Potato Research
https://doi.org/10.1007/s11540-024-09772-7

The Sprout Inhibitor 1,4-Dimethylnaphthalene Results in Common Gene Expression Changes in Potato Cultivars with Varying Dormancy Profiles

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Received: 21 February 2024 / Accepted: 11 July 2024

Abstract

Sprout suppression is a crucial aspect of maintaining postharvest *Solanum tuberosum* (potato) tuber quality. 1,4-dimethylnaphthalene (DMN) has demonstrated effective sprout suppression during long-term storage of potatoes. Its mode of action, however, remains unknown, and previous studies utilizing single cultivars preclude identification of a common response to treatment. Thus, the goal of this study was to identify common transcriptomic responses of multiple potato cultivars of varying dormancy lengths to DMN exposure during two dormancy stages. RNA-seq gene expression profiling supported differing sensitivity to DMN treatment dependent upon cultivar and dormancy stage. A limited number of genes with similar expression patterns were common to all cultivars. These were primarily identified in eco-dormant tubers and were associated with cell cycle progression, hormone signaling, and biotic and abiotic stress response. DMN treatment resulted in significant upregulation of members of ANAC/NAC and WRKY transcription factor families. Investigation of affected protein-protein interaction networks revealed a small number of networks responsive to DMN in all cultivars. These results suggest that response to DMN is largely cultivar and dormancy stage-dependent, and the primary response is governed by a limited number of stress and growth-related genes and protein-protein interactions.

Keywords DMN · Dormancy · Gene expression · Potato · Sprout regulation · Tuber storage

Abbreviations

BR Brassinosteroids

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Published online: 31 July 2024

CIPC	Chlorpropham
DMN	1,4-dimethylnaphthalene
EN	Endodormant/endodormancy
EC	Ecodormant/ecodormancy
TFT	Transcription factor target
PPI	Protein-protein interactions

Introduction

Potatoes, which consistently rank among the top 5 crops in the world in terms of production, are stored, processed, and marketed in a dormant state. The quality of the crop is greatest when tubers are in an endodormant (EN) state, a period of arrested growth of the meristem regulated by endogenous signals that prevent growth even under ideal conditions (Lang et al. 1987). During EN, glucose and fructose (reducing sugars) are low, while starch content is high (Hu et al. 2023). As tubers enter ecodormancy (EC), a state regulated by exogenous signals (Lang et al. 1987), metabolic activity increases, reducing sugars accumulate, and meristematic sprouts develop. This activity contributes to decreased fresh weight, the "sweetening" of tubers and the potential loss of marketable products. The transition from EN to EC is governed by factors such as genotype and pre- and post-harvest conditions, although the exact mechanisms regulating the transition remain unknown.

Sprout inhibitors and suppressants are commonly utilized to prolong storage and maintain tuber quality. Globally, the most widely used inhibitor is chlorpropham (CIPC) due in part to its comparatively low cost and its demonstrated efficacy. Use of CIPC is associated with reduced starch degradation and reducing sugar accumulation (Khurana et al. 1985; Yang et al. 1999) and weight loss (Blenkinsop et al. 2002). Most importantly, CIPC maintains commercially acceptable chip and fry color quality standards even after several months of storage (Blenkinsop et al. 2002; Krause et al. 2023). However, its use has raised concerns regarding environmental and health effects, which led to the 2020 decision by the European Union to ban CIPC (Visse-Mansiaux et al. 2021a; Thoma et al. 2022).

1,4-dimethylnaphthalene (DMN), a compound naturally found in potatoes (Meigh et al. 1973), is another effective sprout suppressant (Beveridge et al. 1981) and promising alternative to CIPC (Yang et al. 1999; Nyankanga et al. 2018; Visse-Mansiaux et al. 2021b; Krause et al. 2023). While the mechanism by which DMN suppresses sprout growth remains unknown, recent studies have found that DMN halts cell cycle progression of dormant potatoes after the G1/S phase transition (Campbell et al. 2010, 2012), induces expression of WRKY transcription factors (Campbell et al. 2012; Campbell and D'Annibale 2016), and is associated with upregulation of multiple genes involved in biotic and abiotic stress responses (Campbell et al. 2020).

Although these studies have elucidated upon the response of dormant potatoes to DMN treatment, they were limited in their use of cultivars, each only utilizing a single long-dormancy cultivar. Thus, it remains unknown whether the changes induced are consistent among various cultivars of differing dormancy lengths. The goal of

this study was to describe transcriptomic changes of multiple potato cultivars of varying dormancy lengths following DMN treatment in two stages of dormancy.

Materials and Methods

Plant Material

Field grown potato cultivars were gifted by commercial producer Troyer Farms of Waterford, Pennsylvania, USA, in the fall of 2015, 2021, and 2022. Following harvest, tubers were held at ambient temperature (approximately 15–18 °C) for 1 week to promote wound healing after which they were transferred to Penn State University for long term storage at 7°C with 90% relative humidity. Cultivars used in this study were short dormancy Colomba (2022), medium-long dormancy La Chipper (2015), and long dormancy Lamoka (2021). La Chipper and Lamoka tubers were grown for chipping purposes and Colomba was grown for seed. Tubers were not treated with sprout suppressants prior to or upon receipt.

Treatments

Tubers were treated at two cultivar-dependent stages of dormancy, EN and EC. Tubers were defined as EN upon initial storage, shortly after harvest. To determine the end of EN, and confirm EC status, several untreated tubers of a given variety were periodically removed from storage and incubated at 22°C for 1 week. Termination of dormancy was defined as peeping of tuber meristems following incubation (Campbell et al. 2010).

Each treatment involved approximately eight tubers, placed in a single layer at the bottom of a 9.5 Liter BBL GasPak chamber (MG Scientific, Pleasant Prairie, WI, USA), exposed to either water (control) or DMN at a rate of 67.5 µl per chamber, approximately 7.11 µl per liter of head space, with six replicates per treatment. Chambers were stored at 25°C in dark conditions for two days. Following treatment, tuber meristems were immediately harvested using a 1-mm micro curette, frozen in liquid nitrogen, and stored at −80°C (all cultivars).

RNA-Seq

Total RNA was extracted from frozen meristems using a Zymo Quick-RNA Plant Kit (Orange, California, USA) according to kit protocol. Extracted RNA was quantified on a Thermo Scientific NanoDrop One (Thermo Fisher Scientific, Waltham, MA, USA) and sent to the Penn State University Nucleic Acid Core Facility for quality assessment using an Agilent 2100 Bioanalyzer. Samples were sequenced using an Illumina HiSeq generating 150 bp single-end reads for the 2015 data, and Illumina NextSeq generating 100 bp single-end reads for all 2021 and 2022 data. The 2015 La Chipper analysis was completed using four biological replicates per treatment and dormancy state, while the 2021 Lamoka and 2022 Colomba analyses

were completed for three biological replicates per treatment and dormancy state (Table S1).

Differential expression analysis was performed on the Galaxy (2022) platform, where sequences were assessed for quality using FastQC, then mapped to the double monoploid potato *S. tuberosum* genome assembly version 6.1 (Pham et al. 2020) using RNASTAR. Differential expression analysis was performed using DESeq2 and tested for significance with a q value of <0.05 and a log2 fold change absolute value >1. The double monoploid genome, genome annotation, and GO category annotations were downloaded from the Spud DB database (Hirsch et al. 2014).

Gene Set Enrichment Analysis (GSEA)

The Plant Gene Set Enrichment Analysis (GSEA) Toolkit (Yi et al. 2013) was used to identify enriched gene ontology (GO) biological process terms and transcription factor targets (TFT). Differentially expressed *Solanum tuberosum* genes (DEG) were translated to their associated *Arabidopsis thaliana* locus IDs as defined by the SpudDB working gene models for the current potato genome. These IDs were queried against the *A. thaliana* gene set using Fisher's exact test with Benjamini-Hochberg correction for multiple testing at a value of $q<0.01$. Analysis was performed for each dormancy state for all cultivars, as well as for lists of genes observed with similar expression patterns in all cultivars in a given dormancy state.

