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Status of the methylome in *Solanum tuberosum* tubers treated with sprout inhibitor 1,4-dimethylnaphthalene

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Abstract

The success of the potato industry depends heavily on the storage of the tubers; if potatoes sprout while in storage, the stock is ruined for commercial sale. For this reason, the industry relies on aerosol sprout inhibitors to prevent sprout growth. One such inhibitor is 1,4-dimethylnaphthalene, or DMN. It is found naturally in potatoes and thus is considered a more natural sprout inhibitor, but it is unknown how DMN prevents growth. This study considers epigenetics as a possible mechanism of sprout inhibition, particularly in the form of DNA methylation. DNA methylation is the covalent attachment of a methyl group ($-CH_3$), particularly to cytosines, that inhibits expression of genes downstream of the methylation. Genomic DNA from meristem tissue of potatoes was isolated before undergoing bisulfite conversion and sequencing at Beijing Genomics Institute. Bisulfite conversion elucidates methylation data by chemical conversion of non-methylated cytosines into uracil, ultimately providing a final sequence wherein all remaining cytosines indicate a methylated cytosine. These data were analyzed using the bisulfite sequencing analysis software "bismark" and aligned with an annotated potato genome through the website Comparative Genomics (CoGe). Methylation rates and locations were compared between different treatments, and the genes identified as being under possible methylation control were referenced to previous gene expression data. The genes identified were not consistently up- or downregulated after exposure to DMN, so we conclude that DMN inhibits sprout growth through another cellular process that also alters DNA methylation and gene expression as a result.

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Introduction

Solanum tuberosum and Sprout Inhibitor 1,4-dimethylnaphthalene

Potatoes, or *Solanum tuberosum*, are an extremely important staple in the diets of many cultures, particularly in America where they were recently one of the leading crops in the nation (*Potatoes*, 2018). In contrast to other main crops, potatoes are stored in a highly perishable form that makes them vulnerable to rot, pesticides, and sprouting (Suttle, 2004). Sprouting has a larger impact than many would think; it accompanies many cellular changes in the potatoes, including reduced sugar content and changes in water retention, which are detrimental to the quality of the stores (Suttle, 2004). For this reason, potato farmers not only invest time and money into preventing their crops from being harmed in the field, but also carefully protect their potato stores after harvest.

In addition to pesticides, potato farmers often spray aerosol sprout inhibitors over their massive potato storage units to prevent the detrimental sprouting of eyes on their stores. One such sprout inhibitor is 1,4-dimethylnaphathalene, or DMN. This compound can be found commercially in multiple products from the company 1,4GROUP, Inc, which includes the product 1,4SIGHT that was used in this study (14group.com) (Figure 1). Though DMN is approved by the EPA for use in the United States, it has recently been under consideration in the UK, with an emergency authorization of DMN use passing as recently as December of 2020 (*Potato processors*, 2020).

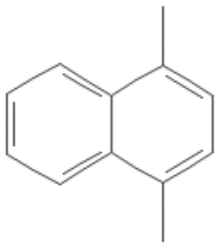

Figure 1: On the left is the chemical structure for 1,4-dimethylnaphthalene (DMN), a commercial sprout inhibitor. The right depicts the commercial logo for the product used in this study, 1,4-SIGHT, distributed by 1,4GROUP, Inc.

Methyl-substituted naphthalenes, including DMN, are found naturally occurring in potatoes, some of which have been known to inhibit sprout growth (Knowles et. al., 2005). DMN is a volatile compound that can be recognized by the average potato-consumer as contributing to the taste and smell of baked potatoes (Knowles et. al., 2004). Because DMN is found naturally in potatoes already, it has been proposed to serve as a safer alternative to other sprout inhibitors. Its naturally derived compound is in contrast to other chemicals like CIPC, which has been found to have highly toxic metabolites (Paul et. al., 2015). Though studies suggest that DMN hormonally inhibits sprout growth, its mechanism of inhibition is unknown (Knowles et. al., 2005).

Earlier studies using RNA-seq to measure gene expression changes in potatoes as a result of DMN exposure indicated that many genes were changing in expression rate; a few of which included GO:0006306, GO:0006346, and GO:0010424, which are involved with DNA methylation, methylation-dependent chromatin silencing, and DNA methylation on cytosine within a CG sequence, respectively (Table 1) (Campbell et. al., 2020). This result suggested that methylation may play a role in DMN's mechanism of sprout inhibition.

Table 1: Gene ontology designations and functions of genes found to have significant changes in expression in *Solanum tuberosum* tubers after exposure to the sprout inhibitor 1,4-dimethylnaphthalene.

