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The sprout inhibitors chlorpropham and 1,4-dimethylnaphthalene elicit different transcriptional profiles and do not suppress growth through a prolongation of the dormant state

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Abstract Chlorpropham (CIPC) and 1,4-dimethylnaphthalene (DMN) are used to control postharvest sprouting of potato tubers. CIPC alters microtubule structure and function resulting in inhibition of cell division. The mechanism of action of DMN is unknown but, because it is a natural product found in potato tubers, there is speculation that it inhibits sprout growth by prolonging the dormant state. To address this issue, the effects of CIPC and DMN on abscisic acid (ABA) content and gene expression in potato tuber meristems were determined and compared to those found in dormant and non-dormant meristems. Dormancy progression was accompanied by a dramatic decline in ABA content and the ABA levels in meristems isolated from CIPC- and DMN- treated tubers were identical to the levels found in nondormant meristems demonstrating that sprout repression is not a function of elevated ABA. Evaluation of transcriptional profiles using cDNA microarrays demonstrated that there were similarities between CIPC- and DMN- treated tuber tissues particularly in transcripts that encode phosphatases and proteins associated with oxygen-related metabolism. Despite these similarities, there were significant differences in transcript profiles derived from treatment with either CIPC or DMN and the dormant state. These results suggested the mechanisms-of -action of DMN and CIPC are distinct and not due to a prolongation of the normal dormant condition.

Keywords Dormancy · Potato · 1,4-dimethylnaphthalene · DMN · Chlorpropham · CIPC · Sprout inhibitors · Microarrays · Transcriptional profiling

Introduction

Dormancy is a complex physiological process that is utilized by plants to survive stress conditions such as drought and exposure to cold. Definitions of various types of dormancy (i.e. endodormancy paradormancy, and ecodormancy) have been established (Lang et al. 1987) and, following harvest, a potato exhibits all three dormant states. Currently, potato is the fourth largest agricultural commodity in the world and storage of this crop is predicated on the ability of harvested tubers to exhibit an endodormant state. Increased storage and shipment of potatoes requires either maintenance of the endodormant state or application of growth suppressors to prevent sprouting. Projection of the future growth of potato as a commodity is expected to be substantial through 2020 with worldwide increases of 1.0 and 1.3% per year (Scott et al. 2000). Therefore, manipulation of the endodormant state of the potato crop, either through mechanical, genetic, or chemical means, will be fundamental to increased

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utilization of this commodity. Breakage of the endodormant state of potato is strictly a time dependent process and does not require a cold treatment, which is common in many perennial plants found in temperate climates. Onset and maintenance of endodormancy in potato tubers has been associated with abscisic acid (ABA) and ethylene (Suttle and Hulstrand 1993). Genetic studies have demonstrated that some genes involved with the extension of the dormant state are linked to elevated ABA levels (Simko et al. 1997). Genes encoding key enzymes involved in ABA metabolism are differentially expressed during dormant progression in potato tubers. (Destefano-Beltran et al. 2006b).

Endodormancy in potato can be shortened by brief exposure to the compound bromoethane (BE) (Destefano-Beltran et al. 2006a) and gene expression analysis following BE treatment shows gene profiles overlap and contain genes similarly altered in expression following natural dormancy termination (Campbell et al. 2008). Chlorpropham (isopropyl 3-chlorophenylcarbamate; CIPC) and 1,4-Dimethylnaphthalene (DMN) are compounds that inhibit sprouting in potato tubers following loss of endodormancy. CIPC is a synthetic compound that modifies spindle formation, inhibits mitosis, and prevents sprouting (Vaughn and Lehnen 1991). DMN is a natural product found in potato tissues that also inhibits sprout growth. The mechanism of action of DMN is unknown but it is thought to be very different from CIPC (Beveridge et al. 1981). Recent reductions in CIPC residue tolerances in fresh market potatoes have renewed interest in the application of DMN in conjunction with CIPC, or alone for sprout control in storage (Kleinkopf et al. 2003).

Gene expression and metabolic profiling have been utilized to describe the transition of poplar meristems as they progress into the dormant state (Ruttink et al. 2007). Microarray analysis has also been used to examine transcript profiles in dormant and nondormant potato tubers (Campbell et al. 2008). In this study, hormone and microarray analyses were conducted in order to ascertain if DMN-treated tuber meristems maintain a dormancy-like physiological state and to gain insight into the effects of sprout-inhibitor treatments on tuber meristem gene expression.

