OCAST

2018 Plant Science Projects

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Development of PSSPGD server - a web server for plant small signaling peptide-encoding gene discovery

PI: Dr. Patrick Xuechun Zhao, Noble Research Institute LLC, Ardmore

Research Area: Genomics and Genetics

OCAST Project: PS18-012

A significant part of the cell-to-cell communication in plants is mediated by small signaling peptides (SSPs) or also called “peptide hormones” or “secreted peptides.” Recently, SSPs have emerged as an important class of regulatory molecules in plants involved in the control of plant growth and development, uptake and utilization of mineral as well as organic nutrients, and also nodule regulation. SSPs thus may be used as “peptide hormone-based fertilizers" for plant/crop improvement.

Methods and tools for small signaling peptides genes in plants however are still lacking, and until now, prediction of SSPs has been made mainly by using the SignalP Server to predict the presence and location of signal peptide cleavage sites. Yet, SignalP does not take into account the size of the proteins or homology with known SSPs.

We propose to develop a PSSPGD Server that will integrate a series of comprehensive and complemental methods for the prediction of small peptides in different plant species. This new prediction server will analyze peptide sizes, position-specific scoring matrix (PSSM) profiles and HMM profiles of known SSP gene families, signal peptide cleavage sites, mono-, di- and tri amino acid composition information and physico-chemical composition information of peptides, and comparison with known SSPs.

Our main objectives for the proposed project include:

- Objective 1: Develop an initial SSP-prediction pipeline for the analyses of small signaling peptides in plants including different criteria such as protein length, prediction of signal peptide cleavage sites, and homology with known SSPs based on HMM models.
- Objective 2: Improve the SSP-Prediction pipeline by the use of machine learning approaches, and validate the results with previous SSP identified in plants (cross-validation).
- Objective 3: Develop the proposed user-friendly and powerful PSSPGD Server - a Web Server for Plant Small Signaling Peptide-encoding Gene Discovery and release the web server for public use.

The major deliverables of the project will include, but are not limit to:

- A publicly available web server namely PSSPGD - a Web Server for Plant Small Signaling Peptide-encoding Gene Discovery.
- A comprehensive database of genome-wide predictions and classifications of plant small signaling peptide-encoding genes.

Elucidating the role of microRNAs in photosynthesis by using closely related C3, C3-C4, and C4 Flaveria species

PI: Dr. Ramanjulu Sunkar, Oklahoma State University, Stillwater **Research Area:** Molecular Biology **OCAST Project:** PS18-014

Photosynthesis is a metabolic process through which plants synthesize carbohydrates in the presence of sunlight using atmospheric CO2 and water. On the basis of the first formed stable Carbon compound (3 carbon molecules containing 3-phosphoglycerate or 4 carbon molecules containing oxaloacetate) after CO2 is incorporated into the Calvin cycle, plants have been classified into C3 or C4 mode of photosynthesis. Additionally, the C4 plants are characterized by the Kranz anatomy (comprising mesophyll cells and bundle sheath cells) coupled with the cooperative moment of the metabolites between these two cell-types and dense leaf venation. The microRNA-mediated gene regulation has emerged as one of the critical modes of gene regulation important for almost all biological processes of a plant life cycle. However, it is unknown whether or not these small regulatory RNAs play a role in photosynthesis, the most important metabolic process.

In plants, most conserved miRNAs regulate transcription factors, thus, indirectly miRNA-guided gene regulation impinges the expression of number of the down-stream genes in a cell type-specific manner. This could be important for the anatomical and biochemical differences that exist between C3 and C4 plants and miRNAs might have played a significant role in the evolution of C4 photosynthesis from the C3 type. A direct way of identifying miRNAs's role in photosynthesis is by comparing their expression profiles in closely related species but differ in their modes of photosynthesis. To address this question, recently we have analyzed miRNAs in the leaves of three Flaveria sps, i.e., Flaveria robusta (C3), Flaveria ramosissima (C3-C4 intermediate) and Flaveria bidentis (C4). Preliminary studies revealed that several miRNAs are differentially expressed in leaves of C3, C3-C4 intermediate and C4 Flaveria spp. suggesting that miRNA-guided gene regulation differs between these different modes of photosynthesis, thus are critical regulators of photosynthesis.

