Evaluation of Switchgrass Germplasm for Early Season Biomass Traits

Lensy Hardy, Vijaya Gopal Kakani Ph.D.
Department of Plant and Soil Sciences, Oklahoma State University, Stillwater, OK 74078

Abstract

A potential path for increasing soil carbon stocks is the development of crop cultivars that input a greater quantity of carbon in the soil through their roots or grow deeper root system. Identifying the root traits to be used in a breeding program to rapidly increase switchgrass biomass production is a major problem. There is little information in the literature on the genotypic variability available for root and shoot traits in the switchgrass collection. A total of 173 switchgrass lines were obtained from the USDA-ARS GRIN collection. The 173 germplasm lines were grown in 10" cone-tainers for 5 weeks. Significant differences were observed for measured root traits in the switchgrass germplasm. Positive correlation was observed between root volume and root diameter, while a negative correlation was recorded between root volume and root length. The genetic variability identified in this research for root traits can be exploited to deploy rapidly for continuous genetic turnover and active land management in marginal lands. The genotypes with high root growth traits can be used to increase soil carbon sequestration and deliver economic net carbon sink with significant economic potential.

Introduction

Switchgrass is a perennial plant that has been a huge topic and beneficial plant in the biofuel industry due to its ability to produce vast amounts of biomass. The relevance of switchgrass has increased as a consequence of the amount of carbon that has been emitted into the atmosphere as a result of burning fossil fuels. Switchgrass is a possible solution to reducing the use of these fossil fuels because of its production of biomass, and decreasing the rate of global warming by sequestering the carbon in the soil. The roots of the grass are the determining factors of the amount of biomass that may be produced and its ability to store the Carbon in the soil. Evaluating the fit conditions/locations for specific genotypes of switchgrass will lead to better results of produced biomass and sequestration of carbon.

Objectives

- To identify the genetic differences for root traits between the genotypes
- To analyze the shoot to root differences

Materials and Methods

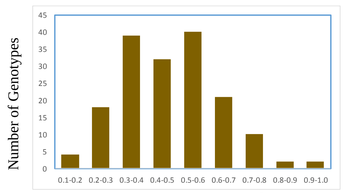
Received 175 switchgrass germplasm lines from USDA-ARS collection and were planted in 10" cone-tainers on June 15th, 2016 in a greenhouse at Oklahoma State University, Stillwater. Replicated same set two more times on June 17th and 21st. Hand watered 1-2 times a day for 5 week growing period. Nutrients were provided through slow-release Osmocote fertilizer fertilizer granules (15-9-12) on June 29th, July 1st and July 4th. Harvested the intact whole roots by hand-washing with water to remove sand and other debris. Organized harvested roots into labeled plastic bags for storage in refrigerator. Washed and scanned roots using WinRHIZO software at 100x resolution and in color. Analyzed collected data from roots. Tables and graphs were developed to present the data.

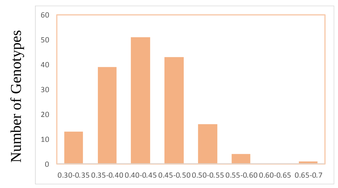
Figure 1 Switchgrass, a perennial grass that produces biomass

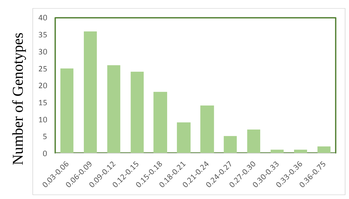
Figure 2 Batch A of switchgrass fertilized

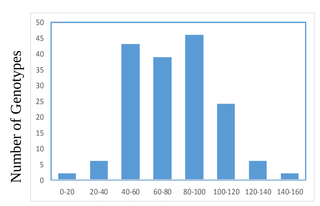
Figure 3 Harvested switchgrass of set A

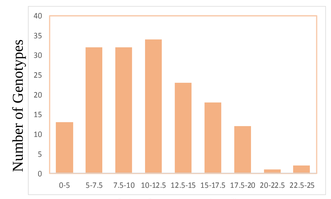
Results

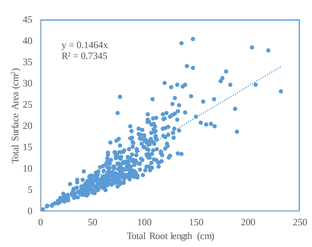


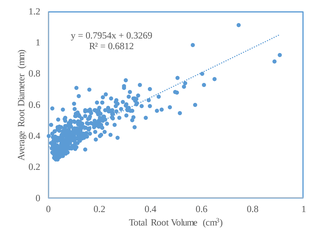




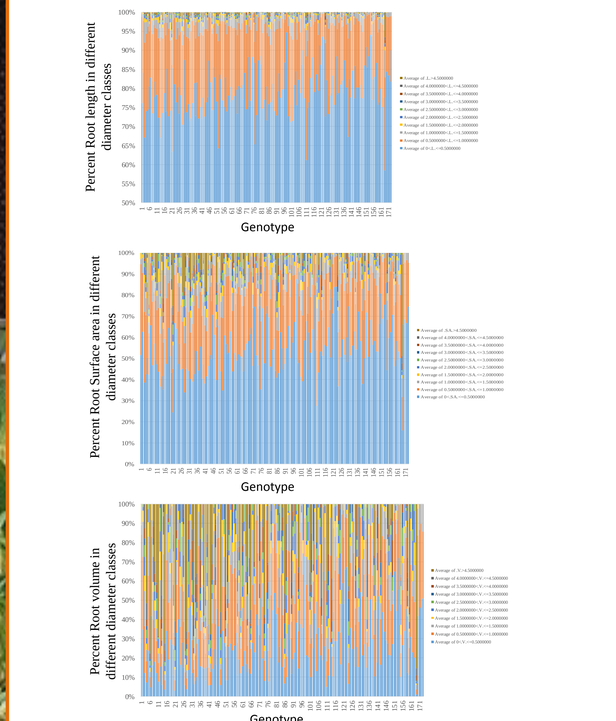








Root Trait	Length (cm)	ProjArea (cm²)	SurfArea (cm²)	AvgDiam (mm)	LenPerVol (cm cm⁻³)	RootVolume (cm³)	Root Len/Root Vol (cm cm⁻³)	Tips	Forks
Length (cm)									
ProjArea (cm²)	**0.87**								
SurfArea (cm²)	**0.87**	1.00							
AvgDiam (mm)	0.26	0.68	0.68						
LenPerVol (cm cm⁻³)	1.00	**0.87**	**0.87**	0.26					
RootVolume (cm³)	0.67	**0.94**	**0.94**	0.83	0.67				
Root Len/Root Vol (cm cm⁻³)	-0.23	-0.58	-0.58	**-0.89**	-0.23	**-0.64**			
Tips	**0.86**	0.68	0.68	0.12	**0.86**	0.48	-0.09		
Forks	**0.83**	0.68	0.68	0.15	**0.83**	0.52	-0.08	0.71	
Crossings	**0.83**	0.69	0.69	0.17	**0.83**	0.53	-0.11	0.68	**0.95**



Discussion

- The extent of genetic variability is indicated by the large range observed for the genotypes.
- The contrasting genotypes identified in this study offer useful plant material for inclusion in switchgrass breeding programs aimed at improving root C sequestration.
- The positive correlation between root volume and average diameter indicates the potential for developing varieties with ability to extract water and nutrients from deeper soil layers.
- The negative correlation between root length and root diameter or root volume suggests demonstrates the potential for improving root traits to sequester more carbon into deeper layers of the soil.

Conclusions

- ✓ We observed significant genotypic variability for root traits.
- ✓ The identified lines with extreme values for the measured traits can be used to identify the genetic markers or control for these root traits.
- ✓ The genetic variability observed in this research during early growth stages can be exploited to improve carbon sequestration, drought tolerance, and resource capture in switchgrass.
- ✓ Further evaluation of root traits in different soil types and multiple location will help to better understand the genetic and environmental control of root traits.

Acknowledgements

We thank Dalton Hahn and Jay Prater for helping in root washing and greenhouse management. Special thanks from Lensy Hardy to NSF and Oklahoma State University for their financial support and opportunity.

CULTIVATION OF MICROALGAE

Andria M. Carpenter[1,2], Giovanni A. Lutzu[2,3], and Nurhan T. Dunford[2,3]

[1]University of Michigan, Department of Chemical Engineering, [2]Oklahoma State University, Biosystems and Agricultural Engineering Department, and [3] Robert M. Kerr Food and Agricultural Product Center

ABSTRACT

With the limited supply of natural gas slowly coming to an end, the search for an alternative and sustainable source of energy is becoming increasingly important. Bioenergy can be a viable alternative to fossil fuel based energy sources. Many different plants can be used to create biofuels; it is now a question as to which is the best.

Microalgae is a promising candidate because of its ability to replicate at a much higher rate and in a smaller amount of space than higher plants. The objective of this project is to study the growth pattern of the algae strain, *Aphanocapsa* sp., and further determine the average maximum biomass concentration and lipid content.

Three bioreactors were inoculated with the *Aphanocapsa* sp. culture in A+ medium. The biomass was analyzed for its proximate composition using Thermogravimetric Analysis (TGA) and standard analytical techniques.

It took 27 days for the strain to reach a high biomass concentration of 5.98g/L. The strain also exhibited a rather low lipid content of 4.58%, which could be increased by using a nitrogen limited growth medium for algae cultivation.

These results suggest that *Aphanocapsa* sp. is a strong strain choice for biomass production, and has the potential to be stronger in terms of lipid content with a change of the medium in which it is cultivated.

INTRODUCTION

Microalgae are microorganisms that have the ability to photosynthesize at a higher efficiency than that of land plants. These microorganisms have the potential to produce biomass that can be used as feedstock for biofuels, feed applications, and pharmaceuticals; while, also, possessing the unique ability to perform waste water clean-up. The benefit of using microalgae as a feedstock for these applications is that it can produce the same amount of biomass as land plants in a much shorter time span and in a notably smaller square footage.

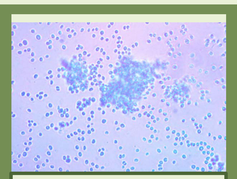
OBJECTIVES

The main objective of this project is to understand the process behind the cultivation of the native to Oklahoma algae strain, *Aphanocapsa* sp. (sp23). Other objectives include developing the biomass production yield and the lipid content yield for the species.