Materials and methods

Plant material

For microarray analysis, potato (*Solanum tuberosum* L cv Russet Norkota) tubers were field-grown, harvested and wound-healed (cured) according to standard agronomic practices. After curing, a sample of tubers was immediately harvested by excising meristems with a 2 mm curette, quick-freezing in liquid nitrogen, followed by storage at −80°C. The remaining tubers were placed in 50-gallon fumigation chambers at 7°C. One sample was treated with air, one with CIPC, and another with DMN (both according to manufacturer's recommendations). After treatment, the chambers were attached to an external ventilation system with a flow rate of 0.14 m^3 per min at >90% relative humidity. The tubers were incubated until the air-treated tubers exited dormancy and exhibited peeping (sprout growth in excess of 2 mm). At this time, meristems were harvested from all tubers as described above.

ABA analysis

Groups of 100 meristems (0.1–0.14 g FW) were allowed to thaw at 4°C in 80% (v/v) aqueous acetone. The tissue was mechanically homogenized (4°C), clarified by centrifugation (10,000×*g* for 15 min), and the supernatant decanted. The pellet was re-extracted in 80% (v/v) aqueous acetone and, after standing for ≥2 h (4°C), was re-centrifuged and the supernatants combined. Depending on the sample, a total of 50–100 ng $^2H_6$-(+)-ABA (OlChemIm Ltd., Olomouc, Czech Republic[1]) was added as an internal standard. The supernatants were dried under a stream of N_2 (40°C) and re-dissolved in 5 ml 1 M formic acid. The acidified extracts were applied to a 150 mg MCX Oasis cartridge (Waters Associates, Milford, MA). The cartridge was washed with 5 ml 1 M formic acid followed by 5 ml methanol. The methanol fraction was taken to dryness under a stream of N_2 (40°C) and was re-dissolved in 10 mM ammonium acetate (pH 5.6). ABA was quantified by high performance liquid chromatography-mass spectrometry (HPLC-MS) using a Thermo Electron Surveyor MSQ system and a 2.1 × 150 mm 5 µm Hypersil Gold Column (Thermo-Finnigan, San Jose, CA). HPLC solvents were: A, 10 mM ammonium acetate (pH 5.6) and B, methanol (0.2 ml/min). Starting conditions were 40% B, a linear gradient to 80% B in 5 min followed by a linear gradient to 100% B in 5 min. Detection and quantification were performed using the MSQ operating in the negative ion, electrospray ionization mode with a probe temperature of 460°C, cone voltage of 30 V, and needle voltage of 4.5 kV. Ions (*m/z*) monitored were: 263 for ABA and 269 for $[^2H]_6$-ABA.

Nucleic acid isolation

Shoot tips from actively growing plants and tuber meristems stored at −80°C were ground to a fine powder in liquid nitrogen and total RNA was isolated using a Ribopure Kit (www.ambion.com). RNA quality was determined using gel electrophoresis and visualization of ribosomal bands on an agarose gel. RNA quantity was determined using spectrophotometry and absorbance at 260 and

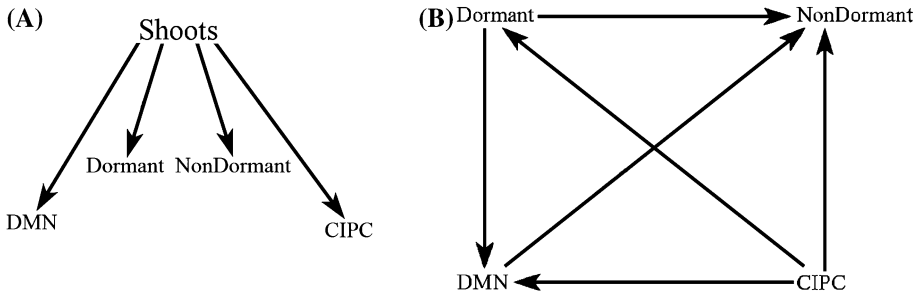

Fig. 1 Experimental design for the microarray experiments. Actively growing potato shoots were used as a reference sample for comparison to dormant meristems, nondormant meristems, or meristems harvested from tissues fogged with DMN or CIPC (**a**). Meristems treated with DMN or CIPC were compared directly to each other or to nondormant and dormant meristems (**b**)

280 nm. RNA was converted to cDNA and labeled using a Superscript Plus Indirect cDNA Labeling System (www.invitrogen.com). Fluorescent probes were hybridized to a TIGR version 4-potato cDNA microarray according to the procedure of DeRisi (http://derisilab.ucsf.edu/microarray/protocols.html) utilizing a Biosciences Lucidea Slidepro Hybridizer (www6.gelifesciences.com). Array TIF images were generated by scanning with an AxonGenepix 4000B (www.axon.com) and gridded using GenSpring 7.3 software (www.home.agilent.com). A total of 10 different comparisons were conducted in duplicate for a total of 20 microarrays hybridized.