Gene Ontology ID	Gene Function
GO:0006270	DNA replication initiation
GO:0000911	cytokinesis by cell plate formation
GO:0008283	cell proliferation
GO:0006306	DNA methylation
GO:0046785	microtubule polymerization
GO:0007018	microtubule-based movement
GO:0051301	cell division
GO:0051567	histone H3-K9 methylation
GO:0006275	regulation of DNA replication
GO:0006346	methylation-dependent chromatin silencing
GO:0010424	DNA methylation on cytosine within a CG ..
GO:0043987	histone H3-S10 phosphorylation
GO:0000910	cytokinesis
GO:0000226	microtubule cytoskeleton organization
GO:0006342	chromatin silencing

DNA Methylation and Dormancy

Epigenetics are cellular processes by which gene expression can be drastically altered without changing the actual genetic code of the DNA. One epigenetic mechanism is DNA methylation, which functions by the enzyme DNA methyltransferase adding a methyl ($-CH_3$) group covalently to a cytosine nucleotide, particularly in groups of cytosines and guanines called CpG islands (Zhang et. al., 2018). This process is conserved between animals and plants, and thus serves as a system that can be analyzed in model plant organisms to possibly better the

understanding of the process in human DNA. Methylation plays an essential role in maintaining genomic stability, controlling chromatin structure, and regulating gene expression (Zhang et. al., 2018).

DNA methylation that occurs in the promoter region, upstream of a gene, can directly inhibit gene expression by physically preventing the transcription activators from binding, or by increasing the binding of transcription repressors (Zhang et. al., 2018). It is also worth noting that crop plants with large genomes, such as *S. tuberosum*, have more genes with promoter methylation compared to plants with smaller genomes, such as *Arabidopsis thaliana* (Zhang et. al., 2018). Promoter regions can be very close to the gene that may be transcribed, such as immediately upstream of the gene within a few dozen base pairs, but they can also be defined as any DNA sequence lying upstream that impacts the transcription of a gene (*Promoter*). In this study, promoter regions were analyzed that were both immediately within the vicinity of the gene and farther upstream.

Increased states of methylation, and thus lowered rates of gene expression, are known to be characteristic of a cell state called dormancy (Suttle, 2004). Cellular dormancy is an essential process in life; it is present in human cells, but it is most easily observed in plants. For example, it is well known that deciduous trees lose their leaves in the winter and do not exhibit any growth until temperatures rise again. Deciduous trees enter dormancy in the winter to defend against lowered temperatures, decreased precipitation, and lack of sunlight. Dormancy, at the cellular level, is a state of lowered metabolic rate and a pause in growth and division. This lack of growth and division is often an effort to conserve resources, as it is in trees during the winter. This state is also characterized by decreased genetic activity, such as translation and transcription, and drastic changes in gene expression (Suttle, 2004). Previous studies have found that, specifically in

potatoes, demethylation of CG islands precedes a tuber's exit from dormancy, after which transcription rises again and cell division resumes (Law & Suttle, 2003). The inhibition of sprout growth caused by DMN mimics dormancy in that there is no growth occurring.

Evidenced in prior research, DMN inhibits sprout growth, possibly by some method of manipulating gene expression via DNA methylation. Because methylation plays a role in dormancy and exposure to DMN alters expression of methylation-related genes, we hypothesized that DMN may be acting by altering methylation status in the genome of *S. tuberosum*, thus inhibiting sprout growth.

Methods

Sample Collection and Treatment

Field grown potatoes (*Solanum tuberosum cv.* Lamoka) were harvested by hand at Glenn Troyer Farms, Waterford PA. We collected 6-8 potatoes of varying size from 30 potato plants and stored them in silk mesh bags in an incubator at 10℃ with 85% relative humidity. The bags were separated by plant; each plant's potatoes are split into two equal groups, one for treatment and one for control. Every plant's potatoes are genetically identical, presenting a unique opportunity to compare individuals that were not different prior to treatment. Once separated into groups, the treatment potatoes were placed in a 9 L chamber with mesh evaporation baskets containing approximately 66 μL of DMN spotted onto Whatman No. 1 filter paper. The same was done with the control group, but with filter paper spotted with water. The samples were left in aa dark holding space for 48 hours at 20℃, which mimics DMN exposure times in typical commercial storage. These trials were performed twice in the fall, resulting in four samples, and three times in the spring, resulting in six samples. The chosen trial times coincided with time points in the potato dormancy stages: endodormancy and ecodormancy. Thus, samples T1-T4 and C1-C4 (T indicating treatment and C indicating control) correspond to potatoes in endodormancy, and samples T5-T10 and C5-C10 correspond to potatoes in ecodormancy. These different stages of dormancy are characterized by the internal cell behavior; though there is no growth in either, endodormancy demonstrates significantly less metabolic activity compared to ecodormancy (Longstroth, 2013).

DNA Isolation

After two days of treatment, the potatoes were removed from the incubation chambers and their apical meristem tissue – from which the tubers would normally grow – was immediately extracted with a curved scalpel. The tissue was removed quickly from each potato to prevent gene expression changes brought about by the trauma of cutting the potato. The meristems were preferentially extracted because the area of the shoot that is suppressed by DMN, the sprouts, arise directly from the meristem. DNA was isolated using the *Quick*-DNA/RNA MagBead kit from Zymo Research (https://www.zymoresearch.com/). Following isolation, we quantified the DNA using a NanoDrop to verify that the samples contained high quality DNA (a 260/280 ratio of approximately 1.8) and enough material to be further sequenced.