Protein-Protein Interaction (PPI) Network Analysis

Network analysis of significant DEGs was performed to describe protein-protein interactions (PPI) using Cytoscape v3.10.1 (Shannon et al. 2003). DEGs were translated to their associated *A. thaliana* locus IDs, as described by SpudDB, and queried against the *A. thaliana* species set in the STRING database version 2.0.1 (Doncheva et al. 2019). Networks with a confidence score >0.90 were visualized.

Results

RNA-Seq Mapping and Gene Expression

Sequencing of the 2015 La Chipper samples produced 165,769,684 raw reads, while sequencing of the 2021 Lamoka and 2022 Colomba samples produced 585,053,964 and 460,312,027 raw reads, respectively. Sequences were assessed for quality prior to mapping, and it was determined that no sequences required trimming based upon FastQC analysis. Following mapping, 101,700,000 (approximately 61.4%) of La Chipper reads, 514,324,979 (approximately 88.2%) of Lamoka reads, and 419,421,856 (approximately 91.1%) of Colomba reads mapped uniquely. Of uniquely mapped reads, 89,400,000 (approximately 87.9%) from La Chipper, 458,110,000 (approximately 89.1%) from Lamoka, and 249,824,650 (approximately 59.6%) from Colomba were uniquely assigned to genomic features. The remaining

reads were unassigned due to failure to map, ambiguities, no features associated with the reads or multi-mapping of the reads.

In total, 7642 DEGs were identified in DMN-treated tubers when compared to controls. Of these, 2310 and 1696 were downregulated and upregulated, respectively, in response to DMN during EN, while 3051 and 3935 were downregulated and upregulated, respectively, during EC. Seven hundred twenty-five genes were uniquely expressed in EN tubers and 3587 were uniquely expressed in EC tubers. Pronounced differences in gene expression patterns were observed between the EN and EC stages of La Chipper and Lamoka tubers. For these cultivars, significantly more genes showed a response to DMN during EC than during EN (Table 1).

Small percentages of genes within each cultivar showed similar expression patterns in both dormancy states. Specifically, approximately 0.10% of La Chipper and 13.6% of Lamoka downregulated genes were common to both dormancy stages, while 0.30% of La Chipper and 11.6% of Lamoka upregulated genes were observed in both dormancy stages (Table S2). Colomba gene expression patterns did not exhibit the large differences between EN and EC that were observed for the other cultivars (Table 1; Table S2), such that 58.1% of genes were downregulated and 59.2% were upregulated in both stages. When considering all three cultivars, 656 genes exhibited the same expression pattern in each dormancy state. Approximately 0.5% (12 genes) of all downregulated genes (Fig. 1a) and 0.9% (16 genes) of all upregulated genes (Fig. 1b) showed similar expression patterns in all cultivars during EN. Of these, only three downregulated genes were observed exclusively during EN, while the rest were observed in both dormancy stages. In contrast, approximately 9.7% (296 genes) of downregulated genes (Fig. 1c) and 9.1% (357 genes) of upregulated genes (Fig. 1d) were common to all cultivars in the EC state, of which 287 and 341, respectively, were exclusive to EC.

A limited number of genes were observed in the subset of genes shared by all EN, and as such, no gene function patterns were observed (Table S3). Numerous patterns were observed in the EC subset, however. Eighteen genes were associated with ethylene response and signaling, of which two were downregulated and 16 were upregulated (Table S3). Six potato homologs within the ethylene response factor (ERF) family (ERF1/AT3G23240; ERF-1/AT4G17500; ERF2/AT5G47220; ERF106/AT5G07580; ERF114/AT5G61890), three HCHIB (AT3G12500) homologs, and three pathogenesis-related 4 (PR4/AT3G04720) homologs were upregulated in DMN-treated tubers. Additionally, 12 potato genes related to cytokinin signaling, brassinosteroid biosynthesis, and cell cycle progression were downregulated following DMN treatment, while five genes related to negative regulation

Table 1 Count and regulation pattern of genes significantly induced or suppressed by DMN treatment in three potato cultivars during two stages of dormancy

	Endodormant		Ecodormant	
Cultivar	Up	Down	Up	Down
La Chipper	47	13	1721	1815
Lamoka	231	179	1516	757
Colomba	1942	2253	2474	1898

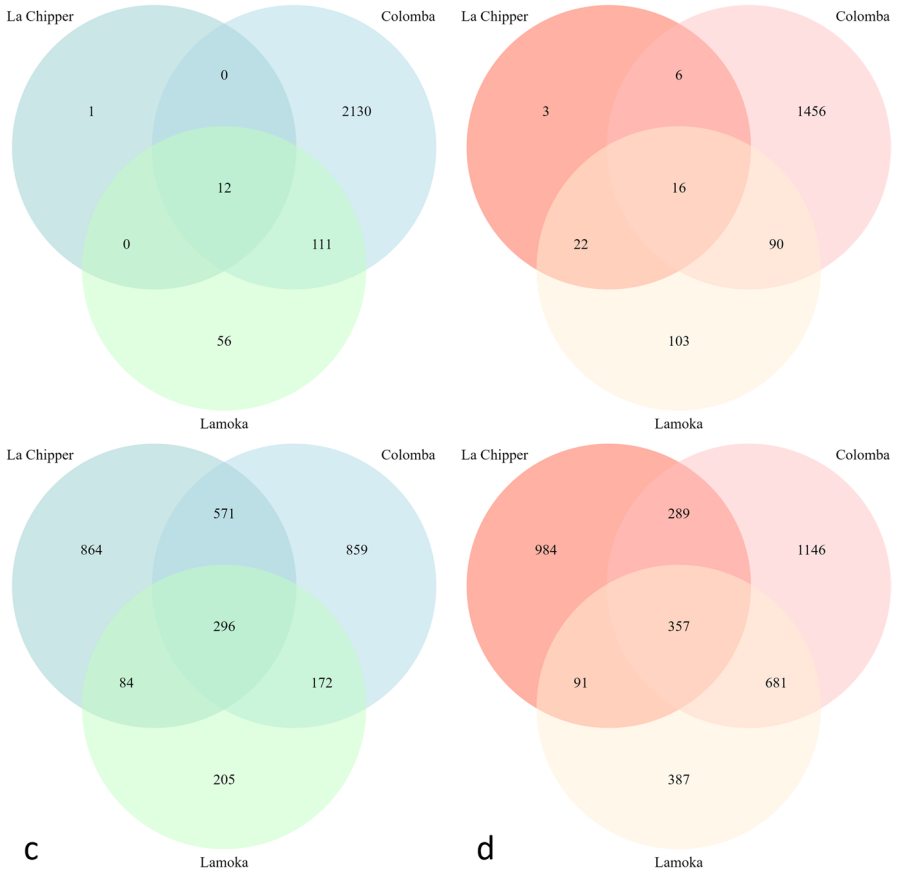

Fig. 1 Statistically significant changes to gene expression of three potato cultivars in response to DMN treatment during two stages of dormancy. Counts shown are downregulated (**a**) or upregulated (**b**) during EN, and downregulated (**c**) or upregulated (**d**) during EC at a significance q-value < 0.05 and a $\log_2$ fold change absolute value > 1

