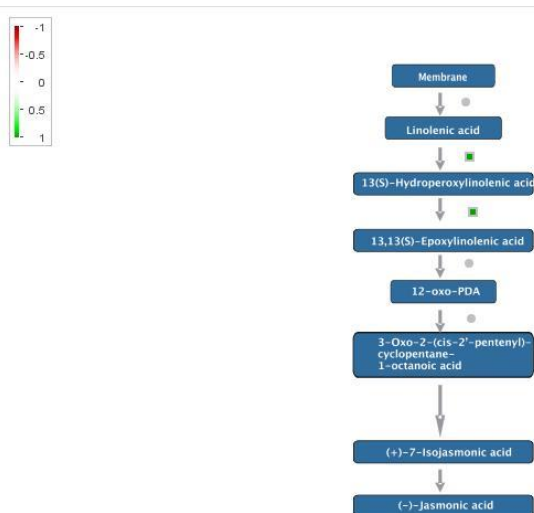


Figure 30 Jasmonic acid synthesis overview of differentially expressed transcripts between early and late senescencing switchgrass leaves on 9-13. Green indicates upregulation of transcripts of the late genotype when compared to the early genotype. Red indicates downregulation of transcripts of the late genotype when compared to the early genotype.

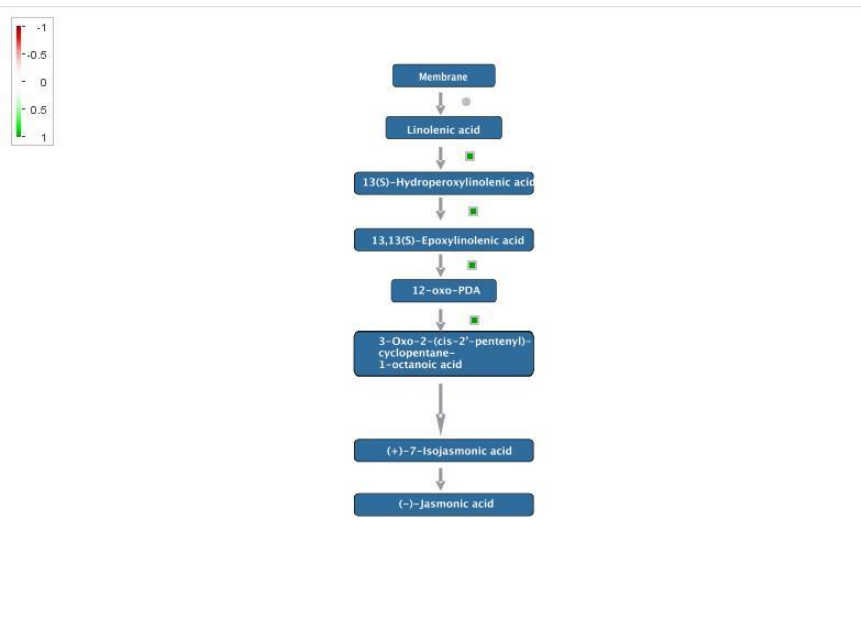


Figure 31 Jasmonic acid synthesis overview of differentially expressed transcripts between early and late senescencing switchgrass crowns on 9-13. Green indicates upregulation of transcripts of the late genotype when compared to the early genotype. Red indicates downregulation of transcripts of the late genotype when compared to the early genotype.

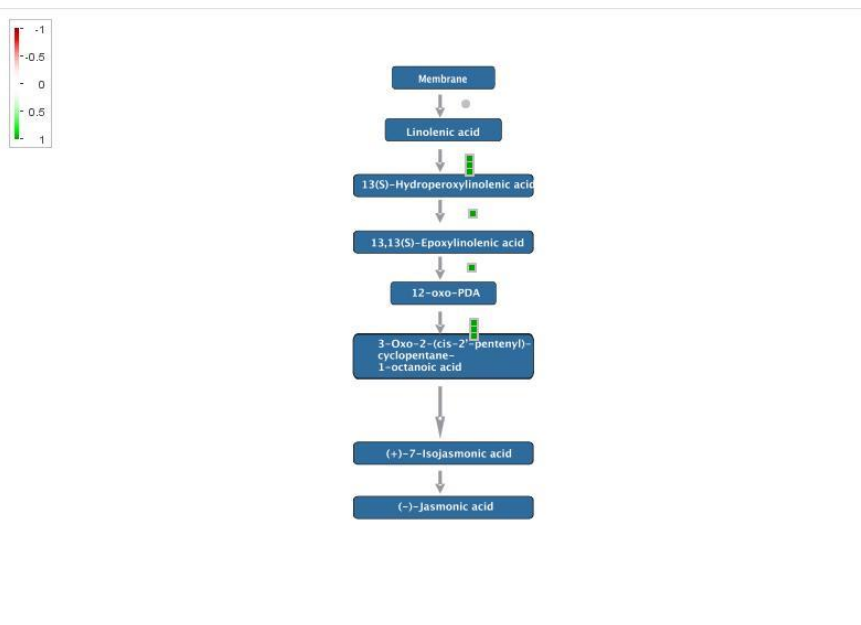


Figure 32 Jasmonic acid synthesis overview of differentially expressed transcripts between early and late senescencing switchgrass leaves on 9-27. Green indicates upregulation of transcripts of the late genotype when compared to the early genotype. Red indicates downregulation of transcripts of the late genotype when compared to the early genotype.