To explore this intriguing observation in more detail, miRNAs and their mRNA targets will be analyzed in bundle sheath cells and mesophyll cells of the Flaveria sps. This study will provide new insights into the role of miRNAs in C3 and C4 photosynthesis and also on the roles of miRNAs in the evolution of C4 photosynthesis from C3 type. The specific objectives are to:

1. Profile miRNAs in leaves of C3, C3-C4 intermediate and C4 Flaveria spp. by deep sequencing small RNAs
2. Profile miRNAs in mesophyll cells and bundle sheath cells of C3, C3-C4 intermediate and C4 Flaveria spp. by sequencing small RNAs
3. Determine the impact of miRNAs on their mRNA targets in C3, C3-C4 intermediate and C4 Flaveria spp. by sequencing degradome libraries.

Pretreatment of switchgrass by fungi-bacteria co-culture for effective saccharification and butanol production

PI: Dr. Babu Fathepure, Oklahoma State University, Stillwater **OCAST Project:** PS18-016 **Research Area:** Biomass Conversion

One of the major challenges in producing cellulosic biofuels is to decrease lignin content for easy access of plant polysaccharides by microbes/enzymes to produce glucose and other sugars for subsequent fermentation to alcohols. Delignification is one of the most costly steps accounting nearly 20% of the total cost involved in cellulosic ethanol production. Most current pretreatment techniques employ physicochemical processes and these pretreatment steps are not only costly but also produce toxic byproducts that inhibit downstream processes. On the other hand, biological processes are cost-effective and sustainable, nonetheless they are slow. To date most studies have focused on fungal ability to degrade lignin, but recently attention is shifted towards understanding the role of bacteria in lignin degradation.

In this project, we propose a different approach in which we will explore fungi-bacteria interactions on the degradation of lignin. Studies have shown that fungi produce a broad range of extracellular enzymes that quickly depolymerize lignin to a mixture of low molecular weight intermediates, while bacteria further degrade partially degraded lignin intermediates using their own oxidative enzymes rendering cellulose/hemicellulose available for saccharification and fermentation.

Our recent studies using a co-culture of Phanerochaete chrysosporium strain RP-78 (fungi) and a lignin-degrading Pseudomonas sp. strain YS-1r (bacteria) inoculated at 1:1 ratio resulted in better lignin degradation in sugarcane bagasse compared to when inoculated with bacteria alone, fungi alone, or at other fungi-to-bacteria ratios. Lignin degradation as measured by decrease in Guaiacyl (G) -to- Syringyl (S) ratio showed almost 50% reduction over un-inoculated control suggesting a significant degradation of lignin. Also, lignin degrading enzymes such as lignin peroxidase and Dyp-peroxidase were expressed maximum in co-cultures compared to other combination of treatments. Overall, our studies have shown promising delignification of plant biomass when treated with a co-culture of fungi and bacteria compared to other treatment conditions. Also, we anticipate production fewer fermentation inhibitors during delignification process as strain YS-1p is capable of metabolizing a variety of toxic lignin intermediates.

In this project, we plan to optimize conditions to achieve higher level of delignification that would result in roughly 4 to 6 % sugars after enzymatic hydrolysis of pretreated switchgrass. Further, we would like to assess the growth and ability of Clostridium acetobutylicum to utilize sugars generated from enzymatic hydrolysis of microbial pretreated plant biomass to produce butanol. We expect that this novel approach has a high potential in the development of a sustainable cost-effective biorefinery for biofuel production from plant biomass.

Forward genetic analysis of cotton fiber development

PI: Dr. Mohamed Fokar. Oklahoma State University, Stillwater **OCAST Project:** PS18-018 **Research Area:** Genomics & Genetics

The production of cotton generates revenues in excess of $6 billion annually, and provides the raw materials to many other industries that generate products worth an additional $120 billion, thereby making cotton the number one value-added crop in the United States. Last year, the state of Oklahoma was ranked the nation's fourth-leading producer of cotton with production exceeding 1.1 million bales. The cotton genome has been sequenced and a rich supply of genomic resources are available for both basic and applied research and breeding both in public and private domains.

However, relatively little work has been carried out on the use of forward genetics to identify and functionally characterize the genes that regulate cotton fiber development and determine the critical characteristics of cotton fibers. Some of the difficulty stems from the fact that the most widely grown cotton species are allotetraploids with extensive gene redundancy, making the identification of qualitative traits difficult. Therefore, we propose a project to develop mutagenized lines of two diploid cotton species that are genetically similar to the ancestral species that gave rise to tetrapolid cotton.

