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Effect of DMN on Genes Associated with Ethylene Signaling and Metabolism in *Solanum tuberosum*

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ABSTRACT

Solanum tuberosum, more commonly known as the potato plant, is the fifth most valuable agricultural crop worldwide. Following harvest, potato tubers can be consumed immediately, but most are stored for later consumption or processing. To maintain the integrity of the crop, chemical inhibitors are often utilized to keep the tubers from sprouting while they are in storage. Recently, in Europe, the main sprout suppressant, CIPC has been banned due to health concerns. Therefore, alternate methods of sprout control, such as ethylene gas and the growth inhibitor 1,4-dimethylnapthalene (DMN), are used in Europe as the primary method maintain tuber quality in storage. How DMN affects the genetic expression of a potato tuber to prevent sprout growth is not yet understood; however, this research suggests that it may affect the ethylene signaling and ethylene biosynthesis pathways. This research uses data from a series of experiments that applied RNA-Seq to examine differential gene expression in tubers treated with DMN. Emphasis was placed on examining transcripts that were involved with ethylene signaling and metabolism.

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LIST OF ABBREVIATIONS

ACC	1-aminocyclopropane-1-carboxylic acid
ACO	ACC Oxidase
ACS	ACC Synthase
AtID	Gene ID for Arabidopsis Thaliana
CTR	Constitutive triple response
DMN	1,4- dimethylnapthalene
EBF	EIN3-binding F-box
EIN	Ethylene insensitive
EIL	EIN3-like
ENAP	EIN2 Nuclear Associated Protein
ERS	Ethylene response sensor
ETR	Ethylene response
SAM	S-adenosyl-L-methionine
StID	Gene ID for *Solanum tuberosum*
T0	"Time Point 0" These samples were removed after two days of treatment and allowed no recovery time.
T14	"Time Point 14" These samples were removed from treatment and allowed 14 days of recovery time

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Chapter 1

Introduction

1.1 Gene Expression

During transcription, RNA copies a DNA strand, then the RNA leaves the cell to create proteins. To Identify the function of each cell and recognize how an organism responds to its environment, it is important to understand which proteins are created and when. Gene expression is defined by Neale and Wheeler as "The amount of mRNA transcribed from an individual gene at any particular time point or within any tissue type" (2019). Some genes are constantly expressed, and others are only expressed during certain periods in an organism's life, such as during growth or when under stress (Brody, 2023). Gene expression can be measured through time, by quantifying the amount of RNA at different time points, to determine when a gene of interest is up- or down-regulated (Segundo-Val & Sanz-Lozano, 2016). When a gene's expression is up-regulated that means its activity is increased, and when it is down-regulated its activity is decreased.

There are different ways to study gene expression, but the method of interest is RNA Sequencing. RNA-Seq uses massive parallel sequencing to measure gene transcription levels in a sample. This method was developed in the mid-2000s and was first used to analyze LNCaP, a prostate cancer cell line (Bainbridge et al., 2006). Then in 2007 it was used to study maize and Arabidopsis thaliana (Emrich et al., 2007) (Weber et al., 2007). RNA Sequencing is still used today because it can measure targeted gene expression levels much faster than other methods. There are many advantages such as high accuracy and high throughput. It also can detect very small changes in gene expression due to a lack of background noise, and a reference genome is not necessary so it can be used to analyze the genomes of novel species (Segundo-Val & Sanz-Lozano, 2016). For these reasons, this research uses RNA Sequencing to analyze the expression of genes in the ethylene signaling and biosynthesis pathways of *Solanum tuberosum*.

1.2 *Solanum Tuberosum*

Solanum tuberosum, informally known as the potato plant, is grown in 130 countries. As of 2016, it had a total crop value of 63.3 billion U.S. dollars (Dolničar, 2021). In fact, the potato crop is the fourth most significant crop on the planet, and can be grown in a variety of geographic locations (Dolničar, 2021). In storage, crop loss is common due to early sprouting or disease. As a potato sprouts, its rate of respiration increases, causing a decrease in mass and an increase in sugar concentration; therefore, the nutritional and commercial value of the tuber decreases after sprouting (Vijay et al., 2018). It is necessary to prevent sprouting to preserve the integrity of the tubers and minimize profit losses for farmers. Sprout suppression is achieved by lengthening the amount of time a potato is in an endo-dormant state or by using growth suppressors (Campbell et al., 2010). The physiological mechanism behind sprout inhibitors is not yet fully understood. As explained later in chapter one, ethylene is an important dormancy regulator; therefore, this study aims to determine the effect of the sprout inhibitor 1,4- dimethylnapthalene (DMN) on the ethylene signaling and metabolism. To understand the basis for this research, the existing research on dormancy, sprout suppressants, ethylene, and ethylene response factors must first be understood.