Bisulfite Conversion and Sequencing

DNA samples were sent to BGI services in Hong Kong for bisulfite conversion and sequencing (https://en.genomics.cn/). Bisulfite conversion and sequencing is regarded as the "gold-standard" for genomic methylation studies because it has the capability to identify methylation sites at single nucleotides (Li and Tollefsbol, 2011). It identifies single base pair methylation locations by converting the DNA with a sodium bisulfite treatment; this causes deamination on unmethylated cytosines, converting them into uracil, and leaves methylated cytosines (5-mC) unchanged. In the polymerase chain reaction (PCR) amplification that follows, the unmethylated cytosines, now uracils, will be recognized as thymines. Thus, the cytosines present in the final sequence are indicative of 5-mC, allowing us to identify precisely where methylation has occurred. Whole genome bisulfite sequencing was conducted to a level of 25X

genome coverage for all samples. This level of coverage allowed for detailed methylome analysis across the entire potato genome.

Genome Mapping

The bisulfite sequencing data were returned from BGI in the form of compressed files (.gz). Due to unforeseen circumstances involving the COVID-19 pandemic and Beijing protests, the analysis was delayed significantly, and samples were sent to multiple locations before being analyzed. For this reason, multiple samples were too degraded for use in this study. This left the only remaining samples from the endodormant period to be samples C3 and T3. From the ecodormant period, the samples C7, C8, C9, and C10 and their corresponding treatment samples were able to be analyzed.

All data analyses that were completed after bisulfite data return were performed through Cyverse (https://www.cyverse.org), an online platform that allows remote access to software and data analyses that would normally require a high-power computer, and its associated website CoGe (http://genomevolution.org). The compressed files (.gz) were first unzipped using the software zcat (version 1.3.5 by Kokulapalan Wimalanathan). The files were split during BGI sequencing due to their size, so each split file was then concatenated using the program “Concatenate Multiple Files” (v. 1.24.1 by user rwalls). The file type was also changed from plain text to fastq format by adding “.fastq” to the end of the file name to allow for analysis using software mentioned below.

These fastq files were processed on the CoGe website; files held on a Cyverse account can be accessed by CoGe for this reason. The bisulfite sequencing data was aligned to an annotated genome (“Solanum tuberosum group Phureja DM1-3 516 R44 (potato): contains anchored and unanchored (chr0) sequences (vPGSC_DM_v4.03_pseudomolecules, id24771): unmasked”)

using the program bismark. Through CoGe, the alignment of bisulfite data to the annotated genome was visualized using JBrowse, which allowed for visual comparison of methylation rate between samples.

A comparison was made between ecodormant control and treatment samples throughout chromosome 1, and locations of significantly different methylation were recorded. These locations were compared to nearby genes (whether protein-coding or not) that followed the corresponding transcription direction, and these genes were cross-referenced with previous RNAseq data (from Campbell, et. al., 2020). This analysis was performed to identify genes that were both under an area of changing methylation as well as having a significant change in gene expression upon exposure to DMN.

Results

Global Methylation Changes

Though individual genes were identified, the global methylation changes across the entire genome were also considered. It was observed that on the chromosomes analyzed, which were chromosome 1 and hypothetical "chromosome 0" which includes unanchored DNA sequences, the treatment samples demonstrated global hypomethylation in comparison to the control samples (Figure 2). This is an odd observation, as typically dormant potatoes demonstrate a higher level of methylation when in dormancy (Law & Suttle, 2003). This global observation, however, only indicates the average overall levels of methylation. Individual areas are still subject to local hypermethylation and could impact gene expression by promoter methylation regardless of overall trends of methylation (as is reminiscent of cancer cells which show global hypomethylation but local hypermethylation).