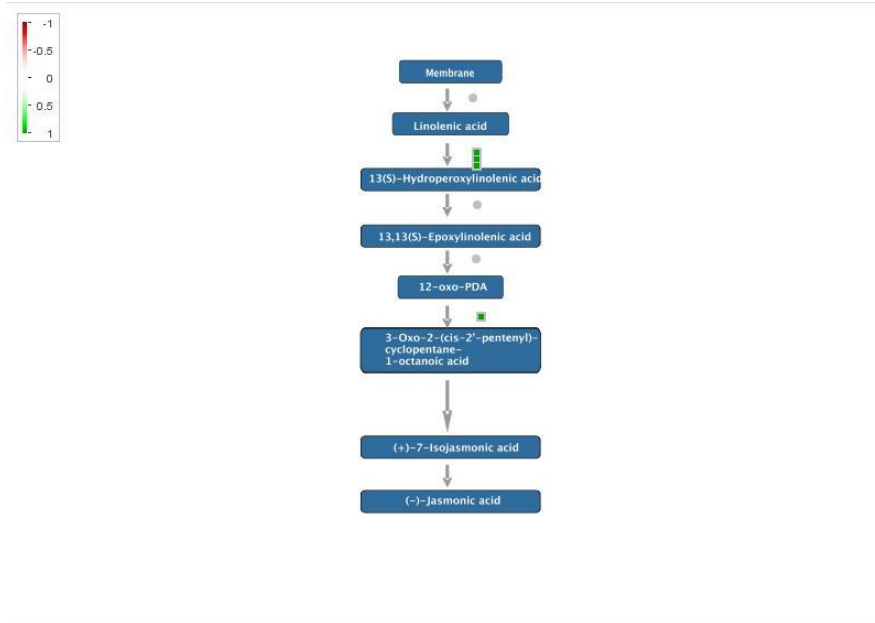


Figure 33 Jasmonic acid synthesis overview of differentially expressed transcripts between early and late senescencing switchgrass crowns on 9-27. Green indicates upregulation of transcripts of the late genotype when compared to the early genotype. Red indicates downregulation of transcripts of the late genotype when compared to the early genotype.

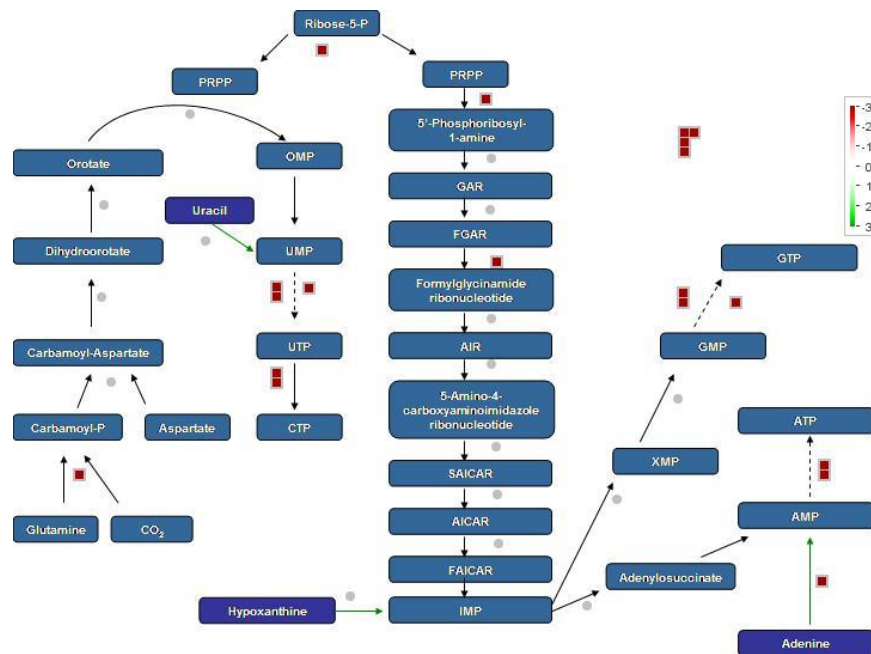


Figure 34 Nucleotide synthesis overview of differentially expressed transcripts of early senescencing switchgrass leaves. Green indicates upregulation of transcripts on 9-27 when compared to 9-13. Red indicates downregulation of transcripts on 9-27 when compared to 9-13.

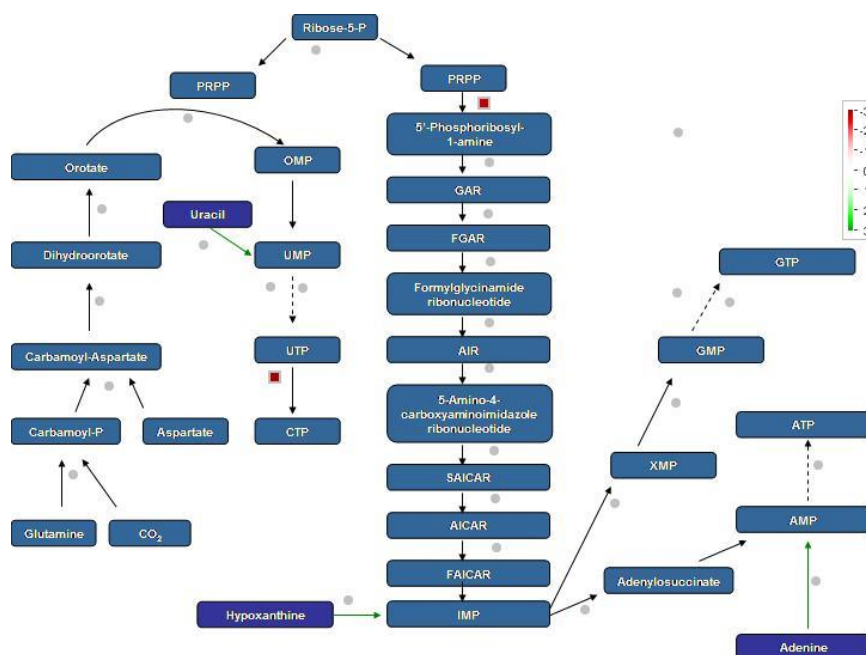


Figure 35 Nucleotide synthesis overview of differentially expressed transcripts of early senescencing switchgrass crowns. Green indicates upregulation of transcripts on 9-27 when compared to 9-13. Red indicates downregulation of transcripts on 9-27 when compared to 9-13.