Microarray analysis

The GeneMaths XT program (Applied Maths, Inc., www.applied-maths.com) was used to construct gene ontologies from differentially expressed genes in the microarray experiment that used shoots as a reference (Fig. 1a). Intensity values derived from hybridization from each treatment were log transformed. Transformed intensities from probes were normalized between arrays and the distribution of the probe intensities was centered. Normalized intensities from technical replicates were averaged and the resulting data was subjected to various statistical analyses including ANOVA and *T*-tests to identify differentially expressed genes. For pathway analysis and identification of over-represented ontologies, ontologies were based on homology of probes to the most similar Arabidopsis gene. Briefly, singletons and, where possible, contig'ed ESTs representing probes present on the array were compared to the Arabidopsis sequence database using the tBlastX algorithm to identify the most likely homologue to any given probe (*p*-value cut off of 10E-5). Ontologies of putative Arabidopsis homologues were used for Gene Set Enrichment Analysis and Sub-Network Analysis using the Pathway Studio Enterprise program suite (Ariadne genomics, Inc), and MIPS functional distribution analysis was through the online program available at http://mips.gsf.de/proj/funcatDB/search_main_frame.html.

The resulting .gpr files from the microarray experiment using woven loop design (Fig. 1b) were analyzed in Bioconductor using the limma package (Smyth 2005). Data files were normalized using loess and analyzed using a reference design with RNA from actively growing potato meristems as the reference (Fig. 1). Comparisons were made without dye swaps using an empirical Bayes method (Smyth 2004). Venn diagrams were constructed by grouping genes with common levels of expression in each treatment using the statistical package R.

Quantitative real-time PCR

Quantitative Real-Time PCR (qRT-PCR) was utilized to compare gene expression values between shoot, dormant, CIPC-treated and DMN-treated tissues. Primers were constructed by obtaining the nucleotide sequence from DFCI-Potato Gene Index and using Applied Biosystems Primer Express 3.0 software on 26 genes whose expression was identified as linked to a specific tissue treatment based on microarray analysis. cDNA template for qRT-PCR was prepared from mRNA isolated from potato meristems as described in the section on nucleic acid isolation. Reactions were run on a StepOneTM Real Time PCR System and data was analyzed using Step One Software v2.0 (www.appliedbiosystems.com) to determine $\Delta\Delta$CT values on a $\log_{10}$ scale. Primers amplifying *ACTIN2* (TC133139) homologs were used as internal controls and to normalize across reactions. *ACTIN2* was chosen as controls because expression was relatively constant across all arrays.

Results

Tubers were classified as dormant if there was no detectable meristem growth following transfer to room temperature for two weeks. This was apparent in tubers examined in September, 2 weeks following harvest, and in tubers assayed in November. Tubers examined in January

exhibited meristem growth in excess of 2 mm and therefore were classified as nondormant.

The ABA content of Russet Norkotah meristems declined by over 75% during the transition between fully dormant and non-dormant (Fig. 2), which is consistent with earlier studies that demonstrated a decline in meristem ABA content during dormancy progression in other varieties (Destefano-Beltran et al. 2006a, and references cited therein). The ABA content of both CIPC- and DMN-treated tuber meristems declined to a comparable extent and was indistinguishable from non-dormant meristems. These results demonstrate that, at a physiological level, both CIPC and DMN-treated tubers resemble their non-dormant counterparts and suggest that growth suppression associated with both of these sprout inhibitors is not an ABA-induced response.

Using cDNA prepared from actively growing shoots as a reference, limma analysis of microarrays identified 5,281 cDNAs exhibiting significant expression differences between dormant, nondormant, and DMN- or CIPC-treated tubers tissues (Supplemental Table 2). Venn diagrams were utilized to compare the similarities between cDNAs that exhibited two-fold changes in expression unique to a treatment (Fig. 3). In comparison to shoots, the majority of genes exhibited expression common to dormant or nondormant meristems and to tissues treated with DMN or CIPC. This similarity in expression was due to decreased expression (relative to shoots) of many transcripts associated with photosynthesis and increased expression of genes associated with tuber development such as patatin. Importantly, there was a high degree of similarity between gene expression patterns of CIPC or DMN-treated meristems and those of either dormant or non-dormant meristems.

