Online Proceedings

POSTER SESSION 2

MGH 241, Easel 163

1:00 PM to 2:30 PM

Identification of Abiotic Stress Responsive Genes in *Zea mays* (Maize) Dependent on MOP1-mediated Epigenetic Regulation and the Plant Hormone ABA

Rachel Christine Calder, Senior, Biology (Bothell Campus)
Mary Gates Scholar
Mentor: Thelma Madzima, STEM - Biological Sciences
Mentor: Jesse Zaneveld, STEM, Division of Biological Sciences, University of Washington Bothell

Zea mays (maize, corn) is an essential crop plant; important to global agriculture and the U.S. economy. However, maize productivity and yield can be drastically affected by abiotic environmental stress. Therefore, a priority for many plant breeding programs is to select for crops displaying phenotypic traits of enhanced tolerance to abiotic stress. A subset of abiotic stresses induce the plant hormone, abscisic acid (ABA). The mediator of paramutation1 (mop1) gene encodes an RNA-dependent RNA polymerase that functions in the RNA-directed DNA methylation (RdDM) pathway. The mop1-1 mutation results in the loss of DNA methylation which in turn causes a variety of genes to be expressed abnormally. We determined how a mutation in a mop1-1 affects RNA expression under abiotic stress by conducting a computational analysis of multiple RNA-seq datasets of stress-treated maize seedlings. We compared RNA-seq data from mop1-1 and WT seedlings treated with exogenous ABA control (no ABA treatment) with a publicly available dataset of WT maize plants treated with heat, cold, drought, salinity, and control (no stress treatment). Genes commonly down-regulated in the four stresses and in MOP1 WT ABA, but up-regulated in mop1-1 ABA represent genes potentially silenced under stress that require MOP1 for gene silencing. The presence of these genes in the given stress treatment allows us to identify the abiotic stress responsive genes that require ABA and MOP1 mediated regulation.

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The Role of the RMR1 Protein in Phenotypic and Transcriptional Responses to Abscisic Acid

Kaheerman (Sabira) Saibire, Senior, Biology (Bothell Campus)
Mary Gates Scholar
Mentor: Thelma Madzima, STEM - Biological Sciences

RNA-directed DNA methylation (RdDM) is an epigenetic pathway that induces transcriptional gene silencing through DNA methylation of target genes. The Zea mays (maize) RMR1 protein has an important role in the RdDM pathway as it functions to assist the process of making double stranded RNAs, required for progression of the pathway. Abscisic acid (ABA) is a plant hormone that accumulates under abiotic stress, and can inhibit seed germination. We determine if the RMR1 protein is involved in epigenetic regulation under abiotic stress by measuring maize phenotype and identifying transcriptional changes in ABA treated (vs. control) rmr1-1 mutants. To determine the effect of the rmr1-1 mutation on maize phenotype, we are measuring shoot length, primary root length and additional roots in maize germinating seeds from a segregating family. The genotype of the individual seeds is determined by amplifying the region of the rmr1 gene that includes the point mutation (C to T) that is on the 4th exon of the gene, followed by Sanger sequencing. The genotype is then associated with the measured phenotype. We are also interested in determining if ABA-induced transcriptional factors are differently expressed in rmr1 mutant compared to wildtype under ABA, and this will be measured by quantitative reverse transcription and PRC(qRT-PCR). This research is in progress and results will be presented. Maize and other crop plants are often negatively affected by abiotic stress, therefore, understanding how epigenetic pathways are involved in these responses is important to agricultural productivity.

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1:00 PM to 2:30 PM

Epigenetic Regulation of the Maize BTF3 Transcription Factor under Drought Stress

Kiana Imani, Senior, Biology (Bothell Campus)
Mentor: Thelma Madzima, STEM - Biological Sciences