MATERIALS AND METHODS

Inoculation of Bioreactors

- Inoculate bioreactors with algae strain
- Bubble gas mix into bioreactors -5% CO_2 / 95% air



Monitor the Cell Growth

- Optical density reading
- pH reading
- Microscopic imaging
- Build growth curve

Harvest Biomass

- Separate the biomass from medium by centrifugation

Characterization Tests

- Dry weight of biomass
- Proximate analysis by TGA (moisture, ash, fixed carbon, volatile matter)

Lipid Extraction

- Cell destruction using bead-beater
- Phase separation
- Removal of organic solvent by evaporation

RESULTS

Figure 1: Average growth rate of sp23 in A+ Medium

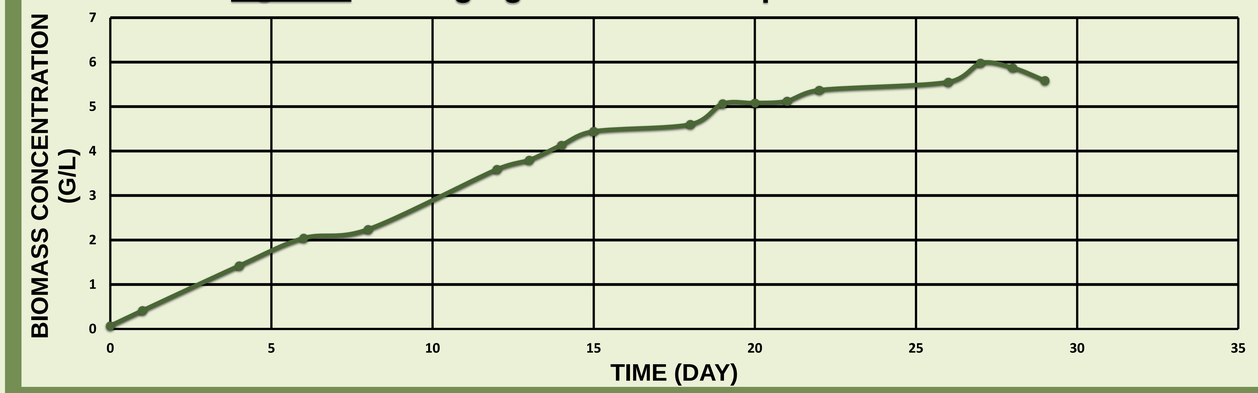


Table 1: Growth Pattern of sp23

Specific Growth Rate (μ)	0.3267
Divisions per Day	0.4713
Doubling time	2.1217
Max Biomass Concentration (g/L), X_{max}	5.9798
Average biomass productivity (g/L · day), ΔX	0.1871

Table 2: Proximate Analysis of Dried Biomass

Moisture %	Volatile Matter %	Fixed Carbon %	Ash %
7.9 ± 1.0	21.4 ± 1.1	34.7 ± 5.7	36.0 ± 5.6

Table 3: sp23 Lipid Extraction Analysis

Average Lipid Content %	4.58 ± 2.58

DISCUSSION / CONCLUSION

- The sp23 algae strain does not demonstrate a lag phase. Instead it goes directly into the exponential phase; which, as shown in figure 1, lasts roughly 15 to 20 days.
- The biomass production of the sp23 strain is high, with an average max concentration of 5.98g/L (Table1).
- The sample exhibited a high ash content of 36.0% ± 5.6 (Table 2). This is caused by a large concentration of salt in the medium used to cultivate the algae. Because the majority of the salt lies on the outside of the algal cells, washing the biomass with deionized water before performing the proximate analysis would lower the salt concentration significantly.
- The average lipid content of 4.58% with a standard deviation of 2.58% was very low (Table 3). This is because the medium used to cultivate the sp23 strain, A+, is rich in nitrogen. High nitrogen content blocks the production of lipid in algal cells.

POTENTIAL IMPACT

Understanding the small scale cultivation of microalgae process is the first step to creating commercial sized production. Knowing the natural biomass production and lipid content yields, will allow us to make the necessary changes to the cultivation procedure which will produce the desired yields for the particular intended use of the algae.

Acknowledgements

I would like to thank the National Science Foundation for funding this research experience for undergraduates. I would also like to thank Oklahoma State University for hosting the REU program.

Metabolic dynamics in switchgrass varieties (*Panicum virgatum* L.)

Ellinor Y. Tai[1,2], Gopal Kakani[2] Ph.D.
[1]Department of Biological Physics, Brandeis University
[2]Department of Plant and Soil Sciences, Oklahoma State University

Abstract

Switchgrass (Panicum virgatum L.) is a warm-season, perennial grass that utilizes C4 photosynthesis. The C4 grasses are ideal for lignocellulosic feedstock biofuel production due to their efficiency at converting available nutrients into carbohydrate molecules via photosynthesis, resulting in a relatively high output of biomass. Switchgrass is an ideal organism for biomass production due to its status as a resilient grass species native to North America. With its capability to produce high yields, natural resistance to pests, and relative ease of establishment, switchgrass is considered a potential source for significant biomass production within the United States. The root system of switchgrass also stabilizes soils and returns nutrients to the ground. In addition, switchgrass sequesters a significant amount of carbon from the environment. The two primary types of switchgrass are lowland and highland, though a wide variety of species exist.

A LI-COR LI-6400XT portable photosynthesis machine was used to collect photosynthetic and fluorescent measurements. These variables were analyzed via their r-squared value. A one-way ANOVA was performed to ensure that data between genotypes was statistically significant. The amount of chlorophyll a, chlorophyll b, and carotenoids was also measured for each genotype. The data could be used to determine how efficient varying genotypes of switchgrass are at converting nutrients and sunlight into biomass. This would assist in determining the ideal genotypes of switchgrass for biomass production for different ecosystems.

Introduction

As demands for energy increases and the available resources for powering the world decreases, we need to invest the time and effort into discovering alternatives to fossil fuels. Biomass has come into play as a major possible significant source of renewable energy. Though biomass cannot completely replace fossil fuels, it can act as a bridge between nonrenewable energy and a sustainable future (Quall 2012). Countries such as Germany have already started utilizing biomass as a major fuel source for heat, electricity, and other purposes. In the United States, biomass is not widely used as an alternative fuel. However, native grasses such as switchgrass (*Panicum virgatum* L.) are viable sources for bioenergy due to their resilience and status as non-invasive species. The two primary varieties of switchgrass are lowland and highland (Cordero et. al 2016). Lowland switchgrass is most often found in wetter climates, and is coarser, taller, and grows faster than the warmer-climate highland variety (van der Tol et. al 2014). Thirteen genotypes of both lowland and highland switchgrass species were planted at the Agronomy Farm in 2009. These plots were the ones examined in this experiment.

Materials and Methods

Photosynthesis (Pn) and fluorescence (Fl) measurements were acquired at the Agronomy Farm at Oklahoma State University via a LI-COR LI-6400XT portable photosynthesis machine. The plants had photosynthetic measurements taken in the middle of the third leaf from top. Multiple leaves of each genotype were sampled, and plots 1 and 3 were used in this project. A one-way ANOVA (analysis of variance) test was performed between trial sets to check for data compatibility. The r-squared value between each variable was also calculated for both photosynthesis and fluorescence measurements.

The amount of pigments (chlorophyll a, chlorophyll b, and carotenoids) in each genotype was calculated (Li et. al 2012). Switchgrass samples of each genotype were collected and plant tissue was immersed in DMSO (Cordero et. al 2016). The liquid from the pigment extractions was then put into a spectrometer and the absorbance at 470 nm, 648 nm, and 664 nm measured. Pigment content was then calculated (Maxwell et. al 1999).

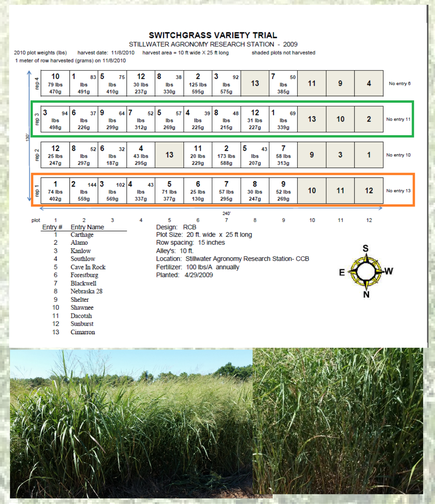


Fig 1| Genotype map and location of switchgrass varieties assessed (Stillwater Agronomy Research Center 2009).

Fig 2| LI-COR 6400XT photosynthesis machine and field site (Oklahoma State University Agronomy Farm)

Data and Results

R-squared values for Photosynthesis

	Photo	Cond	Ci	Fo'	Fm'	Fs	Fv'/Fm'	PhiPS2	PhiCO2	qP	qN	ETR	Trmmol
Photo		0.1293	0.0002	0.0825	0.4253	0.0445	0.6717	0.6062	**0.9951**	0.49140	0.5794	0.6114	0.0091
Cond			0.5044	0.0203	0.0068	0.0207	0.1814	0.1143	0.1354	0.07860	0.1633	0.1095	**0.7904**
Ci				0.0485	0.0061	0.0185	0.0081	0.0019	0.0009	6.00E-05	0.0077	0.0003	0.3333
Fo'					**0.7680**	**0.8374**	0.1225	0.1163	0.0809	0.1742	0.1936	0.1240	0.1188
Fm'						0.6065	0.5018	0.4719	0.4286	0.4936	0.5680	0.4823	0.0446
Fs							0.0871	0.0077	0.0373	0.0158	0.1301	0.0106	0.1166
Fv'/Fm'								0.6891	0.6551	0.5910	**0.965**	0.7185	0.0215
PhiPS2									0.6391	**0.9714**	0.6975	**0.9967**	0.0088
PhiCO2										**0.9714**	0.6975	**0.9967**	0.0088
qP											0.6405	**0.9675**	0.0027
qN												0.7254	0.0153
ETR													0.0087
Trmmol													

Fig 3| R-squared values for photosynthesis variables.