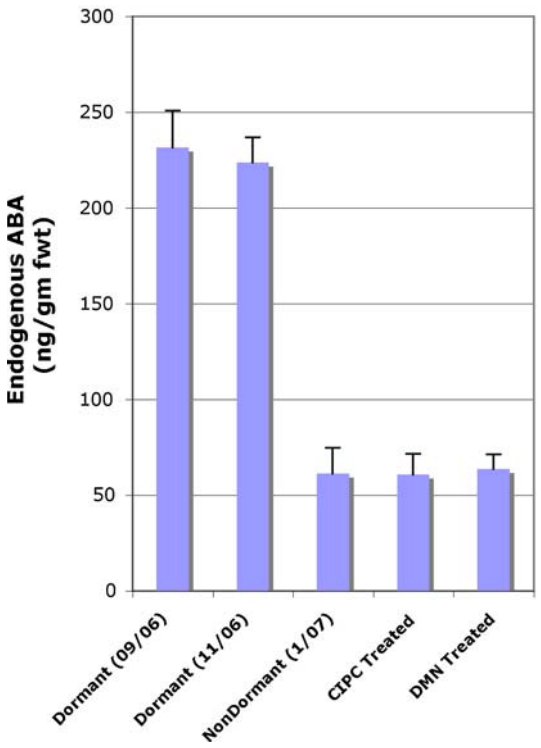

Fig. 2 Endogenous abscisic acid (ABA) levels in dormant tuber meristems, nondormant tuber meristems or nondormant tuber meristems exposed to either CIPC or DMN

Analysis of gene expression

qRT-PCR was used to compare relative gene expression for seven cDNAs in nondormant, DMN, and CIPC treated tissues using dormant tuber meristems as a control (Fig. 4). CIPC resulted in the decrease of five different transcripts: a germin-like protein (TC151520), WD40 (TC144574), MYB transcription factor (TC153242), Prohibitin (TC138720), and CIPK23 (TC134815). These same cDNAs demonstrated down regulation in nondormant and DMN treated tubers. Interestingly, prohibitin, a mitochondrial protein associated with cell proliferation (Merkwith et al. 2008) is down regulated in nondormant tissues yet transcripts increase in meristems under CIPC induced growth suppression.

Characterization of the putative transcripts dotted onto the potato array was based on the Munich Information Center for Protein Sequences (MIPS) classification system (http://www.helmholtz-muenchen.de/mips). Comparison between all treatments and between dormant and nondormant states showed no large changes in any protein group (Fig. 5).

Metabolic profiling and sub network analysis were conducted on array data using active potato shoot meristems as a reference (Supplemental Figures 2–13). Comparisons of genes expressed in DMN and CIPC-treated meristems to the nondormant meristems were examined in more detail in order to determine if these sprout inhibitors have similar mechanisms of action. Similarities were found in transcript profiles from CIPC and DMN-treated tissues

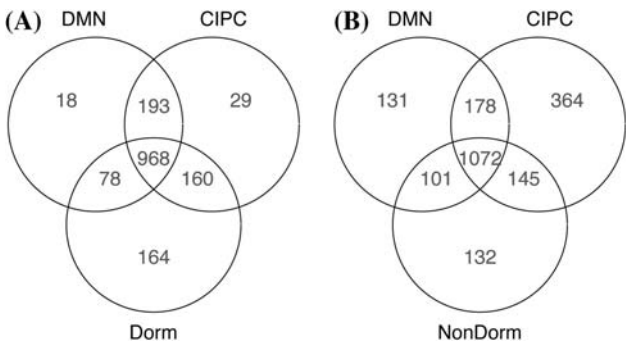

Fig. 3 Venn diagram showing overlapping expression of transcripts between dormant (Dorm) and nondormant (Nondorm) tissues and CIPC and DMN treatments. Data for the Venn diagram was derived from supplemental Table 3