1.3 Dormancy

The viability of a potato crop is dependent on the state of dormancy the tuber occupies postharvest (Lang et al., 1987). Although potatoes experience all three stages of dormancy, endo, para, and ecodormancy, this study focuses on endo and ecodormancy specifically. How long a tuber is dormant depends on the genetic background of the tuber as well as external factors. During endodormancy, sprout growth is inhibited largely by internal factors that are not fully understood (Lang et al., 1987). External conditions only have a small effect on sprouting during this stage. As a tuber transitions into ecodormancy, sprouting is influenced by environmental factors. In this stage, improper storage conditions can heavily influence sprouting (Lang et al., 1987). What is responsible for a tuber's transition from endo

to ecodormancy is not yet understood; therefore, examination of changes in gene expression between dormancy stages may help shed light upon this mechanism.

1.4 Sprout Suppressants

In the United States, sprout suppression is usually achieved via a chemical called chlorpropham (CIPC), which is a sprout suppressant that works by interfering with photosynthesis, protein synthesis, RNA synthesis, and respiration (Vijay et al., 2018). This chemical also inhibits cell division, so CIPC-treated tubers cannot be used for planting. CIPC is very effective in different storage conditions, which explains its widescale use. However, this chemical has been banned in the EU for health and environmental concerns (Epp, 2021). Despite these concerns associated with CIPC, farmers in other countries continue to use the chemical because no other sprout suppressant comes close to the efficacy of CIPC (Vijay et al., 2018). Farmers in the United States are beginning to worry the ban in Europe could mean social pushes for CIPC-free potatoes in the United States (Epp, 2021). DMN and ethylene gas are two alternatives European farmers are turning to. Studies are in progress to understand how these alternatives function. The goal of some of these studies is to find an effective combination of sprout suppressants that can compare to CIPC without the ethical and environmental concerns.

1.4 The Current State of Research on DMN

Campbell et al. (2010) studied the influence of DMN and CIPC on gene expression and the level of abscisic acid (ABA) in meristems. The goal of this research was to determine how these sprout suppressants work to inhibit sprout growth. It is important to note that ABA and DMN are plant hormones, naturally found in potato tubers. His study found that as dormancy progressed, the expression of ABA declined. Expression of ABA in nondormant meristems and CIPC- and DMN- treated meristems were the same. This means that growth suppression from CIPC and DMN does not work by affecting ABA. They

also acknowledge that DMN and CIPC treatment does not extend dormancy, rather it inhibits sprout growth in some other way (Campbell et al., 2010).

1.5 Ethylene and Dormancy

Rylski et al. (1974) researched the effect of ethylene gas on tuber dormancy and sprout growth. They found that when tubers were exposed to ethylene, endodormancy was terminated and the plant was brought into ecodormancy. They also discovered when the tubers were treated with ethylene long term, they did not sprout, but when ethylene treatment was stopped, the tubers immediately began to sprout. They hypothesized this was because ethylene moves a tuber from endo to ecodormancy, and in ecodormancy sprouting is more likely to be influenced by environmental conditions. However, the physiological reasons for the immediate sprouting after stopping treatment was not yet clear (Rylski et al., 1974).

In an attempt to answer this question, researcher Jeffrey Suttle studied the function of endogenous ethylene in endodormancy regulation. His study found that endogenous ethylene does contribute to potato tuber endodormancy and that extracted potato buds produced ethylene during tuber development. When tubers were treated with competitive ethylene antagonist 2,5-norbornadiene, early sprouting occurred. He found that the rate of endogenous ethylene production was higher during the initial stages of endodormancy. Suttle ultimately concluded that endogenous ethylene was necessary to potato tuber endodormancy but its role may only be for the initial stages of endodormancy (Suttle, 1998). This research makes clear the link between ethylene and sprout suppression.

1.6 Ethylene Biosynthesis

Genes in the ethylene biosynthesis pathway were analyzed for their response to DMN. The genes chosen for this study are known to be in the ethylene biosynthesis pathway for *Solanum lycopersicum*, more commonly known as the tomato. This pathway is shown in Figure 1.