R-squared values for Fluorescence

	Photo	Cond	Ci	Fo'	Fm'	Fs	Fv'/Fm'	PhiPS2	PhiCO2	qP	qN	ETR	Trmmol
Photo		0.3600	0.0169	0.0178	0.0959	0.0155	0.2075	**0.831**	**0.999**	0.6995	0.1981	**0.8309**	0.2823
Cond			0.1066	0.0212	0.1066	0.0291	0.1221	0.0019	0.3507	0.2659	0.3004	0.4177	**0.7634**
Ci				0.0153	0.0017	0.0026	0.0665	0.0493	0.0177	0.0233	0.0589	0.0498	0.0623
Fo'					**0.8676**	**0.8014**	0.3159	0.0044	0.0175	0.0002	0.1596	0.2236	0.0231
Fm'						0.6499	0.0766	0.0216	0.5148	0.5193	0.4992	0.3417	0.0281
Fs							0.0612	0.7195	0.0299	0.0211	0.0941	0.0165	0.0464
Fv'/Fm'								0.6925	0.6993	0.6027	**0.9544**	0.0305	0.0214
PhiPS2									0.7199	**0.9743**	0.7152	0.6928	0.0153
PhiCO2										**0.9836**	0.7049	**0.9962**	0.0443
qP											0.6733	**0.9645**	0.0529
qN												**0.8533**	0.0734
ETR													0.0059
Trmmol													

Fig 4| R-squared values for fluorescence variables.

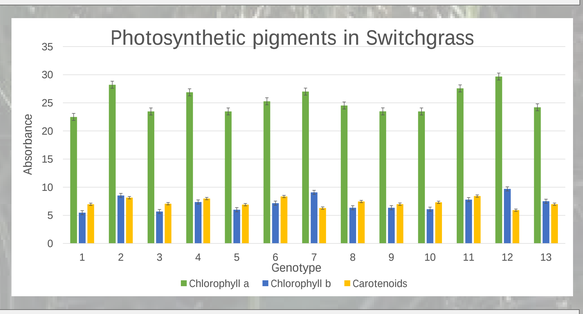


Fig 5| Amounts of chlorophyll a, chlorophyll b, and carotenoids in switchgrass varieties.

Discussion and Conclusion

From the data, there is evidence of correlation between plant stress and photosynthetic rates, with more plant stress (ETR) resulting in lower photosynthesis and fluorescence rates. There is also evidence of a strong statistical correlation between phiCO_2 (CO_2 assimilation) and photosynthesis measurements. qP rates, which measure photochemistry, were also seen to correlate strongly with CO_2 assimilation in terms of both fluorescence and photosynthesis. The Fm' and Fs rates correlated with the Fo' rates, which works out mathematically as they are used to determine the qP rate. Leaf transpiration (Trmmol) was also correlated with stomata conductance (Cond) in both Fl and Pn (Li et. al 2012).

Alamo (genotype 2) and Sunburst (genotype 12) had the highest concentration of pigments. Each genotype expressed a relatively similar amount of Chlorophyll a, Chlorophyll b, and carotenoids. All genotypes of switchgrass exhibited statistically significant ANOVA values between plots.

More consistent data could be acquired by limiting the data collection to one day instead of spreading the data collection over multiple days. More plots could also be sampled. Data should be collected in similar temperatures and after a prolonged time of no precipitation. In addition, as the leaf punches were acquired from the middle of each bundle of leaves, the amount of pigmentation may vary due to mesoderm placement.

Future Directions

In the future, analysis can be done on other variables besides genotype, such as nitrogen usage. The effects of legume crop rotation in the off season could also be investigated to determine how different legumes affect soil and plant quality.

In addition, other photosynthetic variables such as light saturation rate and light compensation point could be measured and taken into account. The biomass of dry switchgrass could also be weighed and compared across genotypes.

References

- Cordero et. al (2016). “Variation in leaf-level photosynthesis among switchgrass genotypes exposed to low temperatures does not scale with final biomass yield”.10.1111/gcbb.12349
- Ecker et. al (2015). “Switchgrass (Panicum virgatum L.) Genotypes Differ between Coastal Sites and Inland Road Corridors in the Northeastern US”. PLoS One. 2015; 10(6): e0130414
- Li et. al (2012). “Photosynthetic and chlorophyll fluorescence regulation of upland cotton (Gossiypium hirsutum L.) under drought conditions”. POJ 5(5):432-437
- Maxwell et. al (1999). “Chlorophyll fluorescence – a practical guide.” 10.1093/jexbot/51.345.659
- Qualls J (2012). “Switchgrass Production Decision tool.” Department of Agricultural & Resource Economics The University of Tennessee
- Stillwater Agronomy Research Station (2009). “Switchgrass Variety Trial”. Oklahoma State University Department of Agriculture.
- van der Tol et. al (2014). “Models of fluorescence and photosynthesis for interpreting measurements of solar-induced chlorophyll fluorescence”. 10.1002/2014JG002713

Acknowledgements

I would like to thank Dr. Kakani for presenting me the opportunity of conducting field research, and also for his guidance throughout this project. Likewise, I express my thanks to Megh Poudel for training me in how to use the LI-COR 6400XT, as well as to Jay for driving me to the research field.

Additionally, I would like to thank the National Science Foundation for this research opportunity, as well as Oklahoma State University for hosting this program.

Isolation and characterization of novel lignin degrading microorganisms from cow and termite guts

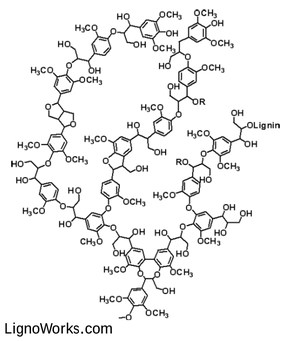
L. Van[1], K. Hennessey[2], B. Fathepure[3]

Department of Biology, Southwestern Oklahoma State University[1]; Department of Biology, George Mason University[2]; Department of Microbiology and Molecular Genetics, Oklahoma State University[3]

Abstract

Using biofuels as an energy source has been of increasing interest, and great effort has been given to find feasible ways in producing fuel ethanol from cellulose. Cellulose is one of three main components of plant cell walls, the other two being hemicellulose and lignin. Lignin is the only component that is not composed of carbohydrate monomers. It is an extremely recalcitrant heteropolymer that binds to cellulose and hemicellulose within the plant cell walls, enhancing the plant's rigidity. In order to free the cellulose and hemicellulose for usage in biofuels, the lignin must be degraded. The process of lignin degradation is difficult, as lignin is composed of an abundance of ether bonds that are formed via radical pathway, causing lignin to be one of the slowest decomposing components of plants. The bacteria within the gut microflora of herbivores have the ability to metabolize lignin, helping its host to digest its food. Despite this knowledge, lignin degrading microorganisms from gut microflora are understudied. Previously, our lab obtained a lignin degrading microbial consortia from rumen fluid and termite guts. We used this enrichment as the source for isolating novel lignin degrading microorganisms. Recently, we successfully isolated an anisoin (a lignin dimer) degrading culture. We have initiated a number of experiments to investigate the isolates ability to degrade a variety of lignin monomers and dimers. In addition, experiments are underway to identify the isolate using 16S rRNA-gene and PCR.

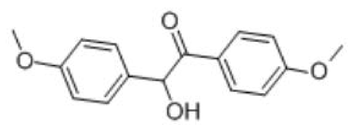
Introduction



From LignoWorks.com

Figure 1: Structure of lignin.

Lignin is a highly complex hetereopolymer whose primary function is to increase plant cell well integrity and to increase resistance from pathogenic attacks. It is formed by the polymerization of a radical reaction pathway. This radical reaction pathway gives lignin its recalcitrant structure, which is resistant to most forms of degradation, including biological. The monomers that lignin is composed of are mainly bonded through ß-aryl ether bonds, di-aryl ether bonds, and biphenyl linkages. All of these bonds are stable and resistant to physical degradation, which obstruct the production of cellulosic ethanol.

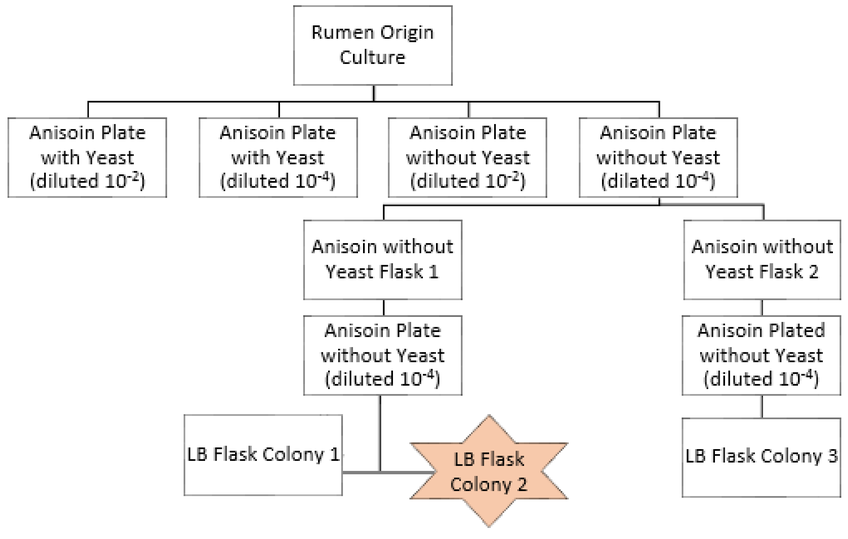


From ChemicalBook.com

Figure 2: Structure of anisoin.

Anisoin is a lignin dimer that was used in the process of isolating the microorganisms from the rumen/termite gut sample. As the structure has similar properties of lignin, microorganisms that can degrade anisoin are presumed to have the ability to degrade lignin.

Materials and Method



Isolation Process: A sample was taken from the rumen of a cow and from termite guts and allowed to grow in a culture over time. Sample from this culture was serial diluted and spread onto four different plates of mineral salt medium (MSM) containing anisoin (with or without yeast). Two different colonies were picked and inoculated into two separate flasks of MSM broth containing anisoin as the carbon source, which were then serial diluted and spread onto MSM agar containing anisoin. From these plates, well-isolated colonies were picked and inoculated into LB broth.

Degradation Studies: Samples from the isolated colony in LB broth were taken and used to inoculate different flasks containing MSM broth, each flask being treated with 9 different lignin-related substrates. After two weeks, growth was determined using folin protein assay.