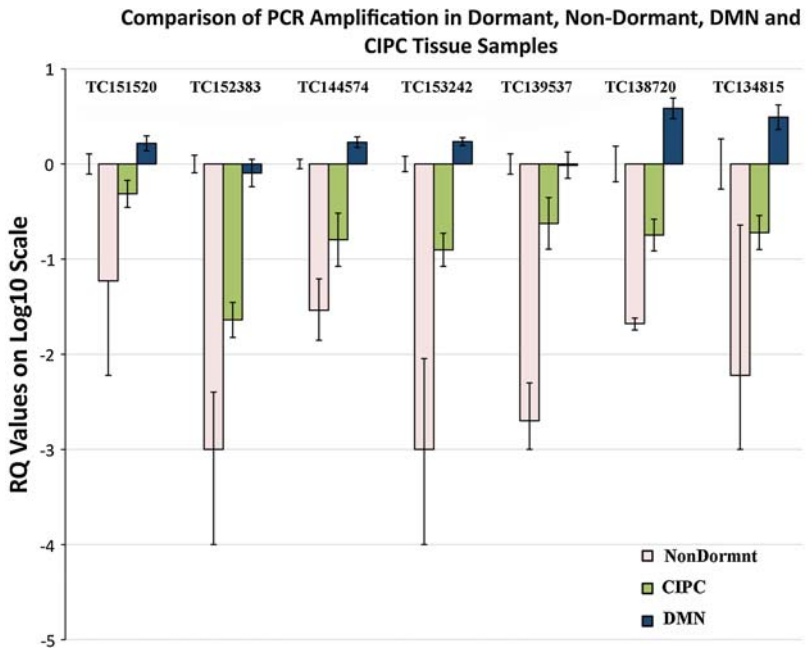

Fig. 4 Comparison of gene expression level using quantitative PCR. Primers to specific accessions from the microarray were used for amplification of cDNA generated from nondormant meristem, DMN- and CIPC-treated meristems. Dormant meristems were set to zero level of expression for comparison. Expression across samples was normalized to amplification products for Actin 2 and adjusted to a $\log_{10}$ scale. Transcripts that exhibited a significant change in expression based on the microarray results were selected for QT-PCR analysis. Expression data and primer sequences can be found in Table 1

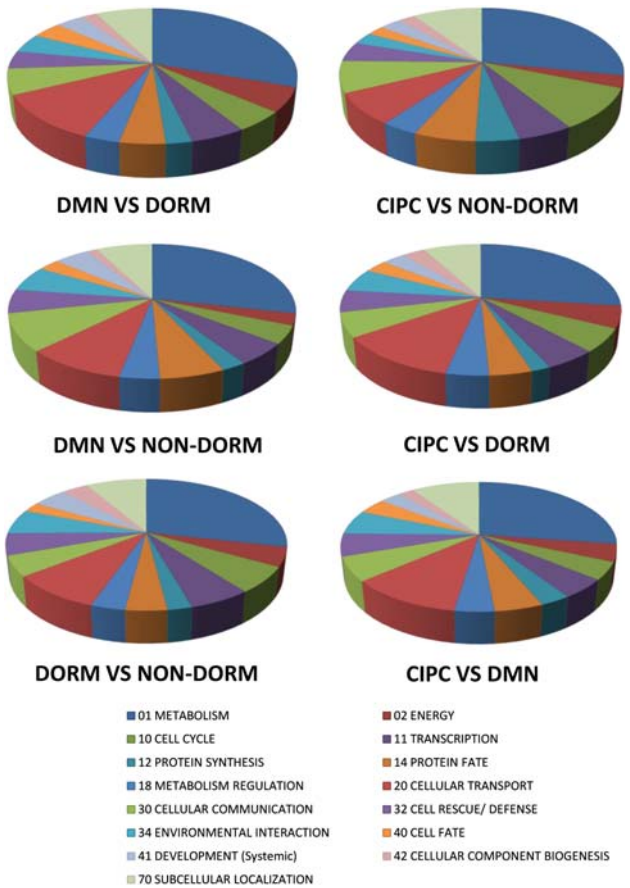

Fig. 5 MIPS classification of transcripts that demonstrate at least a two-fold change between samples used in the microarray experiments

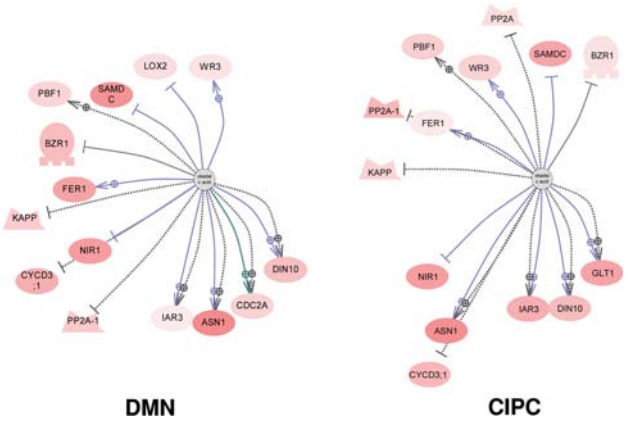

Fig. 6 A portion of the sub network analysis presented in supplemental figures 2 and 4 showing gene products that are putatively affected by okadaic acid that are up regulated by DMN or CIPC compared to nondormant meristems. *Arrows* indicate up regulation and *bars* indicate repression by okadaic acid as reported from the literature. Increased color intensity indicates a greater change in gene expression associated with a specific treatment. *Blue lines* with and without quartered *circle* indicate direct or indirect expression, respectively. The *green line* indicates protein modification. The *grey dashed* and *solid lines* signify regulation. *Lines* containing quartered *circles* indicate an indirect regulation. The *shapes* of the *symbols* indicate protein classes: *crown* (e.g., BZR1), *circle* (e.g., WR3), i (e.g., PPSA-1) indicates transcription factors, general proteins, phosphatases respectively