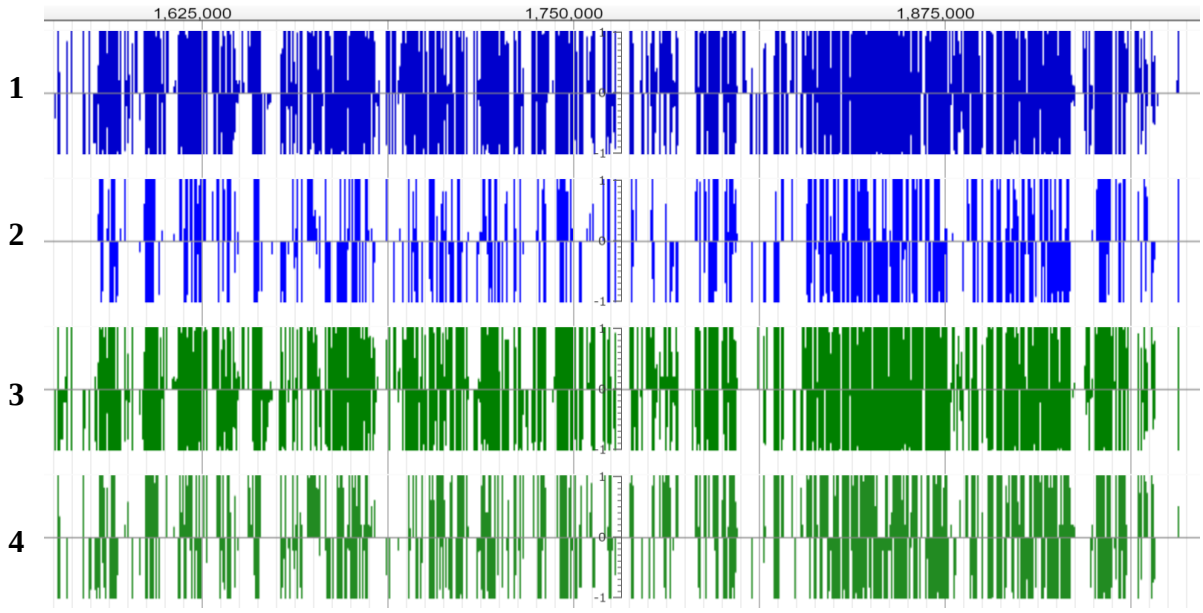

Figure 2: Image of methylation data visualized using JBrowse exemplifying the overall hypomethylation of potato samples treated with 1,4-dimethylnapthalene; this image is on chromosome 1, and base pair location is indicated on the top. Row 1 is control sample 8 (C8), row 2 is treatment sample 8 (T8), row 3 is C9, and row 4 is T9.

The data for these samples are extensive. For this reason, the methylation was analyzed both from a “zoomed out” perspective to identify large-scale methylation changes, as well as a “zoomed in” perspective to identify specific locations of differential methylation between treatments. There were countless locations of individual differences in methylation, but they appear so often that they are likely insignificant in terms of gene expression, and they were seen both between control and treatment and between samples from different plants (Figure 3). For this reason, areas that contained different methylation over a significant area (approximately 6,000 base pairs) were used as possible locations of promoter methylation (Figure 4).

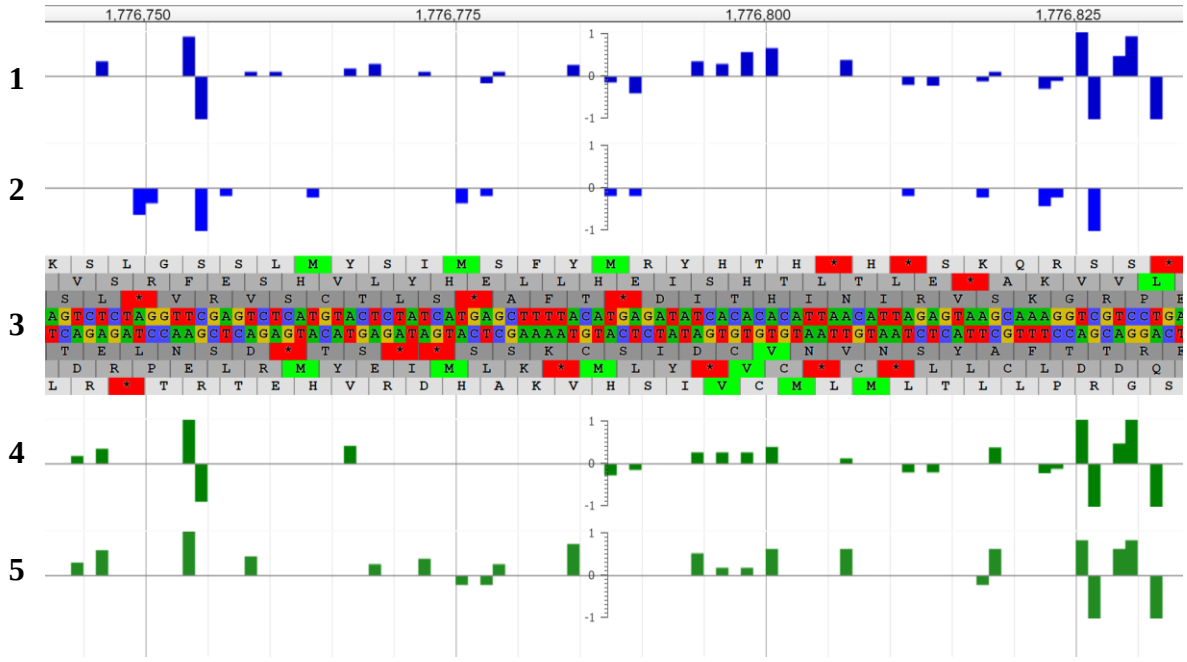

Figure 3: Image of methylation data visualized using JBrowse exemplifying the base pair variation between potato samples, whether control or treated with 1,4-dimethylnaphthalene; this image is on chromosome 1, and base pair location is indicated on the top. Each data point indicates a singular methylated base pair. Row 1 is control sample 8 (C8), row 2 is treatment sample 8 (T8), row 3 is the DNA sequence of that area, row 4 is C9, and row 5 is T9.