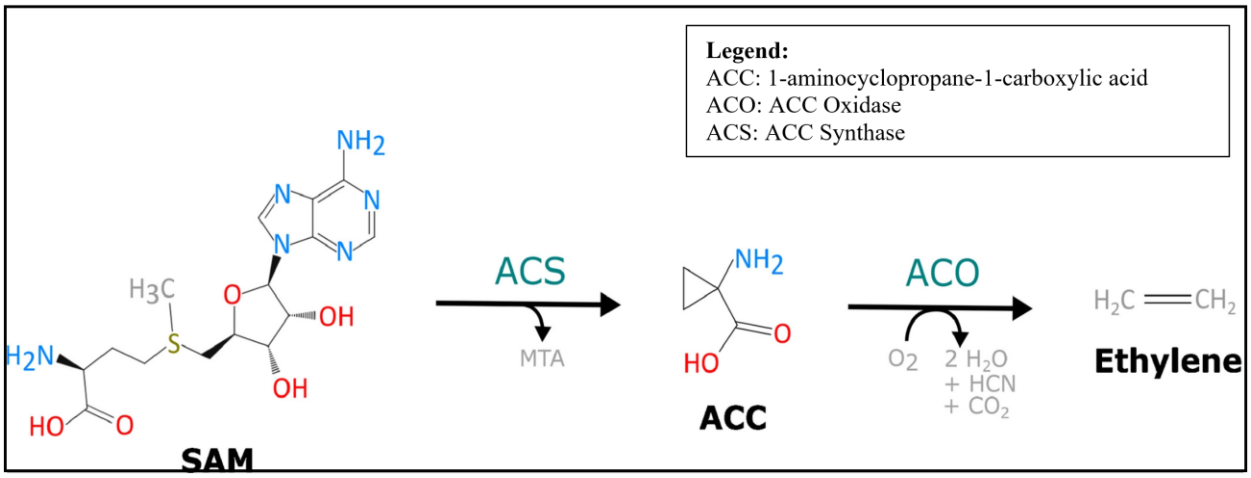

Figure 1. Ethylene Biosynthesis Pathway (Source: Pattyn et al., 2020)

The pathway in Figure 1 begins with ACC synthase (ACS), which are proteins responsible for creating 1-aminocyclopropane-1-carboxylic acid (ACC) from S-adenosyl-L-methionine (SAM). ACC is then turned into ethylene with the help of the enzyme ACC oxidase (ACO) (Pattyn et al., 2020). ACC, ACS and ACO genes were all identified in the data but only ACS and ACO presented significant responses to DMN. It is important to note that stresses such as wounds or temperature changes increase ethylene synthesis. ACO is also believed to be the rate limiting step in ethylene synthesis (Houben & Van de Poel, 2019).

1.7 Ethylene Signaling Pathway

Ethylene is a gaseous molecule that functions like a hormone. Plant growth, development and stress responses are all regulated via the ethylene signaling pathway. The ethylene from the biosynthesis pathway first binds to receptors on the endoplasmic reticulum membrane (Dubois et al., 2018). This is the first step of the ethylene signaling pathway as evidenced in Figure 2. In *Arabidopsis* there are five ethylene receptors including ethylene response 1 (ETR1), ethylene response sensor 1 (ERS1), ETR2, ERS2, and ethylene insensitive 4 (EIN4) (Binder, 2020). These receptors are shown in Figure 2.

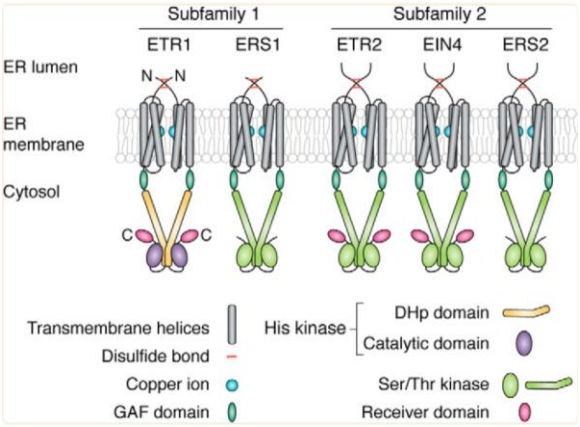

Figure 2. Diagram of Ethylene Receptors (Source: Binder, 2020).

Constitutive triple response 1 (CTR1) and EIN2 interact with ethylene receptors. When ethylene binds to a receptor, EIN2 breaks in two, activates EIN2 Nuclear Associated Protein 1 (ENAP1), which in turn increases the transcriptional activity of EIN3 and EIN3-like 1 (EIL1). These transcription factors then encourage ethylene responses (Binder, 2020). Conversely, CTR1 is a protein kinase that is a negative regulator of ethylene signaling (Zhong & Chang, 2012). When ethylene is not present, it is predicted that CTR1 is activated by the ethylene receptors. CTR1 phosphorylizes EIN2, then EIN2-targeting protein 1 (ETP1) and ETP2 ubiquitinate EIN2 which leads to the degradation of EIN2 (Binder, 2020). Because there is a low level of EIN2, EIN3-binding F-box 1 (EBF1) and EBF2 ubiquitinate the transcription factors, effectively inhibiting ethylene responses (Binder, 2020).

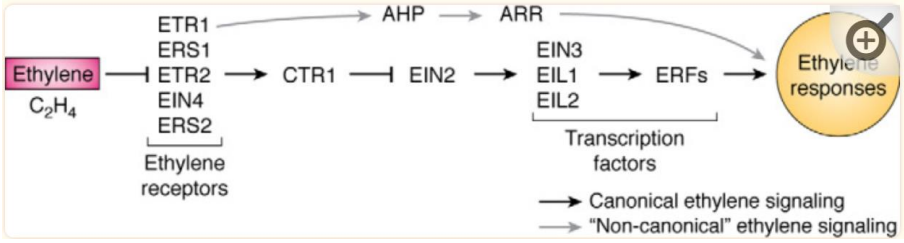

Figure 3. Ethylene Signaling Pathway (Source: Binder, 2020)

The genes from Figure 3 were found in the RNASeq data and analyzed for this research.