Seeds of Gossypium arboreum (AA) and Gossypium raimondii (DD) will be exposed to ethyl methane sulfonate (EMS) and fast neutron radiation at doses sufficient to cause extensive nucleotide substitutions and deletions, respectively. M2 populations will be derived and screening for mutants that affect cotton fiber characteristics will be initiated. M3 seeds will then bulked for mutant cataloging and for distribution to other research and breeding programs around the world. Secondary screening for mutant lines with altered stress tolerance phenotypes will also be carried out.

We anticipate that numerous mutant lines of scientific, agronomic, and industrial importance will be identified and, in future work, the causative lesions will be identified and the role of the affected genes in cultivated cottons will be explored using molecular breeding, transgenic and genome editing approaches.

Stress tolerant cotton

PI: Dr. Randy Allen, Oklahoma State University, Stillwater **OCAST Project:** PS18-025 **Research Area:** Genomics & Genetics

The heat stress response is a conserved stress defense mechanism found in all eukaryotic organisms. This response results in the induced expression of heat shock proteins, leading to stress acclimation. Heat shock transcription factors (HSFs) are the central regulators of the heat shock response and specifically bind to DNA sequences known as heat shock elements, located upstream of stress responsive genes. HSFs have a modular structure with a highly conserved N-terminal DNA binding domain characterized by a helix-turn-helix motif, a C-terminal activator domain, and a bipartite oligomerization domain. Trimerization of HSFs is required for high affinity DNA binding and transcriptional activation.

The heat response is attenuated by heat shock factor binding proteins (HSBP) that interacts with active trimeric HSFs and dissociates them into inactive monomeric units. Several studies showed that suppression of Arabidopsis HSBP improved plant tolerance to heat stress. However, this tolerance was strongly linked to seed abortion. Research in our laboratory showed that partial suppression of HSBP using antisense RNA could ameliorate stress tolerance during heat and dehydration stress without affecting seed development. This acquired tolerance was due, in part, to improved cell membrane stability during stress.

Therefore, we hypothesize that limiting the suppression of HSBP expression vegetative tissues can be used to generate stress tolerant plants while maintaining normal seed production. In this project, we propose to develop transgenic cotton lines in which HSBP amiRNA and HSBP RNAi constructs are expressed under control of a cotton leaf specific promoter derived from the GhrbcS gene. These transgenic plants will be characterized for developmental parameters, gene expression patterns, and physiological responses under controlled greenhouse and growth chamber environments.

In future work, these plants will be tested in the field to determine the effects of these transgenes on agronomic characteristics including plant development, and the yield and quality of both seed and fiber.

The UNIVERSITY of OKLAHOMA

The physiological basis of drought stress responsiveness of switchgrass genotypes with altered cell wall metabolism

PI: Dr. Heather McCarthy, University of Oklahoma, Norman **OCAST Project:** PS18-026 **Research Area:** Energy Crop Production

Switchgrass has garnered increasing interest as a potential biofuel source, due to its high productivity and relatively low water and nutrient needs. However, inefficient biochemical conversion of biomass into biofuels remains a barrier for widespread biofuel production from plant materials. Conversion efficiency is heavily influenced by the composition and structure of the plant cell walls, especially lignin content. Thus, researchers have genetically engineered switchgrass genotypes with lower/altered lignin content that are more easily digestible.

At the same time, these changes may have unintended consequences for plant performance, particularly hydraulic function, as there is generally a positive correlation between tissue lignin content and drought resistance characteristics. However, it is unclear whether concomitant alterations in other cellular properties or changes in whole plant hydraulic architecture could compensate for the effects of reduced or altered lignin. If switchgrass is to become a viable biofuel source, it is critical to understand how alterations to cell wall characteristics intended to improve conversion efficiency may weaken the plant's ability to remain alive and productive under dry conditions.

The objective of this research is to determine how alteration of cell wall lignin content and composition may impact the physiological response of switchgrass to water restrictions. Specially, we will use molecular, anatomical and physiological approaches to: 1) screen existing lignin mutant switchgrass genotypes for greatest sensitivity to drought stress and 2) assess genotype specific physiological and biomass production response to drought. The proposed work will advance basic understanding of the functional consequences of altering grass cell wall properties, which may be applied to future efforts to develop viable switchgrass genotypes for biofuel use.

In the long term, this project will lay the groundwork for further collaboration between the PIs on the linkages between structure and function in switchgrass under stress conditions.