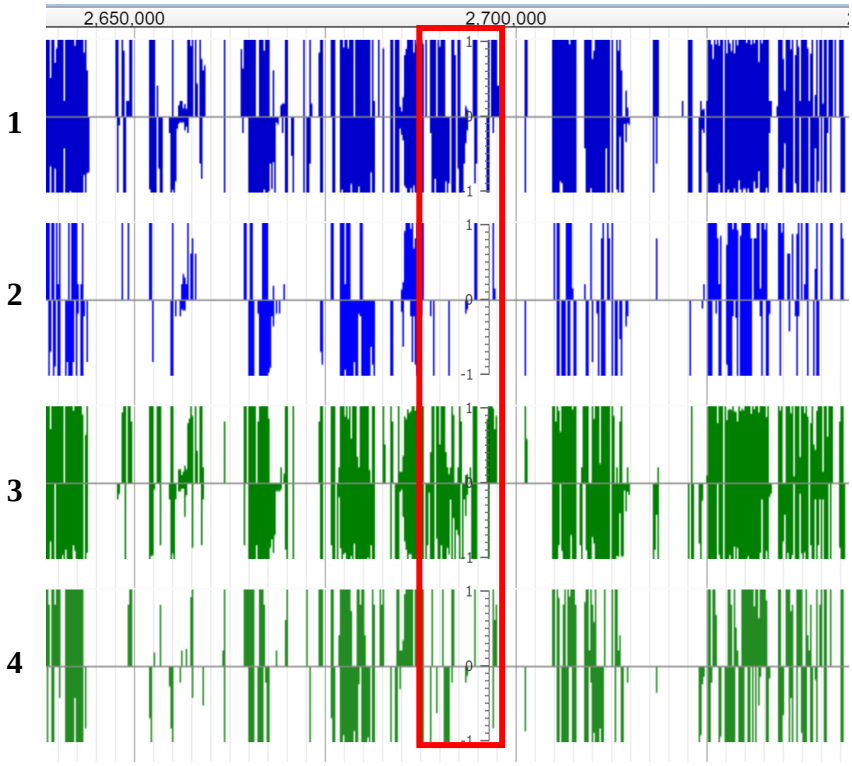

Figure 4: Example of genomic location (indicated by red box) with methylation significantly different enough between treatment and control to be identified as an area of possible promoter methylation, visualized on JBrowse. Row 1 is C8, row 2 is T8, row 3 is C9, and row 4 is T9. Genomic location is chromosome 1 in *S. tuberosum* genome.

Although decreased global methylation does not necessarily indicate that all genes will be hypomethylated, the genes identified in this study were all found to be hypomethylated after treatment with DMN compared to the control samples.

Genes Associated with Promoter Methylation

In addition to the changes in overall methylation, specific locations of different methylation levels between treatment and control were identified (Figure 4). These data were also extensive, but not all locations that appeared to have altered methylation had genes nearby enough, or in the proper transcription direction, to be considered associated with the methylation. The genes chosen

to be further analyzed were within a few thousand base pairs (5,0000 at maximum) of the site of significantly altered methylation and are shown in Table 2.

Table 2: Genes identified as being possibly under the influence of promoter methylation changes in potatoes as a result of exposure to 1,4-dimethylnaphthalene. All genes are on chromosome 1 between base pairs 0 – 23 million.

Gene ID	Gene Function
PGSC0003DMG400021337	J domain-containing protein
PGSC0003DMG400016401	Electron transporter
PGSC0003DMG400016365	Protein kinase domain-containing protein
PGSC0003DMG401016399	KAT8 regulatory NSL complex subunit 2
PGSC0003DMG400016361	Thioredoxin domain-containing protein
PGSC0003DMG400016398	Uncharacterized protein
PGSC0003DMG400016397	Prefoldin subunit 5
PGSC0003DMG402016396	Serine/threonine-protein phosphatase 2A activator
PGSC0003DMG401016396	Dihydroorotate dehydrogenase (quinone), mitochondrial
PGSC0003DMG400016360	3'-N-debenzoyl-2'-deoxytaxol N-benzoyltransferase
PGSC0003DMG400010705	Ubiquitin-protein ligase
PGSC0003DMG400010704	Calcium-dependent protein kinase 8
PGSC0003DMG400010702	Uncharacterized protein
PGSC0003DMG400010701	Protein kinase domain-containing protein
PGSC0003DMG400004429	S2 self-incompatibility locus-linked pollen 3.2 protein
PGSC0003DMG400004430	TPT domain-containing protein
PGSC0003DMG400004431	Uncharacterized protein
PGSC0003DMG400039819	Mannan endo-1,4-beta-mannosidase
PGSC0003DMG400004435	TMEM189_B_dmain domain-containing protein
PGSC0003DMG400044667	Mannan endo-1,4-beta-mannosidase
PGSC0003DMG400004428	Uncharacterized protein
PGSC0003DMG400022714	Minor histocompatibility antigen H13
PGSC0003DMG400022712	PCI domain-containing protein
PGSC0003DMG400028068	Uncharacterized protein
PGSC0003DMG400037473	Uncharacterized protein
PGSC0003DMG400040616	Mucin 5, partial
PGSC0003DMG400006237	Gamma-gliadin
PGSC0003DMG400039577	Uncharacterized protein
PGSC0003DMG400022974	Gag-pol polyprotein
PGSC0003DMG400046500	Uncharacterized protein
PGSC0003DMG400035028	Uncharacterized protein
PGSC0003DMG400015063	ATPase
PGSC0003DMG400015060	Protein ycf2
PGSC0003DMG400015062	Photosystem II CP47 chlorophyll apoprotein

