The effects of sulfur and phosphorous deficiency on 4 types of micrornas essay

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Arabidopsis thaliana is found in the mustard family and is a weed used as a model organism to aid in research. Over the years, research has taken off due to this and other model organisms for many reasons. A short generation time is ideal so that experiments can be conducted in a timely manner. Arabidopsis thaliana only takes about six weeks to grow and is structurally tiny so that space in the lab is conserved. This plant also has a very small genome, the smallest known in plants, and only five pairs of chromosomes. Researchers can easily locate genes if there are only five to choose from.

In turn, it is easier to map out every biological pathway to understand its function in the plant through transformation with foreign DNA. Altering a plant with foreign DNA leads to mutations which can provide information on how genes function in plants (Reece, Urry, Cain, Wasserman, Minorsky, & Jackson, 2011). Three different types of media were used in this experiment to grow the model organism, including; sulfur deficient media, phosphorous deficient media, and full media. Sulfur and phosphorous are macronutrients. Macronutrients are essential elements that plants require in relatively large amounts.

For this reason, growth of the plant will be altered when media is lacking either of these two important nutrients. Phosphorous is a component of nucleic acids, phospholipids, ATP, and several coenzymes and will affect cell number and cell size (Reece, Urry, Cain, Wasserman, Minorsky, & Jackson, 2011). Sulfur is a component of proteins and coenzymes and is usually taken up by the plant, with the help of bacteria and fungi, in the form of sulfate from the soil or hydrogen sulfide gases from the atmosphere (Buhtz, Pieritz, Springer, & Kehr, 2010).

Sulfur deficiency will cause a decreased yield and decreased quality of growth. This occurs because normal light is seen as a light stress because there is a limited energy supply and an extinction of photo-energetic process (Hawkesford, 2000). Full media was used as a control group because plants had all required nutrients for proper growth. The goal of this experiment is to examine how deficient media affects gene expression levels in four different types of microRNAs. MicroRNAs (miRNA), approximately 21 nucleotides in length, target protein-coding mRNAs.

MiRNAs play an important post-transcriptional role in plant development as well as respond to nutrient deficiencies and changes in growth. A young miRNA is the hairpin part of a much longer RNA. There is a double-stranded portion of this hairpin where the mature miRNA is derived from. Also a part of the mature miRNA is the miRNA correspondence; together they make up the larger precursor that dicer-like components will free from the nucleus. This forms the sRNA that is loaded into the RNA-induced silencing complex (RISC).

Here, the RISC recognizes mRNAs with complementary sequences and cleavage takes place here in plants, an important component in the process. The process of identifying miRNA and their target mRNAs has been refined over the years using computational methods. These methods are ideal for finding miRNAs in plants because miRNAs and mRNAs are more complementary as compared to animals (Jones-Rhoades & Bartel, 2004). The following include the four types of miRNAs that were examined in this experiment: miR156, miR395, miR399, and miR398.

MiR395 regulates mRNA associated with sulfate absorption (Hawkesford, 2000). This means that this microRNA's expression level would be affected in the sulfur deficient media. MiR399 is thought to respond in situations of phosphate stress (Lambers & Poot, 2003). MiR398 is also a nutrient stress responder when copper in the soil is greatly reduced(Sunkar, 2010). MiR156 plays a role in vegetative development such as flower production(Wu & Poethig, 2006). The method for examining these microRNAs and their gene expression levels uses qRT-PCR (quantitative real-time polymerase chain reaction).

RNA degrades easily postmortem or with improper handling(Fleige & Pfaffl, 2006). In this method, the RNA must be extracted from the plant. The best way to do this is to break up the plant into mush to release the RNA from the plant cells. RNA is single-stranded and therefore more unstable as compared to DNA. Therefore, reverse transcription must take place so that complementary DNA (cDNA) can be formed for the next part in this method. In reverse transcription, an enzyme called reverse transcriptase is necessary to catalyze the reaction. The polymerase chain reaction amplifies the cDNA.

Items needed for this to take place are primers to serve as attachment sites for synthesis, DNA polymerase to synthesize the strands, fluorescent dye, and free nucleotides. High heat allows for the DNA to be unzipped so that two strands can be formed followed by a cooling cycle to make those two strands into complete copies of DNA. This continues so that amplification of the DNA is exponential. The dye mentioned above acts as an indicator that when plotted on a graph versus time reveals the Ct value. Ct values are

important in calculations to figure out levels of gene expression (M. Axtell, 2012).