particularly in sub-network analysis of cDNAs that encode okadaic acid-sensitive proteins (Fig. 6) and in cDNAs associated with oxygen-dependent reactions (Fig. 7). For example, both CIPC and DMN result in induction of 12 separate genes that are responsive to okadaic acid-including protein phosphatase 2A (*PP2A*), which is a member of the larger family of serine/threonine phosphatases that are responsible for a variety of developmental responses reviewed in (Farkas et al. 2007) and CYCLIN

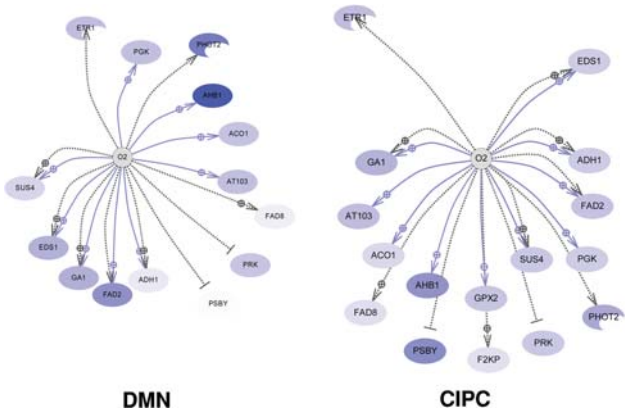

Fig. 7 A portion of the sub network analysis presented in supplemental figures 3 and 5 showing gene products associated with oxygen metabolism that are down regulated by DMN or CIPC compared to nondormant meristems. *Arrows* indicate up regulation and bars indicate repression as reported in the literature. Increased color intensity indicates a greater change in gene expression associated with a specific treatment. The *grey dashed* and *solid lines* signify regulation. *Lines* containing quartered *circles* indicate an indirect regulation. *Blue lines* with and without quartered *circle* indicate direct or indirect expression, respectively. The shapes of the symbols indicate protein classes: *circle* (e.g., SUS4), and *arc* (e.g., PHOT2) indicate general protein and kinase respectively

D3-1 (*CYCD3-1*) which is a key regulator of cell division (Fuerst et al. 1996). The induction of CYCD3-1 in both of these growth repressing treatments suggests that both CIPC and DMN block cell division at a point after the G1 to S phase transition. Despite the diversity of PP2A type proteins in plants (Arino et al. 1993) there is a strong connection between these proteins and ABA signaling and response systems. Although there was no apparent shift in the ABA-regulated signaling following CIPC exposure, sub network analysis indicated that DMN treatment elicited appreciable alteration of transcripts regulated by *ABI1*, *ABI2*, and *ABI3* (supplemental figure 10).

Discussion

Although incompletely understood (especially at the molecular level), potato tuber dormancy progression is associated with marked changes in hormone content and gene expression (Suttle 2004; Campbell et al. 2008). Because of the deleterious effects of sprouting on tuber nutritional and processing qualities, postharvest application of artificial sprout control agents is an integral component of successful potato storage management. CIPC has been used for decades to control tuber sprouting in storage (Kleinkopf et al. 2003). It is generally regarded as a mitotic inhibitor that suppresses sprout growth by direct growth inhibition (Vaughn and Lehnen 1991). Because it occurs naturally in potato tubers and inhibits sprout growth, DMN has recently been introduced as an alternative sprout control agent in storage. Although effective, it's mechanism(s)-of -action is(are) unknown but it has been suggested that it acts by extending the natural period of dormancy via regulation of phytohormones (Kleinkopf et al. 2003). If true, this would be the first compound identified that is capable of lengthening this critical developmental stage and would be of considerable basic and applied interest. In this study, this issue was addressed by comparing the effects of dormancy progression to those elicited by either CIPC or DMN treatment. If DMN acts to extend dormancy, then tissues treated with DMN should more closely resemble tissues isolated from dormant tubers immediately after harvest.