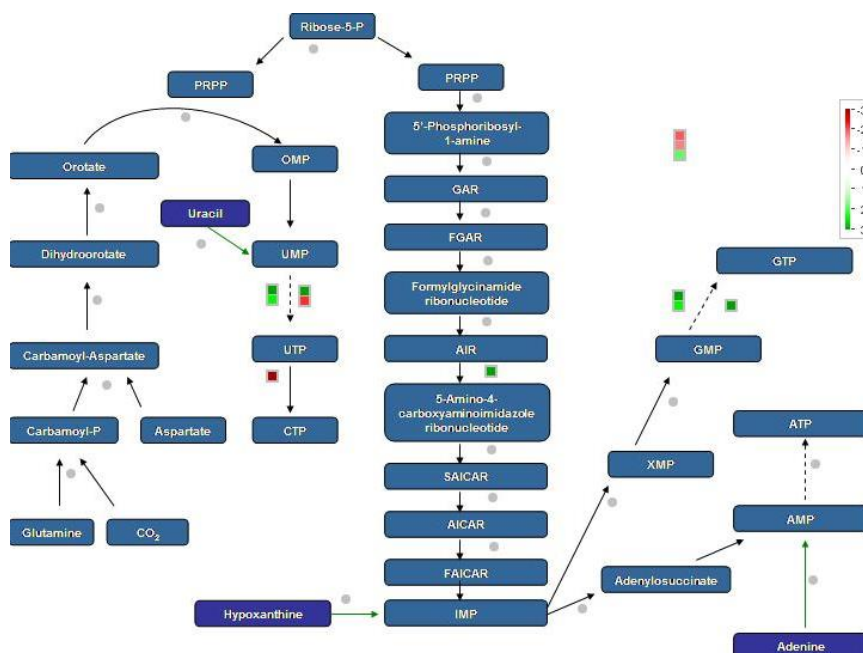


Figure 36 Nucleotide synthesis overview of differentially expressed transcripts of late senescencing switchgrass leaves. Green indicates upregulation of transcripts on 9-27 when compared to 9-13. Red indicates downregulation of transcripts on 9-27 when compared to 9-13.



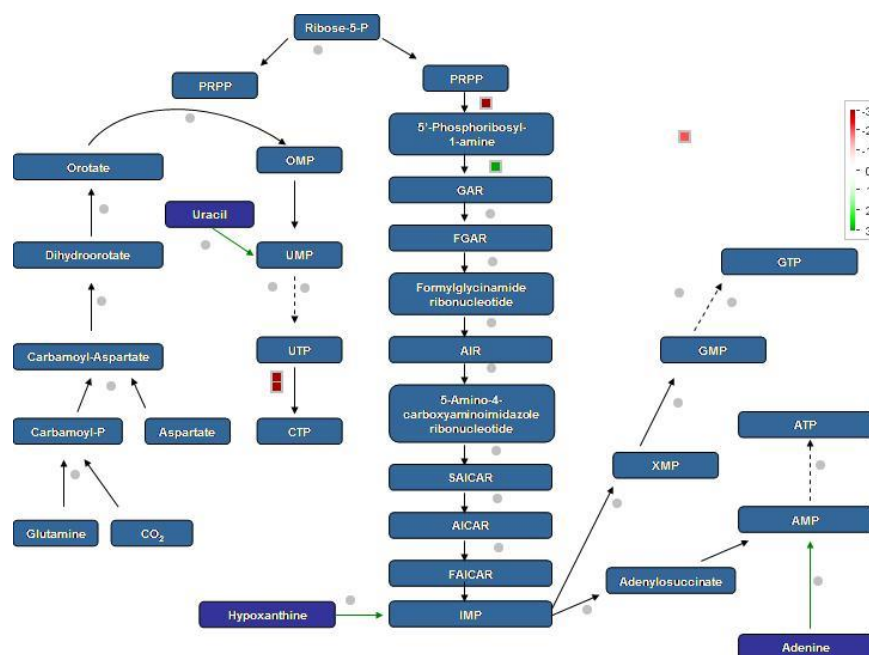


Figure 37 Nucleotide synthesis overview of differentially expressed transcripts of late senescencing switchgrass crowns. Green indicates upregulation of transcripts on 9-27 when compared to 9-13. Red indicates downregulation of transcripts on 9-27 when compared to 9-13.