The *Zea mays* (maize) crop is essential to global agriculture

and food security, and is also vital to the US economy. Like many crop plants, maize is often subjected to extreme environmental stresses, such as drought, extreme cold and high salinity, that negatively impact crop yield. Therefore, it is important to characterize the molecular (epigenetic) changes that occur to a gene under environmental stress conditions in order to manipulate these responses to improve crop yield under stress. We are interested in characterizing the epigenetic regulation of stress-responsive transcription factors under normal (control) and abiotic stress (drought) conditions in maize seedlings. Using bisulfite conversion of genomic DNA, we previously identified that the *Zea mays* basic transcription factor 3 (ZmBTF3) gene displays some variations in methylation patterns in parts of the gene promoter between the two treatments. To confirm these results, we used an alternative method: a methylation sensitive restriction digest technique, which uses methylation sensitive restriction enzymes (MSREs) that cut DNA molecules at precise locations and are sensitive to DNA methylation. Our results show some treatment (control vs. drought) and restriction enzyme (sequence/context) specific methylation patterns. Using these two independent techniques, we identify stress-responsive epigenetic variations (DNA methylation) in the promoter region of the ZmBTF3 gene, previously uncharacterized in maize. Ongoing research includes (i) characterizing the sequence contexts of these methylation patterns between the two treatments, (ii) determining if the observed DNA methylation changes correlate with transcription of the ZmBTF3 gene, and (iii) characterizing the drought response phenotype in maize plants with a mutation in the ZmBTF3 gene. This study will help us gain a better understanding of how the BTF3 gene in maize is regulated through epigenetic modifications.

POSTER SESSION 2

MGH 206, Easel 172

1:00 PM to 2:30 PM

Phenotypic Plasticity and Transgenerational Epigenetic Modification of *Arabidopsis thaliana* in Response to Variable Conditions Predicted with Climate Change

Jackelyn Tolentino Garcia, Senior, Biology (Bothell Campus)
Mary Gates Scholar
Mentor: Cynthia Chang, Biology
Mentor: Thelma Madzima, STEM - Biological Sciences

With our rapidly changing climate, plant communities are predicted to experience more variable conditions. Climate change predicts that there will be longer periods of drought followed by heavy rainfall at unpredictable intervals. With this, plants may experience selective pressures to combat the stress that come with these variable conditions, which ultimately may influence plant evolution. Phenotypic plasticity and epigenetic modification are two factors we believe may strongly influence the adaptative ability of plants. I predict that these variable conditions will promote phenotypic plasticity and epigenetic "memory" (where plants' stressful experience can be inherited from parent to offspring), and potentially help offspring plants cope with the same abiotic stress. We have implemented an experimental design mimicking the predicted variable conditions of climate change. For two generations, we exposed plants to either drought or non-drought conditions. Then in the third generation, we took seeds from parents that experienced those conditions and exposed them to either drought, non-drought, or variable watering in a cross-replicated design. I hypothesize that there is inherited epigenetic memory, and that the different treatment groups will pass memory that will benefits plants if the offspring was exposed to the same condition its parent experienced.

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1:00 PM to 2:30 PM

Chromosomal Mapping of the MOP1-FLAG Transgene in *Zea mays* (Maize)

Ginger L. Lash, Senior, Biology (Bothell Campus)
Mentor: Thelma Madzima, STEM - Biological Sciences

In *Zea mays* (maize), MEDIATOR OF PARAMUTATION 1 (MOP1) is involved in paramutation and epigenetic regulation of gene expression. MOP1 in an RNA-dependent RNA polymerase that functions as part of the RNA-directed DNA methylation (RdDM) pathway, by producing double-stranded RNA molecules that are further processed into small interfering RNAs (siRNAs). These siRNAs are able to trigger DNA methylation which can decrease or silence gene expression. The MOP1-FLAG transgene was created by overexpressing the mop1 gene with the FLAG epitope tag. As a consequence of using agrobacterium-mediated transformation, the chromosomal location of the MOP1-FLAG transgene is unknown. To identify the sequences flanking the transgene, we used an adapter ligation-mediated PCR and cloning technique, as described by O'Malley. To do so, we first digested the MOP1-FLAG DNA with restriction enzymes designed to cut the DNA in specific locations along the genome. We then ligated adapters onto the DNA fragments. Through PCR using gene-specific and adapter-specific primers, we were then able to amplify the fragments that contain MOP1-FLAG and the surrounding genomic sequence, clone and then sequence them. We used the recovered flanking sequence to BLAST search the maize genome in order to identify the maize chromosome that the MOP1-FLAG transgene is on. Chromosomal mapping of the MOP1-FLAG transgene is useful to distinguish between the endogenous mop1 gene in ongoing complementation and chromatin immunoprecipitation (ChIP) experiments.