of cytokinin response were upregulated (Table S3). Of note, expression of cell cycle homologs of F-Box-Like protein 17 (FBL17/AT3G54650), Cell Division Cycle Protein 20.1 (CDC20.1/AT4G33270), and cyclin 3B (CYC3B/AT5G11300), CYCD3;2 (AT5G67260), and CYCB1;1 (AT4G37490) was suppressed by DMN, while expression of three homologs of KISS ME DEADLY 1 (KMD1/AT1G80440) and two of KMD2 (AT1G15670) was enhanced by DMN treatment. Fourteen genes related to auxin signaling and response genes were downregulated, and two were upregulated (Table S3). This included two downregulated homologs of PIN1 (AT1G73590), and one each of indole-3-acetic acid inducible 14 (IAA14/AT4G14550), YUCCA8 (YUC8/AT4G28720), and WUSCHEL-related homeobox 1 (WOX1/AT3G18010). Twenty potato genes were observed to exhibit similar expression patterns in both dormancy states (Table S3). Six genes were downregulated following DMN treatment, of which three were homologs of AT3G45770, a gene related to fatty acid

biosynthesis. The remaining 14 genes were upregulated in both dormancy stages. These were primarily associated with flavonoid and lignin biosynthesis (GT72B1/AT4G01070, HCT/AT5G48930, MYB48/AT3G46130, and TT7/AT5G07990), and stress response (AT2G47710, AtKTI5/AT1G17860, DjC53/AT1G56300, GAD/AT5G17330, and GT72B1/AT4G01070). Three homologs of CYP76G1 (AT3G52970), a gene associated with terpenoid metabolism, were also upregulated following DMN treatment.

Functional Analysis of Differentially Expressed Genes

In EN tubers of individual cultivars, a total of two GO categories related to biological processes were identified as significantly downregulated and 26 as upregulated in response to DMN (Table S4). No significant GO categories were observed in EN La Chipper tubers. However, both downregulated and 24 upregulated terms were shared by the Lamoka and Colomba cultivars. Downregulated GO categories were associated with cell specification and commitment, while upregulated terms were associated with defense and immune responses, as well as response to heat, fungi, light intensity, and biotic, temperature, water, ethylene, jasmonic acid (JA), and abscisic acid (ABA) stimuli. When considering the EN subset, no GO categories were identified as either significantly upregulated or downregulated.

In EC tubers, 28 GO categories were downregulated in total, of which five were shared by all three cultivars, two by Lamoka and Colomba, nine by Lamoka and La Chipper, and 12 by La Chipper and Colomba (Table S4). A total of 162 GO categories were upregulated, of which 101 were observed for all three cultivars. The remaining upregulated GO categories were all shared between two cultivars, such that 40 were shared by Lamoka and Colomba, 17 by Lamoka and La Chipper, and four by La Chipper and Colomba (Table S4). Downregulated terms common to all cultivars were associated with cuticle development, very-long-chain fatty acid metabolic processes, response to UV-B, and response to sucrose and brassinosteroid stimuli. Upregulated GO categories observed in all cultivars were associated with immune, defense, biotic, and abiotic stress responses. Specifically, response to hypoxia, fungus, ethylene, salicylic acid (SA), and JA stimuli, inorganic substances, metal ions, chitin, nitrate, endoplasmic reticulum stress, and oxygen were upregulated. Metabolic processes of ethylene, SA, flavonoid, reactive oxygen species, hydrogen peroxide, and toxins were positively enriched, as were biosynthetic processes of lignin, alkene, ethylene, and SA. Additionally, GO categories associated with aging, senescence, cell death, and MAPK cascade, and signaling pathways of JA, SA, ABA, and ethylene were identified as significantly upregulated.

In the EC gene subset, 18 downregulated and 80 upregulated GO categories were identified in response to DMN (Table S4). Downregulated terms were associated with anatomical structure development and morphogenesis, flower, cuticle, and organ development, developmental processes, very long chain fatty acid and lipid metabolic processes, and multidimensional cell growth. Upregulated terms, on the other hand, were similar to those observed in the individual cultivars and were associated with cell death, response to ethylene, JA, SA and ABA stimuli, response to

salinity, water, and wounding, and SA, phenylpropanoid, and ethylene biosynthesis and metabolism. Additional upregulated terms included defense and immune responses, systemic acquired resistance, and response to biotic stimulus, bacterium, and fungus.

Transcription Factor Target Enrichment Analysis

A total of 18 TFTs were downregulated and 29 upregulated in response to treatment with DMN at a significance cutoff value of $q<0.01$ during EN (Table S4). As with functional analysis of DEGs, no TFTs were significantly down- or upregulated in La Chipper tubers at this stage, and instead, all were shared by Lamoka and Colomba.

In EC tubers, a total of 17 TFTs were downregulated and 63 were upregulated (Table S4). Of the downregulated targets, five were observed in all cultivars, nine in Lamoka and Colomba, two in Lamoka and La Chipper, and one in La Chipper and Colomba cultivars. Of the upregulated targets, 36 were observed in all cultivars, 20 in Lamoka and Colomba, two in Lamoka and La Chipper, and five in La Chipper and Colomba.

AGAMOUS-LIKE15 (AGL15) and AGAMOUS-LIKE63 (AGL63), both members of the MIKC subfamily of MADS-box domain transcription factors, were significantly suppressed following DMN treatment in EN and EC, respectively. Numerous NAC domain protein family members were significantly induced in response to DMN, 10 in EN and 12 in EC, only two of which were observed in both dormancy states, ANAC092 and NAC2. Members of the WRKY gene family were the most highly represented of the upregulated TFTs for both dormancy states. In total, 24 WRKY TFTs were identified, of which 13 were significant during both EN and EC.

No significant TFTs were identified in the EN subset of genes. Seventy-one TFTs were downregulated in the EC subset (Table S4), of which MYB protein family members were the most highly represented. Additional downregulated targets of interest included APETALA 1 (AP1) and AP3 binding sites and AP2 targets, and phytochrome-interacting transcription factor 5 (PIF5) binding sites. One hundred eleven TFTs were upregulated, 30 of which were members of the ANAC/NAC protein family, and 23 of which were from the WRKY gene family. Other noteworthy TFTs included SOMBRERO (SMB), and cryptochrome 2 (CRY2), ethylene-insensitive3 (EIN3) and LEAFY binding sites.

Protein-Protein Interaction Network Analysis

DEGs were translated to their related *A. thaliana* proteins for PPI network analysis. It is important to note that many potato genes currently do not have associated *A. thaliana* genes or proteins, thus analysis was limited to those described to date. Only the largest subnetworks for each condition are reported herein.

The EN and EC gene subsets were used to identify significant PPI networks shared by all cultivars. No PPI networks were identified in down- (Fig. 2a) or upregulated genes (Fig. 2b) of EN tubers. However, a limited number of small networks were identified for down- (Fig. 2c) and upregulated genes (Fig. 2d) in EC tubers.