Chapter 2

Materials and Methods

2.1 Plant Material

Troyer Farms of Waterford, Pennsylvania (PA), USA, gifted Dr. Michael Campbell's lab field grown long dormancy (*Solanum tuberosum* cv Lamoka) potato tubers. The tubers were obtained in October 2021 from a farm in Girard, PA. None of the tubers were treated with sprout suppressants prior to or immediately following harvest. Because of the wet weather conditions in Girard, PA in October, all tubers were dried for two days, then stored at approximately 7°C until use.

2.2 Treatments

Treatments were conducted during endodormancy (December 2021) and early ecodormancy (March 2022). To identify the tubers' dormancy stage, multiple tubers were removed from cold storage and stored at 22°C. If peeping of tuber meristems was observed after one week in these conditions, dormancy was declared to be over (Campbell et al., 2010).

Before each treatment, tubers were washed and air dried. Next, the tubers were prepared following the methods of Campbell et al. (2020). One layer of tubers (approximately eight tubers) were positioned at the bottom of a 9.5 Liter BBL GasPak chamber (MG Scientific, Pleasant Prairie, WI, USA). Next, the tubers were either treated with DMN or water (control) at a rate of 22.5 µl per chamber. Six of these chambers were used which allowed three control replicates and three treated replicates per treatment. After two days of treatment in these chambers at 25°C, half of the tubers' meristems were immediately extracted; this group makes up timepoint 0 (T0). The other half were allowed to recover in open containers for two weeks at 7°C in 24-hour dark conditions. This second group makes up timepoint 14 (T14). To excise the meristems, a 1 mm micro cuvette was used, then they were quick frozen in liquid nitrogen. Meristems were preserved at -80° C.

2.3 Biological Analysis

Total RNA was extracted using a Zymo *Quick*-RNA Plant Kit (Orange, California, USA) according to kit instructions. Next, a Thermo Scientific NanoDrop One (Thermo Fisher Scientific, Waltham, MA, USA) was used for RNA quantification, then samples were sent to the Penn State University Nucleic Acid Core Facility for Illumina sequencing. A bioanalyzer quality assessment was used to determine which samples were selected for full Illumina sequencing. An average of 57,788,488 sequences were produced for the control treatment and an average of 60,617,950 sequence reads were produced for the DMN treatment.

Quality assessment was performed using FastQC with default settings. The quality of the reads did not require trimming. Reads were mapped to the S. tuberosum group Phureja DM1-3 v6.1 genome. The number of reads per gene were counted then the mapped files were analyzed using the DE-Seq2 tool, which scanned for all differentially expressed genes. The genes were filtered for an adjusted p value threshold of less than 0.05 and a log 2-fold change of greater than 1.

2.4 Process for Obtaining Relevant Genes

Because of the large quantity of differentially expressed genes identified in the data, not all of them could be analyzed. Therefore, it was important to identify which genes play a role in the ethylene signaling and biosynthesis pathways. To identify relevant genes, the protein sequences for each protein in Figures 2, 3 and 4 were found using keyword searches in UniProt (https://www.uniprot.org/blast). The amino acid sequences for each of these genes were then entered into SpudDB (http://spuddb.uga.edu/blast.shtml#aln1). A BLASTp search was performed using default settings. At most ten hits were selected for each gene with an arbitrary cutoff of E-05. The general name found on SpudDB was used as an additional classification for each gene. Each table in this paper contains a column

titled “AtID” which holds the generic name assigned to each gene, and another column titled “StID” which holds the specific gene names in the *Solanum tuberosum* genome.

Chapter 3

Results

3.1 Ethylene Biosynthesis Genes

All of the data in this section compares the treated group to the control group during ecodormancy. Because tubers are less responsive to environmental conditions during endodormancy (Lang et al., 1987), very few significant changes were recognized in endodormancy when comparing DMN-treated samples directly to control samples. Two timepoints, one occurring immediately after ecodormant tubers were removed from treatment (T0) and the second occurring two weeks after ecodormant tubers were removed from treatment (T14), are shown.

Four of the identified ACS genes are affected directly by DMN. As shown in Table 1, in ecodormancy, two genes are up regulated in the treated group as compared to the control at T14. Conversely, one gene is downregulated when comparing DMN to the control at T14 and another is downregulated at T0. The downregulation of Soltu.DM.04G032120.1 did not last longer than two weeks. At T14 this gene’s expression returned to the same level as seen in the control.

Table 1. Changes in ACS gene regulation induced by DMN in ecodormant tubers.

AtID	StID	Up	Down
ACS2	Soltu.DM.01G034180.1	T14	
ACSX	Soltu.DM.08G028300.1		T14
ACS4	Soltu.DM.04G032120.1		T0
ACS3	Soltu.DM.02G027270.1	T14	

ACC is converted into ethylene by ACC oxidase (ACO). Three of the five identified ACO4 genes had a significant change in expression from the control group to the treated group. As shown in Table 2,

two genes were upregulated in the treated group compared to the control group at T0, but after two weeks, expression returned to the same levels as in the control. One gene initially showed no change between the control and treated groups, but after two weeks the gene was upregulated when treated with DMN.