Results

From ChemicalBook.com

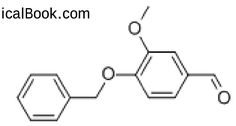


Figure 3: Structure of benzylvanillin,

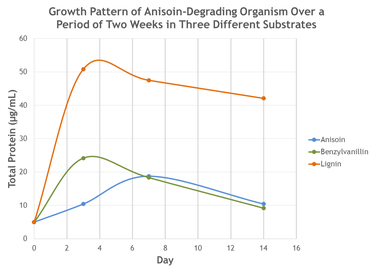
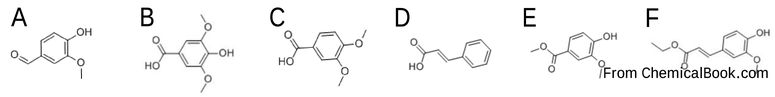


Figure 4: Growth curves of the anisoin degrading organism in MSM broth supplemented with anisoin, benzylvanillin, or lignin as the carbon source.



From ChemicalBook.com

Figure 5: Structures of a) vanillin, b) syringic acid, c) veratric acid, d) trans-cinnamic acid, e) methyl vanillate, and f) ethyl-4-hydroxyl-3-methoxy-cinnamate used to characterize growth capabilities of the anisoin-degrading organism.

Growth of Anisoin-Degrading Organism in Lignin-Related Compounds

Substrate	Total Protein (µg/mL) at Time 0	Total Protein (µg/mL) at Two Weeks
Vanillin	0.833	36.667
Syringic acid	0.833	14.167
Veratric acid	0.833	5.833
Trans-cinnamic acid	0.833	4.167
Methyl vanillate	0.833	9.167
Ethyl-4-hydroxy-3-methoxy-cinnamate	0.833	55.000

Table 6: Growth chart of the anisoin degrading organism in MSM broth containing different lignin-related substrates: vanillin, syringic acid, veratric acid, trans-cinnamic acid, methyl vanillate, and ethyl-4-hydroxyl-3-methoxy-cinnamate over a period of two weeks.

Conclusion

We have successfully isolated a novel microorganism that has the capability of degrading lignin and lignin-related substrates. Identification of the isolate using 16S rRNA-gene and PCR is in progress.

Acknowledgements

Special thanks to Dr. Babu Fathepure for his mentorship throughout this project and to NSF for their financial support to Lyly Van.

Evaluating sorghum germplasm for early season biomass traits

Alexa Cameron and Vijaya Gopal Kakani Ph. D.
Plant and Soil Sciences, Oklahoma State University

Abstract

Soils constitute the largest terrestrial organic carbon pool, estimates at 2400 petagrams of carbon, integrated from the surface to 2 m depth. The main driver for this soil carbon pool is plant roots. Sorghum has been extensively researched as a possible energy crop in the biomass sector, along with switchgrass, corn, and algae. Many varieties of sorghum exist, each growing optimally in differing conditions. By assembling a mini-core collection of 245 varieties and conducting analyses of scanned roots, we were able to determine which varieties established a well-developed root system early in its growing season, yielding more biomass per plant for biofuel conversion. The 245 mini-core germplasm lines were grown in 10" cone-tainers for 5 weeks. Significant differences were observed for measured root traits in the sorghum mini-core germplasm. Positive correlation was observed between root volume and root diameter, while a negative correlation was recorded between root volume and root length. The genetic variability identified in this research for root traits can be exploited to improve carbon sequestration, drought tolerance and/or resource capture in sorghum.

Introduction

As biofuel conversion technologies continue to advance, ever more emphasis is being placed on the feedstocks these processes will use. Sorghum is emerging as an ideal candidate to meet a great portion of the biofuel industry's growing needs. In addition to sorghum's tremendous yield potential, it is also naturally tolerant to drought and heat and requires less fertiliser than corn, allowing it to be grown in areas with marginal rainfall, higher temperatures and with lower inputs. However, little information is available on the root traits that contribute to C sequestration.

Objectives

- Screen mini core collection of sorghum germplasm for root functional properties.
- Identify lines that have higher carbon sequestration rates.

Methods and Materials

Total of 245 genotypes of sorghum received from ICRISAT were planted in 10" cone-tainers on June 15th, 2016 in a greenhouse at Oklahoma State University, Stillwater. Replicated same set two more times on June 17th and 21st. Hand watered 1-2 times a day for 5 week growing period. Nutrients were provided through slow-release Osmocote fertilizer fertilizer granules (15-9-12) on June 29th, July 1st and July 4th. Harvested the intact whole roots by hand-washing with water to remove sand and other debris. Organized harvested roots into labeled plastic bags for storage in refrigerator. Washed and scanned roots using WinRHIZO software at 100x resolution and in color. Analyzed collected data from roots, only first two trials were used for this poster.

Results

- Significant variability was observed for root traits in the mini-core germplasm.
- Both positive and negative correlations were observed for root traits that can be further exploited (Table 1).
- Distribution of root length, surface area and root volume is different diameter classes was significantly different between the genotypes (Fig. 1).
- The larger root diameter fraction contributed the most to the total surface area (Fig. 1).
- The frequency distribution (Fig. 2) figures show the potential root traits to be exploited for improving C sequestration in sorghum.
- For root length, most of the genotypes were in the range of 100-300 cm.
- For total surface area, majority of the genotypes ranged from 125 to 225 cm^2.
- Specific root length, root length to root volume was dominated by genotypes with 0.5 to 2.0 cm cm^{-3}.

Table 1. Correlation analysis between different root traits.

Root Trait	Length (cm)	ProjArea (cm^2)	SurfArea (cm^2)	AvgDiam (mm)	LenPerVol (cm cm^{-3})	RootLentoRootVol (cm cm^{-3})	RootVolume (cm^3)	Tips	Forks
Length (cm)									
ProjArea (cm^2)	0.19								
SurfArea (cm^2)	0.19	1.00							
AvgDiam (mm)	-0.66	0.40	0.40						
LenPerVol (cm cm^{-3})	**1.00**	0.19	0.19	-0.66					
RootLentoRootVol (cm cm^{-3})	0.48	-0.57	-0.57	-0.61	0.48				
RootVolume (cm^3)	-0.54	0.56	0.56	**0.97**	-0.54	-0.60			
Tips	**0.85**	0.13	0.13	-0.52	0.85	0.47	-0.42		
Forks	**0.92**	0.48	0.48	-0.47	0.92	0.20	-0.31	0.74	
Crossings	**0.95**	0.27	0.27	-0.57	0.95	0.38	-0.45	0.77	**0.96**

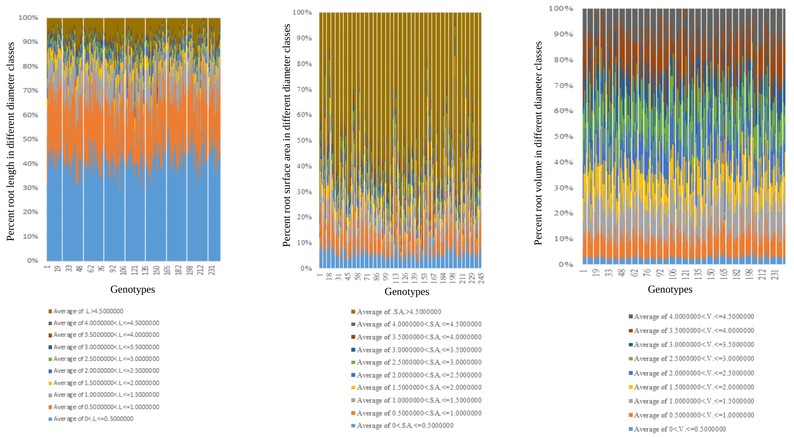


Figure 1. Percent (A) total rot length, (B) total root surface area and (C) total root volume in different root diameter classes.

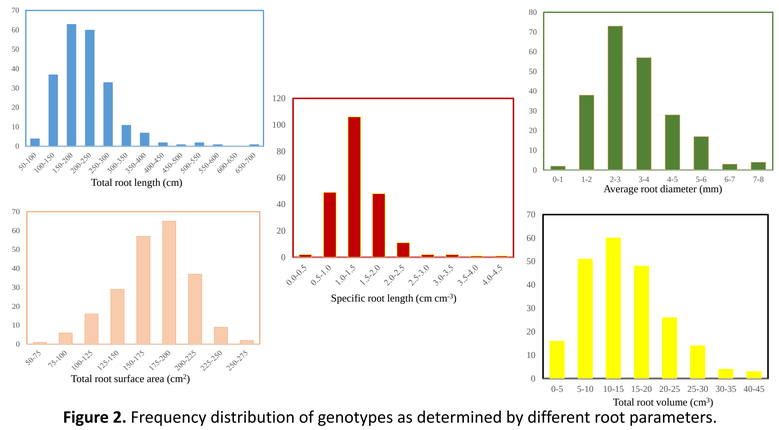


Figure 2. Frequency distribution of genotypes as determined by different root parameters.

Discussion

- ❖ The extent of genetic variability is indicated by the large range observed for the genotypes.
- ❖ The contrasting genotypes identified in this study offer useful plant material for inclusion in sorghum breeding programs aimed at improving root C sequestration.
- ❖ The positive correlation between root volume and average diameter indicates the potential for developing varieties with ability to extract water and nutrients from deeper soil layers.
- ❖ The negative correlation between root length and root diameter or root volume suggests demonstrates the potential for improving root traits to sequester more carbon into deeper layers of the soil.

Conclusions

- ✓ We observed significant genotypic variability for root traits.
- ✓ The identified lines with extreme values for the measured traits can be used to identify the genetic markers or control for these root traits.
- ✓ The genetic variability observed in this research during early growth stages can be exploited to improve carbon sequestration, drought tolerance, and resource capture in sorghum.
- ✓ Further evaluation of root traits in different soil types and multiple location will help to better understand the genetic and environmental control of root traits.

Contact

Alexa Cameron
Oklahoma State University
alexa.cameron@okstate.edu
(816) 799-2473

Acknowledgements

We thank Dalton Hahn and Jay Prater for helping in root washing and greenhouse management. Special thanks from Alexa Cameron to NSF and Oklahoma State University for their financial support and opportunity.

The isolation and characterization of lignin degrading bacteria from rotting wood.

K. Hennessey[1], L. Van[2], B. Fathepure[3]

Department of Biology, George Mason University; Department of Biology, Southwestern Oklahoma State University; Department of Microbiology and Molecular Genetics, Oklahoma State University.