PGSC0003DMG400041353	Uncharacterized protein
PGSC0003DMG400043498	GH10 domain-containing protein
PGSC0003DMG400003334	Uncharacterized protein
PGSC0003DMG400044917	Uncharacterized protein
PGSC0003DMG400035112	Uncharacterized protein
PGSC0003DMG400037877	Uncharacterized protein
PGSC0003DMG400040250	Uncharacterized protein
PGSC0003DMG400039042	Uncharacterized protein
PGSC0003DMG400034914	Uncharacterized protein
PGSC0003DMG400042449	Uncharacterized protein
PGSC0003DMG400043615	Uncharacterized protein
PGSC0003DMG400039841	Uncharacterized protein
PGSC0003DMG400043380	Uncharacterized protein
PGSC0003DMG400044471	Uncharacterized protein
PGSC0003DMG400035023	Uncharacterized protein
PGSC0003DMG400040329	Uncharacterized protein
PGSC0003DMG400034423	CBS domain-containing protein
PGSC0003DMG400039188	Uncharacterized protein
PGSC0003DMG400044637	Uncharacterized protein
PGSC0003DMG400005432	STAG domain-containing protein
PGSC0003DMG400005431	Uncharacterized protein
PGSC0003DMG400046697	Uncharacterized protein
PGSC0003DMG400038575	Uncharacterized protein
PGSC0003DMG400039427	Uncharacterized protein

Gene functions were identified using PANTHER (http://www.pantherdb.org/). Many proteins were found to be uncharacterized in the potato genome, but they may have orthologs found in other species. Of the 58 genes included, 28 genes were found to have a known function in *S. tuberosum*.

Gene Expression Changes as Result of Exposure to DMN

Gene identities were also compared to previous RNAseq data of potatoes exposed to DMN at different periods of dormancy. The RNAseq data compared gene expression between dormant

and nondormant tubers as well as differences in expression between exposure to DMN and water as a control. Because our study focused on the possible genetic changes after exposure to DMN, not the changes between stages of dormancy, the chosen RNA-seq data used to support the methylation data were those that only compared between control and treatment potatoes. Of the 58 genes identified in Table 2 as being under possible methylation control, 21 genes were found to have changes in expression between control and treatment, and 11 genes were found to have at least one significant change in expression ($p < 0.05$). Of the genes identified to have expression changes, it was not consistent as to whether the expression changes were that of upregulation or downregulation (Table 3). Three genes had insufficient evidence to make a strong conclusion regarding whether they were up- or downregulated after exposure to DMN; these were marked as "Inconclusive" on Table 3.

Table 3: *S. tuberosum* genes identified as being possibly under the control of promoter methylation and change in gene expression upon exposure to 1,4-dimethylnaphthalene. Conclusions were based on the number of values found to be up- or downregulated and the values that were significant. The higher value between the "Upregulated" and "Downregulated" column has been highlighted for each gene, and genes concluded to be upregulated were highlighted green, with downregulated highlighted red.

Gene ID	Upregulated	Downregulated	Significant Values	Conclusion
PGSC0003DMG400021337	14	4		Upregulated
PGSC0003DMG400016401	17	7		Upregulated
PGSC0003DMG400016365	7	2	2 up	Upregulated
PGSC0003DMG401016399	9	9		Inconclusive
PGSC0003DMG400016361	3	6	1 down	Downregulated
PGSC0003DMG400016398	5	4	1 up	Upregulated
PGSC0003DMG400016397	0	9	2 down	Downregulated
PGSC0003DMG402016396	8	10	2 down, 2 up	Downregulated
PGSC0003DMG401016396	2	7	5 down	Downregulated
PGSC0003DMG400016360	2	7		Downregulated
PGSC0003DMG400010705	14	4	2 up	Upregulated

PGSC0003DMG400010704	3	6	1 up, 3 down	Downregulated
PGSC0003DMG400010701	5	4		Upregulated
PGSC0003DMG400004429	5	4		Upregulated
PGSC0003DMG400004431	8	10	1 up	Downregulated
PGSC0003DMG400022712	5	13		Downregulated
PGSC0003DMG400028068	4	5	2 down	Downregulated
PGSC0003DMG400006237	1	2		Inconclusive
PGSC0003DMG400022974	2	4		Downregulated
PGSC0003DMG400015060	6	3	3 up, 1 down	Upregulated
PGSC0003DMG400005432	1	1		Inconclusive

All genes identified in Table 3 were under the possible control of areas that were de-methylated after exposure to DMN; for this reason, the gene expression would be expected to increase, but as indicated in Table 3, the results varied.