Table 2. Changes in Ethylene Formation Enzyme (ACO4) regulation induced by DMN in ecodormant tubers

AtID	StID	Up	Down
ACO1	Soltu.DM.07G016750.1	T0	
ACO4	Soltu.DM.07G016780.1	T14	
ACO2	Soltu.DM.12G023340.1	T0	

3.2 Normal Expression of Ethylene Signaling Pathway Genes through Dormancy States

To study the effect of a sprout suppressant on gene expression, it is important to understand what is happening to the genes as dormancy progresses, without the application of the suppressant. Therefore, the data in this section focuses only on ethylene signaling pathway genes in the control group. Early in the ethylene signaling pathway, most of the genes do not show a change in regulation when directly comparing the treated and control groups. Therefore, all of the data in this section analyzes expression levels in eco compared to endodormancy after a two-week recovery period. The controlled and treated groups of the ethylene signaling pathway will be directly compared in Chapter 3.4.

Out of the thirteen unique ethylene receptor genes, three demonstrated significant changes in the control group. As shown in Table 3, as dormancy progresses, the receptor genes are upregulated in eco compared to endodormancy after two weeks of recovery time. None of the genes were downregulated.

Table 3. Changes in ethylene receptor gene regulation in controlled samples at T14 as induced by dormancy progression.

AtID	StID	Control
ETR2	Soltu.DM.07G022640.1	No Change
ETR2	Soltu.DM.06G014700.1	Up
ETR2	Soltu.DM.09G026120.1	Up
EIN4	Soltu.DM.11G007790.1	Up

The next genes analyzed in the pathway are the transcription factor genes named EIN3-like (EIL). Of the top ten unique EIL genes, four showed significant changes in the data, but as shown in Table 4, these changes were not observed in the control.

Table 4. Changes in ethylene transcription factor gene regulation in controlled samples at T14 as induced by dormancy progression.

AtID	StID	Control
EIL1	Soltu.DM.01G002310.1	No Change
EIL2	Soltu.DM.01G006210.1	No Change
EIL3	Soltu.DM.01G035980.1	No Change
EIL4	Soltu.DM.03G012570.1	No Change

Ethylene Response Factors (ERFs) are downstream of these transcription factors. As shown in Table 5, only three of those eighteen ERF genes displayed any expression changes when comparing eco to endodormancy in the control.

Table 5. Changes in ERF gene regulation in controlled samples at T14 as induced by dormancy progression.

AtID	StID	Control
ERF1	Soltu.DM.05G020900.1	No Change
ERF1	Soltu.DM.01G031000.2	No Change
ERF1	Soltu.DM.06G019590.1	Up
ERF1	Soltu.DM.06G024040.1	No Change
ERF2	Soltu.DM.08G024160.1	Up
ERF2	Soltu.DM.08G024150.1	No Change
ERF2	Soltu.DM.08G000970.1	No Change
ERF4	Soltu.DM.07G020090.1	No Change
ERF4	Soltu.DM.12G022800.1	No Change
ERF4	Soltu.DM.02G016660.1	No Change
ERF5	Soltu.DM.03G014560.1	No Change
ERF5	Soltu.DM.03G014570.1	No Change
ERF5	Soltu.DM.03G014580.2	No Change
ERF5	Soltu.DM.04G008930.1	Down
ERF5	Soltu.DM.11G001300.1	No Change
ERF5	Soltu.DM.08G024170.1	No Change
ERF	Soltu.DM.04G027450.1	No Change
ERF	Soltu.DM.03G014550.1	No Change

3.3 Response of Ethylene Signaling Pathway Genes to DMN through Dormancy States

The data in this section focuses only on ethylene signaling pathway genes in the treated group. Expression levels during ecodormancy are compared to expression levels during endodormancy at T14.

When treated with DMN, receptor gene expression is upregulated in only one gene and downregulated in another as shown in Table 6. In the control, as shown in Table 3, none of the genes were downregulated. It is important to note however, when the treated and control groups were compared directly, there was not a significant change between the groups, with the exception of Soltu.DM.09G026120.1. In ecodormancy, after a two-week recovery period, this gene is upregulated in DMN compared to the control. Therefore, DMN only has a significant effect on one of the thirteen unique ethylene receptor genes, meaning that overall, DMN does not have a significant effect on ethylene receptor genes.

Table 6. Changes in ethylene receptor gene regulation in treated samples at T14 as induced by dormancy progression.

AtID	StID	DMN
ETR2	Soltu.DM.07G022640.1	Down
ETR2	Soltu.DM.06G014700.1	No Change
ETR2	Soltu.DM.09G026120.1	Up
EIN4	Soltu.DM.11G007790.1	No Change

Of the top ten unique transcription factor genes downstream in the pathway, four showed significant changes in the data. As shown in Table 7, when treated with DMN, three of the genes are downregulated in as dormancy progresses, and one is upregulated. None of the genes showed a significant change in expression when comparing the treated group directly to the control group.