ABSTRACT

In the ever present search for a solution to the ever present modern question of how to meet the world's ever growing energy demands, biofuels, particularly that derived from plant biomass, one of several possible solutions. However, biofuels, like any other resource to be tapped by humans, have several issues with their practical use in meeting our socital, economic and environmental demands. Key to the issue of biofuels are their economic viability and net energy return in comparison to fossil fuels as current so called 1st generation biofuels such as corn ethanol and waste biodiesel often falter in both aspects and have serious limiting factors in practicality. 2nd generation biofuels promise a much more viable replacement of fossil fuels as they better meet both economic and socital demands required of a fuel source. Key to reaching this viability is the effective treatment of tough biomass before it is transformed into various liquid fuel and other products. The plant's cellulose in particular are the key for ethanol production and as such the opening of the plant's cell wall is the primary goal of biomass pretreatment. The cell wall is made of three polymers, cellulose, hemicellulose and lignin. Lignin is the plant's ultimate structural polymer providing defense, rigidity, and incredible resistance to most forms of degradation. This key polymer is a bottleneck in lignocellulose ethanol production for which no organism that can degrade the polymer in a commercially acceptable way is known.

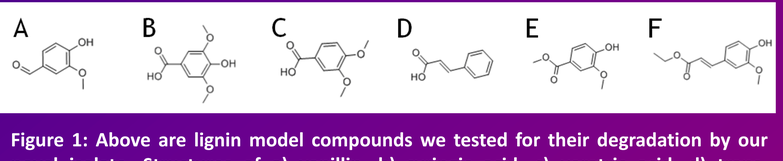


Figure 1: Above are lignin model compounds we tested for their degradation by our novel isolate. Structures of a) vanillin, b) syringic acid, c) veratric acid, d) trans-cinnamic acid, e) methyl vanillate, and f) ethyl-4-hydroxyl-3-methoxy-cinnamate used to characterize growth capabilities of the benzyl vanillin-degrading organism.

INTRODUCTION

Lignin is a tough heteropolymer, or a polymer composed of two or more monomers, that provides rigidity and resistance to the plant. Unlike the two other polymers that compose a plant's cell wall, cellulose and hemi-cellulose, lignin is created not by common reaction mechanisms such as acid-base, substitution, addition, etc. but rather it polymerized by a radical reaction pathway. It is due to this radical reaction pathway that gives lignin its resistance to most forms of degradation, including biological, as this reactions are uncommon in the natural world and as such the enzymes capable of reversing the polymerization reaction via radical reaction are uncommon. Additionally, the bonds between the three lignin monomers, p-coumaryl alcohol, conferyl alcohol and sinapyl alcohol, are mainly β-aryl ether bonds (~40%), di-aryl ether bonds (~20%), and biphenyl linkages (~20%) all of which are very stable bonds that provide little energy upon breakdown and are resistant to physical breakdown and are more or less chemically inert. These properties seriously impede the production of useful biobased energy products from ~~crop~~ biofuel feedstocks such as switchgrass ~~and sorghum~~ and dramaically increase the cost of pretreatment, as ideally a bioreactor that was capable of mostly total depolymerization would be the most cost effective means of pretreatment. While various research efforts have gone into developing feedstock plant strains that produce less lignin and more cellulose and hemicellulose, it is not possible nor desirable to completely remove the production of this key plant polymer. Currently, highly effective lignin degrading fungi do exist, however, none are phenotypically applicable to a bio reactor scheme of pretreatment. Additionally, due the need for peroxides and the inherent nature of the reactions involving lignin many radical chemicals are produced in its degradation requiring a separate pretreatment phase for lignin.

Lignin can serve as both a carbon and energy source for some bacteria, and these lignin degrading microbes mainly derive their energy through the breakdown of the aromatic ring of the lignin monomers. These chemical reactions are often carried out by laccase enzymes or peroxidase enymes as both are capable of mediating radical reactions through the carrying of free electrons from one place to another.

METHODOLOGY

1) Plate original culture of micro organisms on to selective mineral salt agar containing only one lignin model compound as the sole carbon source.
2) Pick individual colonies and inoculate into a mineral salt flask containing the same model compound as the sole carbon source.
3) Replate the flask growth on to multiple plates each using a serial dilution in order to isolate different organisms that were likely in the same colony forming unit on the original plate.
4) Repeat steps two and three until cultures on plates are found to be pure. This should be confirmed from known colony and cell characteristics.
5) Identify the organism using 16S rRNA-gene and PCR method.
6) Explore the organisms ability to degrade lignin using numerous other known lignin model compounds Tests for degradation ability are performed by adding a known amount of liquid inoculum to liquid mineral salt media containing the target compound then estimating the number of viable cells in the source flask through any established means and then, using the same method, estimate the number of cells in the test flask after a set period of time.

RESULTS

- We After successfully isolated a pure culture bacteria that degrade benzylvanillin as the sole carbon source.
- The isolate was found to degarde various lignin model compounds such as Anision, Benzyl Vanillin, Lignin, Vanillin, Syringic acid, Veratric acid, Trans-Cinnaminic acid, Methylvanillate and Ethyl-4-Hydroxyl-3-Methoxy-Cinnamate.
- An investigation of the inoculated flasks showed a total loss of undissolved substrate in flasks containing non-water soluble compounds .
- A folin protein assay was used to estimate the initial population levels and the population levels in inoculated flasks after two weeks in order to determine relative growth abilities on each of the substrates. (Figures 3 and table 1)
- Flasks inoculated with Benzyl Vanillin, Anision and Lignin were sampled ~every 3-4 days and populations estimated using the folin protein assay. (Figure 3)
- Absorbance values were converted into protein concentrations using a standard BSA curve to calculate protein concentration from the slope of the line.

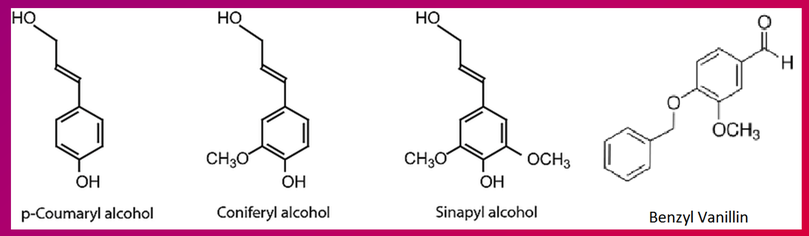
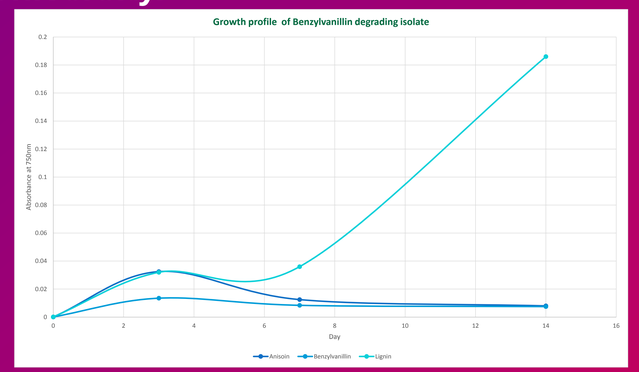


Figure 2: Above are the lignin monomers and the lignin model compound Benzyl Vanillin which was the selective substrate used in this study. Each plant species uses different proportions of the three monomers yielding different compositions of lignin specific to each plant species . This alters the strength of different lignin compounds and as such the source of a lignin compound can affect the results of studies like this.



Growth of Benzilvanillin Degrading Organism on other Lignin Related Compounds

Substrate	Protein Absorbance at Time 0	Total Protein Intial µg/mL	Protein Absorbance at 2 Weeks	Total Protein Two Weeks µg/mL
Vanillin	0.00002	0.017	0.007	5.833
Syringic acid	0.00002	0.017	0.008	6.667
Veratric acid	0.00002	0.017	0.001	0.833
Trans-cinnamic acid	0.00002	0.017	0.004	3.333
Methyl vanillate	0.00002	0.017	0.013	10.833
Ethyl-4-hydroxy-3-methoxy-cinnamate	0.00002	0.017	0.075	62.5

Figure 3: Growth of the Benzylvanillin-degrading strain on benzylvanillin, lignin and anisoin as the sole carbon source. The isolate showed reference to alkali lignin as the carbon source.

Table 1 : Ability of benzylvanillin-degrading isolate to degrade various lignin-related compounds. in all substrates was check with visual turbidity comparisons and the Folin protein assay. The assay results confirm the increased levels of protein in solution associated with increased cell growth.

Observation

The benzylvanillin-degrading organism isolated from the green stream was shown to be capable of metabolizing several lignin model compounds proving. Future genetic identification of the organism and characterization of its physiciological features and a study of the products of its lignin metabolism are suggested.

REFERENCES

Brown, M. E., & Chang, M. C. (2014). Exploring bacterial lignin degradation. *Current Opinion in Chemical Biology*, *19*, 1–7.

Bugg, T. D., Ahmad, M., Hardiman, E. M., & Singh, R. (2011). The emerging role for bacteria in lignin degradation and bio-product formation. *Current Opinion in Biotechnology*, *22*(3), 394–400.

Compton, S. J., & Jones, C. G. (1985). Mechanism of dye response and interference in the Bradford protein assay. *Analytical Biochemistry*, *151*(2), 369–374. http://doi.org/10.1016/0003-2697(85)90190-3

DeAngelis, K. M., Allgaier, M., Chavarria, Y., Fortney, J. L., Hugenholtz, P., Simmons, B., … Hazen, T. C. (2011). Characterization of trapped lignin-degrading microbes in tropical forest soil. *PLoS One*, *6*(4), e19306.

Díaz, E., Jiménez, J. I., & Nogales, J. (2013). Aerobic degradation of aromatic compounds. *Current Opinion in Biotechnology*, *24*(3), 431–442.

Haider, K., Trojanowski, J., & Sundman, V. (1978). Screening for lignin degrading bacteria by means of 14C-labelled lignins. *Archives of Microbiology*, *119*(1), 103–106.

Sapan, C. V., & Lundblad, R. L. (2015). Review of methods for determination of total protein and peptide concentration in biological samples. *PROTEOMICS-Clinical Applications*, *9*(3–4), 268–276.

PubChem National Institutes of Health chemical database.

ACKNOWLEDGEMENTS

Special thanks to Dr. Babu Fathepure for his mentorship throughout this project and to NSF for their financial contributions to the lab.

Identifying Novel Stress Specific miRNA Expressions in *Sorghum bicolor* (L.)