Discussion

We had hypothesized that DMN suppressed sprout growth by increasing DNA methylation across the *Solanum tuberosum* genome; this was supported by previous research in which genes associated with methylation had altered expression upon exposure to DMN (Campbell et. al., 2020), as well as the observation that potatoes beginning to sprout demonstrate an overall decrease in genomic methylation (Law & Suttle, 2003). The results of this study, however, do not support this hypothesis, but rather suggest a much more complex role of methylation in relation to gene expression and the effect of DMN.

The results indicated that the genes identified as being possibly under the control of methylation had a wide range of functions, many of which did not have an identified function in potatoes at all (Table 2). Of the genes that were identified as having changes in expression after exposure to DMN, the changes in expression were not consistent (Table 3). This indicates that the genes identified as being downregulated in Table 3 did not have expression dependent on the identified methylation upstream, so that methylation area is likely not a promoter region for those genes. The genes identified as being upregulated in Table 3 may be connected to the identified areas of significantly changed methylation, but further research would have to be conducted in order to definitively connect the possible promoter regions to their associated genes. Additionally, these genes may have been demethylated as a result of the global methylation, rather than by specific demethylation of select genes. If they were demethylated in a non-selective fashion, the gene products may not play a significant role in DMN's sprout inhibition.

Methylation is not the only factor that affects the gene expression of a cell. The process may also include the action of cellular signaling that results in the inhibition or activation of

pathways that lead to changes in gene expression, or other epigenetic mechanisms like histone modifications or small non-coding RNA (snRNA) regulation. Methylation also plays a role in maintaining genomic integrity, especially in higher order plants like potatoes (He, Chen, & Zhu, 2011).

Additionally, gene-body methylation may play a contrasting role to the silencing ability of promoter methylation. Gene-body methylation, in both plants and animals, has been shown to promote gene expression through acting against the repressive action of the Polycomb group proteins (He, Chen, & Zhu, 2011). Thus, the global hypomethylation observed in DMN-exposed potatoes in this study may also be resulting in changes in gene expression through this mechanism rather than, or in addition to, changes via promoter methylation.

Polycomb repressive complex (PRC) proteins serve an important role in development and function to alter the chromatin structure via trimethylation of histone H3K27, resulting in transcriptional silencing that carries over even through cell division (Holec & Berger, 2012). Very recently, the over-expression of specific PRC proteins has also been found to play a role in a potato's exit from dormancy by repressing the transcription of genes essential to tuberization (Kumar et. al., 2021). Previous RNAseq data from potatoes exposed to DMN revealed changes in gene expression of genes associated with chromatin modifications, including those involved in "histone H3K9 methylation," "methylation-dependent chromatin silencing," and "chromatin silencing" (Table 1) (Campbell et. al., 2020).

This study identified eight genes that were upregulated and connected to a possible promoter region that was de-methylated as a result of exposure to DMN. Future study could aim to identify definitively if these genes are connected to the identified regions of altered methylation, indicating that those regions do serve as promoter regions for the genes. Additionally, future

research should be conducted to analyze changes in expression of PRC proteins, as well as the Polycomb complex's possible role in DMN induced sprout inhibition.

Conclusion

Though exposure to 1,4-dimethylnaphthalene induces gene expression changes in *S. tuberosum*, our study indicates that DMN is likely not acting through solely altering DNA methylation. DMN exposure did result in global hypomethylation, but the changes in gene expression were not consistently upregulated, indicating that the hypomethylation may not affect the expression level of many significant genes. Rather, DMN may be acting through other methods of altering gene expression, such as through histone modification by the Polycomb repressive complex or through cell signaling pathways, if DMN acts hormonally.

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ACADEMIC VITA

EMILY EISS

EDUCATION

The Pennsylvania State University, Erie, PA — Expected 5/2021
Bachelor of Science in Biology — Schreyer Scholar
Focus on Cellular and Molecular Biology and Biochemistry — Behrend Honors Program
Minors in Psychology and Chemistry

RESEARCH / LABORATORY EXPERIENCE

Status of the methylome in *Solanum tuberosum* tubers treated with sprout inhibitor 1,4-dimethylnaphthalene (2019-current)
- Advisor: Dr. Michael Campbell
- Schreyer Honors College senior thesis project.
- Observed changes in potato methylome as a result of exposure to 1,4-dimethylnaphthalene, a chemical that induces dormancy in potato tubers by unknown methods.
- Performed tissue collection, DNA isolation, and analysis of bisulfite sequencing data using BISMARK via CoGe.
- Scheduled to present at 2021 Sigma Xi Undergraduate Research Conference, 2021 Undergraduate Exhibition through Penn State University Park, and the 2021 Potato Expo.