Table 7. Changes in ethylene transcription factor gene regulation in treated samples at T14 as induced by dormancy progression.

AtID	StID	DMN
EIL1	Soltu.DM.01G002310.1	Up
EIL2	Soltu.DM.01G006210.1	Down
EIL3	Soltu.DM.01G035980.1	Down
EIL4	Soltu.DM.03G012570.1	Down

Ethylene Response Factors (ERFs) are downstream of the transcription factors in Table 7. ERF1, 2, 4 and 5 were identified in the dataset and had significant responses to the presence of DMN after a two-week recovery period. As shown in Table 8, five of the genes are upregulated and six are downregulated. Only seven of the eighteen identified ERF genes do not show a significant change when comparing eco to endodormancy of the treated group.

Table 8. Changes in ERF gene regulation in controlled samples at T14 as induced by dormancy progression.

AtID	StID	DMN
ERF1	Soltu.DM.05G020900.1	No Change
ERF1	Soltu.DM.01G031000.2	Up
ERF1	Soltu.DM.06G019590.1	Up
ERF1	Soltu.DM.06G024040.1	No Change
ERF2	Soltu.DM.08G024160.1	Up
ERF2	Soltu.DM.08G024150.1	No Change
ERF2	Soltu.DM.08G000970.1	Up
ERF4	Soltu.DM.07G020090.1	Down
ERF4	Soltu.DM.12G022800.1	Down
ERF4	Soltu.DM.02G016660.1	No Change
ERF5	Soltu.DM.03G014560.1	No Change
ERF5	Soltu.DM.03G014570.1	Down
ERF5	Soltu.DM.03G014580.2	Down
ERF5	Soltu.DM.04G008930.1	Down
ERF5	Soltu.DM.11G001300.1	No Change
ERF5	Soltu.DM.08G024170.1	Down
ERF	Soltu.DM.04G027450.1	No Change
ERF	Soltu.DM.03G014550.1	Up

3.4 Direct Comparison of the Gene Expression of ERFs in the Treated and Control Groups

ERFs are the first group of genes in the ethylene signaling pathway to have a significant response to the presence of DMN. The data in Table 9 compares the expression of the treated group to the control group during ecodormancy at two timepoints (T0 and T14). The benefit to analyzing two timepoints when directly comparing the treated group to the control group is it shows which expression changes occur immediately then disappear, which occur immediately and last for two weeks, and which appear only after a two-week recovery period.

Table 9. Changes in ERF gene regulation induced by DMN in ecodormant tubers

StID	AtID	Up	Down
Soltu.DM.05G020900.1	ERF1	T0	
Soltu.DM.01G031000.2	ERF1		T0
Soltu.DM.06G019590.1	ERF1	T14	
Soltu.DM.06G024040.1	ERF1	T14	
Soltu.DM.08G024160.1	ERF2	T0	
Soltu.DM.08G024150.1	ERF2	T0, T14	
Soltu.DM.08G000970.1	ERF2	T14	
Soltu.DM.07G020090.1	ERF4		
Soltu.DM.12G022800.1	ERF4		
Soltu.DM.02G016660.1	ERF4	T0, T14	
Soltu.DM.03G014560.1	ERF5		
Soltu.DM.03G014570.1	ERF5		
Soltu.DM.03G014580.2	ERF5		
Soltu.DM.04G008930.1	ERF5	T0	T14
Soltu.DM.11G001300.1	ERF5		
Soltu.DM.08G024170.1	ERF5		
Soltu.DM.04G027450.1	ERFX	T0	
Soltu.DM.03G014550.1	ERFX		

It is clear from the data in Table 9 that the ERFs do have a significant response to DMN. At T0, six genes were upregulated and one was downregulated when comparing DMN to the control. At T14, three of the six genes were no longer upregulated, and therefore returned to the same level of expression as the control. Two of the six genes maintained a higher level of expression. Three additional genes were upregulated and one additional gene was downregulated after two weeks of recovery time.

Chapter 4

Discussion

4.1 Regulation of Genes in the Ethylene Biosynthesis Pathway

1-aminocyclіopropane-1-carboxylic acid (ACC) synthase (ACS) is responsible for using SAM to create ACC, a direct precursor of ethylene and central in the ethylene biosynthesis pathway. This research shows that expression of ACS is not affected by DMN in endodormancy, but in ecodormancy, there are significant differences in expression between the control and treated groups. This is expected because as a tuber enters eco-dormancy, it is more likely to respond to environmental conditions. There is not a clear pattern of up-regulation or down-regulation for the identified ACS genes. But three of the four genes do show significant changes in expression only after a two-week recovery period. This research agrees with previous studies on the expression of ACS in *Solanum lypercosum*. One study proved that four ACS genes, which were also differentially expressed, play a role in ripening (Barry et al., 2000). A potato is further along in dormancy when it enters ecodormancy, so it makes sense that these genes that contribute to ripening are showing significant changes in expression during ecodormancy.