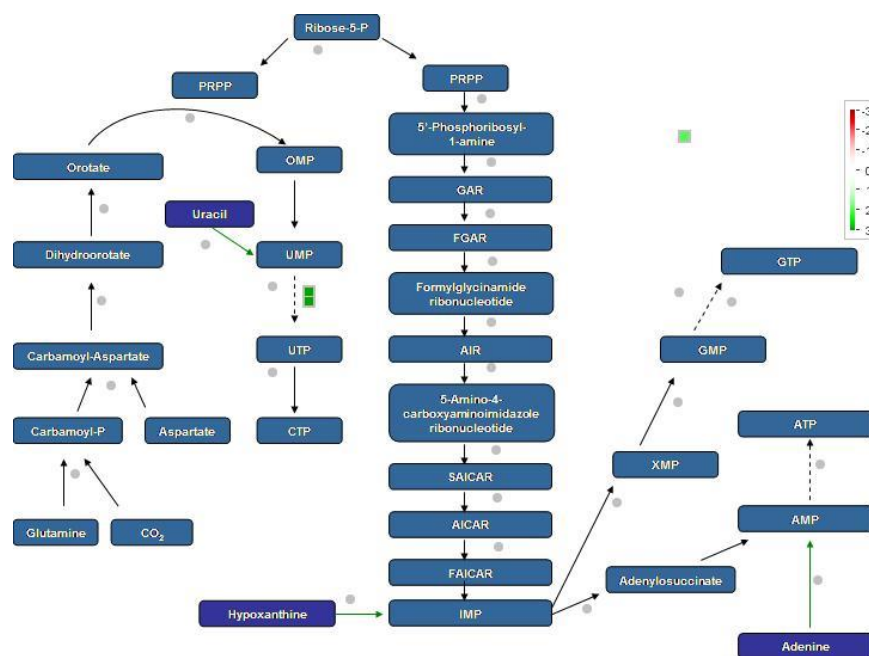


Figure 38 Nucleotide synthesis overview of differentially expressed transcripts between early and late senescencing switchgrass leaves on 9-13. Green indicates upregulation of transcripts of the late genotype when compared to the early genotype. Red indicates downregulation of transcripts of the late genotype when compared to the early genotype.

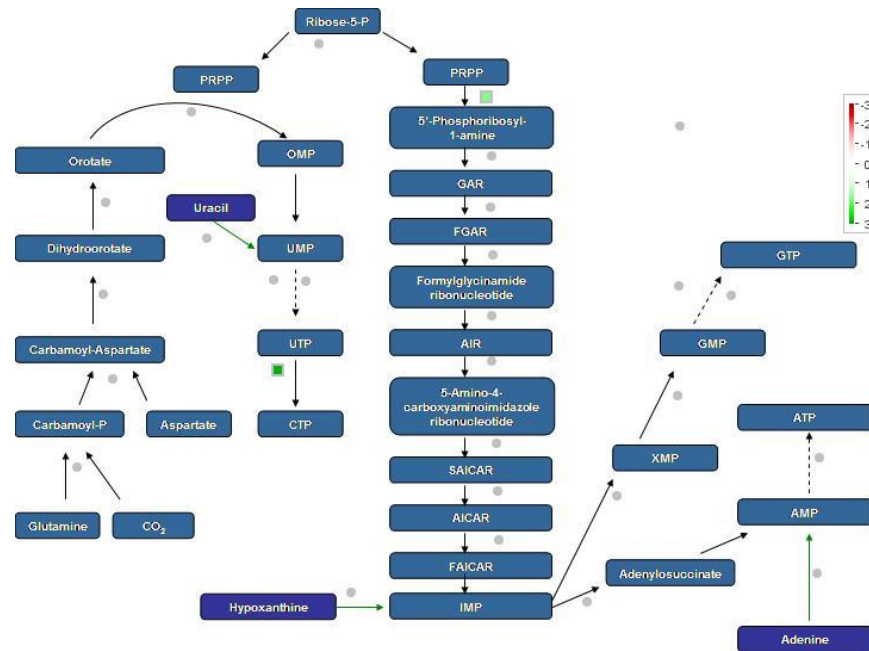


Figure 39 Nucleotide synthesis overview of differentially expressed transcripts between early and late senescencing switchgrass crowns on 9-13. Green indicates upregulation of transcripts of the late genotype when compared to the early genotype. Red indicates downregulation of transcripts of the late genotype when compared to the early genotype.

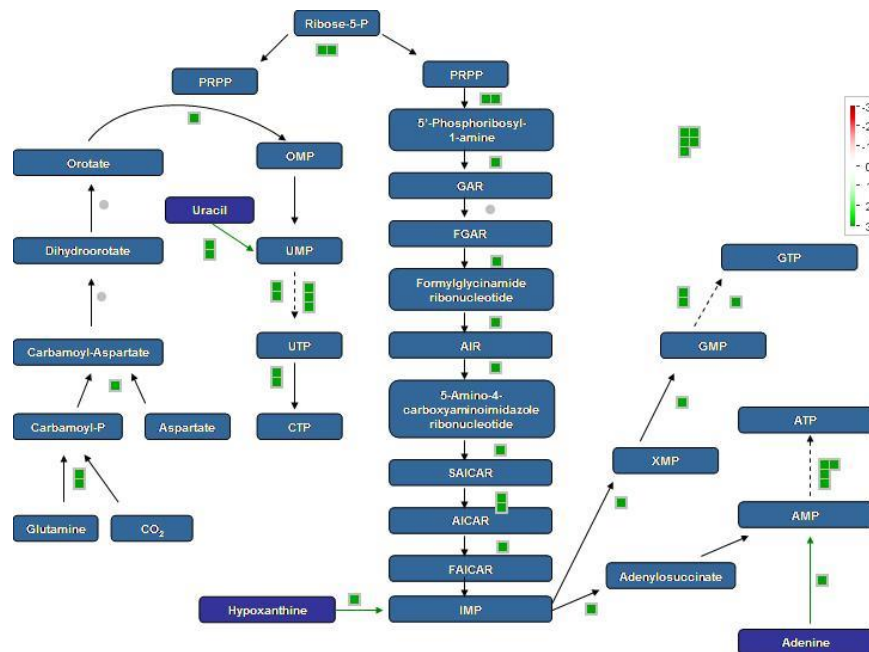


Figure 40 Nucleotide synthesis overview of differentially expressed transcripts between early and late senescencing switchgrass leaves on 9-27. Green indicates upregulation of transcripts of the late genotype when compared to the early genotype. Red indicates downregulation of transcripts of the late genotype when compared to the early genotype.