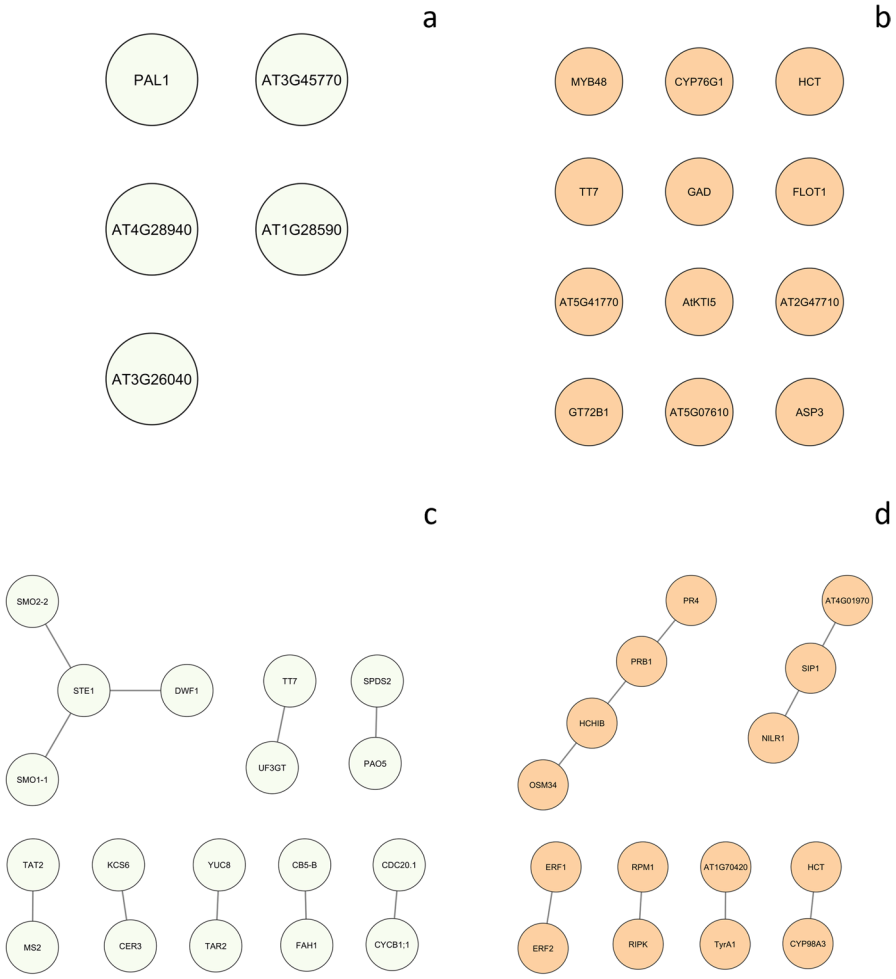

Fig. 2 PPI networks shared by three potato cultivars following DMN treatment during two stages of dormancy. Shown are downregulated (**a**) and upregulated (**b**) endodormant networks, and downregulated (**c**) and upregulated (**d**) ecodormant networks

The largest downregulated network was associated with brassinosteroid biosynthesis (Fig. 2c), while smaller two-protein networks were associated with flavonoid and anthocyanin, auxin, and wax biosynthesis, polyamine synthesis and conversion, and cell cycle progression (Fig. 2c). The largest upregulated and second largest networks were associated with pathogen and stress response, respectively (Fig. 2d).

PPI networks of individual cultivars were also considered. During EN, no networks were identified from downregulated genes of La Chipper (Fig. 3a). However, a network related to cell cycle progression was observed during EC for this cultivar (Fig. 3b). EN Lamoka tubers had a two-protein network related to flavonoid biosynthesis (Fig. 3c) and a larger network associated with photosynthesis in EC (Fig. 3d). Colomba had the largest downregulated network during EN, which was composed of clusters related to cell cycle progression, and sterol, flavonoid, and phenylpropanoid

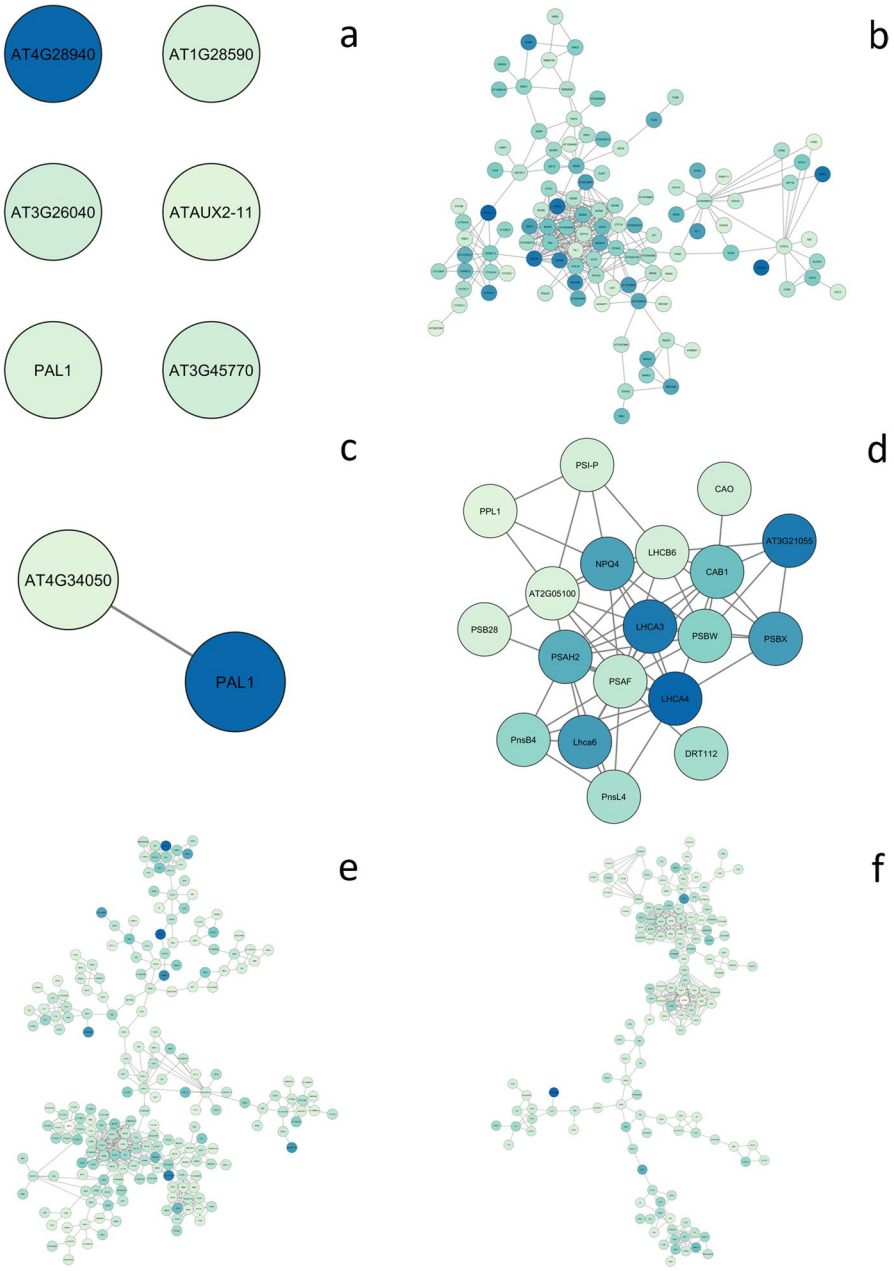

Fig. 3 PPI subnetworks downregulated in response to DMN. The largest subnetworks are portrayed for endodormant (**a**) and ecodormant (**b**) La Chipper, endodormant (**c**) and ecodormant (**d**) Lamoka, and endodormant (**e**) and ecodormant (**f**) Colomba cultivars. All networks identified using Cytoscape with confidence scores >0.90

biosynthesis (Fig. 3e). Similarly, EC Colomba tubers had a network composed of clusters related to cell cycle progression and sterol, flavonoid and phenylpropanoid biosynthesis, as well as photosynthesis (Fig. 3f).