ACC is converted into ethylene by ACC oxidase (ACO). Specifically, ACO4 was found in the data, which is also known as an ethylene forming enzyme. All of the ACO4 genes that showed a significant response to DMN were upregulated in the treated group compared to the untreated group. Two of the three genes were upregulated immediately after removal from treatment, then after two weeks their expression returned to the same level as the control. One of the three genes were upregulated only two weeks after removal from treatment. It is also important to note that again, expression between the control and treated groups did not differ until ecodormancy. None of the ACS nor ACO genes maintained a significant positive or negative expression response to DMN through both timepoints. Therefore, it seems that the effects of DMN on these genes does not last or it takes two weeks for the response to be significant.

4.2 Regulation of Genes in the Ethylene Signaling Pathway

In the ethylene signaling pathway, there were few significant changes between the control group and treated group with the exception of the ethylene response factors. However, there are some interesting differences between treatments as dormancy progresses. Quantitatively, when looking at same genes, DMN experienced more differential gene expression between dormancy stages than the control. However, when the two treatments are directly compared within a dormancy stage, there was not a significant change in gene expression. Something significant is happening in the DMN-treated tubers, but it is not significantly different from the control. Therefore, these changes need to be further studied.

Two of the five ethylene receptors, ETR2 and EIN4 were found to experience significant regulation as dormancy progressed. In the ethylene signaling pathway, either ethylene can bind to one of these five receptors, or its negative regulator, CTR1, will interact with the receptors. Whether these genes are upregulated or downregulated can reveal the tendency of the receptors to bind (Patel, 2021). In the control, when comparing eco to endodormant states, the receptor genes are upregulated. Conversely, when examining the treated group, one gene is upregulated, one is downregulated, and two show no significant change. Therefore, in the control, the receptors are more sensitive to binding in ecodormancy compared to endodormancy. This same level of readiness to bind is not shown in the treated group when comparing eco and endodormancy. The higher sensitivity of the receptor genes in ecodormancy could perhaps explain why most of the genes later in the signaling and biosynthesis pathways are showing the most changes in the ecodormant state.

Downstream transcription factors receive signals from genes upstream. These transcription factors are either tagged for degradation if ethylene did not bind to the receptors, or they begin signaling to downstream transcription factors if ethylene did bind to the receptors (Binder, 2020). The data shows that these genes are downregulated in ecodormancy compared to endodormancy when treated with DMN. This indicates that downstream ethylene responses are repressed in the treated samples as dormancy progresses. Nevertheless, it is important to note that there is not a significant difference between

expression levels when comparing ecodormant treated samples directly to ecodormant untreated samples. There is also not a significant difference between expression levels when comparing endodormant treated samples directly to endodormant untreated samples. Therefore, if DMN does cause downregulation as ecodormancy progresses it is not significantly different from the expression levels seen in the control.

4.3 Comparing the Regulation of ERFs in the Treated Group to the Control Group

Up to this point in the ethylene signaling pathway, few genes showed a significant change in regulation when comparing the control group directly to the treated group. However, DMN causes upregulation in most of the ethylene response factor genes that showed significant changes. This upregulation occurs during ecodormancy, at both timepoints. This agrees with previous research that found upregulation in genes playing a role in stress and defense responses in ecodormancy when treated with DMN (Campbell et al., 2020). DMN is known to cause a stress response in potatoes, so it makes sense that the ERFs experience up-regulation in ecodormancy. Two genes to note are Soltu.DM.08G024150.1 and Soltu.DM.02G016660.1 because they remain upregulated in both timepoints of ecodormancy. These two genes could have stronger expression levels when exposed to stressful conditions.

4.4 Limitations

There were quite a few limitations to this study and more research is necessary. First, only one potato cultivar (*Solanum tuberosum* cv Lamoka) was analyzed for the study; therefore, these results may only apply to that one cultivar. A subsequent study should analyze multiple different types of cultivars to determine the applicability of these results to other cultivars. Additionally, more treatments between endo and early ecodormancy would provide more information about gene expression as dormancy progresses. Few conclusions can be drawn from treatments at two dormancy states with a large gap of time between

the two treatments. Significant gene expression changes could occur between these two dormancy stages that this study does not capture.

Another limitation is that this study does not mimic the amount of DMN treatments that would occur in a commercial setting. In a commercial setting, tubers are treated with DMN in endodormancy, then those same tubers are treated again. In this study, the ecodormant tubers were only treated with DMN once. Multiple treatments with DMN can cause DMN levels to spike higher and last longer. Future studies could examine how multiple DMN treatments on the same tubers affect gene expression.