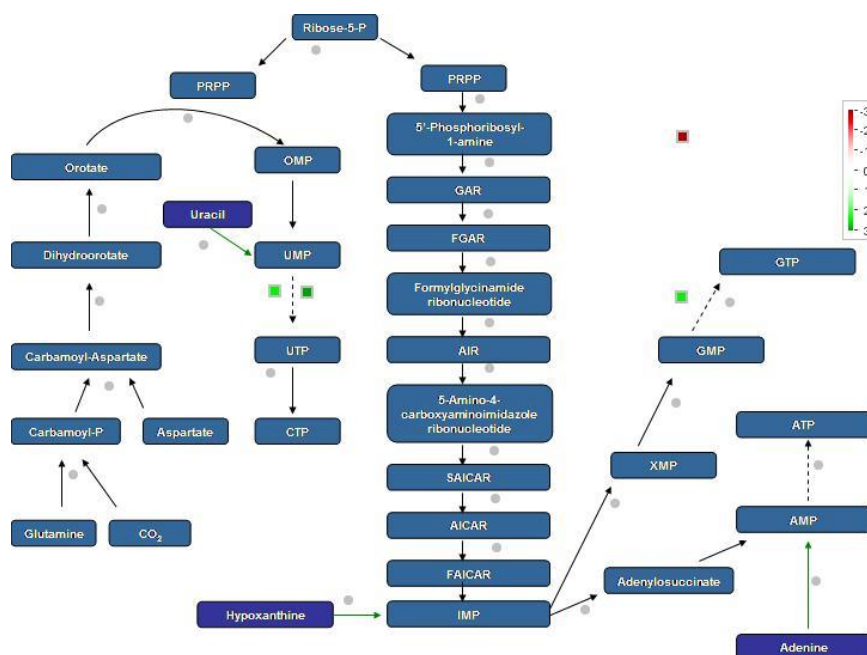


Figure 41 Nucleotide synthesis overview of differentially expressed transcripts between early and late senescencing switchgrass crowns on 9-27. Green indicates upregulation of transcripts of the late genotype when compared to the early genotype. Red indicates downregulation of transcripts of the late genotype when compared to the early genotype.

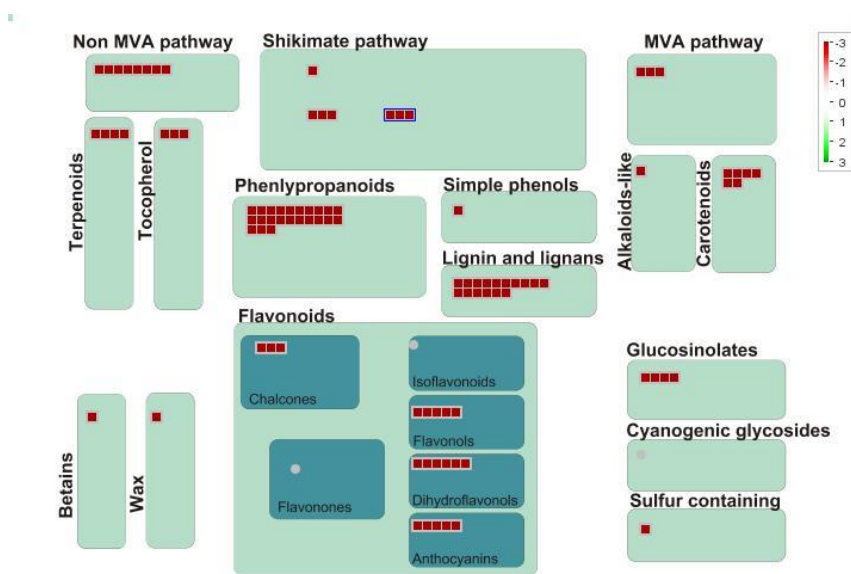


Figure 42 Secondary metabolism overview of differentially expressed transcripts of early senescencing switchgrass leaves. Green indicates upregulation of transcripts on 9-27 when compared to 9-13. Red indicates downregulation of transcripts on 9-27 when compared to 9-13.

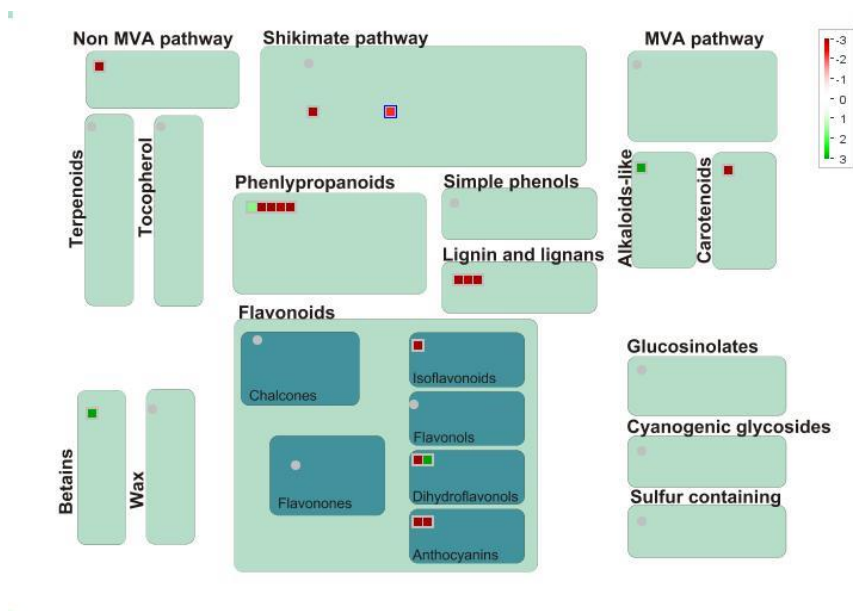


Figure 43 Secondary metabolism overview of differentially expressed transcripts of early senescencing switchgrass crowns. Green indicates upregulation of transcripts on 9-27 when compared to 9-13. Red indicates downregulation of transcripts on 9-27 when compared to 9-13.