The results presented in this study, demonstrate that at both a physiological and molecular level, tubers treated with DMN do not resemble their dormant counterparts. First, the decline in ABA content that accompanies natural dormancy progression is not affected by DMN treatment (Fig. 2), although there does appear to be common modulation of ABA signaling in both DMN and naturally dormant buds (see below for discussion). Second, DMN and CIPC treated tuber meristems share a larger number of transcripts that exhibit two-fold changes in expression in comparison to dormant tissues (Fig. 3). Collectively, these results suggest that DMN does not act as a dormancy-extending agent but rather inhibits sprout growth by other undefined mechanisms.

Recent studies have shown that a homologue of the auxin response factor 6 (*ARF6*) can be used as a marker of meristem activity in potato tubers as dormancy cessation occurs and growth begins (Faivre-Rampant et al. 2004). However, *ARF6* homologs were not present on the TIGR 10 K array and expression of this transcript could not be measured in this study. However, CIPC did alter expression of *ARF1* and *ARF2* homologues (Supplemental data figure 4). There was no similar expression change associated with DMN suggesting a potential difference in mechanisms of action of the two sprout inhibitors.

Both CIPC and DMN have similar effects on transcripts encoding okadaic acid-sensitive phosphatases (Fig. 6). For example, both sprout inhibitors induce expression of protein phosphatase 2A-1 (*PP2A-1*). There is a large diversity of *PP2A* genes in Arabidopsis with a multiplicity of functions reviewed in (Farkas et al. 2007) but over expression of the gene GRC1 results in an increase in *PP2A* and loss of seed dormancy via regulation of the cell cycle. Therefore, DMN and CIPC induction of *PP2A-1* suggest a connection between sprout inhibitors and regulation of cell division. This confounding increase in a transcript inducing cell division is also highlighted by the induction of *CYCD3-1* in both CICP and DMN treated tissues. However, although this suggests that the G1 to S

phase transition of the cell cycle is initiated, the lack of growth suggests that these two growth inhibitors prevent growth through mechanisms that are downstream of this process.

Both DMN and CIPC affect the expression of genes responsive to oxygen-associated signaling and a decrease in expression of genes involved in glycolytic processes. Both of these observations would be consistent with reduced oxidative stress in the CIPC and DMN treated tissues (Fig. 7, supplemental figures 1 through 4). For example, there is a decrease in alcohol dehydrogenase (*ADH1*) expression by both sprout inhibitors. Application of hydrogen cyanamide (HC) to grape buds results in the breakage of endodormancy via sub lethal oxidative stress induction, and a decrease in transcripts encoding proteins associated with oxygen metabolism, particularly catalase (Ophir et al. 2009; Scott et al. 2000; Blenkinsop et al. 2002). DMN and CIPC treatments alter the expression of transcripts associated with oxygen metabolism thus; there are parallels between oxidative stress-induced dormancy cessation by HC in grape and the interaction of DMN and CIPC to suppress sprout growth in potato. DMN and CIPC treatments result in the down regulation of transcripts for alcohol dehydrogenase (ADH) and it has been demonstrated that ethanol can break apical bud dormancy in potato tubers possibly through changes in redox potential of tissues induced by ADH (Claassens et al. 2005). The most surprising observation here is that naturally dormant buds are not showing a significant decrease in oxidative stress responses, suggesting that the naturally dormant buds are experiencing oxidative stress in a manner similar to non-dormant buds. Although both CIPC and DMN may actively prevent oxidative stress from occurring and thus could block growth induction by preventing perception of oxidative stress, a completely different signaling process must be preventing the oxidative stress from breaking dormancy