Robert Mann[a,b] and Ramanjulu Sunkar Ph.D[b]

[a]School of Life Sciences, Arizona State University, Tempe, AZ 85281

[b]Dept. of Biochemistry & Molecular Biology, Oklahoma State University, Stillwater, OK 74078

Abstract

Micro RNAs (miRNAs) are 20-22 nucleotides long small RNAs and emerged as major regulators of gene expression at the posttranscriptional level. They regulate the expression of target messenger RNAs either by degrading the mRNA or preventing protein synthesis. The role of miRNA has been strongly linked to plant growth and development but also immensely important for adaptation to stress conditions. In this study, miRNA profiles were created for Sorghum exposed to drought or heat or simultaneous heat and drought stresses with the goal of determining miRNA expression profile of conserved and novel miRNAs. My role is limited to characterizing novel miRNAs and their expression profile under stress in sorghum. Using a minimum of a one fold (log2) change in expression, we 30 novel miRNA sequences. Increased expressions were found in 14 novel miRNAs (t18261875, t19538716, t2176785, t2298404, t8395717, t4678508, t16574179, t18939320_1(2), t19260627, t8265032, t17581965, t20411345, t2288983, t6515920). Conversely 17 novel miRNAs (t16574179, t14715744, t8253978, t8530652, t13354128, t15855227, t3056609, t38256, t6304272_1(3), t822606, t9660393_2, t9711961, t151143_1(2), t13194263_1(2), 16312634, 16428447, t9258583_1) were identified with decreased expression rates. We identified both similarities and dissimilarities in the miRNA expression responses to the three stress conditions. The heat and combined stress conditions were quite similar, with drought and the combined stress conditions being similar to a lesser degree.

Introduction

Sorghum is not only an important bioenergy crop species but also a good model system to study the molecular mechanisms important for stress tolerance because it can tolerate abiotic stresses such as drought and heat very well compared to many other crop plants. Prior to my joining, Sunkar lab has been studying the expression profiles of miRNAs in response to drought, heat or combination of these two stresses together in sorghum in order to understand the miRNA-dependent gene regulation important for stress tolerance in sorghum. Prior to my joining the lab also has determined conserved miRNA expression profile under these stress conditions but not novel miRNAs. My role as an REU researcher was limited to identifying novel miRNAs on the basis of hairpin structure predictions for the precursors as well as determining their expression profiles based on sequencing of small RNA libraries that the lab has sequenced.
The effects of the combined stress condition on miRNA expression is of interest because of the correlation frequently found between heat and drought conditions.

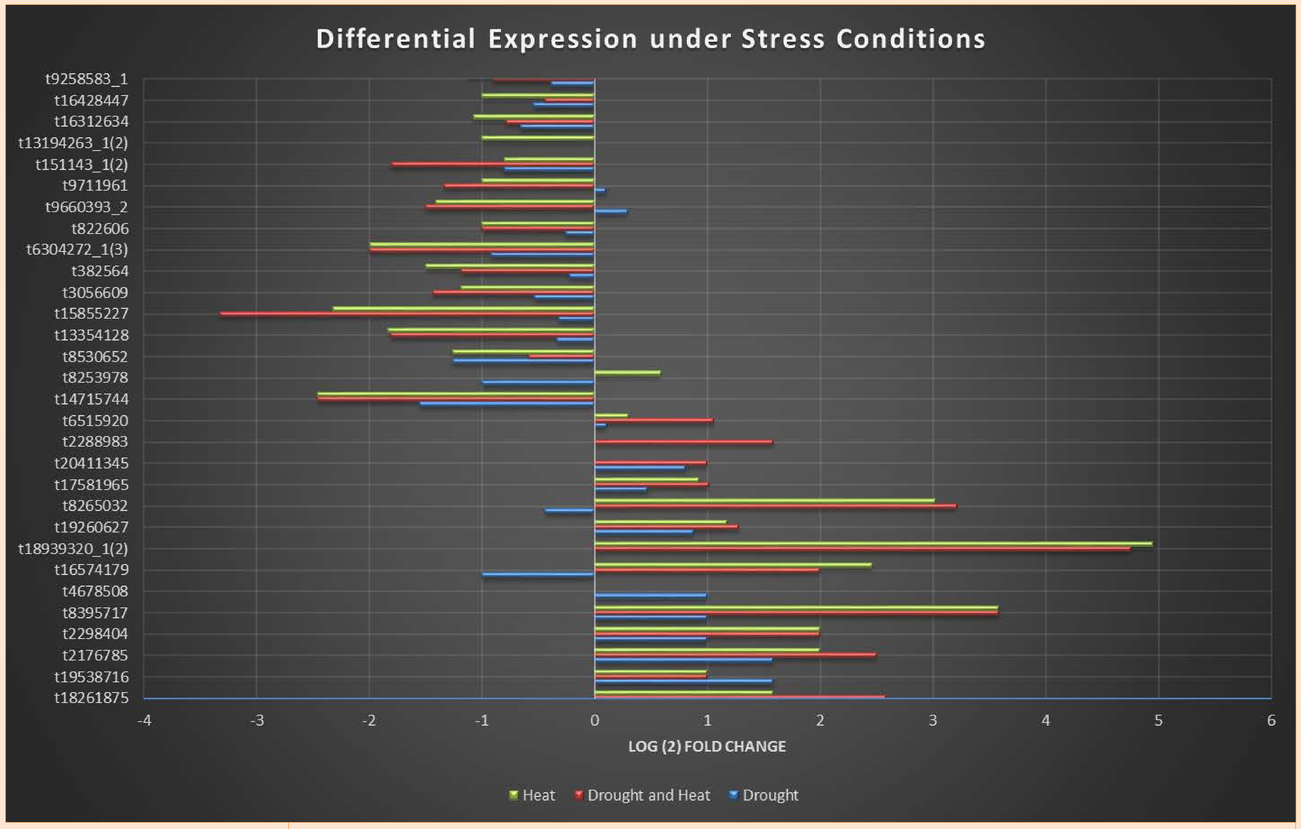


Figure 1: Fold-change in miRNA expression under each stress condition; relative to control group

Hairpin Structure Predictions

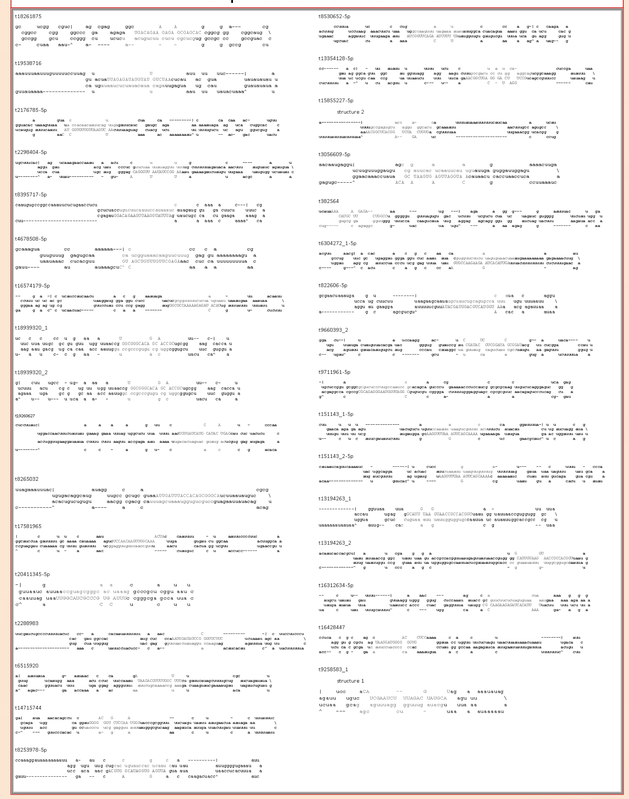


Figure 2: Predicted hairpin structures for novel miRNAs. Capital bases are the miRNA sequence bases.

Methods & Materials

Novel miRNAs identification by predicting hairpin structure for the precursors
Putative novel miRNA sequences were identified via high throughput sequencing. By mapping this sequence to Sorghum genome, precursors sequences (longer genomic regions around the mapped miRNA) were isolated and used for hairpin structure predications with the Mfold Web Server, hosted by The RNA Institute, College of Arts and Sciences, State University of New York at Albany. Predictions without any secondary folds were discarded.

Identification of stress-specific differential expression
Normalized read counts and associated novel miRNAs were uploaded to analytical/statistical software Microsoft Excel. The miRNA read counts extracted from the control and each stress sample were compared. Changes in read frequencies were calculated and compared using $\log_2()$ values, with a single fold change set as the minimum threshold for differential expression.

Results

Differential regulation was observed for 30 novel miRNA sequences
Overall, differential expression was similar under all stress conditions for t18261875, t19538716, t2176785, t2298404, t8395717, and t14715744. All but the later were upregulated, with the t14715744 being downregulated by all three stress conditions.
However, we also found stress specific expression for the remaining 24 miRNAs.
Only miRNAs t4678508, t16574179, t8253978, and t8530652 were specifically regulated by drought.
The combined stress condition specifically regulated 17 miRNAs, 8 miRNAs (t16574179, t18939320_1(2), t19260627, t8265032, t17581965, t20411345, t2288983, and t6515920) upregulated and 9 miRNAs (t13354128, t15855227, t3056609, t382564, t6304272_1(3), t822606, t9660393_2, t9711961, and t151143_1(2)) downregulated.
Heat stress specifically regulated 17 miRNAs, upregulating 4 miRNAs (t16574179, t18939320_1(2), t19260627, t8265032) and downregulating 13 miRNAs (t8530652, t13354128, t15855227, t3056609, t382564, t6304272_1(3), t822606, t9660393_2, and t9711961).

Discussion & Conclusion

We identified 30 novel miRNAs in sorghum with differential expression under drought and/or heat stresses. Multiple miRNAs were differentially regulated by all three stress treatments, however, majority of the miRNA identified had stress-specific responses. The combination of the stresses did induce unique miRNA expression in 5 miRNAs, nearly all of which were up regulated.
One-third of the identified miRNAs were differentially regulated by the drought stress condition, majority of which had increased expression.
Heat stress induced differential expression in over two-thirds of the identified miRNAs. Decreased expression of the miRNA was found in over 60% of the heat-affected miRNAs.
The combined stress conditions was found to largely mirror the heat stress responses, with two-thirds of the identified miRNAs were commonly regulated by heat and combined drought and heat stress treatments.
The general lack of similarity between drought and combined stress suggests that sorghum is significantly more responsive to heat stress than drought.