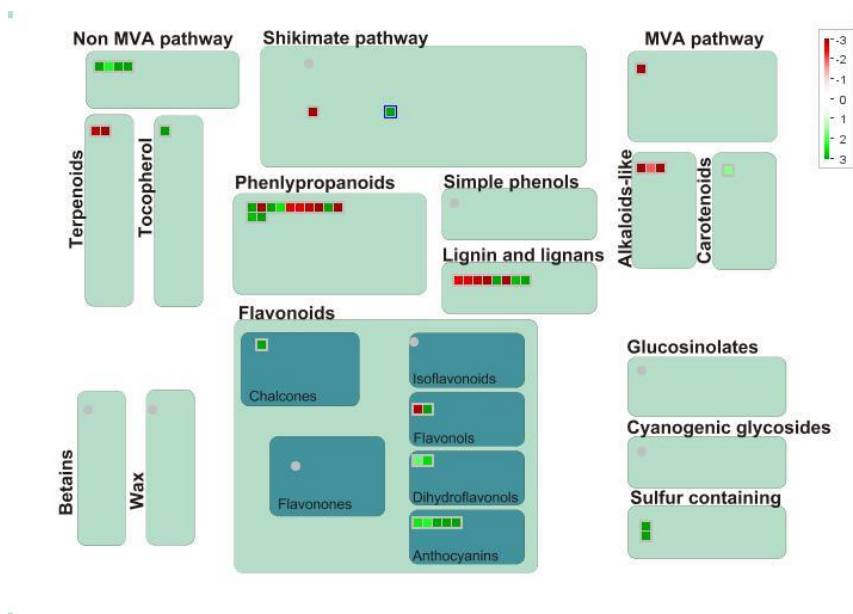


Figure 44 Secondary metabolism overview of differentially expressed transcripts of late senescencing switchgrass leaves. Green indicates upregulation of transcripts on 9-27 when compared to 9-13. Red indicates downregulation of transcripts on 9-27 when compared to 9-13.

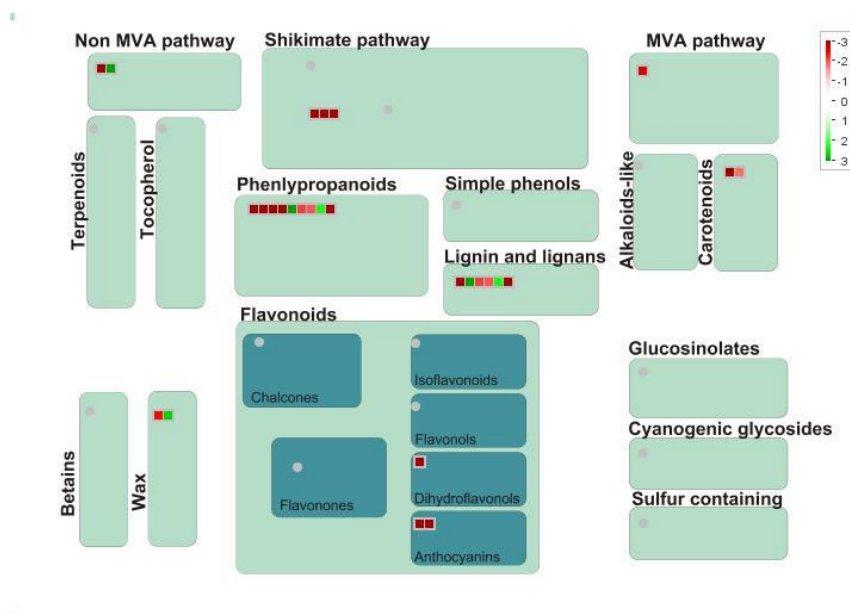


Figure 45 Secondary metabolism overview of differentially expressed transcripts of late senescencing switchgrass crowns. Green indicates upregulation of transcripts on 9-27 when compared to 9-13. Red indicates downregulation of transcripts on 9-27 when compared to 9-13.

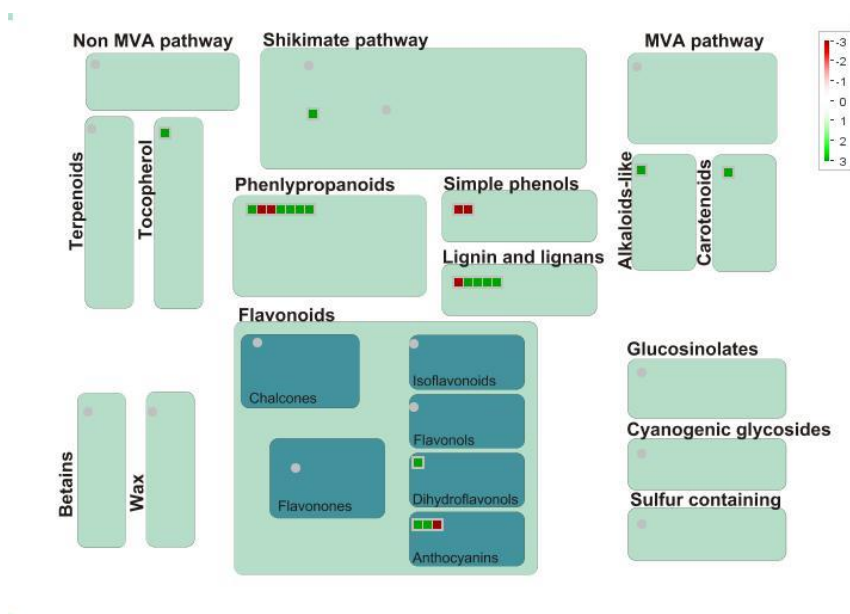


Figure 46 Secondary metabolism overview of differentially expressed transcripts between early and late senescencing switchgrass leaves on 9-13. Green indicates upregulation of transcripts of the late genotype when compared to the early genotype. Red indicates downregulation of transcripts of the late genotype when compared to the early genotype.