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Preliminary study of genetic diversity in Grindelia ciliata, a promising biofuel crop native to Oklahoma

PI: Dr. Abigail Moore, University of Oklahoma, Norman **OCAST Project:** PS18-027 **Research Area:** Genomics & Genetics

How do species change genetically in response to drought? Oklahoma is in the center of a strong east-west precipitation gradient, corresponding to the transition between eastern deciduous forest in the east to tallgrass and mixed grass prairie to shortgrass prairie in the west. Given that precipitation in this area is more uniform north to south, there is a broad front across which adaptation can occur, which could potentially allow locally adapted genes to be shared between populations.

I propose to examine the evolution of drought tolerance using Grindelia ciliata, Spanish Gold, in the Asteraceae or sunflower family. This widespread annual plant has a distribution that is centered in Oklahoma, and extends into Kansas, Texas, and New Mexico. Its wide distribution across Oklahoma will allow us to use the highly accurate precipitation and soil moisture data from the Oklahoma Mesonet for our study, making it an ideal region and plant to study drought responses.

Grindelia ciliata is a species of interest for development as a biofuel crop. Unlike most biofuel crops today, which are grown for the production of ethanol or biodiesel, the genus Grindelia has a very high content of diterpene acids (7–15% by weight in wild plants, Adams et al. 2016), which are a form of bio-crude. Although it is not currently commercially grown, G. ciliata has a particularly high promise as a biofuel crop for Oklahoma as it already grows naturally all across the drier two thirds of the state without supplementary water, in addition to having a very high bio-crude content.

Greater knowledge of the regions of the genome involved in drought tolerance in G. ciliata will be extremely valuable in selecting improved genotypes for a breeding program, as well as pinpointing important loci for increasing drought tolerance in these breeding lines. In addition, although directly examining diterpene content is beyond the scope of this study, we will look at the genetic variation present in or near genes in the terpene synthesis pathway. This will provide us with valuable preliminary data to support a larger collaborative proposal that will integrate genetic, biochemical, and growth data to select the best genotypes for diterpene production to begin preliminary crop development.

This study will be facilitated by the genetic resources that are being developed for Grindelia ciliata. Its genome is currently being sequenced by Dovetail Genomics, a leading commercial genome sequencer. I propose to use the RADseq method to sequence many small loci from throughout the genome. Having a sequenced genome on which to place the loci allows us to examine the way patterns of genetic diversity and selection change across the genome, in addition to allowing us to pinpoint which genes could be under selection, even when the loci we sequence are merely near, instead of in, those genes.

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Unraveling genes underlying dual-purpose wheat seedling drought and heat tolerance using automated phenotyping platforms

PI: Dr. Xuefeng Ma, Noble Research Institute LLC, Ardmore **OCAST Project:** PS18-028 **Research Area:** Genomics & Genetics

Drought and heat stresses during the seedling stage are the most common abiotic factors affecting winter wheat in the Southern Plains of the U.S. where wheat is often seeded early for cool-season grazing until February and then for grain, known as dual-purpose. Generally in Oklahoma and Texas, wheat grown for animal grazing needs to be planted at least 2-3 weeks earlier than wheat planted for grain production only, allowing for good fall forage production. However, early planting often coincides with drought and heat stresses that may affect seed germination, seedling growth and development, eventually resulting in reduced forage and grain yield. Therefore, breeding for seedling drought and heat tolerance in wheat is crucial for early planting to secure cool-season forage production.

Over the years, phenotyping wheat seedling drought and heat tolerance under field conditions has been challenging because of the complexity of the traits and environmental interactions. Currently, phenotyping these traits is done manually using hand-held meters which is very tedious, time-consuming and not amenable to large scale phenotyping. In this regard, a more efficient and high throughput phenotyping platform is needed. Therefore, the goal of this project is to use an automated imaging phenotyping system to capture plant traits (e.g chlorophyll fluorescence) displayed by seedlings subjected to drought and heat stresses; and to improve breeding efficiency using marker-assisted selection.

To achieve these objectives, three recombinant inbred line (RIL) mapping populations, which have already been genotyped, will be evaluated for seedling drought and heat tolerance in controlled environments (growth chamber and greenhouse) using both automated imaging phenotyping platform and manual methods. The effectiveness of the automated imaging phenotyping will be estimated and optimized by correlating to manual phenotyping data. Genome-wide association studies will be conducted to discover QTL associated with seedling drought and heat tolerance.

Specially, the project will aim: (1) to assess the potential of using an automated imaging phenotyping platform to assess seedling drought and heat tolerance; (2) to map QTL and identify SNP markers for marker-assisted selection (MAS) of seedling drought and heat tolerance during wheat breeding and (3) to identify stress tolerant genotypes for breeding integration.