Acknowledgments

NSF for funding the Bioenergy REU Program

Vegetation Response After the Removal of Eastern Redcedar (*Juniperus virginiana* L.)

Janet L. Deardorff, Jacob H. Dyer, and Rodney E. Will

Department of Natural Resource Ecology and Management, Oklahoma State University, Stillwater, OK 74078

Introduction

Woody plant encroachment is an important factor contributing to prairie ecosystem degradation in the mid-western United States (Briggs et al. 2005, Alford et al. 2012). Many factors such as fire exclusion, drought, and increased grazing have contributed to the success of woody species invasion in prairies and savannas (Allen and Breshears 1998, Briggs et al. 2002, 2005, DeSantis et al. 2011). Eastern redcedar (*Juniperus virginiana* L.), has become a dominant woody invader in tallgrass and mixed prairie ecosystems (Briggs et al. 2005, Alford et al. 2012). When present in an ecosystem, *J. virginiana*, decreases herbaceous vegetation richness and evenness (Limb et al 2010), and by forming dense stands it can affect other environmental factors such as litter decomposition, soil temperature, and light availability to understory vegetation (Norris et al. 2001, Pierce and Reich 2009).

Studies investigated multiple management methods for the removal of *J. virginiana* from prairie and savanna ecosystems (Schmidt and Stubbendieck 1993, Ortmann et al. 1998, Morton et al. 2010). One method is manually removing *J. virginiana* and utilizing it as a biofuel, specifically ethanol and butanol (Olukoya et al 2014, Lui et al. 2015). Removing *J. virginiana* and using it as a biofuel feedstock will be important because it will contribute to the control of it's invasion into prairies and savannas. Removal of *J. virginiana* from the ecosystem causes a rapid increase in herbaceous plant diversity (Alford et al. 2012). After the removal of *J. virginiana*, we must monitor and understand what vegetation will come back in order to better achieve prairie restoration.

Objective

The objective of this study was to compare the vegetation community dynamics in a *J. virginiana* cleared watershed after the removal of *J. virginiana* with that of two *J. virginiana* encroached watersheds and an intact prairie watershed.

Hypotheses

I hypothesized that there will be a higher species richness and diversity in the prairie and *J. virginiana* cleared watersheds than that of the two *J. virginiana* encroached watersheds and that the vegetation in the *J. virginiana* cleared watershed will resemble the prairie watershed more than that of the two *J. virginiana* encroached watersheds.

Methods

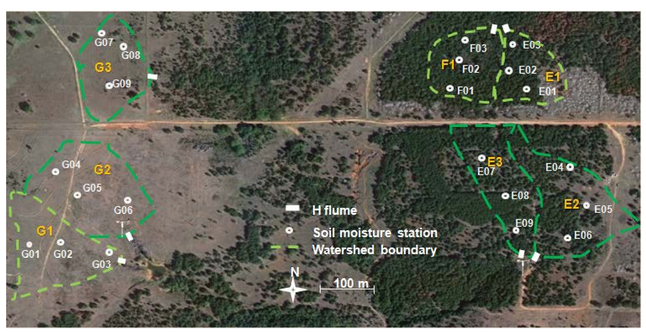


Figure 1: Study site at Cross Timbers Experimental Range (CTER) located in Payne County, Oklahoma (36°03'46.73" N, 97°11'03.33" W)

Vegetation response was monitored in one *J. virginiana* cleared watershed, two *J. virginiana* encroached watersheds, and one prairie watershed. In each watershed, twenty random points were selected based on previous vegetation and soil studies. At each point, a 0.25 m^2 quadrat was placed 2m due north of the point and marked with a flag. Within each quadrat, all plant species were identified and percent cover measurements (to the nearest 1%) were taken. Other variables such as % grazed and substrate were observed. The data were analyzed with the Shannon Index, Jaccard Index, Coefficient of Community Similarity, ANOVA (SAS 9.4), and Duncan's Multiple Range Test.

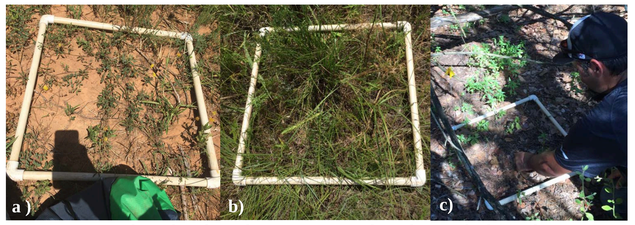


Figure 2: A quadrat taken from the *J. virginiana* cleared watershed (a), the prairie watershed (b), and a *J. virginiana* encroached watershed (c).

Results

The *J. virginiana* cleared watershed (RCCUT) had the highest diversity (H'= 3.32) followed by the prairie watershed (Prairie) (H'= 3.18), *J. virginiana* encroached watershed one (RC1) (H'= 3.17) and *J. virginiana* encroached watershed two (RC2) (H' = 2.99) (Figure 3a) RC1 had the highest evenness (J = 0.92) followed by RC2 (J = 0.91), RCCUT (J = 0.89) and the Prairie watershed (J = 0.86) (Figure 3b) . RC1 and RC2 had the highest coefficient of community similarity (CC = 0.54) and RC1and the prairie watershed (CC = 0.33) were the least similar (Figure 3c).

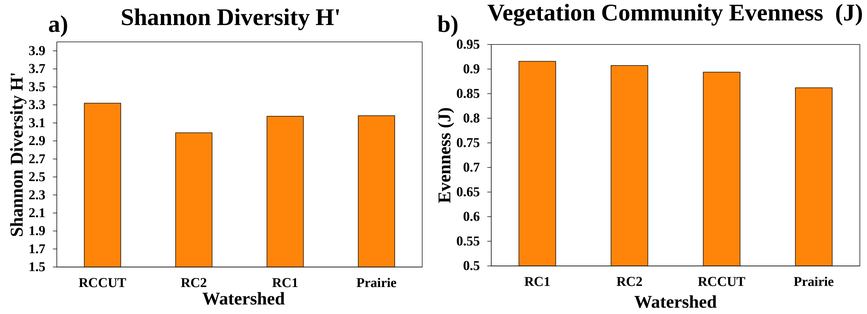


Figure 3: Shannon Diversity (a), Evenness (b), and Community Similarity (c) values for *J. virginiana* encroached watershed one (RC1), *J. virginiana* encroached watershed two (RC2), the *J. virginiana* cleared watershed (RCCUT), and the prairie watershed (Prairie).

RCCUT had the highest species richness per quadrat followed by the prairie watershed RC1 and RC2 ($p < 0.001$). Both RC1 and RC2 were grouped together based on species richness, while RCCUT and the prairie watershed were different from the other watersheds (Figure 4a). The prairie watershed had the highest percent vegetation cover per quadrat followed by RCCUT , RC2, and RC1 ($p < 0.001$). RC1 and RCCUT were grouped together, while RC2 and the prairie watershed made up their own individual groups (Figure 4b). The dominant substrate in RC1, RC2, and RCCUT was *J. virginiana* litter. The dominant substrate in the prairie watershed was prairie litter (Figure 4c).

Acknowledgements

I would like to thank the National Science Foundation for funding this research experience and Dr. Gopal Kakani for additional guidance and supplying switchgrass seed.

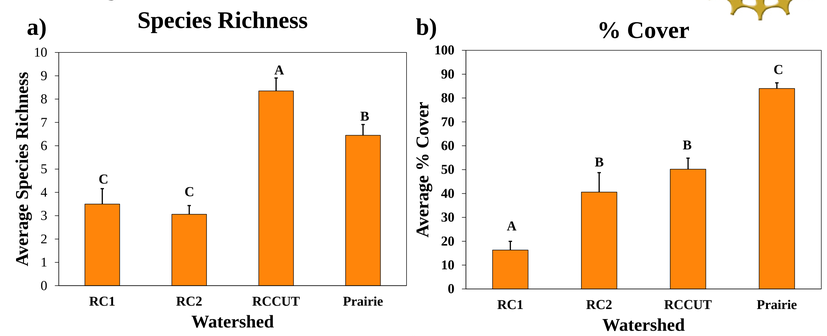


Figure 4: Average Species Richness (a), Average % Cover (b), and Substrate (c) values for *J. virginiana* encroached watershed one (RC1), *J. virginiana* encroached watershed two (RC2), the *J. virginiana* cleared watershed (RCCUT), and the prairie watershed (Prairie).

Conclusions

RCCUT had the highest species diversity (H') and richness while RC1 and RC2 had a lower species diversity and richness. Though RCCUT had a higher species richness, it had a lower community evenness which is probably due to the fact that watershed was disturbed and could be more readily taken over by weedy species. RCCUT also more closely resembled RC2 than the prairie watershed which was hypothesized. This is probably due to the close proximity between RCCUT and RC2. The RCCUT watershed vegetation is quickly recovering after the removal of *J. virginiana* and is producing a greater vegetation biomass than that of RC1 and RC2.

Additional Work

We also need to monitor and understand the potential land productivity for utilization as an agricultural or biofuel feedstock crop such as switchgrass. Switchgrass, *Panicum virgatum*, is one of the leading dedicated energy crops in the United States because of its ability to efficiently use water, its low nitrogen and phosphorus requirements, and its high productivity (Morris et al. 1982, Thomas and Lucey 1987, Stout et al. 1988, Jung et al. 1990, Samson and Omielan 1992, McLaughlin and Kszos, 2005) Few studies have examined polyculture plantings of *P. virgatum* with nitrogen fixing legume species. We are examining, with a field and greenhouse study, the potential productivity when planting a dedicated biofuel feedstock such as switchgrass as a monoculture and as a polyculture with other grass and legume species.