Table 1 Gene expression of selected accessions from the microarray data

Expression levels and TC Accessions for QT-PCR							
TC number	Notes	Expression levels in specific arrays					
		DM/C	ND/C	D/C	DM/D	ND/D	DM/ND
TC142974	Thaumatin At4g11650	3.381524	0.19475	−0.1635	3.21803	0.0312	3.576272
TC134222	Osmotin-like At4g11650	3.521764	−0.17245	0.6921	4.21391	0.5197	3.349314
TC144128	Osmotin-like At4g11650	3.054264	0.0221	0.7456	3.79983	0.7677	3.076369
TC136785	Germin AT5G39150	2.813055	0.3127	0.1628	2.97588	0.4755	3.125751
TC151520	Germin AT5G39100	2.359912	0.47988	0.2656	2.62555	0.7455	2.839791
TC146021	Cation efflux family protein AT1G51610	1.172269	0.26044	−1.345	−0.1727	−1.0845	1.432708
TC154238	Class II chitinase AT3G12500	3.361326	−0.56121	0.4944	3.85568	−0.0669	2.800117
TC137764	Cytochrome P450 At3g52970	2.173352	−1.23541	−0.7285	1.44486	−1.9639	0.937941
TC148124	CYP72A15 electron carrier/iron ion binding/ monooxygenase AT3G14690	2.690755	0.26582	−0.082	2.60879	0.1838	2.956573
TC148742	DNaJ-like heat shock protein AT2G17880	2.204025	0.04032	−0.2371	1.96688	−0.1968	2.244344
TC144574	Multicopy supressor of IRA1-2 (MSI2) AT2G16780	−1.20132	−0.67403	−0.0418	−1.2431	−0.7159	−1.87535
TC141076	YLS9 (Yellow-leaf-specific gene 9) AT2G35980	2.403432	−0.28396	0.8656	3.26907	0.5817	2.119472
TC153242	ATMYB48 (MYB111) Reg Flavanol Biosyn AT3G46130	0.735723	2.31132	−0.6058	0.12997	1.7056	3.04704
TC161369	Myb DNA binding protein 111 AT3G46130	0.793214	0.60602	−0.5194	0.27381	0.0866	1.399232
TC139932	Myb DNA binding protein 111 AT3G46130	0.665345	2.29219	−0.2646	0.4007	2.0275	2.957534
TC141516	Adenylate succinate lyase (purine synthesis) AT1G36280	0.197715	0.34495	−0.4926	−0.2949	−0.1477	0.542669
TC154002	Plastid-specific 30S ribosomal protein 3 AT1G68590	1.470626	0.28208	0.2537	1.72432	0.5358	1.752709
TC152383	RD22 AT5G09530	0.020248	2.59515	−1.2133	−1.193	1.3819	2.615394
TC145755	ARR4 response regulator (circadina clock reg) AT1G10470	0.738781	−0.56976	−0.1849	0.55388	−0.7547	0.169018
TC138720	PHB3 Mito morphol. Prohibitin (cell cycle progression) AT5G40770	1.189455	0.16138	1.0036	2.19305	1.165	1.350836
TC139537	ATPRB1 (A. thaliana pathogenesis-related protein 1) AT4G33720	2.557941	0.6953	1.0109	3.56886	1.7062	3.253237
TC134815	CBL-interacting serine/threonine protein kinase AT5G01820	0.537437	−0.44148	0.8504	1.38782	0.4089	0.095956

All expression levels are compared on a $\log_2$ scale. tBLASTx analysis was conducted to determine similarity to the Arabidopsis genome and function was assigned when e-values were less than 10^{-5}

in naturally dormant buds. It is noteworthy that expression of genes involved in the cell cycle are down in naturally dormant buds, but are up in both CICP and DMN treated buds relative to the non-dormant buds. The reduced cell cycle activity could result from the increased ABA levels in the naturally dormant buds since ABA is known to induce cell cycle inhibitors such as KIP-RELATED PROTEINS (KRPs) (Wang et al. 1998). Dormancy progression in potato is associated with a reduction in the ABA content of tuber tissues (Destefano-Beltran et al. 2006b). Treatment of potato tubers with DMN and CIPC results in sprout inhibition without the elevated ABA levels associated with dormant tissues indicating that growth suppression is not a result of presence this phytohormone. However, DMN results in the induction of transcripts encoding for proteins involved with ABA-controlled metabolic processes regulated by products of *ABI1*, *ABI2*, and *ABI3* (Finkelstein and Somerville 1990) (Table 1). The alteration of transcripts that encode for proteins associated with the ABA signal transduction pathway by DMN, without appreciable concurrent changes in ABA levels, might involve stress or osmotic-induced modification of gene expression. This is supported by DMN alteration of transcript levels encoding for the stress induced osmotin and taumautin. Comparable ABA levels were found in both CIPC or DMN growth suppressed tissues but CIPC treatment did not result in alteration of the ABA associated transcripts common to DMN exposure. This demonstrates that growth suppression by CIPC and DMN functions through different mechanisms, and that DMN treatment differentially affects components of the ABA signal transduction pathway.

Results demonstrate that neither hormone content nor gene expression patterns in growth-arrested meristems resemble those associated with endodormancy and therefore neither sprout inhibitor acts to extend the natural period of tuber dormancy. Further, both CIPC and DMN treatments elicit some similar and some distinct gene expression patterns indicative of overlapping and non-overlapping mechanisms of action. In conclusion, the sprout-inhibiting actions of CIPC and DMN are not associated with maintenance of dormancy-related ABA content or gene expression and therefore do not appear to reflect an extension of tuber dormancy *per se*. The distinct patterns of gene expression in CIPC and DMN-treated meristems suggests different mechanisms of action for these two classes of sprout inhibitors that, in the case of DMN, may involved components of the ABA signal transduction pathway.

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