The goal of this experiment is to test how nutrient stress affects microRNA expression in four different types of microRNAs. This information is important because it provides an understanding of gene expression that can be applied to other situations. For example, if the model organism shows that certain microRNAs respond to nutrient stress, then maybe the knowledge of this can allow trees to grow better if nutrient stress were a factor. We plated grew Arabidopsis thaliana plants on three types of media to extract RNA for synthesis using qRT-PCR. I predict that miR395 will increase its expression level on the sulfur lacking media.

I reached this hypothesis by researching the miR395 family and finding that it responds in sulfur deficient media (Jones-Rhoades & Bartel, 2004). Materials/Methods Approximately 25 Arabidopsis thaliana seeds were plated on three different types of media that include the following: sulfur deficient media, phosphorus deficient media, and full media. Growth of these plants took approximately three weeks. RNA extraction was carried out. To extract RNA the miRNeasy Mini Kit from Qiagen was used. Once the RNA was extracted from the plants, pulsed reverse transcription took place.

This was done using the iScript Advanced cDNA Synthesis Kit for qRT-PCR from Bio-Rad. qRT-PCR was run using a 96-well plate shared by the class. The samples plated were made using the miScript SYBR Green PCR Kit from Qiagen. Analysis of the data from qRT-PCR uses a few equations. The first equation was used to find the difference between the control Ct value and

the experimental Ct value. ? Ct= Ctcontrol-Ctexperimental(Equation 1: This shows the equation used to calculate change in Ct) The next equation was used to calculate the efficiency that can be plugged into the equation to find the relative abundance of a sample.

First, a plot of Ct versus the log10 dilution was made so that slope was found. The following equation gave efficiency: E= 10(-1S) (Equation 2). Then, normalized relative abundance was calculated using this equation: RAn= Etarget? Ct target/(Ereference)? Ct reference (Equation 3). The normalized relative abundance value is the abundance of microRNA in the nutrient stress media relative to the full media. By using a normalized relative value, it allows for error to be eliminated and shows how the microRNA differs in situations of stress as compared to no stress. Results

I examined miR399 in low sulfur conditions. My Ct values for the no template control reaction was not undetermined or above 36. Instead, they were 9. 7 and 32. 7. This showed contamination in these specific wells. The reason the wells should be undetermined or above 36 is because there is no DNA, it is only water. Therefore, fluorescence will never reach the set threshold and be considered undetermined or it will take over 36 cycles to reach the threshold meaning that there was a minute amount of DNA in the well. If values show fluorescence, then there was contamination in the well.

The number 9. 7 means that it only took 9. 7 cycles to reach the threshold; this is a high amount of contamination. The value 32. 7 shows a small amount of DNA, but still enough to signify contamination. The RA normalized value for miR399 was 0. 232843211. Figure 1 shows the changes in the

accumulation of microRNA in the Arabidopsis thaliana seedlings according to each type of nutrient-deficient media in comparison to full media. So, miR156 is one or close to one in both deficient media types which shows that the expression level did not change in comparison to full media.

MiR395 showed a drastic change in expression level for both media types. Low phosphate had a decrease in expression level while low sulfur increased expression level 1000 fold. MiR398 showed a decrease in expression level in both media types. MiR399 showed an increase in expression level in the low phosphate media while expression level stayed the same in the low sulfur media. Figure 1: This chart shows normalized relative abundance values according to each miRNA type on two types of media. Figure 1: This chart shows normalized relative abundance values according to each miRNA type on two types of media.

Section 18 collected data to show the levels of miRNA on different types of media. MiR156 remained the same compared to Figure 1 on the low phosphorous medium, while expression increased 10 fold on the low sulfur medium (Figures 1&2) MiR395 remained fairly constant on the low phosphorous medium which differs from the control data (Figures 1&2). The expression level increased by almost 10 million fold on the low sulfur medium as compared to the control data which only increased by 1000 fold (Figures 1&2). Again, miR398 differs from the control data on both types of media (Figures 1&2).

Low phosphorous shows no expression level changes while low sulfur increases by almost 1000 fold (Figure 2). MiR399 increases by 10 fold on low

phosphorous medium and decreases on low sulfur medium (Figure 2). Figure 2: Section 18 expression levels according to media type. Figure 2: Section 18 expression levels according to media type. Normalized relative abundance values for microRNAs according to the entire Bio 240W class can be seen in Figure 3. Like the other two experiments show, miR156 remains the same with no change in expression level in low phosphorus medium.

However, miR156 in the low sulfur medium also does not change, differing from the section 18 data (Figures 2&3). MiR395 remains the same in the low phosphorous medium while increasing by 1000 fold in the low sulfate medium (Figure 3). Increasing by 1000 fold in low sulfur matches the control data, but differs from section 18 (Figures 1, 2, 3). Remaining the same in the low phosphorous medium is only different from the control data which decreases (Figures 1&3). MiR398 doesn't change in the low phosphorous medium which is similar to section 18 data (Figures 2&3).