Chapter 5

Conclusion

This study shows that DMN does have a significant effect on the ethylene signaling and biosynthesis pathways, but this chemical does not significantly affect these pathways until ecodormancy. In the ethylene biosynthesis pathway, ACS genes are differentially expressed in ecodormancy when comparing the treated to untreated samples, which agrees with previous research on the role of ACS on ripening. On the other hand, ACO genes displayed a significant positive response to the presence of DMN during ecodormancy. None of the ACS nor ACO genes maintained a significant positive or negative expression response to DMN through both timepoints. Therefore, it seems that the effects of DMN on these genes does not last, or it takes two weeks for the response to be significant. Studies with more timepoints could be conducted to determine how long these responses last.

While the ethylene signaling pathway did not show significant responses to DMN at the beginning of the pathway, at the end of the pathway, a majority of the ethylene response factor genes identified in this study were upregulated in the treated group compared to the control group during ecodormancy. Two of these genes, Soltu.DM.08G024150.1 and Soltu.DM.02G016660.1 were upregulated in ecodormancy through both timepoints, and should therefore be subject to further studies. The genes that were directly affected by DMN throughout the entire ethylene signaling and biosynthesis pathways are summarized in Table 10.

Table 10. Summary of genes directly affected by the presence of DMN during ecodormancy. This compares DMN-treated samples to control samples at both timepoints across the entire pathway.

Pathway	StID	Up	Down
ACS	Soltu.DM.01G034180.1	T14	
	Soltu.DM.02G027270.1	T14	
	Soltu.DM.04G032120.1		T0
	Soltu.DM.08G028300.1		T14
↓			
ACO	Soltu.DM.07G016750.1	T0	
	Soltu.DM.12G023340.1	T0	
	Soltu.DM.07G016780.1	T14	
↓			
Receptors	Soltu.DM.09G026120.1	T14	
↓			
EIL	NA		
↓			
ERF	Soltu.DM.05G020900.1	T0	
	Soltu.DM.08G024160.1	T0	
	Soltu.DM.08G024150.1	T0, T14	
	Soltu.DM.02G016660.1	T0, T14	
	Soltu.DM.04G008930.1	T0	T14
	Soltu.DM.04G027450.1	T0	
	Soltu.DM.06G019590.1	T14	
	Soltu.DM.06G024040.1	T14	
	Soltu.DM.08G000970.1	T14	
	Soltu.DM.01G031000.2		T0

This study also found that earlier in the ethylene signaling pathway, DMN did not have a significant effect on the expression of most genes. However, the genes in the treated samples seem to be expressed at differently in the treated and control samples as dormancy progresses. The samples treated with DMN seem to experience different gene changes as time progresses, at least during the timepoints in this study. The genes that were affected by the progression of dormancy are summarized in Tables 11 and 12. Table 11 summarizes the genes in the control that were upregulated or downregulated in eco compared to endodormancy. Table 12 summarizes the genes in the treated group that were upregulated or downregulated in eco compared to endodormancy.

Table 11. Control sample genes with significant expression changes at T14 induced by dormancy progression. The ethylene signaling pathway is highlighted on the left in blue.

Ethylene Signaling Pathway		
Pathway	**Upregulated Genes**	**Downregulated Genes**
ERS1, ETR1, ERS2, ETR2, EIN4	Soltu.DM.06G014700.1	NA
	Soltu.DM.11G007790.1	
	Soltu.DM.09G026120.1	
↓		
EIN3, EIL1, EIL2	NA	NA
↓		
ERFs	Soltu.DM.06G019590.1	Soltu.DM.04G008930.1
	Soltu.DM.08G024160.1	

Table 12. Treated sample genes showing changes in expression from endo to ecodormancy at T14. The ethylene signaling pathway is highlighted on the left in blue.

Ethylene Signaling Pathway		
Pathway	**Upregulated Genes**	**Downregulated Genes**
ERS1, ETR1, ERS2, ETR2, EIN4	Soltu.DM.09G026120.1	Soltu.DM.07G022640.1
↓		
EIN3, EIL1, EIL2	Soltu.DM.01G002310.1	Soltu.DM.01G006210.1
		Soltu.DM.01G035980.1
		Soltu.DM.03G012570.1
↓		
ERFs	Soltu.DM.01G031000.2	Soltu.DM.07G020090.1
	Soltu.DM.06G019590.1	Soltu.DM.12G022800.1
	Soltu.DM.08G024160.1	Soltu.DM.03G014570.1
	Soltu.DM.08G000970.1	Soltu.DM.03G014580.2
	Soltu.DM.03G014550.1	Soltu.DM.04G008930.1
		Soltu.DM.08G024170.1

In conclusion, this study shows that DMN may play a role in regulating dormancy by affecting the expression of genes in the ethylene signaling and biosynthesis pathway.

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- Troubleshooted technical issues and gave direction for repair while components were in process
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- Regularly communicated with customers to discuss state of components and ship dates
- Assigned initial directions for repair after components passed initial inspection

TECHNOLOGY AND TUTORING INTERNSHIP EXPERIENCE
Larson Texts, Inc. Erie, PA
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- Tutored students from grade six to college level
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