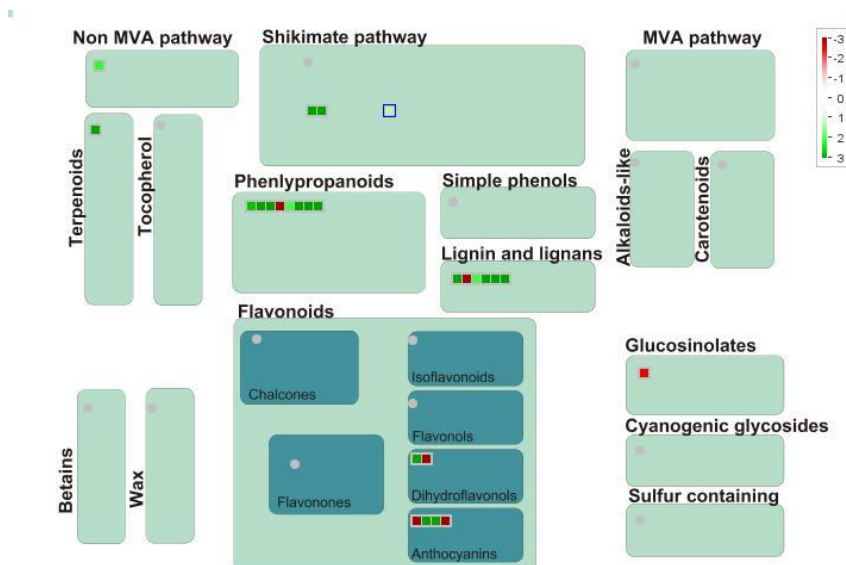


Figure 47 Secondary metabolism overview of differentially expressed transcripts between early and late senescencing switchgrass crowns on 9-13. Green indicates upregulation of transcripts of the late genotype when compared to the early genotype. Red indicates downregulation of transcripts of the late genotype when compared to the early genotype.

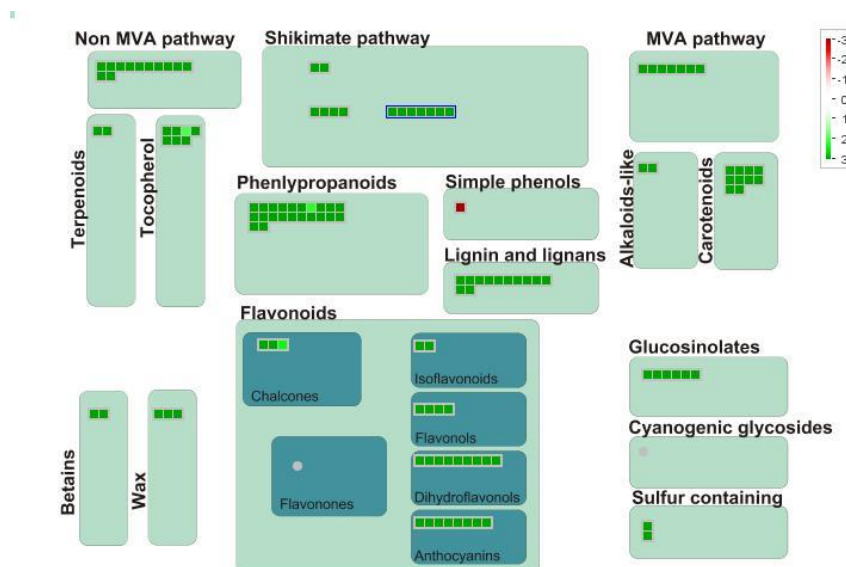


Figure 48 Secondary metabolism overview of differentially expressed transcripts between early and late senescencing switchgrass leaves on 9-27. Green indicates upregulation of transcripts of the late genotype when compared to the early genotype. Red indicates downregulation of transcripts of the late genotype when compared to the early genotype.



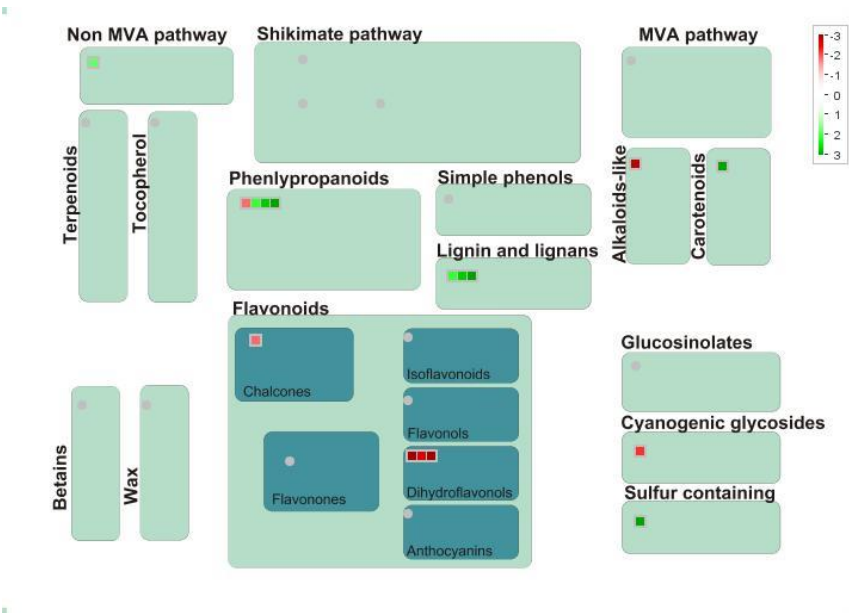


Figure 49 Secondary metabolism overview of differentially expressed transcripts between early and late senescencing switchgrass crowns on 9-27. Green indicates upregulation of transcripts of the late genotype when compared to the early genotype. Red indicates downregulation of transcripts of the late genotype when compared to the early genotype.