There is a significant decrease in the low sulfur medium which counters the almost 1000 fold increase in the section 18 data (Figures 2&3). MiR399 is consistent in all three experiments, increasing by 10 fold in the low phosphorous medium (Figures 1, 2, 3). The low sulfur medium shows no change in expression level which matches the control data, but differs from the decrease in expression found in the section 18 data (Figures 1, 2, 3). Figure 3: Bio 240W expression levels of different microRNAs according to media type.

Figure 3: Bio 240W expression levels of different microRNAs according to media type. Discussion The data collected above showed a few major trends

across all three data sets. These include the following: miR156 remained fairly constant with little to no changes in expression for both media types, miR399 up-regulated under phosphorous stress and did not change expression level under sulfur stress, and miR395 was significantly up-regulated in low sulfur conditions. When microRNA is up-regulated, the opposite occurs for the mRNA that the microRNA is targeting.

For example, a process in metabolism for a plant that requires much energy can take place when all nutrients are available with no problems. However, under nutrient stress the plant must conserve energy in order to stay alive. So, microRNAs come into play by degrading the mRNA that makes the proteins for the unnecessary process. Understanding this, the up-regulation or down-regulation of certain miRNAs can give clues as to what pathways they regulate. An interesting trend is that miR156 remains fairly constant with no changes in expression under both stresses.

This implies that the pathway that miR156 controls is not concerned with plant metabolism and nutrition. Reading literature on the subject, it mentioned that miR156 deals with the phase change from juvenile to adult and flowering that occurs with that change. MiR156 is down regulated as the plant grows while its target mRNA SPL3 is up-regulated (Wu & Poethig, 2006). This was supported in a review that stated that miR156 is associated with development and not included in the group that is poplar-specific and stress induced (Sunkar, Chinnusamy, Zhu, & Zhu, 2007).

To examine this further, an experiment comparing a plant at different stages in life could be analyzed for the ration of miR156 and SPL3. The up-

regulation of miR399 under phosphorous stress implies that this microRNA needs to down regulate a target mRNA so the plant can survive. These specific target genes are referred to as At3g54700 (Phosphate transporter) and At2g33770 (Ubiquitin conjugating enzyme-E2, UBC24) (Sunkar et al. , 2007). Low P causes changes in root architecture from a single long root in high P conditions to more fibrous roots. This occurs because the lant is " searching" for more phosphorous so it needs a larger surface area (book). There is an inverse relationship between miR399 and UBC because the down regulation of UBC allows for the changes in root architecture to be made for conditions with low phosphorous (Sunkar et al. , 2007). Finally, miR395 is a major player in regulation under sulfate stress. This microRNA targets three sulfate assimilation enzymes: APS1, APS3, and APS4. These enzymes are catalysts for the absorption of sulfate by the roots. When these enzymes are down regulated, miR395 is up-regulated to maintain sulfur homeostasis in the plant.

Another target is the sulfate transporter that transports sulfate from roots to the shoots, AST68 (Sunkar et al. , 2007). This validates my hypothesis that miR395 is up-regulated under sulfate stress. There are many possible sources of error with this experiment. Pipetting is a difficult art. If the proper amounts of substance are not pipetted, the Ct values can be incorrect. If too much DNA is pipetted, the Ct value will be too small because it will take less cycles for the DNA to fluoresce. Any calculations after Ct value calculations will be incorrect; therefore, the normalized relative abundance values will be skewed.

This could account for Section 18 having such a huge spike, 10 million fold, in miR395 under sulfate stress as compared to only increasing 1000 fold in the control data (Figures 1&2). Another source of error is cross contamination. The Ct values for the no template control should be undetermined or high. In some situations, the Ct value for miR399 in low sulfur had no template control values that indicate contamination. Another source of error could be from not having a good enough RNA yield in the very first step of mashing up the plant tissue.

Enough RNA is needed in order to do reverse transcriptase to form cDNA for qRT-PCR. Understanding genetic pathways in a model organism could provide insight to genetic pathways in other organisms. If a certain pathway for growth was similar in the model organism and another, then growth could be optimized for different environments. MicroRNAs and their involvement in nutrient stress could provide researchers and scientists with cures to diseases that involve lack of nutrients as well as a better understanding of the intricacies of molecular genetics. (Reece, Urry, Cain, Wasserman, Minorsky, & Jackson, 2011)