A simple two-protein network was identified from upregulated genes of EN La Chipper tubers, which was associated with anthocyanin production (Fig. 4a). Three

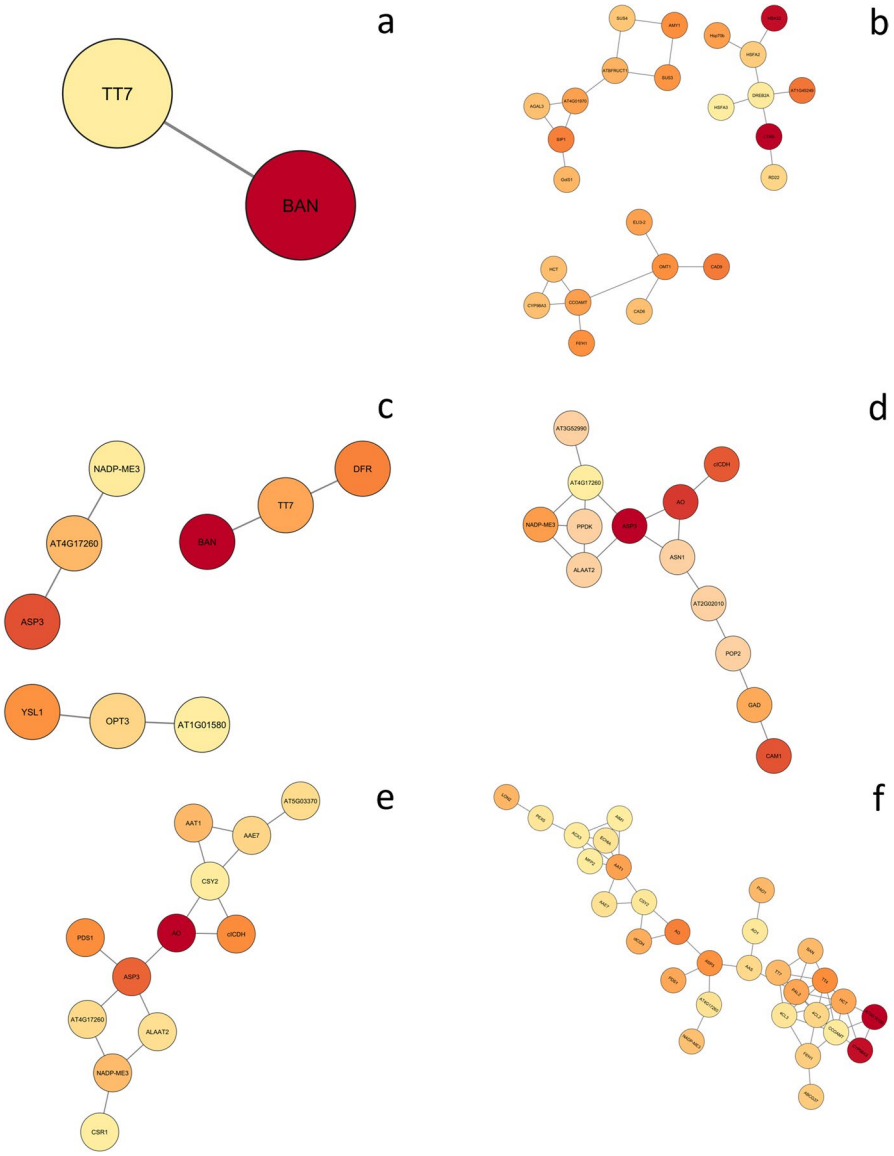

Fig. 4 PPI subnetworks upregulated in response to DMN. The largest subnetworks are portrayed for endodormant (**a**) and ecodormant (**b**) La Chipper, endodormant (**c**) and ecodormant (**d**) Lamoka, and endodormant (**e**) and ecodormant (**f**) Colomba cultivars. All networks identified using Cytoscape with confidence scores >0.90

networks of equal size were observed during EC for this cultivar. These networks were associated with starch production and abiotic stress response, water and temperature stress, and lignin biosynthesis (Fig. 4b). EN Lamoka tubers produced three small three-protein networks. These were associated with anthocyanin production, metal ion transport, and glycolysis (Fig. 4c). The largest subnetwork observed for this cultivar during EC was composed of proteins related to glycolysis and stress response (Fig. 4d). The EN subnetwork of Colomba tubers was primarily composed of proteins associated with stress response and glycolysis (Fig. 4e), while the EC subnetwork had clusters associated with JA biosynthesis, glycolysis, and stress response (Fig. 4f).

Discussion

Differentially Expressed Genes Vary by Cultivar and Dormancy State in Response to DMN

Gene expression changes in response to DMN were both cultivar and dormancy state-dependent, though cultivation year, the effect of which could not be directly assessed, may also have played a role. Both La Chipper (2015) and Lamoka (2021), which are medium-long to long dormancy varieties respectively, exhibited low response to DMN during EN, while Colomba (2022), a short dormancy variety, exhibited pronounced response to the treatment. In contrast, La Chipper and Lamoka showed increased response during EC, while the response of Colomba during this stage was similar to that observed during EN. These differences in gene expression levels and response suggest the short dormancy Colomba may have never fully entered an EN state following harvest. Additionally, although thousands of genes responded to DMN, particularly in the EC state, these were primarily cultivar specific and only a small fraction of genes exhibited similar expression patterns in all cultivars. These results agree with previous research which demonstrated differential response of tubers to DMN treatment dependent upon dormancy state (Campbell et al. 2020). Therefore, to elucidate the primary effect of DMN, the following discussion will be limited to responses which were common to all cultivars.

Ethylene Response Is Significantly Induced by DMN

Ethylene has been paradoxically shown to both suppress meristem growth and shorten tuber dormancy, with consistent exogenous exposure suppressing growth (Rylski et al. 1974; Prange et al. 1998) and cessation of exposure resulting in growth resuming almost immediately (Rylski et al. 1974; Foukaraki et al. 2016). In this study, DMN exposure during EC induced expression of numerous ethylene response (ERF1, ERF-1, ERF2, ERF106, PR4) and signaling (HCHIB, also known as PR3) genes but was not found to induce genes with confirmed roles in ethylene biosynthesis. While the lack of genes related to ethylene biosynthesis supports previous research which observed only a small transient increase in ethylene production following DMN treatment (Suttle 2003), it is important to note that ethylene

concentration was not quantified in the present study and changes to its biosynthesis, therefore, cannot be confirmed. Future studies to assess ethylene concentrations following DMN exposure may help to elucidate the potential relationships between DMN and ethylene biosynthesis and response.

Although the mechanisms by which ethylene suppresses sprout growth remain unknown, exogenous exposure has been observed to induce expression of genes related to stress and defense (Yang et al. 2020; Tosetti et al. 2021). ERF (Thirugnanasambantham et al. 2015) and PR genes (Islam et al. 2023) have known roles in abiotic and biotic stress response, and expression of multiple genes from both families has been observed to increase following ethylene exposure (Yang et al. 2020). No previous studies of dormant tubers treated with DMN have noted changes to ERFs. However, DMN treatment has been observed to induce a stress response (Campbell et al. 2010, 2012, 2020; Campbell and D'Annibale 2016) and increase expression of PR4 and PR5 (Campbell et al. 2020). Together, this suggests that DMN and ethylene act on some of the same pathways to suppress sprout development.

DMN Affects Cell Cycle Signaling, Cell Division, and Expansion

Previous studies of dormant tubers exposed to DMN treatment have observed changes in expression of genes related to cell cycle regulation (Campbell et al. 2010, 2012, 2020; Campbell and D'Annibale 2016). Here, we observed downregulation of gene sets involved in anatomical structure development and morphogenesis, developmental processes, and multidimensional cell growth. This included several genes involved in cell cycle regulation, including CDC20.1, CYC3B, CYCB1;1, CYCD3;2, and FBL17, as well as two genes involved in cytokinin signaling, KMD1 and KMD2, and four involved in auxin biosynthesis, signaling and response, PIN1, IAA14, YUC8, and WOX1. Importantly, these genes exhibited similar expression patterns in all three evaluated EC cultivars, highlighting the potential importance of these genes in sprout growth suppression induced by DMN treatment.