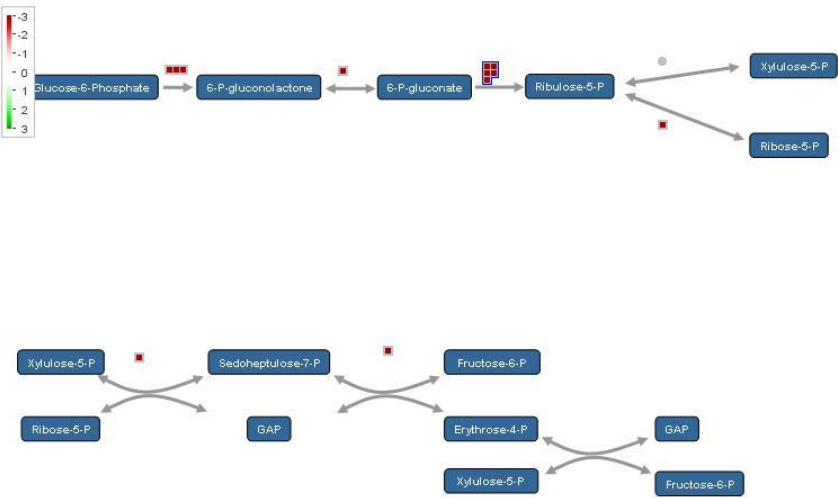


Figure 50 Pentose phosphate pathway overview of differentially expressed transcripts of early senescencing switchgrass leaves. Green indicates upregulation of transcripts on 9-27 when compared to 9-13. Red indicates downregulation of transcripts on 9-27 when compared to 9-13.

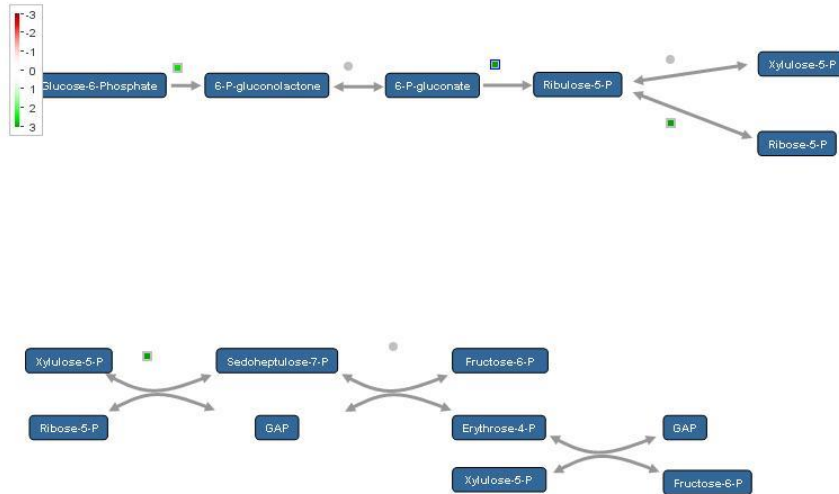


Figure 51 Pentose phosphate pathway overview of differentially expressed transcripts of late senescencing switchgrass leaves. Green indicates upregulation of transcripts on 9-27 when compared to 9-13. Red indicates downregulation of transcripts on 9-27 when compared to 9-13.

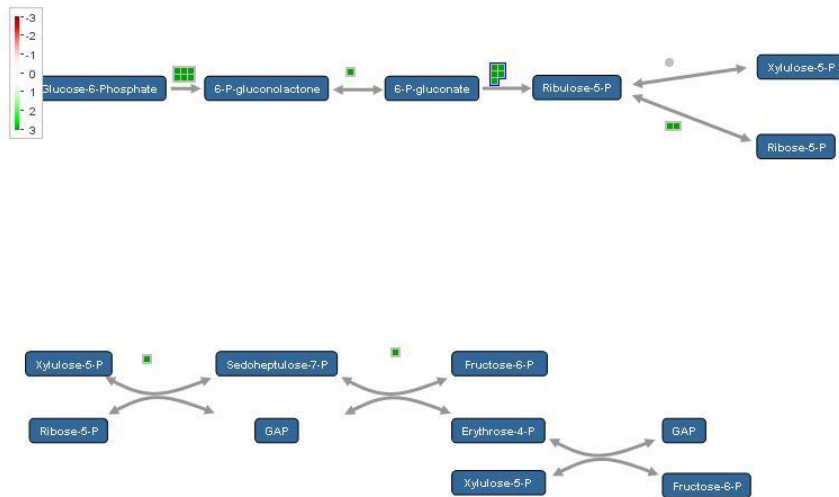


Figure 52 Pentose phosphate pathway overview of differentially expressed transcripts between early and late senescencing switchgrass leaves on 9-27. Green indicates upregulation of transcripts of the late genotype when compared to the early genotype. Red indicates downregulation of transcripts of the late genotype when compared to the early genotype.



*Table 1 Total number of reads per sample.*

Sample	Number of Reads
2SGER1C	39,108,414
2SGER1L	47,342,024
2SGER2C	31,675,449
2SGER2L	35,523,154
2SGER3C	44,779,582
2SGER3L	40,832,793
2SGLR1C	34,905,768
2SGLR1L	39,125,560
2SGLR2C	33,705,315
2SGLR2L	39,128,625
2SGLR3C	34,900,415
2SGLR3L	45,181,491
6SGER1C	37,446,539
6SGER1L	38,228,258
6SGER2C	32,726,182
6SGER2L	41,731,871
6SGER3C	40,370,410
6SGER3L	33,838,607
6SGLR1C	38,283,364
6SGLR1L	38,167,833
6SGLR2C	45,885,770
6SGLR2L	41,916,956
6SGLR3C	40,565,582
6SGLR3L	38,961,498
<b>Total</b>	<b>934,331,460</b>

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