Population genetics of spotted salamanders (*Ambystoma maculatum*) across a fragmented landscape (2018-2020)
- Advisor: Dr. Adam Simpson
- Analyzed microsatellite information from populations of spotted salamanders in different locations to determine speciation
  and population dynamics impacted by environmental changes.
- Performed tissue collection, DNA isolation, multiplex PCR, manual genotyping of capillary electrophoresis peaks, and data analysis with FSTAT.
- Presented virtually at the 2020 Undergraduate Exhibition through Penn State University Park; was scheduled to present at 2020 Sigma Xi Undergraduate Research Conference (cancelled due to pandemic).

Genetic Barcoding: A Survey of Invertebrate DNA and Analysis of Methods (2018-2019)
- Advisor: Dr. Adam Simpson
- Performed DNA extraction, purification, and sequencing; culminated in a set of local invertebrates identified by their genetic barcode of the cytochrome oxidase c gene.
- Presented at 2019 Sigma Xi Undergraduate Research Conference (Erie, PA).

GRANTS AWARDED

Status of the methylome in *Solanum tuberosum* tubers treated with sprout inhibitor 1,4-dimethylnaphthalene
- Penn State Behrend Undergraduate Student Academic Year Grant; $1200 — 2020
- Erickson Discovery Grant; $3500 — 2020
- Summer Undergraduate Remote Research Grant; $3200 — 2020
- Summer Undergraduate Research Fellowship (cancelled due to COVID-19); $4000 — 2020
- Schreyer Honors College Grant; $300 — 2019
- Penn State Behrend Undergraduate Student Academic Year Grant; $1200 — 2019

Population genetics of spotted salamanders (*Ambystoma maculatum*) across a fragmented landscape
- Penn State Behrend Undergraduate Student Academic Year Grant; $1200 Fall 2018

Genetic Barcoding: A Survey of Invertebrate DNA and Analysis of Methods
- Penn State Behrend Undergraduate Student Academic Year Grant; $1200 Spring 2018

LEADERSHIP / SERVICE EXPERIENCE

Behrend Honors Student Association, President (2018-current) 2017-current
- Coordinated club activities, including requesting catering, reserving meeting spaces, and co-planning with other clubs.
- Recruited new members by speaking at Honors events throughout academic year.

Beta Beta Beta – Biology Honor Society, Rho Omicron Chapter President 2019-current
- Communicated with club members to coordinate events and recruitment at campus-wide level.
- Facilitated and encouraged academic and extracurricular development of club members, including research and community service activities.

Science Ambassador 2020-current
- Served as a representative for Penn State Behrend's School of Science to prospective students.
- Contributed significant hours of service to the School of Science through outreach events to various age groups (from pre-school to late high school).

Laboratory Teaching Assistant 2018
- BIOL 220W: Populations and Communities under Dr. Adam Simpson.
- Assisted professor in answering student questions and grading assignments.

EMPLOYMENT

Peer Tutor; Penn State Behrend Learning Resource Center 2019-current
- Tutored students of all academic years in science, mathematics, and writing.
- Participated as a writing tutor in specialized research project focused on improving the writing methods of plastic engineering students (NSF Grant Award No. 2013496).

Behavioral Health Technician; Millcreek Community Hospital – LECOM Health 2019-2020
- Promoted from Observation Tech in fall 2019.
- Provided direct patient care and support in psychiatric in-patient setting, such as explanation of medication, emotional support, and providing protection for patients (from self or others).
- 800+ hours of patient observation and care since May 2019.

In-Home and Community Support Staff; Barber National Institute 2018-current
- Provided 1-1 assistance to a college-age individual with autism to work on progressing toward his personal goals in education, independence, and working toward his career.

Resident Assistant; Penn State Behrend Residence Life 2018-2020
- Oversaw a residential hall floor of approximately 40 undergraduate students in an honors-specific residence hall.
- Planned programs according to residence life curriculum to enrich student experience and build community.

HONORS AND AWARDS

- Council of Fellows Undergraduate Research Award	2021
- Academic Excellence in Biology Award	2021
- Phillips Scholarship for Schreyer Scholars	2020
- Outstanding Writing Tutor Award	2020
- Kochel Leadership Scholarship	2019-2020
- Murry L Meiselman Scholarship	2019
- Accepted to Schreyer Honors College	2019
- Certificate of Completion from Penn State Behrend's Honors Program	2019
- Aaron Meehl Biology Award (Outstanding Rising Junior in Biology)	2019
- Robert M. and Elizabeth Q. Mehalso Scholarship	2019
- Elizabeth Smiley Scholarship of The Corry Community Foundation	2017, 2020
- Arloween Zurn Todd Leadership Scholarship	2017, 2019
- Chancellor's Scholars Program Scholarship	2017-2020
- Dean's List, Pennsylvania State University (>3.5 semester GPA)	2017-2020
- Terry Parker Memorial Scholarship of The Corry Community Foundation	2017
- Edward M. and Irene Fisher-Allen Scholarship	2017
- Virgil G. Curtis Scholarship	2017
- Corry Area High School Class of 2017 Salutatorian	2017