Although previous research utilizing DMN suggested the sprout suppressant blocks cell division after the G1/S phase transition (Campbell et al. 2010), current results suggest that it suppresses cell cycle progression at multiple stages. CYCD3;2 (Swaminathan et al. 2000) and FBL17 (Noir et al. 2015), genes which regulate the G1/S phase transition, were downregulated following DMN exposure. CYCD3;2, an important rate-limiter of cytokinin response, plays a role in the regulation of cell number in developing lateral organs (Dewitte et al. 2007) and may be especially important for vascular development (Collins et al. 2015). Reduced expression of CYCD3;2 contributed to upregulation of KIP-Related Protein 1 (KRP1), KRP2, E2Fa, E2Fb, cyclin-dependent kinases (CDK) and CDK subunits in *Dimocarpus longan* embryos (Zhao et al. 2022), and loss of FBL17 function induced DNA-damage responses and contributed to KRP2 accumulation in *A. thaliana* (Gentric et al. 2020). Previous research has identified significant increases in expression of KRP1 and KRP2 following DMN exposure (Campbell et al. 2012), and although the current study did not observe significant changes to these genes across all cultivars, decreased expression of genes involved in their regulation was a common response. CDC20.1 (Kevei et al. 2011), CYC3B, and CYCB1;1 (Ito

2000), which play important roles in regulation of G2/M phase transition, were also downregulated in all cultivars following DMN treatment. CDC20, which interacts with anaphase promoting complex subunits, is co-expressed with its B-type cyclin targets, including CYCB1;1 (Yang et al. 2017). Reduced expression of CYCB1;1 was related to reduced growth and yield parameters in tomato (Liu et al. 2018) and delayed cellularization and reduced viability of rice (Guo et al. 2010).

Cytokinin levels and sensitivity are low in dormant potatoes and increase with dormancy release (Turnbull and Hanke 1985; Suttle 2004) to promote cell division. In the current study, two negative regulators of cytokinin signaling were significantly upregulated in response to DMN. KMD1 and KMD2 are thought to directly interact with and destabilize type-B *Arabidopsis* response regulators (ARR), specifically ARR1 and ARR12 leading to cytokinin insensitivity (Kim et al. 2013a, b). Increased expression of KMD1 and KMD2, as well as KMD4, has been documented to occur as floral development ceases and overexpression of KMD2, specifically, was found to accelerate meristem arrest in *A. thaliana* (Martínez-Fernández et al. 2020). These findings were consistent with previous research in which overexpression of KMD1 and KMD2 resulted in a dwarf phenotype and premature termination of primary root growth (Kim et al. 2013a). Taken together, expression of KMD1 and KMD2 plays an important role in regulation of meristem growth and development.

Like cytokinin, auxin concentration is crucial for cell division and expansion (Perrot-Rechenmann 2010), but its role in potato dormancy is not well understood. However, an increase in free and conjugated IAA concentration was seen in tuber meristems at dormancy release (Sorce et al. 2000), and genes related to auxin biosynthesis, transport, and signaling upregulation have been observed in dormant potatoes after treatment with GA3 (Hartmann et al. 2011). In EC tubers treated with DMN, we observed down regulation of YUC8, a key enzyme in auxin biosynthesis, as well as PIN1 and WOX1, regulators of auxin transport. WOX1, WOX3, and WOX5 have been implicated as having potential redundant roles in auxin biosynthesis necessary for leaf expansion through positive regulation of YUC genes (Zhang et al. 2020), and overexpression of WOX1 was correlated with significant upregulation of PIN1 and AUX1 expression at the shoot apical meristem of *A. thaliana* (Nakata et al. 2018). Localization of PIN1 is crucial for proper organogenesis and positioning in both the root and the shoot (Vernoux et al. 2000; Heisler et al. 2010; Krogan et al. 2016). The results of this study suggest DMN halts cell cycle progression and expansion through negative regulation of auxin and cytokinin signaling in potato meristems. Further studies quantifying auxin and cytokinin levels following DMN treatment are needed to elucidate whether the observed changes in hormone signaling are correlated with changes to endogenous concentrations.

DMN Elicits Water Stress Response in Dormant Tubers

Previous research has observed increases in transcripts and gene sets related to multiple biotic and abiotic stress responses following DMN exposure (Campbell et al. 2010, 2012, 2020). In the current study, DMN treatment induced numerous stress-related

biological processes. Importantly, DMN treatment resulted in upregulation of gene sets related to cell death, and responses to salt and water. These results support previous work which identified transcripts associated with salt stress, water deprivation (Campbell et al. 2010, 2012), and osmotic stress (Campbell et al. 2012, 2020) in DMN-treated tubers. DMN treatment also induced biological processes related to hormonal response. Specifically, responses to SA, JA, ABA, and ethylene were upregulated following treatment. Similar results were observed when DMN elevated gene sets associated with SA and JA (Campbell et al. 2020). JA and SA are important regulators of plant defense signaling that experience considerable positive and negative crosstalk (Clarke et al. 2000; Tamaoki et al. 2013; Caarls et al. 2015). ABA and ethylene are known to be involved in dormancy induction and maintenance (Suttle and Hultstrand 1993; Li et al. 2021), as well as response to biotic and abiotic stresses (Cao et al. 2006; Yan et al. 2016; Gietler et al. 2020). All these hormones have also been found to play important roles in drought response (Manavella et al. 2006; Arraes et al. 2015; Sharma et al. 2017; Wang et al. 2021). Tubers lose weight during storage from transpiration and respiration activity, which is partially mitigated by sustaining low temperatures and high relative humidity to reduce the vapor pressure difference at the surface of the tubers. Tubers treated with DMN experience less weight loss in storage than untreated tubers (Krause et al. 2023), suggesting reduced water loss. Because DMN is a volatile organic compound that coats the surface of the tuber when applied, it is likely affecting transpiration and vapor pressure perception, signaling the occurrence of a potential water deficit and the need for water conservation.

Defense and Stress Response-Related Transcription Factor Targets Are Induced by DMN

In this study, 111 TFTs were commonly upregulated in all cultivars in response to DMN exposure, of which nearly half were members of the NAC and WRKY families. Both NAC and WRKY are major plant-specific transcription factor families with important roles in biotic and abiotic stress response as well as diverse physiological and developmental processes (Chen et al. 2017; Singh et al. 2021). To date, approximately 110 NAC (Singh et al. 2013) and 79 WRKY genes (Zhang et al. 2017) have been identified in *Solanum tuberosum*.

In a study identifying and describing potato NAC genes and their stress-related responses, salt and drought stresses induced the most genes, followed by heat (Singh et al. 2013). In the current study, 30 ANAC/NAC TFTs were upregulated in response to DMN, 11 of which have been previously found to be induced by drought, heat, salt, wound, and pathogen stresses and hormone or chemical exposure (Table 2; Singh et al. 2013). Of these, ANAC017 and VND6 (ANAC101) both increased expression when exposed to drought, heat, salt, or ABA stress (Singh et al. 2013). NST1 (ANAC043) increased when exposed to drought or salt, and ANAC096 increased when exposed to heat or salt stress (Singh et al. 2013).