References

Alford et al. (2012). *Ecological Applications*
Allen and Breshears (1998) *Proc. Natl. Acad. Sci.*
Briggs et al. (2002) *Ecosystems*
Briggs et al. (2005) *BioScience*
DeSantis et al. (2011) *Ecology and Management*
Jung et al. (1990) *Agronomy Journal*
Limb et al. (2010) *Rangeland Ecology & Management*
Lui et al. (2015) *Bioresource Technology*
McLaughlin and Kszos (2005) *Biomass and Bioenergy*
Morris et al. (1982) *Agronomy Journal*
Morton et al. (2010) *Rangeland Ecology & Management*
Norris et al. (2001) *Can. J. Bot*
Olukoya et al. (2014) *Bioresource Technology*
Ortmann et al. (1998) *Journal of Range Management*
Pierce and Reich (2009) *Biol. Invasions*
Samson and Omielan (1992) *The Thirteenth North American Prairie Conference*
Schmidt and Stubbendieck (1993) *Journal of Range Management*
Stout et al. (1988) *Soil Science Society American Journal*
Thomas and Lucey (1987) *In the Proceedings of the 1987 American Forage & Grassland Conference*

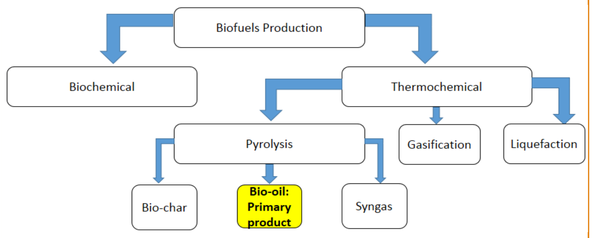
The Effects of Heating Rate on the Pyrolysis of Eastern Redcedar

Will Smith, Ajay Kumar, Redemptus Tshikesho, Zixu Yang

Biosystems and Agricultural Engineering, Oklahoma State University

Background

Solid biomass can be converted to liquid and gaseous fuels by a number of biochemical and thermochemical processes. One such thermochemical process is pyrolysis, which is the thermal decomposition of solid biomass in the absence of oxygen to produce products that are primarily liquid. This liquid is referred to as bio-oil and is a complex, heterogeneous mixture of hundreds of compounds with potential for fuel and chemical applications. [1] There are many challenges that must be overcome in order for bio-oil utilization to be fully realized. One such challenge is to better understand the effect of different parameters, such as heating rate, on the pyrolysis products.



Heating rate is a fundamental parameter of biomass pyrolysis. The effect of heating rate on pyrolysis yields has been investigated extensively. Most research has focused on measuring the yields of the three primary pyrolysis products: bio-oil, syngas, and biochar. Pyrolysis is typically classified as either slow, fast, or flash pyrolysis. Many researchers have investigated the relationship between heating rate and pyrolysis products. Thermogravimetric analysis (TGA) of ground rapeseed was used to demonstrate that higher heating rates result in higher rates of solid mass loss. [2] One experiment used a pyroprobe to show that increasing the heating rate had no statistically significant effect on the pyrolysis of pine wood or switchgrass. Despite increasing the heating rate from 50 °C/s to 2000 °C/s, the real heating rate of the biomass, as measured by a thermocouple inserted into the sample, remained constant at around 50 °C/s. It was also reported that no significant change in the yield of bio-oil compounds was detected. [3]

Pyrolysis Process	Solid Residence Time (s)	Heating Rate (K/s)	Particle Size (mm)	Temp. (K)	Product Yield (%)		
					Oil	Char	Gas
Slow	450–550	0.1–1	5–50	550–950	30	35	35
Fast	0.5–10	10–200	<1	850–1250	50	20	30
Flash	<0.5	>1000	<0.2	1050–1300	75	12	13

Chart Source: [1]

Objectives

1. Investigate the relationship between pyrolysis heating rate and bio-oil composition.
2. Examine possible interactions between primary biomass components when undergoing pyrolysis.
3. Investigate the relationship between heating rate and biochar surface characteristics.

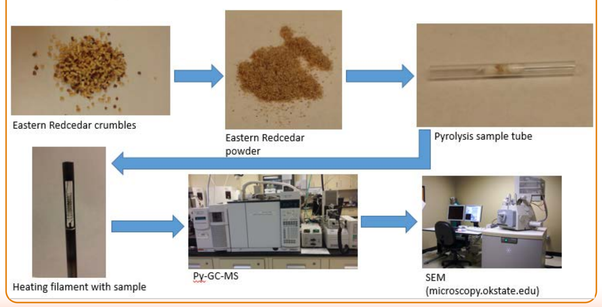
Materials & Methods

Bio-oil analysis

Eastern redcedar crumbles were purchased from Forest Concepts, LLC (Auburn, WA, USA), milled (Thomas-Wiley Model 4 lab mill), and sieved using an average hole diameter of 212 µm. Approximately 300 µg of ground redcedar were inserted into a quartz pyrolysis tube. The sample tube was then inserted into a pyroprobe (CDS Analytic, Inc. 5200 HPR) and heated at a controlled rate to a final temperature of 500 °C, which was held for a duration of 90 s. The heating rates used were: 0.25, 250, 500, 750, and 1000 °C/s. Condensable gases were automatically injected into a gas chromatograph-mass spectrometer (GC-MS, Agilent 7890A GC, Agilent 5975C MS) using a trap and purge system with helium as a carrier gas. Compounds were identified via comparison of MS spectra with the NIST library, and GC percent area data were used to quantitatively analyze product composition. The number of peaks detected by the GC was also recorded. In addition to redcedar, samples of cellulose (Microcrystalline, Fisher Scientific) and lignin (Alkaline, Fisher Scientific) were also analyzed using Py-GC-MS.

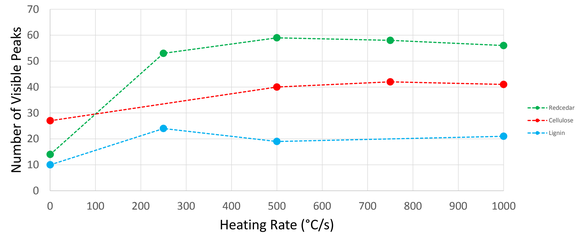
SEM analysis

After pyrolysis in the pyroprobe, the solid biochar left over from the five redcedar trials was observed at multiple magnifications using a scanning electron microscope (SEM, FEI quanta 600) at the Oklahoma Technology and Research Park, Stillwater, OK.

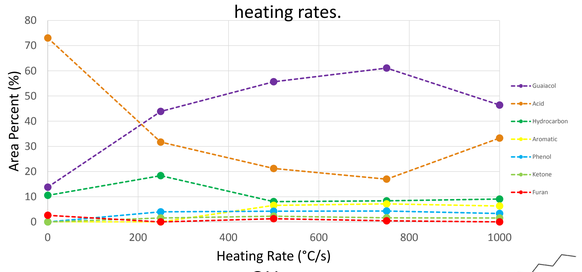


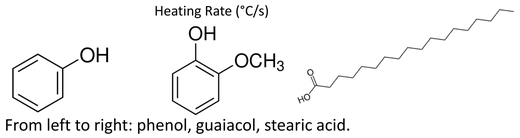
Results (Py-GC-MS)

Number of visible peaks for GC of bio-oil compounds from pyrolysis of eastern redcedar, cellulose, and lignin at 500 °C with various heating rates.



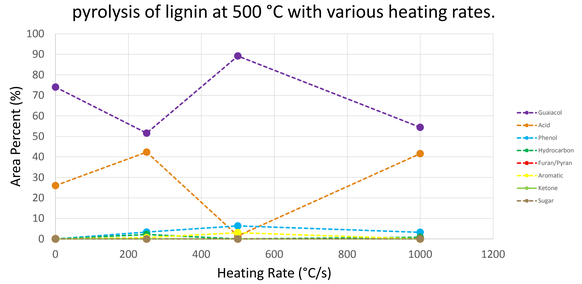
Normalized percent area for GC of bio-oil compounds from pyrolysis of eastern redcedar at 500 °C with various heating rates.



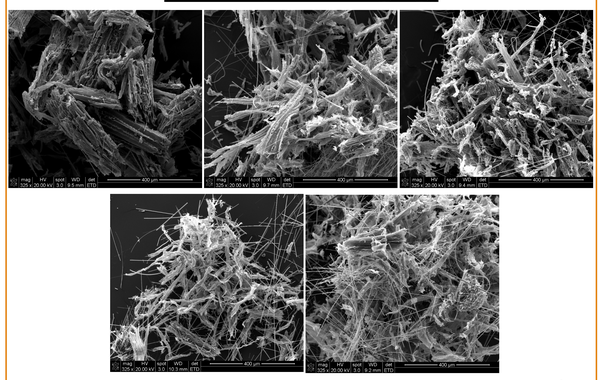


From left to right: phenol, guaiacol, stearic acid.

Normalized percent area for GC of bio-oil compounds from pyrolysis of lignin at 500 °C with various heating rates.

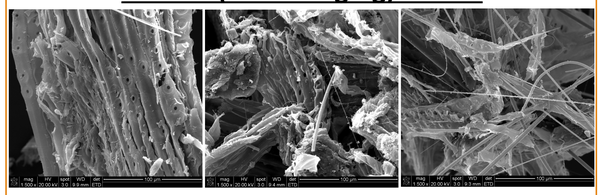


Results (SEM Imaging)



X325 From left to right: 0.25, 250, 500, 750, 1000 °C/s

Results (SEM Imaging) Cont'd



X1500 From left to right: 0.25, 500, 1000 °C/s

Conclusions

- The number of compounds in redcedar, lignin, and cellulose bio-oil increases with heating rate until 500 °C/s.
- When observing number of compounds produced, cellulose and lignin reaction pathways appear not to interact significantly.
- Guaiacol and acid, the two most prominent types of redcedar pyrolysis products, demonstrate a loosely inverse relationship with respect to heating rate.
- Guaiacol production is at its highest around 750 °C/s while acid production is at its lowest.
- Lignin appears to be a significant source of the trend displayed by redcedar, with cellulose contributing to the production of a smaller, but more varied amount of products with little change with respect to heating rate.
- SEM indicates that increasing heating rate to 500 °C/s corresponds to greater redcedar fragmentation.
- Pyrolysis appears not to create pores in redcedar, but instead "shreds" the woody structure.

References

1. Jahirul, Mohammad; Rasul, Mohammad. Biofuels Production through Biomass Pyrolysis – A Technological Review. Energies 2012; 5:4952-5001.
2. Haykiri-Acma, H.; Yaman, S. Effect of Heating Rate on the Pyrolysis Yields of Rapeseed. Renewable Energy 2006; 31:803-810.
3. Thangalazhy-Gopakumar, Suchithra; Adhikari, Sushil. Influence of Pyrolysis Operating Conditions on Bio-Oil Components: A Microscale Study in a Pyroprobe. Energy Fuels 2011; 25:1191-1199.

Acknowledgments

Special thanks to Dr. Gopal Kakani and Dr. Pamela Abit for organizing and running this program. I am also grateful to Oklahoma State University Department of Plant & Soil Sciences and National Science Foundation for allowing me to experience this learning opportunity.