Of 72 potato WRKY genes, 63%, 69%, and 74% were found to be upregulated in response to salt, heat, and drought stresses, respectively (Filiz and Kurt 2021). In the current study, 23 WRKY genes were upregulated in response to DMN exposure,

Table 2 Putative abiotic stress responses of ANAC and WRKY transcription factor targets upregulated following DMN exposure

TFT	Stress	Species	Reference
ANAC017	Drought, heat, salt, ABA	*S. tuberosum*	Singh et al. 2013
ANAC018	*P. infestans*	*S. tuberosum*	Singh et al. 2013
ANAC043	Drought, salt, chemical (BTH)	*S. tuberosum*	Singh et al. 2013
ANAC042	Wound	*S. tuberosum*	Singh et al. 2013
ANAC047	Wound, chemical (BTH)	*S. tuberosum*	Singh et al. 2013
ANAC050	Heat	*S. tuberosum*	Singh et al. 2013
ANAC053	Drought	*S. tuberosum*	Singh et al. 2013
ANAC071	Heat	*S. tuberosum*	Singh et al. 2013
ANAC083	GA3	*S. tuberosum*	Singh et al. 2013
ANAC096	Heat, salt	*S. tuberosum*	Singh et al. 2013
ANAC101	Drought, heat, salt, ABA	*S. tuberosum*	Singh et al. 2013
WRKY3	Drought	*S. commersonii*	Villano et al. 2020
WRKY6	Drought, heat, salt	*S. tuberosum*	Zhang et al. 2017
WRKY6	Drought	*S. commersonii*	Villano et al. 2020
WRKY8	Drought, heat, salt	*S. tuberosum*	Zhang et al. 2017
WRKY8	Drought, heat	*S. commersonii*	Villano et al. 2020
WRKY15	Drought	*S. commersonii*	Villano et al. 2020
WRKY18	Drought	*S. commersonii*	Villano et al. 2020
WRKY20	Drought, heat	*S. tuberosum*	Zhang et al. 2017
WRKY22	Drought, heat, salt	*S. tuberosum*	Zhang et al. 2017
WRKY22	Drought, salt	*S. tuberosum*	Filiz and Kurt 2021
WRKY27	Drought, heat, salt, SA	*S. tuberosum*	Zhang et al. 2017
WRKY28	Drought	*S. commersonii*	Villano et al. 2020
WRKY29	Salt	*S. tuberosum*	Filiz and Kurt 2021
WRKY45	Heat	*S. commersonii*	Villano et al. 2020
WRKY50	Heat	*S. commersonii*	Villano et al. 2020
WRKY55	Drought, heat	*S. commersonii*	Villano et al. 2020
WRKY65	Heat	*S. tuberosum*	Filiz and Kurt 2021
WRKY70	Drought	*S. commersonii*	Villano et al. 2020
WRKY75	Drought	*S. commersonii*	Villano et al. 2020

16 of which have documented upregulation in response to salt, heat, drought, SA, or a combination of these stresses in potato species (Table 2; Zhang et al. 2017; Villano et al. 2020; Feliz and Kurt 2021). Expression of WRKY8 increased in response to drought and heat stress (Zhang et al. 2017, Villano et al. 2020) and WRKY6 increased in response to drought stress (Zhang et al. 2017, Villano et al. 2020) in *S. tuberosum* and *S. commersonii*, while expression of WRKY22 increased in response to drought and salt in *S. tuberosum* (Zhang et al. 2017; Filiz and Kurt 2021). These results suggest that DMN significantly induces multiple biotic and abiotic stress responses, the most highly represented of which is response to drought stress.

DMN Response Involves Limited Protein-Protein Interaction

EN tubers treated with DMN did not exhibit changes to subnetworks. However, individual peptides did change in expression across cultivars following DMN treatment. Five peptides were downregulated by DMN in the EN subset (Table 1). Two of these peptides were associated with defense response, oxidative stress, wounding, and response to JA. JA has been linked to tuber initiation (Pelacho and Mingo-Castel 1991; Koda et al. 1991), but it is unclear if that developmental response is linked only to tuberization and not also to the onset of EN or tuber growth arrest. The downregulated genes associated with JA response may suggest a change in JA levels within tubers treated with DMN. DMN also resulted in the downregulation of a peptide with similarity to At3g45770, which has been assigned to the GO function of fatty acid biosynthesis and fatty acid metabolism. DMN is a highly hydrophobic molecule, and it is expected to interact with lipid structures, which may alter lipid metabolism. Twelve peptides were upregulated by DMN in the EN subset (Table 3) with no discernible subnetwork changes occurring. There was an increase in peptides with functions related to lipid metabolism (CYP76G1, At2g47710) and one involved with changes in membrane structure (FLOT1). Two of the peptides have GO biological process assignments involving flavonoid biosynthesis (HCT, TT7). The increase in expression of the transcription factor MYB48 by DMN has interesting implications. MYB transcription factors have been linked to a myriad of biological processes including response to biotic stress (Lee et al. 2001), flavonoid biosynthesis (Zhao et al. 2013), and axillary meristem initiation (Müller at al. 2006). More specifically, over expression of MYB48 from *Zea mays* in *Arabidopsis* resulted in increased drought tolerance (Wang et al. 2017). Drought is an environmental signal that induces dormancy and growth arrest in multiple perennial plants (Volaire et al. 2023).

In EC gene subsets, eight subnetworks were downregulated after DMN exposure (Table 3). The subnetwork SMO2-2:SMO1-1:STE1:DWF1 is involved with sterol and brassinosteroid (BR) biosynthesis. BRs function as growth promoting phytohormones and as regulators of abiotic stress in plants (Li et al. 2021). Thus, the suppression of BR synthesis pathways by DMN in meristems that have lost EN suggests a possible mechanism of growth inhibition in potato tubers. Previous work by Korableva et al. (2002) showed that application of the brassinosteroid 24-epibrassinolide (EB) to potato tubers increased endogenous ABA and ethylene levels and prolonged tuber dormancy. Thus, it appears that DMN suppression of growth and sterol biosynthesis occurs through an alternate mechanism than the brassinolide EB.

Downregulation of TT7:UF3GT by DMN suggests a suppression of the flavonoid biosynthetic pathway. Flavonoid biosynthesis has multiple roles in plant development including response to stress, protection from oxidative burst, regulation of cell cycle and growth (Kumar et al. 2018). It is unclear if the DMN suppression of proteins associated with the flavonoid pathway has a clear linkage to suppression of meristem activity and sprout growth in potato. The suppression of PAO5:SPDS2 subnetwork by DMN suggests an alteration of polyamine biosynthesis following DMN exposure. Downregulation of the KCS6:CER3 subnetwork by DMN should result in a decrease in long chain fatty acid production and

Table 3 Biological processes of the largest identified protein-protein interaction subnetworks altered by DMN exposure of endodormant tubers

Regulation	Protein	Biological process
Down	PAL1	Defense response, drought recovery. Lignin and salicylic acid catabolic processes, phenylpropanoid biosynthetic process, pollen development, and response to UV-b, oxidative stress, and wounding
Down	At1g28590	Defense response, organonitrogen compound catabolic process, response to jasmonic acid
Down	At3g45770	Fatty acid biosynthetic process and metabolism
Down	At4g28940	Aromatic compound, organic cyclic compound and secondary metabolite biosynthetic processes, cell differentiation, leaf senescence, plant epidermis development, root morphogenesis, and response to water deprivation
Down	At3g26040	HXXXD-type acyl-transferase family protein
Up	MYB48	Regulation of DNA-template transcription
Up	CYP76G1	Lipid biosynthetic process, positive regulation of RNA metabolic process, regulation of developmental process and DNA-template transcription, tissue development
Up	HCT	Lignin biosynthetic process, positive regulation of flavonoid biosynthetic process
Up	TT7	Flavonoid biosynthetic process, response to UV and auxin
Up	GAD	Aromatic compound, organic cyclic compound and secondary metabolite biosynthetic processes, cell differentiation, cell wall organization or biogenesis, defense response to other organism, macromolecule and oxoacid metabolic processes, plant epidermis development, response to bacterium, inorganic substance and organic cyclic compound, root morphogenesis
Up	FLOT1	Cellular response to hypoxia, endocytosis, membrane invagination
Up	At5g41770	mRNA spicing, via spliceosome, spliceosomal complex assembly
Up	AtKTI5	Response to insect
Up	At2g47710	Cellular catabolism process, cellular lipid and monocarboxylic acid metabolic processes, organonitrogen compound catabolic process, positive regulation of lipid metabolic process, response to hypoxia
Up	GT72B1	Lignin biosynthetic process, response to toxic substance, xenobiotic catabolic and metabolic processes
Up	At5g07610	F-box protein, biological process
Up	ASP3	Leaf senescence, nitrogen compound metabolic process

Table 4 Biological processes of the largest identified protein-protein interaction subnetworks altered by DMN exposure of ecodormant tubers

Regulation	PPI network	Protein	Biological process
Down	SMO2-2:SMO1-1:STE1:DWF1	SMO2-2	4-alpha-methyl-delta7-sterol oxidation, sterol biosynthetic process
		SMO1-1	4,4-dimethyl-9beta, 19-cyclopropylsterol oxidation, embryo development, sterol biosynthetic process
		STE1	Brassinosteroid and sterol biosynthetic processes
		DWF1	Brassinosteroid biosynthetic process, lignin metabolic process, plant-type secondary cell wall biogenesis, response to light stimulus, unidimensional cell growth
	TT7: UF3GT	TT7	Flavonoid biosynthetic process, response to UV and auxin
		UF3GT	Anthocyanin-containing compound biosynthetic process, cyanidin 3-O-glucoside metabolic process
	PAO5:SPDS2	PAO5	Spermine catabolic process
		SPDS2	Spermidine biosynthetic process
	TAT2:MS2	TAT2	L-phenylalanine and tyrosin catabolic processes, amino acid metabolic process
		MS2	Methionine biosynthetic process
	KCS6:CER3	KCS6	Cutin-based cuticle development, response to light stimulus and cold, unidimensional cell growth, very long-chain fatty acid metabolic and wax biosynthetic processes
		CER3	Alkane, cutin and wax biosynthetic processes, pollen sperm cell differentiation
	YUC8:TAR2	YUC8	Regulation of auxin biosynthetic process, brassinosteroid-mediated signaling pathway, response to cytokinin and ethylene, somatic embryogenesis
		TAR2	Cotyledon development and vascular tissue pattern formation, flower, gynoecium, shoot system and lateral and primary root development, embryo development ending in seed dormancy, maintenance of root meristem identity, phloem or xylem histogenesis, positive gravitropism, response to ethylene and cellular response to nitrogen levels, defense response to bacterium, indoleacetic acid biosynthetic and amino acid metabolic processes
	CB5-B:FAH1	CB5-B	Alkane and very long-chain fatty acid biosynthetic processes
		FAH1	Negative regulation of programmed cell death, fatty acid and very long-chain fatty acid metabolic processes
	CDC20.1:CYCB1.1	CDC20	Positive regulation of anaphase-promoting complex-dependent catabolic process, protein ubiquitination
		CYCB1.1	Regulation of cell growth and cyclin-dependent protein serine/threonine kinase activity, response to gamma radiation, mitotic cell cycle phase transition

Table 4 (continued)

Regulation	PPI network	Protein	Biological process
Up	OSM34:HCHIB:PRB1:PR4	OSM34	Pseudogene of ATOSM34 (osmotin-like protein). Functions in ABA signaling
		HCHIB	Defense response to fungus, jasmonic acid and ethylene-dependent systemic resistance, ethylene-mediated signaling pathway, cell wall macromolecule catabolic process
		PRB1	Response to ethylene, jasmonic acid, salicylic acid, bacterium, herbivore and wounding, defense response to other organism,
		PR4	Response to ethylene, herbivore and virus, systemic acquired resistance, defense response to bacterium
	NILR1:SIP1:AT4G01970	NILR1	Defense response to nematode, multidimensional cell growth, seed germination
		AT4G01970	Response to oxidative stress
		SIP1	Root morphogenesis
	ERF1:ERF2	ERF1	Defense response, ethylene-activated signaling pathway, jasmonic acid-mediated signaling pathway, regulation of DNA-templated transcription
		ERF2	Cell division, ethylene-activated signaling pathway, induced systemic resistance, jasmonic acid-mediated signaling pathway, phloem or xylem histogenesis, positive regulation of DNA-templated transcription
	RPM1:RIPK	RPM1	Defense response, plant-type hypersensitive response
		RIPK	Defense response to bacterium, protein phosphorylation
	AT1G70420:TyrA1	AT1G70420	Cellular response to organic substance, defense response to bacterium, positive regulation of DNA-templated transcription, regulation of anatomical structure morphogenesis, cellular component organization, defense response, developmental growth and reproductive processes, response to lipid, organic cyclic compound, oxidative stress, red or far red light, and temperature stimulus, unidimensional cell growth
		TyrA1	Tyrosine biosynthetic and metabolic processes
	HCT:CYP98A3	HCT	Lignin biosynthetic process, positive regulation of flavonoid biosynthetic process
		CYP98A3	Coumarin, flavonoid, lignin and phenylpropanoid biosynthetic processes

cuticular waxes. An additional fatty acid associated subnetwork, CB5-B:FAH1, was also found to be downregulated by DMN. Suppression of cuticular, long-chain fatty acids has been shown to be associated with drought stress (Negin et al. 2023). A more direct link between DMN and cell division can be found in the suppression of the subnetwork CDC20.1:CYCB1.1. CDC20 is part of the anaphase-promoting complex (Willems and De Veylder 2022) and B-type cyclins are associated with the mitosis promoting factor.

Six PPI subnetworks are induced or upregulated by DMN across all EC potato cultivars (Table 4). The network OSM34:HCHIB:PRB1:PR4 involves protein interactions that center around phytohormones signaling and stress signaling. OSM34 encodes for an osmotin-like protein that is a positive regulator of ABA signaling (Park and Kim 2021). HCHIB encodes for a fungal pathogen induced endochitinase that also elevates genes controlling ethylene levels (Kappagantu et al. 2017). PRB1 and PR4 are pathogenesis related proteins associated with ethylene response pathways (Kaur et al. 2022). The remaining PPI subnetworks (NILR1:SIP1:AT4G01970, ERF1:ERF2, RPM1:RIPK, and AT1G70420:TyrA1) are associated with stress responses mitigated by ethylene and JA. The induction of PPI stress associated networks may be a function of ethylene response. Application of ethylene has been used to control sprouting in stored potato tubers (Prange et al. 1998), which may be part of the mechanism of action for DMN. A caveat to this concept is that the DMN treatment used in this study was for a single application whereas commercial use of ethylene in stores to control sprouting is through continuous application.

Conclusion

No previous study has evaluated the effect of DMN on multiple potato cultivars of differing dormancy profiles. Here, we have affirmed that DMN treatment elicits differing responses dependent upon cultivar and dormancy state with EC tubers being more responsive than EN tubers. DMN was observed to suppress numerous genes related to cell-cycle progression and plant development and induce genes and TFTs related to multiple biotic and abiotic stress responses. Specifically, response to water stress was strongly induced, suggesting DMN may alter water movement and perception. Treatment with DMN resulted in thousands of genes changing expression, but genes common to all three cultivars were limited, and of those, fewer were identified as acting in PPI networks. Thus, DMN results in the global response of tubers to a perceived stress, but a small number of genes and protein interactions govern the primary response.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11540-024-09772-7.

Acknowledgements We would like to thank Craig Praul of the Penn State Genomic Core Facility for his help and advice throughout this project. We would also like to thank Mark Troyer for providing the tubers used in this study, Jessie Johnson for her assistance in harvesting tubers, and Keegan Rupp and Ian Costello for technical assistance.

Author Contribution EPD and MAC performed experiments and composed the manuscript. EPD performed all relevant data analyses and compiled data for publication.

Funding This work was supported by a grant from the 1,4Group of Meridian, ID and by the Lake Erie Regional Grape Research and Extension Center.

Data Availability Raw sequencing reads were submitted to the NCBI SRA database under the accession of PRJNA1064110.

Declarations

Conflict of Interest Part of this work was supported by the 1,4Group who manufacture and sell the sprout inhibitor DMN. The authors declare no competing interests.

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